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In Vitro effect of angelica (*Angelica archangelica* L.) hydrolate on soybean (*Glycine max* L. Merr.) and winter wheat (*Triticum aestivum* L.) seed germination

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SUMMARY

Soybean (*Glycine max* L. Merr.) and winter wheat (*Triticum aestivum* L.) are among the key agricultural crops for which germination and early growth are crucial for achieving high yields. In this study, the biostimulant potential of *Angelica archangelica* hydrolate, a by-product obtained during essential oil distillation, was evaluated on soybean and winter wheat seeds. Seeds were treated with hydrolate solutions at different concentrations (0.25%, 0.5%, 1%, 5%, and 10%), and germination tests were conducted under controlled laboratory conditions. The results show that hydrolate treatments significantly increased seedling length in both crops compared to the control. The highest growth was recorded at the 10% concentration for both soybean (5.77 cm) and wheat (3.13 cm). Lower concentrations also improved growth, though with less pronounced effects. These findings confirm that *A. archangelica* hydrolate positively influences germination and early seedling development, suggesting its potential use as a natural biostimulant in sustainable agriculture. Since hydrolates are environmentally safe and underexplored in agricultural practice, further research under field conditions is necessary to optimize their application and fully exploit their potential in promoting plant growth.

Keywords: hydrolate, *Angelica archangelica* L., seed germination, soybean, winter wheat.

INTRODUCTION

Soybean (*Glycine max* L.) is one of the most important leguminous plant species worldwide, primarily due to the highly favourable chemical composition of its grain, which contains about 40% protein and approximately 20% oil. Owing to this composition, soybean has a very wide range of applications – it is used in human and animal nutrition, the pharmaceutical and food industries, and many other sectors of the economy (Popović, 2010). Recent estimates from the Statistical Office of the Republic of Serbia indicate that in 2024, the area under soybean cultivation in Serbia was 206 485 hectares. Winter wheat (*Triticum aestivum* L.), due to its agronomic adaptability, broad cultivation range, and ease of storage and long-term preservation is, together with rice, one of the most important staple foods for the majority of the global population and the primary source of carbohydrates in many countries (Jaćimović et al., 2017). In Serbia, in 2023 the total area under winter wheat was 682 246 hectares (Statistical Office of the Republic of Serbia). The seed germination phase is one of the most important periods in the soybean and winter wheat life cycle, as it determines the success of sprouting, the formation of a uniform plant stand, and the subsequent crop yield. Seed quality and vigour, along with optimal environmental conditions (temperature, moisture, soil structure), directly affect the rate and success of germination (Abd Ghani et al., 2023). In modern agricultural production, increasing attention is given to the use of natural biologically active substances that can contribute to sustainable development and reduce the use of chemical agents. Among these, essential oils are a significant group of bioactive plant compounds, known for their antimicrobial, antioxidant, and biostimulating properties (Oğuz et al., 2023; Costa et al., 2024). *Angelica archangelica* L. is an aromatic medicinal plant from the Apiaceae family whose native range is in the northern temperate region of Europe, as well as the Himalayas (Aćimović et al., 2022; Das et al., 2023). This herbaceous aromatic plant is commercially cultivated in Italy, Germany, Finland, Hungary, and several other countries (Paroul et al., 2002). As a biennial or perennial plant, in the first year of growing, it produces a leaf rosette, forming thick roots which are extracted at the end of the growing season (October - November). The goal of its production is to obtain a high-quality essential oil which is predominantly found in the root. This essential oil has a wide range of applications in various industries, and it is most often obtained by steam or water distillation (Lazarević et al., 2023). In addition to essential oils, several byproducts are produced during distillation. Among them are hydrolates, which may also be useful and appliccable in various industries (Aćimović et al., 2020). The use of *A. archangelica* hydrolates in agriculture, as a by-product in essential oil distillation process and a potential plant biostimulant, has not yet been sufficiently investigated. However, it is known that plant extracts, which often contain phytohormones, flavonoids, and terpenoids, including hydrolates, can significantly affect physiological processes such as water absorption, enzyme activation, and increased chlorophyll synthesis, resulting in improved germination and growth (Tavares et al., 2022). As environmentally friendly alternatives, plant hydrolates can contribute to sustainable agriculture (Truzzi et al., 2021). Given that hydrolates are often

used for human consumption, their application in agriculture provides new opportunities for the development of green technologies.

