Arch Biol Sci. 2025;77(1):5-14 https://doi.org/10.2298/ABS241126001J

The bioherbicidal potential of hemlock water extracts

Vladan D. Jovanović[1], Stefan R. Stanojević[2], Slavica S. Dmitrović[2,*], Jasmina M. Nestorović Živković[2], Bogdan R. Nikolić[3], Ivana Č. Dragićević[4] and Zlatko S. Giba[4]

[1]*Institute of Pesticides and Environmental Protection*, Banatska 31b, 11080 Belgrade, Serbia

[2]*Institute for Biological Research "Siniša Stanković", National Institute of the Republic of Serbia, University of Belgrade*, Bulevar despota Stefana 142, 11108 Belgrade, Serbia

[3]*Institute for Plant Protection and Environment*, Teodora Drajzera 9, 11000 Belgrade, Serbia

[4]*Faculty of Biology, University of Belgrade*, Studentski trg 16, 11000 Belgrade, Serbia

***Corresponding author**: slavica.dmitrovic@ibiss.bg.ac.rs

Received: November 26, 2024; **Revised**: December 12, 2024; **Accepted**: December 23, 2024; **Published online**: January 13, 2025

Abstract: Studying plant species rich in bioactive compounds, including allelochemicals, could lead to the development of biodegradable and environmentally safe herbicides. In this study, a water extract of hemlock (*Conium maculatum* L.), known to be rich in alkaloids, was used to investigate its effects on the germination of *Amaranthus retroflexus* L., *Ambrosia artemisiifolia* L., *Stellaria media* (L.) Vill., and *Lactuca sativa* L., providing valuable insights into its phytotoxic properties. Among the *C. maculatum* water extracts (*Cm*WEs) tested, that of dry leaves showed the strongest inhibitory effect on the germination of all species analyzed. The *Cm*WE of dry leaves (50%) completely inhibited seed germination of all species tested. The *Cm*WE of dry stems (50%) reduced seed germination in *S. media*, *A. retroflexus*, and *A. artemisiifolia* by 20% to 89%, whereas the germination of *L. sativa* was less affected (9%). In addition, a *Cm*WE of dry stems (5%) selectively inhibited ragweed germination (45%), while the germination of *Lactuca sativa* was not significantly affected. The *Cm*WE of dry leaves (20%) inhibited early root and shoot growth of *S. media* more effectively than suppressing seed germination. Moreover, the dry leaves and inflorescences of *C. maculatum* demonstrate strong allelopathic properties even after two years of storage, highlighting the long-term potential of *C. maculatum* as a source of natural herbicides.

Keywords: *Conium maculatum*, water extract, seed germination, early seedling growth, allelopathy

Abbreviations: *C. maculatum* water extract (*Cm*WE)

INTRODUCTION

The reduction in crop yields and the high costs of weed control are major problems in agricultural production systems. As the global population is projected to exceed 9 billion by 2050, it is crucial that food production systems avoid significant yield losses caused by weed competition [1,2]. Typically, the spread of weeds is suppressed by physical or chemical measures. Currently, farmers mainly use chemical herbicides due to their selectivity, affordability, and easy availability. Synthetic herbicides can lead to water and soil pollution and endanger the soil microbiome, plants, animals, and humans. However, the long-term use of synthetic herbicides has led to the occurrence of herbicide-resistant weed species. Currently, 273 weed species have developed resistance to herbicides, affecting 101 crops across 72 countries. These weeds have developed resistance to 21 of the 31 known herbicide modes of action, involving 168 different herbicides (https://www.weedscience.org). To reduce the harmful, long-term environmental impact of synthetic herbicides, interest in bioherbicides has increased significantly. Biologically active compounds/formulations offer an eco-friendly alternative as they control weeds through natural mechanisms, reducing the environmental impact of conventional chemical herbicides. Significant resources are being invested in research into natural herbicides.

Allelopathy is increasingly recognized as an important ecological mechanism that impacts plant dominance, shapes plant communities in natural

How to cite this article: Jovanović VD, Stanojević SR, Dmitrović SS, Nestorović Živković JM, Nikolić BR, Dragićević IČ, Giba ZS. The bioherbicidal potential of hemlock water extracts. Arch Biol Sci. 2025;77(1):5-14.

ecosystems, and influences plant productivity in agricultural systems [3,4]. Many allelochemicals can serve as natural, biodegradable herbicides, offering a completely different mode of action compared to synthetic herbicides. Although the efficacy and specificity of many allelochemicals remain unknown or limited, they represent a suitable alternative to synthetic herbicides [2,5]

The invasive biennial weed *Conium maculatum* L. (family Apiaceae) is a toxic plant naturally widespread across Europe, Asia, and Africa, and introduced to North and South America, Australia, and New Zealand [6-9]. It is characterized by a strong, pervasive, and musty odor [7]. It is also a common weed in Serbia [10], growing in pastures, farms, and ruderal area. Due to its rapid growth, toxicity, and high seed production, *C. maculatum* spreads easily to cultivated fields. It also can colonize disturbed places easily and can displace native plants in natural habitats.

