

## THE GERMINATION OF *Triticum aestivum* L. AND *Pisum sativum* L. SEEDS TREATED WITH WALNUT LEAF AQUEOUS EXTRACT

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**ABSTRACT.** Allelopathy determines the dynamics of germination, development and growth of plant species in the environment. Therefore, in this study, the allelopathic effect of an aqueous extract isolated from walnut (*Juglans regia* L.) leaves (concentrated extract, 1:2, 1:4 and 1:8) on the germination percentage, mean germination value and germination rate of wheat (*Triticum aestivum* L.) and peas was monitored (*Pisum sativum* L.). Both tested species were sensitive to juglone, but the percentage of germination was significantly lower in wheat (by 80%) compared to peas, so it can be said that this species is more sensitive. The length of roots and shoots of both tested species decreased in proportion to the increase in the concentration of the extract. Monitoring of this biological phenomenon could help to understand the changes in the environment caused by allelochemicals.

**Keywords:** allelopathy, germination, growth, walnut extract, peas, wheat

### INTRODUCTION

Allelopathy is a natural phenomenon during which organisms (plants, algae, bacteria and fungi) release allelochemicals that affect the dynamics of germination, growth, survival and reproduction of other organisms in the environment (MALLIK and INDERJIT, 2002). Allelochemicals are known to be present in many plant species and to be released into the rhizosphere by a variety of mechanisms, including residue degradation, leaf and litter leaching, evaporation and root excretion (MALLIK and INDERJIT, 2002; SCOTT and SULLIVAN, 2007; TERZI, 2008; KOCAÇALIŞKAN and TERZI, 2001). One of the oldest examples of allelopathy is the inhibitory effect of walnut (*Juglans nigra* L.) on other plant species in the environment. The chemical that causes allelopathy in walnuts is juglone (5-hydroxy-1,4naphthoquinone) (ERCISLI *et al.*, 2005). Juglone has been isolated from many plants of the walnut family (*Juglandaceae*), including *J. nigra*, *J. regia* L. and others. A non-toxic reduced form without color, which is called a harmless hydrojuglone, when exposed to air or another oxidizing substance, it oxidizes into a poisonous form, juglone. Rain washes the juglone from the leaves into the soil, and so plants growing near the walnut adopt juglone through the roots (KOCAÇALIŞKAN and TERZI, 2001; SCOTT and SULLIVAN, 2007; TERZI, 2008). Most crops are sensitive to juglone toxicity, so it is extremely important to choose tolerant species for planting in walnut-associated sites.

Studies by KOCAÇALIŞKAN and TERZI, (2001) showed that the growth and germination of tomato, cucumber and alfalfa were inhibited by juglone extract, and that melon germ growth increased under this treatment. Plants sensitive to the presence of walnuts include many vegetable species such as tomato, potato, pea, cucumber, watermelon, beans, corn, wheat, soybeans, alfalfa and many ornamental plants (KOCAÇALIŞKAN *et al.*, 2019) while some plant species, as onions or some maize cultivars, are tolerant to juglone (CVETIĆ ANTIĆ and MILENKOVIĆ, 2015).

The effect of juglone on seed germination and seedlings growth has not yet been sufficiently studied. Therefore, this study aimed was to examine the allelopathic effect of an aqueous extract isolated from walnut leaves on wheat (*Triticum aestivum* L.) and pea (*Pisum sativum* L.) seed germination and seedlings growth.

## MATERIALS AND METHODS

Plant material was collected during the summer taking the leaves from 7-year-old walnut trees, to obtain the extract, because the younger trees do not contain enough juglone to cause toxicity. Aqueous extracts were prepared from fresh, then dried walnut leaves according to the method described by NORSWORTHY (2003). Walnut leaves were dried in an oven at a temperature of 70 °C for 48 h. Then, 100 g of dry plant material was immersed in 1000 mL of distilled water and left at room temperature for 24 h, after which the mixture was filtered through filter paper to obtain a 10% walnut leaf aqueous extract.

The treatment was as follows: control – distilled water (C), undiluted (concentrated) extract – 10% (CE), diluted in 1:2 ratio (3.3%), diluted in 1:4 ratio (2%) and diluted in 1:8 ratio (1.25%). The aqueous extracts were stored at 4 °C until the start of the experiment. Seed material of two test plant species, common wheat (*Triticum aestivum* L.) and pea (*Pisum sativum* L., cv. Petit Provencal) was used in the experiment. As the first, the seeds surface was sterilized with 1% sodium hypochlorite for five minutes, and then washed several times with distilled water. Then, 30 seeds of wheat or pea were transferred with tweezers to Petri dishes with filter paper moistened by distilled water (control) or prepared treatments (4 ml of each prepared was used for pea and 1 mL for wheat). Each treatment was repeated three times. The prepared Petri dishes were placed for germination in a thermostat at 26 °C.