Therefore, the aim of this research was to evaluate the effect of different concentrations of *A. archangelica* hydrolate on two major field crops: soybean and winter wheat – by assessing their seed germination, with a particular focus on germination rate. Although the use of plant hydrolates in agriculture is still insufficiently studied, this research should provide initial indications that seed treatment with *A. archangelica* hydrolate could positively affect germination and growth, opening opportunities for further research and application of this natural biostimulant.

MATERIAL AND METHODS

The hydrolate of *A. arhangelica* was obtained during the process of steam-distilling the essential oil from the root of this plant, which was cultivated in the experimental field in the Kujavica village (44°4022N; 19°47'09" E) in the 2020/2021 growing season. The distillation process was carried out in a semi-industrial unit for steam distillation as follows: the dried root of *A. arhangelica* was ground and immediately distilled over a period of 4 hours (European Pharmacopoeia). The plant material was placed in the distillation vessel, which was hermetically sealed, and steam supplied from an external high-pressure boiler passed upward through the plant matter. The water vapor carrying the volatile substances moved through a pipeline from the vessel to the condenser and cooler, and was finally collected in a Florentine glass flask (height 1 m and diameter 20 cm, open to drain the hydrolate). After completion of the 4-hour distillation, the essential oil was decanted from the Florentine flask and placed into a separatory funnel. Anhydrous sodium sulfate was added to the funnel to remove traces of water from the essential oil, which was then filtered and stored in a dark glass bottle until further analysis. The hydrolate was drained from the Florentine flask through filter paper into a sterile plastic bottle and stored in the fridge until further analysis.

The effect of the hydrolate of *A. archangelica* was tested on seeds of soybean cultivar Apollo, and winter wheat cultivar Solehio, from the 2024 production year. The seeds were properly stored and kept in the laboratory of the Research and Development Institute Tamiš and used for further experimental work.

Effects of the tested hydrolate on germination were evaluated on soybean and winter wheat seeds using a modified method (Lazarević et al., 2023). Seed germination was carried out in germination chambers of the Seed Laboratory of the Research and Development Institute Tamiš, under controlled conditions at a constant temperature of 20 - 22°C and 60% humidity. The seeds were surface-sterilized with 2% sodium hypochlorite for 2 minutes, after which they were placed on filter paper in a laminar flow cabinet to dry. The tested seeds were treated with the hydrolate of *A. archangelica* at the following concentrations: 0.25%, 0.5%, 1%, 5%, and 10%, while the control involved the use of distilled water. The treatment was performed

by immersing the tested seeds in the previously prepared hydrolate solutions and distilled water for 10 minutes. After treatment, the seeds were transferred to glass Petri dishes (150 × 25 mm) lined with filter paper moistened with distilled water. The experiment was set up on the 19th March, 2025 in five replicates with 50 seeds per replicate for each concentration. Results were recorded after seven days. Length of the seedlings (cm) was measured.

One-factorial ANOVA was conducted to determine the effect of tested hydrolate on winter wheat and soybean seedling length. Multiple comparisons of treatment means were performed using Duncan's test, at a significance level of 5%. Results are presented graphically using a boxplot, with statistically significant differences between treatments highlighted in different lowercase letters. Analyzes were performed using the R Project for Statistical Computing, Version 4.3.2, 2023-10-31ucrt (https://www.R-project.org/).

RESULTS AND DISCUSSION

A one-way analysis of variance (Table 1) established a statistically highly significant ($P < 0.01$) effect of the treatment (different concentrations of *A. archangelica* hydrolate solution) on soybean seedling length (Figure 1). A Duncan's post-hoc test was conducted at a 5% significance level to perform multiple comparisons of mean values among different treatments. The greatest seedling length was achieved with the application of a 10% *A. archangelica* hydrolate solution (5.77 cm), which differed significantly from the values obtained under other treatments. The seedling lengths obtained with treatments using hydrolate solution concentrations of 0.50% (5.34 cm), 1% (5.34 cm), and 5% (5.2 cm) did not differ significantly from each other. The smallest seedling length was recorded in the control treatment with distilled water (3.42 cm), which differed significantly from the values obtained with all other tested concentrations of *A. archangelica* hydrolate solution.