Plant toxins are chemical substances that are naturally produced as secondary metabolites. These compounds, also known as phytochemicals, are synthesized in different parts of the plant and are transported to different organs and tissues, where they are stored [11]. Various pharmacological effects of *C. maculatum* tinctures and extracts are well documented, including anti-herpetic, antispasmodic, sedative, analgesic, and anticancer properties [9]. However, their effects on plant organisms, especially weeds, are still insufficiently explored. Allelochemicals represent a broad spectrum of low molecular weight compounds such as alkaloids, polypeptides, terpenoids, flavonoids, glycosides, and tannins [12]. Each of these substances affects the interactions between plants and other organisms in their environment. All plant parts of *C. maculatum* (both vegetative and reproductive) contain the toxic piperidine alkaloids, although their concentration may vary depending on the plant tissue, growth stage, and environmental conditions. Coniine, N-methyl-coniine, conhydrine, pseudoconhydrine, and γ-coniceine are some of the piperidine alkaloids found in *C. maculatum* responsible for its toxicity [7,13]. The mode of action of alkaloids is determined by their chemical structure. These compounds can alter enzyme activity, leading to changes in cellular functions, including disruptions in DNA replication and repair mechanisms [11,14].

Redroot pigweed or redroot amaranth (*Amaranthus retroflexus* L.) is a globally distributed weed found in over 70 countries in 60 different crops [6]. It is considered the third most dominant dicotyledonous weed species worldwide [15] and is particularly widespread in Serbia, where it is classified as an economically harmful invasive species [16]. *A. retroflexus* is propagated by seeds that remain viable in the soil for at least 6-10 years [17,18]. In addition to its harmful effect, which is reflected in crop yield reductions, *A. retroflexus* is host to pathogens and pests, is toxic to livestock, and produces pollen that causes allergic reactions in humans and animals [19,20]. *Ambrosia artemisiifolia* L., commonly known as ragweed, is an invasive species originating from North America. In Serbia, ragweed was first recorded in 1953 and is currently classified as an allochthonous neophyte in its invasive stage [16,21]. The high germination rate, rapid initial growth, high photosynthetic rate, and allelopathic properties of *A. artemisiifolia* contribute to its competitiveness and rapid spread [22,23]. In addition to their ecological and economic impacts, ragweed pollen is one of the most potent allergens known to cause severe allergic reactions in humans [24,25]. Chickweed (*Stellaria media* (L.) Vill.) is native to Europe but has spread worldwide. It is a troublesome weed in agricultural fields and often grows in disturbed areas. *S. media* is known for its rapid growth and ability to adapt to a wide range of environmental conditions [26]. *Lactuca sativa* L., commonly known as lettuce, is a leafy vegetable that is widely used in the human diet worldwide, including Serbia. It is rich in vitamins, essential minerals, and fiber.

This work aimed to investigate the potential phytotoxic properties of water extracts of *C. maculatum* (leaves, stems, and inflorescences) on the germination of three weed species (*A. retroflexus*, *A. artemisiifolia*, and *S. media*), which are widely distributed in agricultural fields in Serbia, as well as on the germinations of a commonly cultivated crop plant, lettuce (*L. sativa*). We also investigated the effect of water extracts of *C. maculatum* leaves on the early development of *S. media* seedlings by measuring some growth parameters.

MATERIALS AND METHODS

Plant material

The seeds of three weed species: redroot pigweed (*Amaranthus retroflexus* L.), ragweed (*Ambrosia artemisiifolia* L.), and chickweed (*Stellaria media* (L.) Vill.), were collected in 2022 in the wider area of Belgrade, Serbia, and subsequently cleaned from the rest of the plant material using sieves with different pore diameters. The dry seeds were stored in the dark at room temperature until the beginning of the experiment. The seeds of lettuce (*Lactuca sativa* L. cv. 'May Queen'), (Bioprodukt, Čačak, Serbia) were commercially purchased. These untreated seeds had an expected germination rate of 95% and were produced in 2022 in Serbia. Before the experiments, the seeds of *A. retroflexus* and *A. artemisiifolia* were stratified at a temperature of 4±1°C for 2.5 and 6 weeks, respectively. *C. maculatum* plants were collected in 2021 and 2023 in the wider area of Belgrade, Serbia.