After germination, the influence of different concentrations of walnut leaf aqueous extract on the percentage of germination, mean germination time (MGT), seed germination rate (GI) and growth parameters (root length and height of wheat and pea sprouts) were monitored.

The percentage of germination was monitored for 3, 5 and 7 days, and was calculated according to the following formula:

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

Mean Germination time (**MGT**) was calculated by the formula (ELLIS and ROBERTS, 1981):

$$\text{MGT} = \Sigma (\text{D} \times \text{n}) / \Sigma \text{n}$$

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

**Germination index (GI)** was calculated according to the following formula (KHANDAKAR and BRADBEER, 1983):

$$\text{Germination index} = (\text{n}_1 / \text{d}_1 + \text{n}_2 / \text{d}_2 + \text{n}_3 / \text{d}_3 + \text{Nn} / \text{n}) \times 100$$

where **n** is the number of germinated seeds germinating 1, 2, 3... n days after setting up the experiment, **d** – number of days.

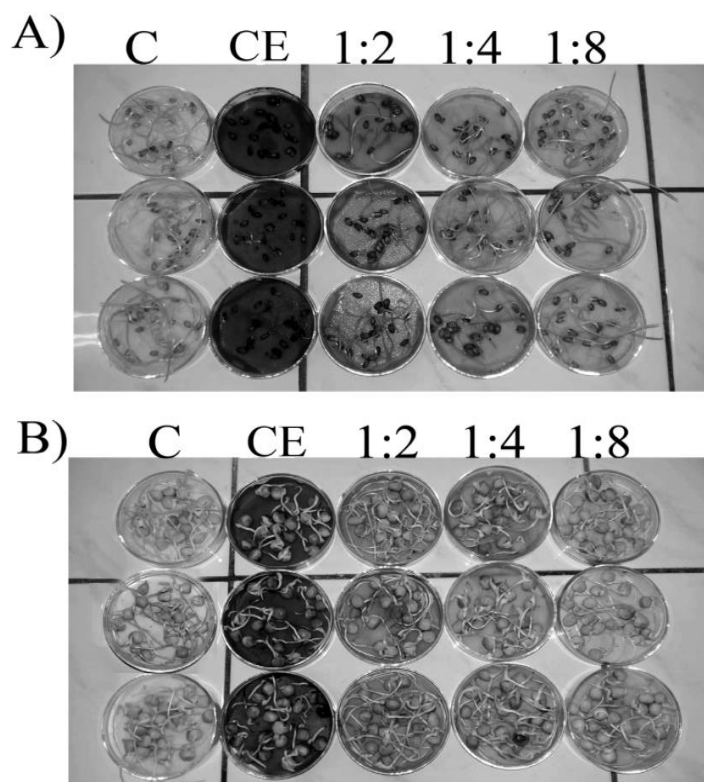


Figure 1. Allelopathic effect of different concentrations walnut leaf aqueous extract on seeds of wheat (A) and pea (B).

The results of all analyzes are presented as an average of three replicates  $\pm$  standard deviation (SD). Statistical processing of the obtained results was done in the OriginPro 8 program, while the differences between the average values of the results were determined by analysis of variance (One-Way ANOVA), and the Tukey test at the significance level of 5% ( $p < 0.05$ ) was used to assess statistical significance.

## RESULTS AND DISCUSSION

Stress caused by juglone is called allelochemical stress, which causes a stressful situation in many plants (KOCAÇALIŞKAN and TERZI 2001; TERZI, 2008). Juglone is a well-known compound present in walnut leaves and it is known as inhibitor of the germination of various plant species, which we also noted in this paper. The results obtained in our experiments showed that the tested species were sensitive to the walnut leaf aqueous extract, but with different reactions depending on the applied concentration.

Walnut leaf aqueous extract inhibited the germination of wheat seeds in all treatments compared to the control (Figures 2A). The percentage of wheat germination increased from the third to the seventh day, with the highest percentage recorded in the control, and the lowest in the highest concentration of the extract (CE and 1:2). Significant statistical differences were observed between control and applied treatments during monitoring of wheat seed germination percentage (Figure 2A).

