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Original scientific paper

ALLELOPATHIC INFLUENCE OF JUGLANS REGIA L. AQUEOUS EXTRACT ON THE GERMINATION AND THE GROWTH OF LETTUCE AND TOMATO

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ABSTRACT

The allelopathic effect of the common walnut *Juglans regia* L. was examined by bioanalysis of the extract on seed germination and growth of lettuce (*Lactuca sativa* L.) and tomato (*Solanum lycopersicum* L.). Aqueous extracts of leaves of different concentrations (concentrated extract, 1:2, 1:4 and 1:8) were made to determine the allelopathic potential, and distilled water was used as a control. The obtained results showed that the lettuce is more sensitive, and the tomato is more tolerant to the applied concentrations of the aqueous extract of juglone. All applied concentrations inhibited lettuce germination, while lower concentrations had stimulating effect on growth. In tomatoes, the inhibitory effect was fond in concentrated solution and 1:2, while lower concentrations had a stimulating effect. Allelopathy is a unique phenomenon in nature that is relatively new in science, and the results of this experiment are not only important in botanical research, but also have application in practice.

Key words: allelopathy, walnut, *Lactuca sativa* L., *Solanum lycopersicum* L.

INTRODUCTION

Allelopathy is a biological phenomenon that refers to the positive or negative, direct or indirect impact of one plant, fungus or microorganism on another, by creating chemical compounds (allelochemicals) that are released into the environment (Rietweld, 1983; Rice, 1984; Scott & Sullivan, 2007). Allelochemicals are released from plants by a variety of mechanisms that include degradation of plant debris, leaching, evaporation from leaves and stems, and excretion from roots (Rice, 1984; Kocacalaliskan & Terzi, 2001; Scott & Sullivan, 2007; Inderjit & Malik, 2011). The allelopathic influence of the black walnut (*Juglans nigra*) and its relatives is one of the oldest examples of allelopathy (Davis, 1928; Rice, 1984). It is caused by the phenolic compound juglone isolated from many species of the walnut family (including the common walnut), whose colorless, reduced, non-toxic form of hydrojuglone is abundant in the leaves, walnut shells, stem and roots (Terzi, 2008). The release of allelopathic substances plays an important role in the distribution, population density and reproduction of plants, especially for plants in the natural environment and agroecosystem. Application of allelopathic principles in practice can reduce the overuse of pesticides and fertilizers, increase crop growth and yield, and can serve as a weed control measure (Scott & Sullivan, 2007; Inderjit & Malik, 2011; Rodino et al., 2016).

Most plant cultures are sensitive to juglone toxicity (Rietveld, 1983; Terzi, 2008; Scott & Sullivan, 2007). Previous research has shown that black walnut (*Juglans nigra* L.) secretes juglone which prevents the growth of tomatoes and alfalfa (Lambers et al., 1998). Jose & Gillespie (1998) report that maize (*Zea mays* L.) and soybean (*Glycine max* L. Merr.) are sensitive to black walnut juglone. Juglone from walnuts (*Juglans regia* L.) also has an inhibitory effect on strawberries, reducing fruit yield, average fruit weight, root weight (Ercisli et al., 2005). Qin et al. (2011) noted that allelochemicals from walnuts negatively affect the germination of tomatoes, cabbage, beets and lettuce, and that the germination of corn increases.

This study is based on the fact that juglone has an inhibitory effect on the growth of vegetable crops, so understanding the mode of action is of particular importance for agriculture. Therefore, the aim of the study was to examine the effect of different concentrations of *Juglans regia* L. leaf extract on germination and initial growth stages of tomato (*Solanum lycopersicum* L.) and lettuce (*Lactuca sativa* L.).

MATERIAL AND METHODS

Seven-year-old walnut leaves were used and samples were collected in the first week of August, because according to some literature data, the concentration of juglone was determined to be the highest in the last week of July and the first week of August (Tekintas et al., 1988). Aqueous extracts were prepared from fresh, then dried walnut leaves according to the Norsworthy (2003) method. The leaves were dried for 48 h in an oven at 70 °C. Later, we immersed 100 g of dry plant material (ratio 1:10) in 1000 ml of distilled water and kept it at room temperature for 24 h. After 24 h, the upper layer of suspension was decanted and filtered through quantitative filter paper. The following concentrations were used in the experiment; walnut leaf extract (undiluted); walnut leaf extract (diluted 1:2), walnut leaf extract (diluted 1:4) and walnut leaf extract (diluted 1:8). Distilled water was used for control.