Preparation of *C. maculatum* water extract (*Cm*WE)

The water extract of fresh leaves of *C. maculatum* was prepared immediately after plant collection. In contrast, the water extracts of dry leaves and stems were obtained after drying the plants at a temperature of 20-22°C until a constant dry weight was achieved. The *C. maculatum* plants collected in 2021 were dried at a temperature of 20-22°C and stored in the dark at room temperature for two years before the leaves, stems, and inflorescences were separated and the water extracts were prepared. The crushed leaves, stems, and inflorescences were immersed in distilled water (1:10, weight (g): volume (mL)) in Erlenmeyer flasks (250 mL), which were kept in the dark for 24 h in a growth chamber. The water extracts were then filtered through filter paper (Ahlstrom-Munksjö, Helsinki, Finland) and diluted with distilled water to 50%, 20%, 10%, and 5% concentrations.

Seed germination

The seeds of *A. retroflexus*, *A. artemisiifolia*, *S. media*, and *L. sativa* were germinated in 60-mm diameter glass Petri dishes on a layer of filter paper moistened with 2 mL of distilled water or the tested solutions, until reaching final germination percentages. Seeds were illuminated for 15 min per day and were counted as germinated when a radicle of 1 mm or longer was observed. *A. retroflexus* and *A. artemisiifolia* seeds were germinated at 25±1°C, and *S. media*, and *L. sativa* at 18±1°C. The experiments were conducted three times, using 50 seeds with each repetition.

The percentage of inhibition was determined using the following formula:

$$\% \text{ Inhibition} = (1-T/C)\times 100.$$

In this equation, T is the parameter measured in the treated samples, and C is the parameter measured in the control samples.

Growth of *S. media* seedlings in the water extract of *C. maculatum* dry leaves

The seeds of *S. media* were germinated in 150-mm diameter glass Petri dishes on a layer of filter paper moistened with 10 mL of distilled water or the tested solutions (*Cm*WE of dry leaves at concentrations of 20%, 10%, and 5%) for 7 days at 18±1°C. The seeds were exposed to light for 15 min per day. The experiments were repeated three times, using 30 seeds for each repetition. Growth parameters (root and shoot length) were measured on the 5th, 6th, and 7th days after the start of the experiment.

Statistical analysis

Statistical analysis was performed using STATGRAPHICS software, version 4.2 (STSC Inc. and Statistical Graphics Corporation, Rockville, Maryland, USA). Percentage data were arcsine-transformed before analyzing the statistical significance of differences between means. Data was subjected to one-way analysis of variance (ANOVA), and the significance of differences between means was assessed using Fisher's LSD test at a confidence level of $P \leq 0.05$.

RESULTS

Effects of water extracts of *C. maculatum* prepared from different plant parts (leaves, stems, and inflorescence) on the germination of seeds of three weed species (*A. retroflexus*, *A. artemisiifolia*, and *S. media*) and a crop species (*L. sativa*)

This study examined the effects of water extracts (from 5% to 50%) derived from various parts of *C. maculatum* plants (leaves, stems, and inflorescences) on the germination dynamics and final germination rates of three weeds (*A. retroflexus*, *A. artemisiifolia*, and *S. media*) and a leafy vegetable, *L. sativa*, compared to a water control (Figs. 1-4). The research compared the impact of *C. maculatum* water extracts (*Cm*WEs) of fresh and dry leaves, dry leaves and stems, and dry stems from plants that were either freshly harvested or stored for two years, on the germination of *A. retroflexus*, *A. artemisiifolia*, *S. media*, and *L. sativa* seeds. The study also compared the effects of *Cm*WEs from dry leaves of freshly harvested and two-year-stored plants, as well as from dry leaves, stems, and inflorescences of plants stored for two years, on the germination of *S. media*.