Table 1. Analysis of variance for seedling length in soybean from seeds treated with different *A. archangelica* hydrolate treatments.

Source of variance	Df	Sum of Squares	Mean Square	F-value	P-value
Treatments	5	14.601	2.920	59.059**	0.000
Error	18	0.890	0.049		
Total	23	15.491			

In the case of winter wheat, the application of one-way analysis of variance (Table 2) showed that there was a highly significant effect of the applied treatments ($P < 0.01$) on seedling length. A multiple comparison of treatment means was performed using Duncan's post-hoc test. The highest seedling length value was recorded with the application of 10% hydrolate (3.13 cm), which differed significantly from the values obtained with other treatments and the control. Treatments with 0.25% and 0.50% hydrolate resulted in almost identical seedling length values (2.85 and 2.86 cm, respectively), while the application of more concentrated

hydrolate solutions (1% and 5%) led to smaller seedling length values (2.64 and 2.68 cm). The smallest seedling length value was obtained in the control treatment (1.48 cm).

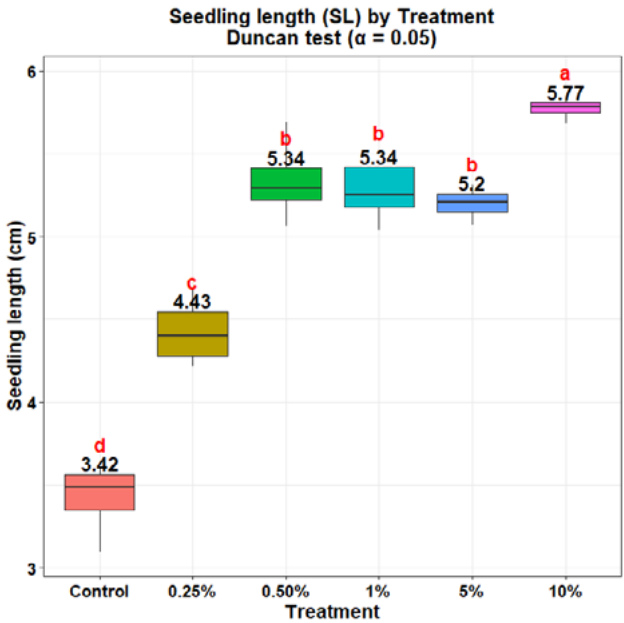

Figure 1. Mean seedling length of soybean under different *A. archangelica* hydrolate treatments (different lowercase letters indicate statistically significant differences at α = 0.05).

Table 2. Analysis of variance for seedling length in wheat from seeds treated with different *A. archangelica* hydrolate treatments.

Source of variance	Df	Sum of Squares	Mean Square	F-value	P-value
Treatments	5	6.655	1.331	106.951**	0.000
Error	18	0.224	0.012		
Total	23	6.879			

The obtained results show that *A. archangelica* hydrolate has a positive effect on seedling length in both soybean and winter wheat. Lazarević et al. (2023) reported that this hydrolate positively affects the length of the hypocotyl and epicotyl in corn, with higher values observed in treatments with higher concentrations of hydrolate solutions, which is consistent with our results. In the case of wheat, it can be observed that the two higher concentrations of hydrolate resulted in shorter seedling lengths compared to the two lowest tested concentrations. A decrease in seedling length with increasing hydrolate concentration was also noted by Lazarević et al. (2023), who tested *A. archangelica* hydrolate on seeds of the weed species *Amaranthus retroflexus*. In that case, the values decreased from 14.5 cm at the lowest to 13.4