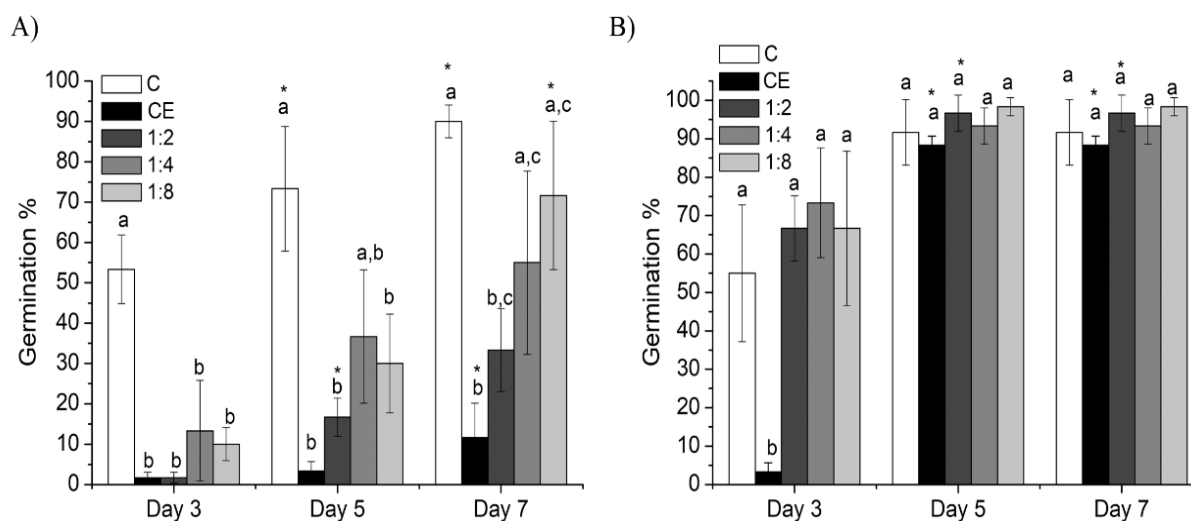


Figure 2. Germination % of wheat (A) and pea (B) seeds treated with different concentrations of walnut leaf aqueous extract. Different lowercase letters *a*, *b*, *c* indicate a statistically significant difference between treatments in the same period, while the symbol \* indicates a statistically significant difference in the same treatment that occurred over time (day 5 or 7) compared to day 3 ( $p < 0.05$ ).

Compared to wheat, a higher percentage of germination was recorded in pea in all treatments, and it was noticed that germination was the lowest on the third day, while on the fifth and seventh it was increased (Figure 2B). The inhibitory effect of walnut leaf aqueous extract on pea germination was determined at the lowest dilution (CE and 1:2), while at a dilution of 1:4 and 1:8 no inhibitory effect was observed, but germination was the same as in the control group. The obtained data indicate that no significant statistical differences were found in peas during the study period, except between the control and concentrated walnut leaf aqueous extract (CE).

Not any walnut leaf aqueous extract concentration led to a statistically significant change of MGT in comparison with control, both in wheat and in pea (Figure 3A).

The germination index (GI) of pea was significantly higher compared to wheat (Figure 3B), and it can be noticed that it was higher in the control treatment in both tested species. In wheat, GI was maximal at the lower extract concentrations (1:4 and 1:8) and minimal at the higher extract concentrations (CE and diluted in ratio 1:2). The concentrated walnut leaf aqueous extract had the greatest effect on the reduction of the GI, with wheat being more pronounced in relation to peas (Figure 3B). The pea GI was higher in the extract concentrations of 1:2, 1:4 and 1:8, while the minimal was recorded in the CE. It is also observed that the GI of peas in the control variant was lower compared to the treated pea seeds, even though those differences were not statistically significant. For the wheat a significant statistical difference in GI was registered between the control and the applied treatments, while between the individual applied concentrations of walnut leaf aqueous extract was not recorded. On the other side, for the pea different value of GI between CE and other applied treatments was recorded (Figure 3B).