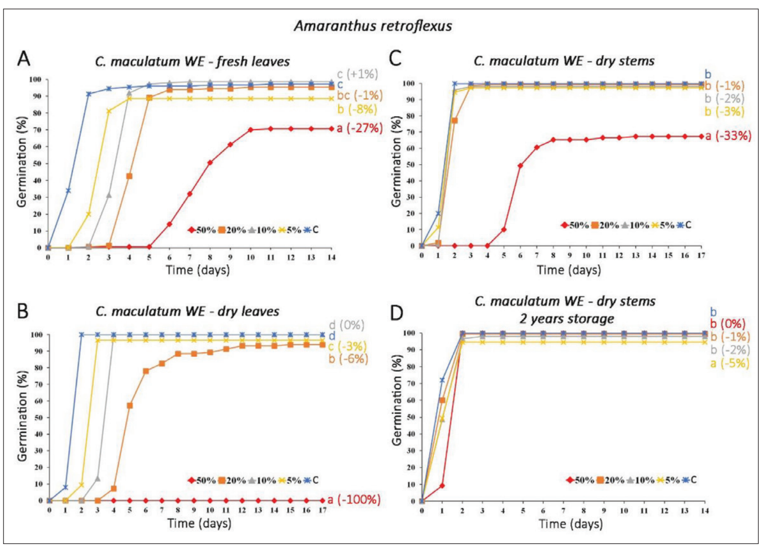

Fig. 1. *Amaranthus retroflexus* seed germination (%) after treatment with *Conium maculatum* water extract (5%, 10%, 20%, and 50%) of **A** – fresh leaves, **B** – dry leaves, **C** – dry stems, **D** – dry stems 2 years storage. Control treatment (C) was performed using distilled water. The values with the same letter indicate statistically homogeneous groups (P≤0.05), according to Fisher's LSD test; percentage inhibition (−)/stimulation (+) compared to the control.

The strongest significant inhibitory effect on the final germination of *A. retroflexus* had 50% *Cm*WE of dry leaves which inhibited germination 100%, then 50% *Cm*WE of dry stems and 50% *Cm*WE of fresh leaves which inhibited germination 33% and 27%, respectively, compared to the control (Fig. 1 A,B,C). The 50% *Cm*WE of 2-year-stored dry stems did not affect *A. retroflexus* final germination compared to the control (Fig. 1D). Increasing the applied concentrations of *Cm*WE from 5% to 20% of fresh leaves and dry leaves delayed germination by one day (Fig. 1 A,B). The significant inhibition of final germination percentage of *A. artemisiifolia* was observed at 50% *Cm*WE of fresh leaves (45%), 50% and 20% *Cm*WEs of dry leaves (99% and 43%, respectively), and 50%, 20%, 10%, and 5% *Cm*WEs of dry stems (from 89% to 23%) compared to the control (Fig. 2 A,B,C). The *Cm*WE of 2-year-stored stems had no significant effect on *A. artemisiifolia*'s final germination percentage at all applied concentrations (from 5% to 50%), while the 5% *Cm*WE of 2-year-stored stems stimulated final germination (6%) compared to the control (Fig. 2D).

Complete inhibition (100%) of *S. media* germination was detected at 50% *Cm*WE of dry leaves and 50% *Cm*WE of 2-year-stored dry inflorescences, while a high inhibition (89%) was achieved with 50% *Cm*WE of 2-year-stored dry leaves compared to the control (Fig. 3 B,C,F). Significant moderate inhibition of final germination (24%, 20%, and 17%) was observed at 50% *Cm*WE of fresh leaves, 50% *Cm*WE of dry stems, and 20% *Cm*WE of dry leaves, respectively, compared to the control (Fig. 3 A, B,D). Concentrations of 5% and 10% of all *Cm*WEs tested did not significantly alter the final germination percentages, except for the *Cm*WE of fresh leaves, where inhibition was 7% for both treatments (Fig. 3). With increasing concentrations of all *Cm*WEs, a delay in germination is observed compared to the control (Fig. 3). A slight stimulation of germination (from 1% to 4%) occurred with 5% *Cm*WEs of dry leaves, dry stems, 2-year-stored dry stems, and 2-year-stored dry inflorescences, as well as with 10% *Cm*WEs of dry stems and 2-year-stored dry stems (1% each) and 20% *Cm*WE of 2-year-stored

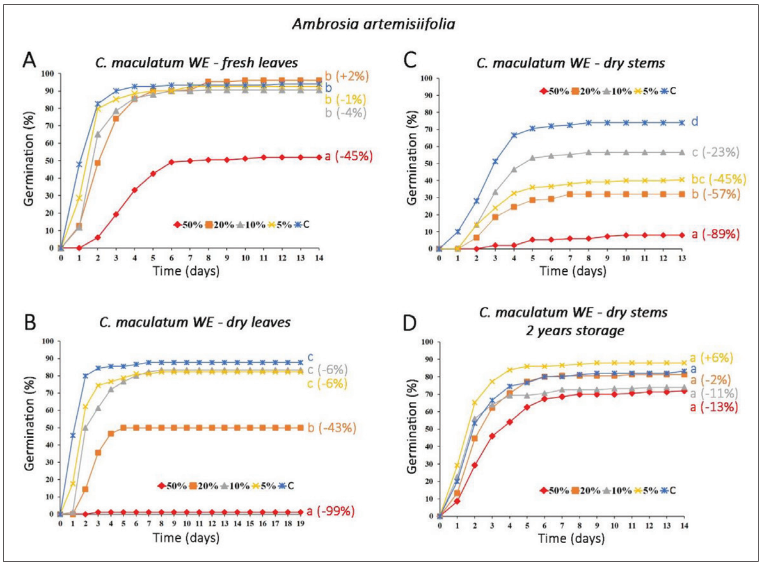

Fig. 2. *Ambrosia artemisiifolia* seed germination (%) after treatment with *Conium maculatum* water extract (5%, 10%, 20% and 50%) of **A** – fresh leaves, **B** – dry leaves, **C** – dry stems, **D** – dry stems 2 years storage. Control treatment (C) was performed using distilled water. The values with the same letter indicate statistically homogeneous groups (P≤0.05), according to Fisher's LSD test; percentage inhibition (−)/stimulation (+) compared to the control.