In the experiment, we used the seed material of two test plant species, tomato (*Solanum lycopersicum* L.) and lettuce (*Lactuca sativa* L.). The seeds were first surface sterilized with 1% sodium hypochlorite for 5 minutes, and then washed several times with distilled water. In each sterilized petri dish, 2 layers of filter paper were placed, in which we then transferred 30 salad and tomato seeds with tweezers and 2 ml of each prepared extract. Each treatment was repeated three times. The vessels were left in a thermostat at 26 ºC in the dark. The percentage of germination was monitored 3, 5 and 7 days from the beginning of the experiment.

At the end of the experiment, the influence of different concentrations of aqueous juglone extract isolated from walnut leaves on the germination percentage, mean germination time (MGT), seed germination rate (GI) and growth parameters (root length and height of tomato and lettuce shoots) was monitored.

The percentage of germination was calculated by the following formula:

Germination% = Number of germinated seeds / Total number of seeds at the beginning of the experiment × 100

Mean Germination time (MGT) - calculated by the formula (Elis & Roberts, 1981):

$$MGT = \Sigma (Dn) / \Sigma n$$

where n is the number of seeds that germinated on day D, and D is the number of days since germination began

Germination rate (GI) was calculated according to the following formula (Khandakar & Bradbeer, (1983)

$$GI = n1 / d1 + n2 / d2 + n3 / d3 + Nn/n \times 100$$

where n = number of germinated seeds germinating 1, 2, 3… n-days after setting up the experiment, d = number of days

All data were statistically processed in the program SPSS 20.0 (Statistical Package for the Social Sciences). The analyzes 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

Aqueous extracts of juglone significantly inhibited the germination of tomato seeds and lettuce compared to the control. The obtained results showed that there was no germination of tomatoes on the third day, while in lettuce it occurred but in a smaller percentage. Treatment of tomato seeds (5 and 7 days) with juglone extract (concentrated extract and 1:2) significantly inhibited germination (while 50%), while lower concentrations (1:4 and 1:8) partially stimulated seed germination. The germination of lettuce seeds in the control variant averaged 78%, while in the treatment with the aqueous extract it ranged from 5% in the concentrated extract, 30% in the extract of 1/2 and 50-56% in the variant with the extract of 1:4 and 1:8. The allelopathic effect of aqueous juglone extract was more reflected in lettuce germination with significant statistical differences between control and applied concentrations (Figure 1A and B).

In the control treatment, the average Germination Time (Mean Germination Time - MGT) of tomatoes was the fastest and was 6 days. The results indicate significant differences between control and treatment (1/2 and 1/4) on average germination time (Figures 2A and B).

Compared with the control, the concentrated inhibitor solution had the greatest inhibitory effect, while at lower concentrations of MGT tomatoes it was similar to the control. Also, it is noticed that the average germination time was longer in tomatoes compared to lettuce. MGT of lettuce was 5.2 days, with statistically significant differences found between control and applied aqueous extracts (Figures 2A and B).