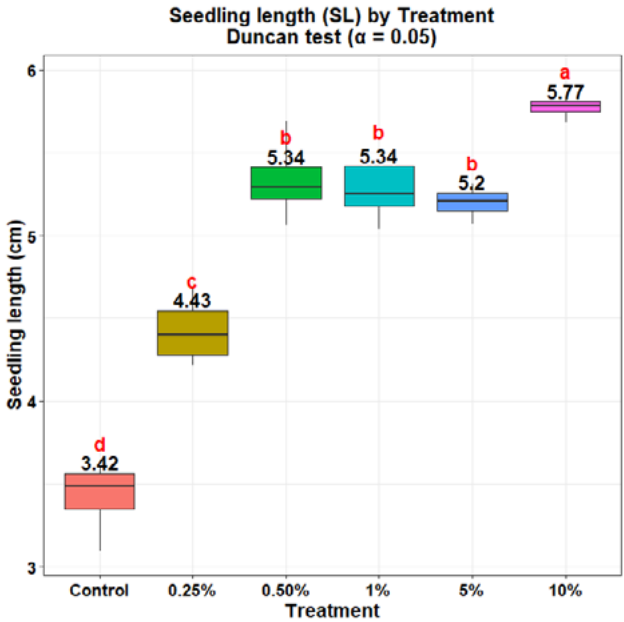

Figure 2. Mean seedling length of winter wheat under different *A. archangelica* hydrolysate treatments (different lowercase letters indicate statistically significant differences at α = 0.05).

cm at the highest tested hydrolate concentration. In general, there are not many studies on this topic – the effect of hidrolates in different agricultural practices. Research has mostly focused on essential oils, but not to the same extent on secondary by-products obtained during the distillation process, such as hydrolats. Our results show the potential use of hydrolats in agriculture, indicating that further research is necessary, both under controlled conditions and in open field conditions.

CONCLUSION

Results of this study demonstrate that *A. archangelica* hydrolate has a significantly positive effect on the germination and early growth of soybean and winter wheat seedlings. The application of hydrolate at different concentrations led to an increase in seedling length compared to the control, with the most pronounced effect observed at the 10% concentration for both crops. These findings indicate that *A. archangelica* hydrolate, a by-product of essential oil distillation, possesses biostimulant properties that can enhance seedling development and could be effectively used in sustainable agricultural practices. Given the limited number of studies addressing the role of plant hydrolates in agriculture, this research provides an important contribution and forms a basis for future investigations. Further studies should focus on

understanding the mechanisms of hydrolate action, optimizing concentrations for different crops, and testing their effects under field conditions. Overall, the use of *A. archangelica* hydrolate represents a promising, eco-friendly approach that supports the development of green technologies and the reduction of synthetic chemical inputs in crop production.

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LITERATURE

Abd Ghani, R., Jolánkai, M., Omar, S., Khalid, N., Tarnawa, Á.: Influence of temperature and variety on seed germination of soybean (*Glycine max* L. Merr.) at different germination times. Acta Agraria Debreceniensis, 2, 5 - 12, 2023.

Aćimović, M., Rat, M., Pezo, L., Lončar, B., Pezo, M., Miljković, A., Lazarević, J.: Biological and chemical diversity of *Angelica archangelica* L. – case study of essential oil and its biological activity. Agronomy, 12, 1570, 2022.

Aćimović, M., Tešević, V., Smiljanić, K., Cvetković, M., Stanković, J., Kiprovski, B., Sikora, V.: Hydrolates - By-products of essential oil distillation: chemical composition, biological activity and potential uses. Advanced Technologies, 9 (2), 54 - 70, 2020.

Costa, J. P., Nasser, V. G., Macedo, W. R., Santos, M. F. C., Silva, G. H.: The biostimulant potential of clove essential oil for treating soybean seeds. Agriculture, 14 (7), 1202, 2024.

Das, S., Chaudhari, A. K., Singh, V. K., Dwivedy, A. K., Dubey, N. K.: *Angelica archangelica* essential oil loaded chitosan nanoemulsion as edible coating for preservation of table grape fruit against *Botrytis cinerea* contamination and storage quality deterioration. Postharvest Biology and Technology, 205, 112482, 2023.