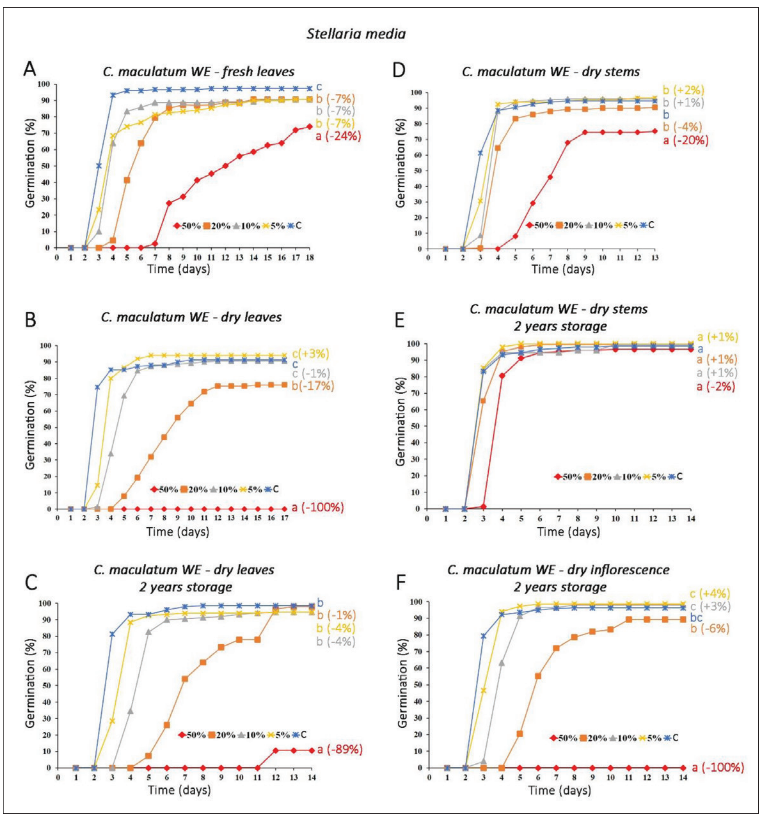

Fig. 3. *Stellaria media* seed germination (%) after treatment with *Conium maculatum* water extract (5%, 10%, 20%, and 50%) of **A** – fresh leaves, **B** – dry leaves, **C** – dry leaves 2 years storage, **D** – dry stems, **E** – dry stems 2 years storage, **F** – dry inflorescence 2 years storage. Control treatment (C) was performed using distilled water. The values with the same letter indicate statistically homogeneous groups (P≤0.05), according to Fisher's LSD test; percentage inhibition (−)/stimulation (+) compared to the control.

dry stems (1%) compared to the control (Fig. 3B,D,E,F).

The germination of *L. sativa* was completely inhibited with 50% *Cm*WE of dry leaves, while moderate inhibition (15% and 9%, respectively) was observed with 50% *Cm*WEs of fresh leaves and dry stems compared to the control (Fig. 4 A,B,C). Germination was not significantly affected by any other *Cm*WE treatment (Fig. 4). Additionally, a slight stimulation of final germination (3%) was noted with 10% *Cm*WE of dry leaves, compared to the control (Fig. 4B).

Effects of *C. maculatum* water extracts of dry leaves on *S. media* early seedling growth

The study focused on the effects of *Cm*WE from dry leaves on the early growth of *S. media* seedlings, as these seeds do not require stratification. Previous experiments revealed that *Cm*WE of dry leaves had the strongest inhibitory effect on the germination of various species tested. In this experiment, the influence of *Cm*WE of dry leaves (5%, 10%, and 20%), with distilled water as a control, on the early growth of seedlings was investigated by measuring the shoot and root lengths of *S. media* seedlings from the 5^{th} to 7^{th} day.