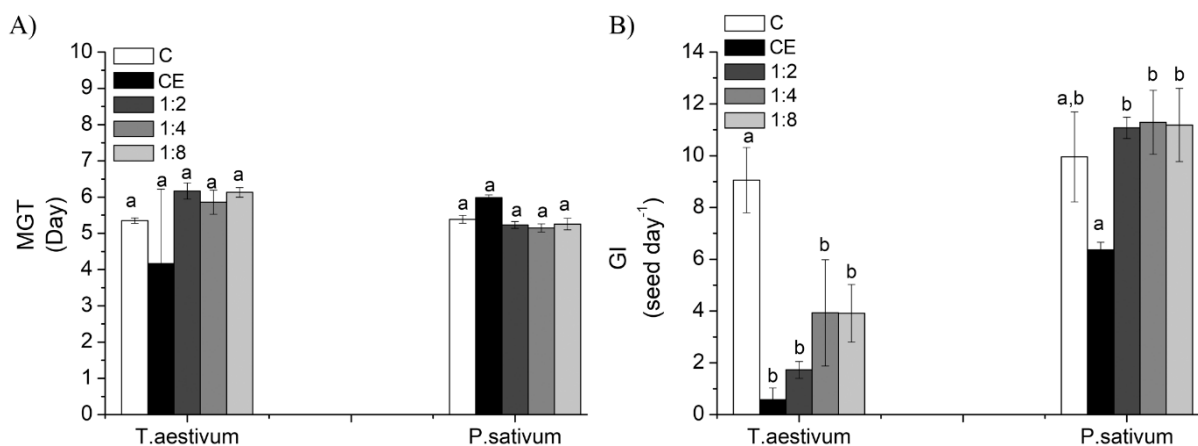


Figure 3. Mean germination time (MGT) (A) and germination index (GI) (B) of the tested species in the control and at different concentrations of walnut leaf aqueous extract.

Different lower case letters *a*, *b*, indicate a statistically significant difference between treatments of one test species ( $p < 0.05$ )

PAVIĆEVIĆ (2013) in his research noted that juglone has an inhibitory effect on wheat germination, which we also noted in this paper. Different results for wheat in which germination was not inhibited by juglone were recorded by KOCAÇALIŞKAN and TERZI (2001), even though these results could be dependent on cultivar. On the other hand, according to our results in pea experiments only concentrated extract inhibited germination, while other concentrations (1:2, 1:4 and 1:8) had, surprisingly, a certain stimulating effect on germination. Based on that, it can be concluded that *P. sativum* is less sensitive species to allelochemicals present in walnut leaves than *T. aestivum*. This data could be useful for application in plant cultivation. In the same study, the allelopathic effects of juglone did not affect the germination of wheat, barley, corn, watermelon, radish, and beans, but the growth of seedlings was inhibited (KOCAÇALIŞKAN and TERZI, 2001). On the other hand, in the TERZI's study (2008) juglone and diluted extracts of walnut juice reduced the germination and growth of cucumber seedlings, while they did not show any inhibitory effect on melon seeds. The reason for different results could be the testing of different wheat and pea varieties of, but also different temperatures and light conditions (DASS *et al.*, 2005; KUMAR *et al.*, 2015).

The physiological role of allelochemicals is still not sufficiently elucidated. Allelochemicals can be beneficial to one, but harmful to another plant, which we also noted in this paper. ORCUTT and NILSEN (2000) consider that certain plant species are more tolerant of allelochemicals because of possessing ability to reduce the intake of allelochemicals through the roots, and to localize or detoxify them. Our research has shown that it depends on the type, concentration of juglone and the duration of treatment. The research of MATVIENKO *et al.* (2001) showed that certain plant species release enzymes that regulate the biosynthesis of compounds in which hydrojuglone becomes less toxic. Such plants have tolerance to juglone-induced stress, which is probably one of the reasons for the different behavior of the studied species to juglone treatment. ISLAM *et al.* (2014) by monitoring the allelopathic effect of *Chenopodium murale* on the germination rate of wheat, corn, chickpea and mung beans, found it was maximal in the lowest concentration of the extract and minimal in the highest concentration of the extract, which can be related to the results obtained in our study in both studied species.

Walnut leaf aqueous extract (concentrated extract, 1:2, 1:4 and 1:8) significantly disrupted growth parameters. Figures 4A and 4B show the mean values of root and seedlings length of both tested species. Root growth was significantly reduced under the influence of

walnut leaf aqueous extract in both test species (Figures 4A and 4B). In that sense lower influence was recorded at pea seeds since only in CE treatment root growth was significantly reduced. In wheat experiments it was observed that the largest root length was in control (13.75 cm), while the lowest was found in CE (0.62 cm). In pea experiments, the root length was greatest at a lowest concentration of 1:8 (6.56 cm), while in concentrated extract it was significantly reduced (2.38 cm). A significant statistical difference was found in the control and concentrated and walnut leaf aqueous extract diluted to 1:2, while in the treatment of 1:4 and 1:8 no significant statistical difference was found. The maximum height of wheat seedlings was determined in control (7.83 cm), while in pea it was recorded in a dilution of 1:4 (1.91 cm). Plant growth decreased in proportion to the increase of walnut leaf aqueous extract concentration, but the extract concentrations of 1:2 and 1:8 stimulated the growth of pea, even though these values were not significantly higher. In wheat, a significant statistical difference was observed between control and CE and walnut leaf extract diluted to 1:2. In contrast to wheat, no significant statistical difference was found in pea between the control and the applied treatments, with the differences in growth being evident between the control and the CE.