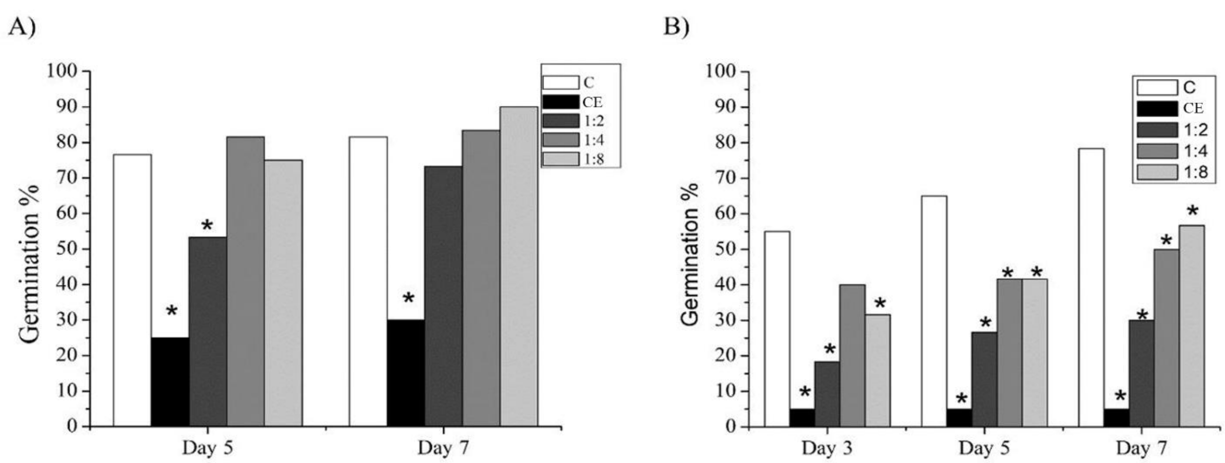

Figure 1. Germination (%) of tomato seeds (A) and lettuce (B) treated with different concentrations of aqueous juglon extract. The symbol* indicates a statistically significant difference between control and treatment in the same period ($p < 0.05$).

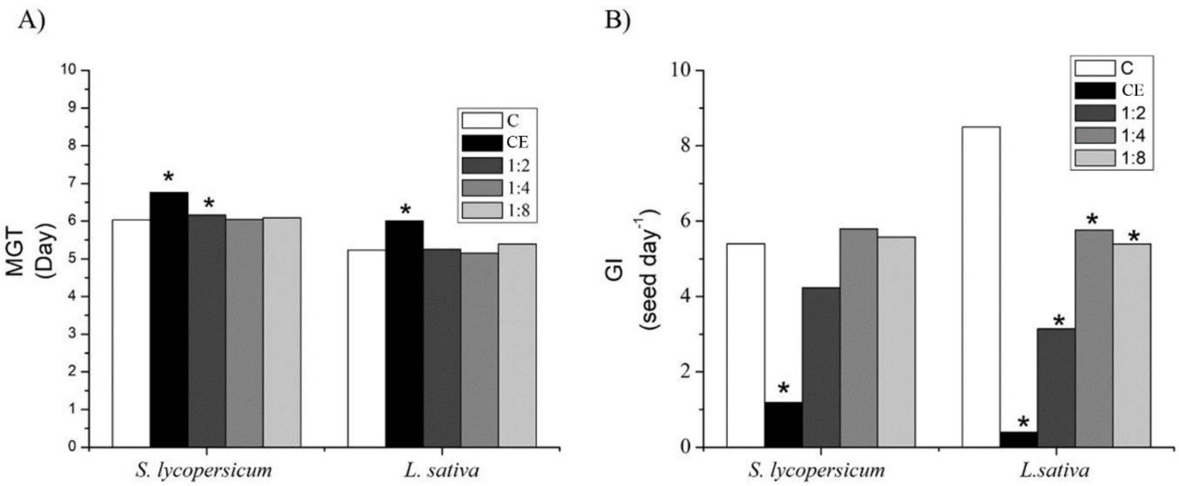

Figure 2. Mean germination time (MGT) (A) and germination index (GI) (B) of tested species in control and at different concentrations of Juglans regia L. extract The symbol* indicates a statistically significant difference between control and applied treatments in one tested species
($p < 0.05$).

The applied concentrations of aqueous extract of juglone significantly influenced the germination index of tomato and lettuce seeds. Juglone treatment (concentrated and 1:2) significantly reduced the tomato seed germination index, while lower concentrations (1:4 and 1:8) had a stimulating effect.

The leaf extract had a greater effect on reducing the germination index of lettuce compared to tomatoes. Lettuce germination index was the highest in the control and then decreased proportionally with decreasing extract concentration. Significant differences were noted in GI of lettuce between control and applied concentrations of walnut extract.

Root and shoot growth is one of the parameters on which juglone has a great influence (Tekintas et al., 1988). Concentrated juglone extract inhibited tomato root growth approximately three-fold, while lower concentrations promoted growth to a lesser extent (Figures 3A and B). All applied concentrations of test extract inhibited lettuce root growth (Figures 3A and B) relative to the control variant. The maximum length of lettuce root was measured in the control treatment and was 2.90 cm. In the concentrated extract, the root length was significantly inhibited compared to other applied concentrations. At lower concentrations (1:2 and 1/:4) the average length of lettuce root was half as short, while at a concentration of 1:8 it was slightly shorter than the control. An interesting observation in this paper are the blackened root tips of tomatoes and lettuce during germination in juglone extracts (Figure 4A and B). Although the reason for this is still unknown, it is thought to be related to the sensitivity of the roots to juglone (Terzi, 2008).