The length of *S. media* shoots was significantly reduced at the highest applied concentration of *Cm*WE of dry leaves (20%), with a decrease from 39% to 14% between the 5^{th} and 7^{th} day, compared to the control. In contrast, lower concentrations of the *Cm*WE of dry leaves (10% and 5%) led to a significant increase in the length of *S. media* shoots (Fig. 5A). The length of the roots after treatment with *Cm*WE (20%) was significantly less. The inhibition of elongation ranged from 86% on the 5^{th} day to 87% on the 7^{th} day. In comparison, the treatment with *Cm*WE at a lower concentration (10%) inhibited

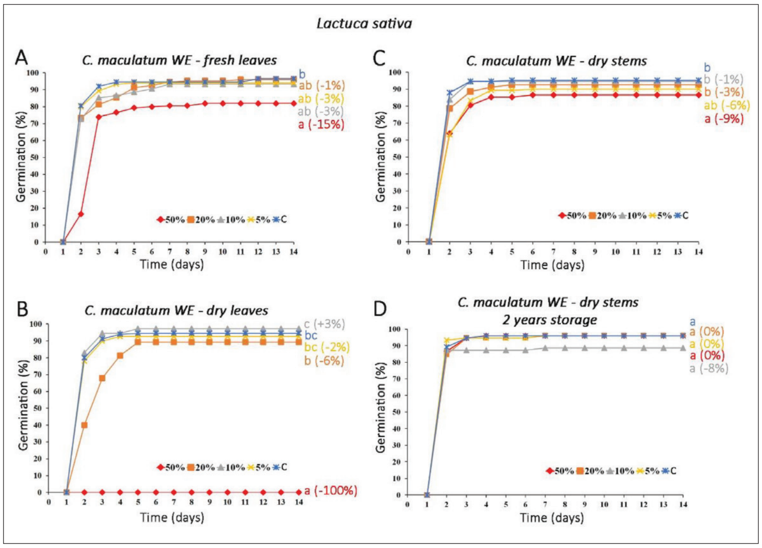

Fig. 4. *Lactuca sativa* seed germination (%) after treatment with *Conium maculatum* water extract (5%, 10%, 20%, and 50%) of **A** – fresh leaves, **B** – dry leaves, **C** – dry stems, **D** – dry stems 2 years storage. Control treatment (C) was performed with distilled water. The values with the same letter indicate statistically homogeneous groups (P≤0.05), according to Fisher's LSD test; percentage inhibition (–)/stimulation (+) compared to the control.

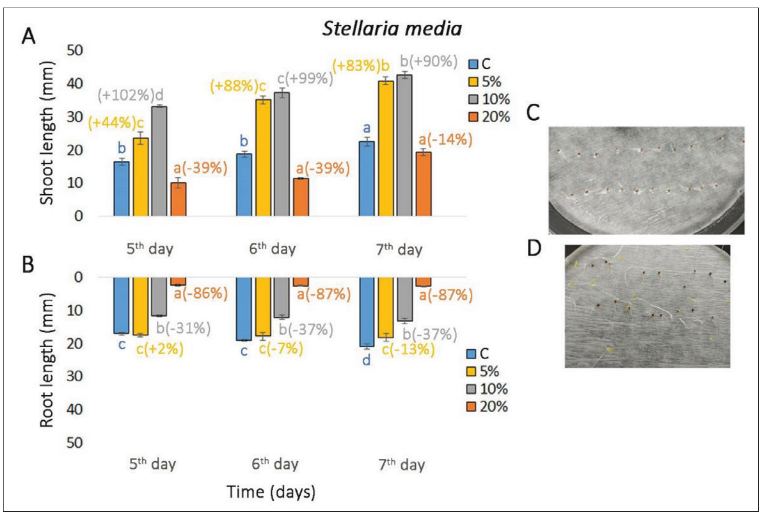

Fig. 5. *Stellaria media* early seedlings growth. **A-B** Seedlings growth parameters from 5th to 7th days after the beginning of treatment with *Conium maculatum* water extract of dry leaves (5%, 10%, and 20%): **A** – shoot length (mm), **B** – root length (mm). **C-D** Phenotypic appearance of control seedlings on the 2nd day (**C**) and 6th day (**D**) after the beginning of the experiment. The values with the same letter indicate statistically homogeneous groups (P≤0.05), according to Fisher's LSD test; percentage inhibition (–)/stimulation (+) compared to the control.

root length by 31% on the 5th day to 37% on the 7th day, compared to the control (Fig. 5B).

DISCUSSION

Allelochemicals are produced, distributed, and stored in various parts of a plant, depending on the species and the specific compounds involved. Plants release allelochemicals into their environment via several mechanisms that influence the growth and development of nearby plants and other organisms. These mechanisms include leaching from the aerial parts, exudation from the roots, volatilization into the atmosphere, and decomposition of plant residues in the soil [27,28].