Jaćimović, G., Aćin, V., Crnobarac, J., Latković, D.: Biološke i agroekološke osnove proizvodnje pšenice. Biljni lekar, 44, 391 - 408, 2017.

Lazarević, J., Aćimović, M., Pezo, L., Lončar, B., Konstantinović, B., Popov, M., Šovljanski, O., Travičić, V., Sikora, V., Vujisić, Lj.: Chemical composition and *in vitro* biological activity of angelica root and hop strobile essential oils and hydrolates. Waste and Biomass Valorization, 15 (2), 867 - 883, 2023.

Oğuz, M. Ç., Oğuz, E., Güler, M.: Seed priming with essential oils for sustainable wheat agriculture in semi-arid region. PeerJ, 11, e15126, 2023.

Paroul, N., Rota, L., Frizzo, C., Atti dos Santos, A. C., Moyna, P., Gower, A. E., Serafini, L., Cassel, E.: Chemical Composition of the Volatiles of Angelica Root Obtained by Hydrodistillation and Supercritical CO_2 Extraction. Journal of Essential Oil Research, 14 (4), 282 - 285, 2002.

Popović, V.: Agrotehnički i agroekološki uticaji na proizvodnju semena pšenice, kukuruza i soje. Doktorska disertacija, Poljoprivredni fakultet, Univerzitet u Beogradu, Beograd, str. 62 - 65, 2010.

R Foundation for Statistical Computing: R Project for Statistical Computing; Version 4.3.2 (2023-10-31ucrt); R Foundation for Statistical Computing: Vienna, Austria, 2023. Available online: https://www.R-project.org/ (Accessed May 05th, 2025).

Tavares, C., Gameiro, J., Roseiro, L., Figueiredo, C.: Hydrolates: a review on their volatiles composition, biological properties and potential uses. Phytochemistry Reviews, 21, 1661 - 1737, 2022.

Truzzi, E., Benvenuti, S., Bertelli, D., Francia, E., Ronga, D.: Effects of biostimulants on the chemical composition of essential oil and hydrosol of lavandin (*Lavandula x intermedia* Emeric ex Loisel.) cultivated in Tuscan-Emilian Apennines. Molecules, 26 (20), 6157, 2021.

In Vitro efekat hidrolata angelike (*Angelica archangelica* L.) na klijavost semena soje (*Glycine max* L. Merr.) i ozime pšenice (*Triticum aestivum* L.)

REZIME

Soja (*Glycine max* L. Merr.) i ozima pšenica (*Triticum aestivum* L.) predstavljaju važne poljoprivredne kulture, čija su klijavost i rani porast ključni za postizanje visokih prinosa. U ovom istraživanju je ispitivan biostimulativni potencijal hidrolata *Angelica archangelica*, sporednog proizvoda dobijenog tokom destilacije etarskog ulja, na semenu soje i pšenice. Semena su tretirana rastvorima hidrolata različitih koncentracija (0,25%, 0,5%, 1%, 5% i 10%), a testovi klijanja sprovedeni su u kontrolisanim laboratorijskim uslovima. Rezultati su pokazali da tretmani hidrolatom značajno povećavaju dužinu klijanaca kod obe kulture u poređenju sa kontrolom. Najveći porast zabeležen je pri koncentraciji od 10% kod soje 5,77 cm, a kod pšenice 3,13 cm. Niže koncentracije takođe su imale pozitivan efekat, ali slabijeg intenziteta. Dobijeni rezultati potvrđuju da hidrolat *A. archangelica* pozitivno utiče na klijanje i rani razvoj biljaka, ukazujući na njegov potencijal kao prirodnog biostimulatora u održivoj poljoprivredi. Kako su hidrolati ekološki bezbedni i nedovoljno istraženi u poljoprivrednoj praksi, potrebna su dalja istraživanja u poljskim uslovima radi optimizacije njihove primene i potpunog iskorišćenja njihovog potencijala za unapređenje rasta biljaka.

Ključne reči: hidrolat, *Angelica archangelica* L., klijavost semena, soja, pšenica.