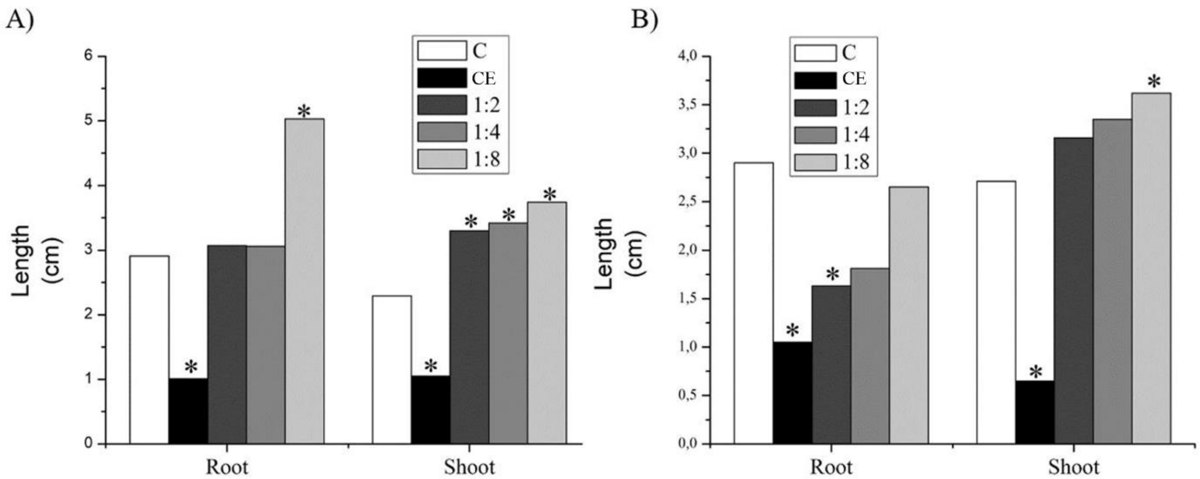

Figure 3. Influence of different concentrations of *Juglans regia* leaf extract on root length and seedlings height in tomato (A) and lettuce (B). The symbol * indicates a statistically significant difference between control and applied treatments in one tested species ($p < 0.05$).

The allelopathic effect on pony growth in treatments with aqueous juglone extracts depended on the concentration applied. The highest concentration completely inhibited the growth of lettuce and tomato seedlings, compared to the control. In contrast, the applied concentrations of juglone (1: 2, 1: 4 and 1: 8) in our experiment partially stimulated the growth of tomato and lettuce shoots compared to the control variant.

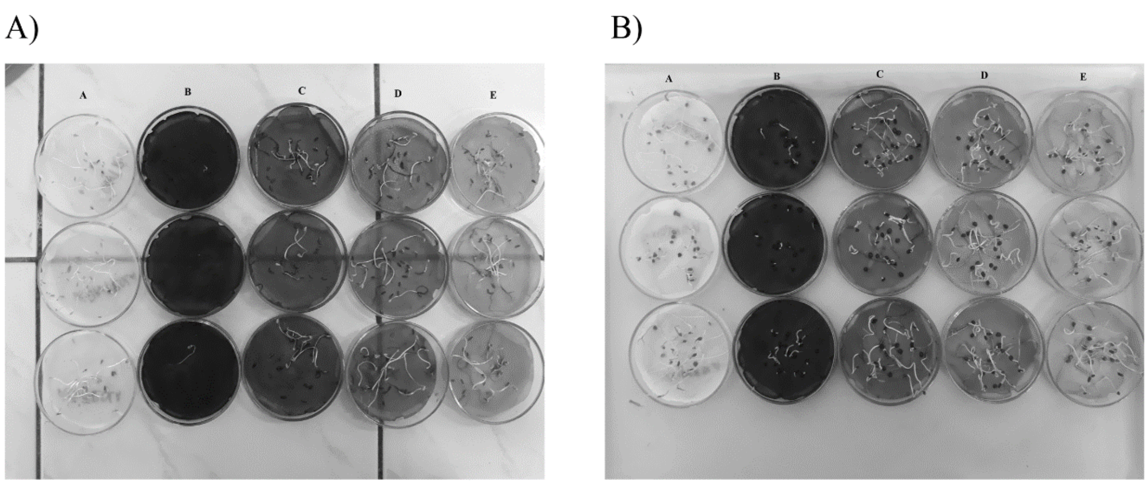

Figure 4. Allelopathic effect of different concentrations of juglone extracted from walnut leaves (A-control; B-concentrated extract (CE); C-1:2; D-1:4; E-1:8) on tomatoes (A) and lettuce (B)