Water extracts of plants generally contain a range of water-soluble compounds, such as polysaccharides, glycosides, flavonoids, tannins, phenolic acids, certain alkaloids, proteins, vitamins, minerals, and organic acids. Conventionally prepared water extracts of many plants are increasingly being used for weed control due to their environmental benefits and effectiveness. These extracts are biodegradable and can be applied selectively to certain weed species without harming the crop. They are also inexpensive to produce. Unlike organic solvents, water leaves no harmful residues, thereby reducing the risk of toxicity in the environment.

Conium maculatum possesses a remarkable ability to synthesize piperidine-type alkaloids, including coniine, N-methyl-coniine, conhydrine, pseudoconhydrine, and *γ*-coniceine, which are synthesized by the cyclization of an eight-carbon chain derived from four acetate units and are structurally similar to nicotine. Among these, *γ*-coniceine serves as a precursor for the other hemlock alkaloids. They are present in all parts of the plant, including the vegetative organs, flowers, and fruits. The concentration and composition of these alkaloids vary depending on the plant variety (genotype), ecological conditions, and developmental stage of the plant, as well as diurnal changes [7,29,30]. The exact sites of synthesis and accumulation of piperidine alkaloids remain unclear. Research by Corsi and Biasci [31] found alkaloids in the root and shoot of seedlings, but in mature plants, they are more prominent in cells of elongation zones, as well as in the secretory ducts of vegetative organs, flowers, and fruits. The alkaloid content of *C. maculatum* varies among different parts of the plant: roots contain from 0% to 0.5%, shoots 0.02% to 0.7%, and leaves 0.3% to 1.5%. Flowers usually contain around 1.0%, while unripe fruits have a higher alkaloid concentration from 1.6% to 3.0%, which decreases as the fruit ripens, dropping to between 0.2% and 1.0%. The seeds contain between 0.02% and 0.9% alkaloids [7,29].

The predominant piperidine alkaloids in *C. maculatum*, coniine, and N-methylconiine, exist in the free base form – neither ionized nor water-soluble in plant tissue. These properties contribute to their volatility and the plant's characteristic odor. Essential oils of *C. maculatum* from Italy (Sicily) contained mostly sesquiterpene (*E*)-caryophyllene in the leaves, and 1-butylpiperidine and monoterpene myrcene in the inflorescences [32]. Essential oils from the leaves and flowers of *C. maculatum* collected in Serbia contained 23 and 57 compounds, respectively [30]. The dominant components in leaves and flowers were sesquiterpene germacrene D, and monoterpene *β*-ocimene. The extract of *C. maculatum* also contained flavonoids, coumarins, polyacetylenes, and vitamins [30]. Piperidine alkaloid-rich water extracts of *Prosopis* sp. leaves, bark, and roots inhibited the growth of *Mimosa tenuiflora* seedlings, whereas water extracts of leaves inhibited the germination and development of *Cynodon dactylon* seedlings, and water extracts of leaves, stems and roots inhibited the growth and development of wheat [33].

The results of our study showed that the *Cm*WE of dry leaves caused the strongest inhibition of *A. retroflexus* germination compared to the other tested *Cm*WEs (from fresh leaves, dry stems, or 2-year-stored dry stems). Previously, similar studies were conducted with the water extract obtained from the fresh leaves of artichoke thistle (*Cinara cardunculus* L.), which is rich in the sesquiterpene aguerin B, and the sesquiterpene lactones, grosheimin, and cynaropicrin, on the germination of *A. retroflexus*. The study used 40% and 80% water extracts, and the germination of *A. retroflexus* was inhibited by an average of 58% in both cases compared to the control [34]. In agreement with our results, Yarnia et al. [35] demonstrated that the leaf extract of *Sorghum bicolor* (L.) Moench had the strongest inhibitory effect on the germination of *A. retroflexus*, while the stem extract was the least effective, both at a concentration of 20%. Furthermore, the water extract of the aerial parts of mugwort (*Artemisia vulgaris* L.), which contains terpenoids and flavonoids among other bioactive compounds, inhibited the germination of *A. retroflexus* seeds [36].