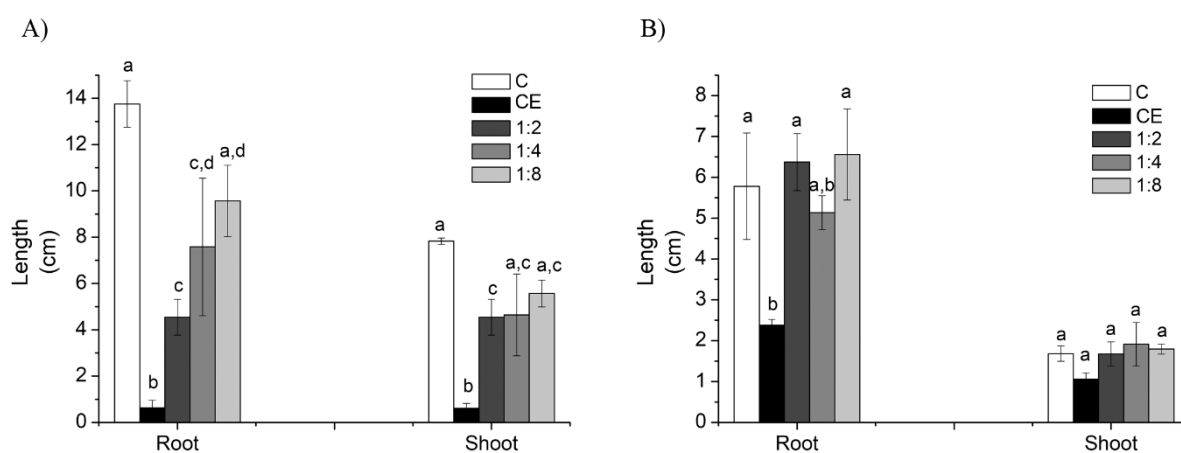


Figure 4. Influence of different concentrations of walnut leaf aqueous extract on root length and seedlings height in wheat (A) and pea (B). Different lower case letters *a*, *b*, *c*, *d* indicate a statistically significant difference of the measured parameter between treatments of one examined species ( $p < 0.05$ ).

Allelopathic effects accelerating growth are usually rare (MALLIK and WILLIAMS, 2005). The results of our study show that there are stimulatory (low concentrations) and inhibitory (high concentrations) effects of aqueous extract of walnut leaves on the growth of wheat and peas. At pea, root growth was stimulated by lower concentrations of juglone extract (1:4 and 1:8). Similar results on the influence of juglone were obtained by KOCAÇALIŞKAN and TERZI (2001 in tomatoes, cucumbers, wheat, barley, corn and beans. In other studies, soybeans have been reported to be extremely sensitive, with root growth significantly inhibited by juglone. TERZI (2008) in his study noted the inhibitory effect of juglone on cucumber, while melon was tolerant. In our study when it comes to seedlings, compared to the control all applied concentrations inhibited wheat growth, while in peas the lowest concentrations had a stimulating effect (1:4 and 1:8). In general, the juglone effect was more reflected in the growth of seedlings of both species than in the growth of roots. This results in the fact that if wheat and peas are found near walnuts, their growth will likely be endangered and later the yield will be reduced. ÇIÇEK and TILKI (2007) in their research state that larger seeds have a higher survival rate (80%) and develop a larger diameter root, while smaller seeds have a significantly lower

survival rate (71%), which can be related to the results obtained in this paper since the pea has larger seeds than wheat.

The allelopathic effect of walnut leaf aqueous extract pronounced in both studied species (wheat and pea), which reacted differently depending on the applied concentration. In our study, the wheat germination was inhibited in all treatments, and was shown to be more sensitive to walnut leaf aqueous extract. In pea, only the concentrated extract inhibited germination, while lower concentrations did not express a negative effect. The growth of wheat seedlings was inhibited at all concentrations of walnut leaf aqueous extract, while in pea lower concentrations promoted growth. Therefore, it is obvious that the allelopathic effect depends on the applied concentration and the studied species. The data obtained in this paper are useful for comparing the susceptibility of species to walnut leaf aqueous extract, especially in the first stages of growth and development, which is of great importance for the cultivation of these species under field conditions.

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