DISCUSSION

Most literature data indicate that the effect of juglone on plants is mostly toxic, and that it is an obstacle in the germination and growth of seeds of many species (Rietveld, 1983; Rice, 1984; Tekintas et al., 1988; Qin et al., 2011). Juglon extracts isolated from the leaves of *Juglans regia* caused a decrease in seed germination, a decrease in germination index, prolonged germination time and led to poorer initial growth of young plants. The results of the research in this paper confirmed the fact that the allelopathic effect in the first stages of germination is more pronounced and more toxic compared to the later stages of growth (Fig. 1A and B). It was observed that both investigated species were sensitive to juglone, with significant differences in the effect on germination, which are attributed to different concentrations and its action. Tomato germination and growth was most inhibited by concentrated juglone, while lower applied concentrations partially stimulated growth. On the other hand, in relation to tomatoes, all applied concentrations of juglone had a strong inhibitory effect on lettuce germination. Previous studies (Qin et al., 2011) have found that juglone inhibits the germination of tomatoes, cabbage, beets and lettuce, and stimulates the germination of corn. Juglon has also been found to inhibit the germination of garden cress, tomato, cucumber, alfalfa, radish and watermelon seeds (Kocaçalışkan & Terzi, 2001; Terzi & Kocaçalışkan, 2009) in earlier researches. On the other hand, Terzi (2008) and Kocaçalişkan et al. (2008) showed in their research that melon is very tolerant of juglone. The germination rate was maximal in the control variant and lowest in the concentrated juglone solution, to decrease proportionally with decreasing extract concentration. Therefore, it is obvious that the allelopathic effect depends primarily on the applied concentration. This has been confirmed in other studies (Rietveld, 1983; Kocaçalışkan & Terzi, 2001; Terzi, 2008; Islam et al., 2014).

In both studied species, root and shoot growth was inhibited in the concentrated *Juglans regia* extract compared to the control variant. Lower concentrations (Figures 3A and B) to some extent stimulated tomato root and shoot growth. Under the influence of juglone, there was a decrease in root and shoot growth of many plants, including soybean (Jose & Gillespie, 1998), tomato (Milewska-Hendel, 2017), eggplant and pepper (Kocaçalışkan et al., 2019), cucumber (Terzi et al., 2003), and also for some trees and shrubs (Funk et al., 1979). Contrary to these studies, Kocaçalışkan et al. (2008) and Terzi (2008) did not record an inhibitory effect on melon germination and growth under the influence of juglone extract. Given the fact that allelochemicals may be beneficial for one species and harmful for others and that this depends on the type and concentration of allelochemicals and the duration of treatment (Rice, 1984; Scott & Sullivan, 2007), it is assumed that this is one of the reasons for the different responses of the studied species to juglone treatment. Rietveld (1983) in their study of 16 plant species showed that juglone concentration is very important in determining the detrimental effect on plant growth and that this effect is different on seed germination and plant growth depending on the applied concentration. In their research, they have shown that lower concentrations of juglone (in the range of 10^{-4} M to 10^{-6} M) stimulate the germination of some species. In most experiments, a concentration of 10^{-3} M juglone is very detrimental to plant growth (Rietveld, 1983; Milewska-Hendel, 2017). The same was observed in this paper, i.e. the positive effect of lower concentrations of juglone on germination and plant growth and the harmful effect of higher concentrations.

CONCLUSION

The results of the research in this paper showed that the influence of the aqueous extract of *Juglans regia* leaves varied depending on the applied concentration and the examined species. Both species were sensitive to juglone, but lettuce proved to be far more sensitive to all applied concentrations. The results of the research showed that there are suppressive effects of higher concentrations (concentrated and 1:2) on germination and growth of tomato and lettuce roots and sprouts, which would ultimately lead to lower yields when growing this vegetable in the immediate vicinity of walnut trees. Knowing this, as well as the fact that most vegetable crops are sensitive or susceptible to the influence of juglone, the plants should be grown far from the place where walnut trees grow.

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