Among the *Cm*WEs tested, those of dry leaves and dry stems showed the strongest inhibitory effect on the germination of *A. artemisiifolia*. This is consistent with studies on cover crops from the Brassicaceae family, such as white mustard (*Sinapis alba* L.), and radish (*Raphanus sativus* L.), which have shown that their water extracts can completely inhibit the germination and early growth of *A. artemisiifolia* [37]. Additionally, Kazinczi et al. [38] demonstrated that water extracts of sunflower shoots and roots inhibited *A. artemisiifolia* germination at higher concentrations while stimulating early seedling development at lower concentrations. This aligns with our finding that low concentrations of *Cm*WE of dry leaves stimulated shoot elongation in *S. media* seedlings. We observed the phenomenon of hormesis during the germination of *L. sativa* after treatment with the *Cm*WE of dry leaves, applied at a lower concentration, indicating a potential for growth enhancement. Further research is needed to determine the optimal concentrations for promoting the germination and growth of these edible and nutritious plants. On the other hand, the highest applied concentration of the *Cm*WE of fresh and dry leaves resulted in significant inhibition of *L. sativa* germination, which is in agreement with Mahmoodzadeh et al. [39], who found that water extracts of the aerial parts of *Cannabis sativa* inhibited the germination of *L. sativa* seeds. Furthermore, Sbai et al. [40] revealed the inhibitory effect of aerial parts of *Apium graveolens* L. on *L. sativa* germination and growth. Lettuce seeds, as well as *A. retroflexus* and *A. artemisiifolia*, were also highly sensitive to the essential oil of *N. rtanjensis*, which is rich in the iridoid monoterpenoid nepetalactone [41,42].

In addition to the *Cm*WEs already analyzed, *Cm*WEs of dry leaves and dry inflorescences stored for

2 years were also tested for their effects on the germination of *S. media* seeds. Unlike the *Cm*WEs from dry stems, which did not significantly affect germination in the tested species (except *A. retroflexus* at a 10% concentration), the highest concentrations of *Cm*WEs of 2-year-stored dry leaves and dry inflorescences (50%) strongly inhibited *S. media* germination. This indicates that the bioactive compounds responsible for the plant's phytotoxic effects may remain stable over time in certain parts of the plant, further supporting the potential of *C. maculatum* as a long-lasting source of natural herbicides. Higher concentrations of *Cm*WE of dry leaves (20%) had a strong inhibitory effect on both *S. media* early shoot and root growth. Lower concentrations of *Cm*WE of dry leaves (10% and 5%) promoted shoot growth but still inhibited root elongation, particularly at 10%, compared to control. Notably, the highest concentration of *Cm*WE of dry leaves (50%) entirely suppressed the germination of *S. media*, while 20% *Cm*WE of dry leaves significantly affected early seedling development, inhibiting root and shoot growth more effectively than seed germination. This indicates that 20% *Cm*WE of dry leaves has a stronger phytotoxic impact on the post-germination phase, making it a promising candidate for *S. media* seedling control. This finding aligns with the results of Kazinczi et al. [38], who conducted a similar bioassay using sunflower extracts on the germination and seedling growth of *A. artemisiifolia*. Similar to our study, Sturm et al. [43] showed the inhibitory effect of sunflower water extracts on the germination and root length of *S. media* seedlings. In addition, Jovanović et al. [44] demonstrated the inhibitory effect of water extract of *Xeranthemum cylindraceum* on the germination of *S. media*.

A particularly important finding of this study is that 5% *Cm*WE of dry stems inhibited *A. artemisiifolia* germination (45%), highlighting this extract as the most environmentally friendly for controlling ragweed germination. Furthermore, 20% *Cm*WE of dry stems, 20% *Cm*WE of dry leaves, and 50% *Cm*WE of fresh leaves effectively inhibited ragweed germination by 57%, 43%, and 45%, respectively, while lettuce seed germination was not significantly inhibited at treatments with 20% *Cm*WE of dry stems and leaves. Additionally, a 50% *Cm*WE of dry stems inhibited seed germination of *S. media*, *A. retroflexus*, and *A. artemisiifolia* by 20% to 89%, while the germination of *L. sativa* was less affected, showing only a 9% reduction. *Cm*WE of dry leaves (20%) was more effective in inhibiting early root and shoot growth of *S. media* than in suppressing seed germination. All these properties could be valuable for integrated weed control strategies. However, further studies are needed to determine the optimal concentration of hemlock extract that increases herbicide efficacy while minimizing impacts on non-target species and ecosystems. Future research should focus on the identification and characterization of bioactive compounds contained in extracts from different plant parts, as well as the evaluation of their potential presence in non-target plants, and the determination of their degradation rate after exposure. The development of standardized extraction methods and safety protocols will be key to advancing the practical use of hemlock-based bioherbicides as an environmentally friendly alternative to synthetic chemical herbicides.

It is important to note that while hemlock can offer some weed suppression benefits, it is also toxic to humans and animals [7]. Thus, the use of hemlock for weed suppression should be approached with caution and implemented only in controlled environments where its toxic properties can be properly managed. Furthermore, its invasive characteristics and potential ecological consequences must be thoroughly assessed before considering it as a method for weed control.

Funding: This work was financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract numbers 451-03-66/2024-03/ 200214; 451-03-66/2024-03/ 200007; 451-03-66/2024-03/ 200010; 451-03-65/2024-03/ 200178).

Author contributions: Conceptualization, VJ, SS; methodology, VJ, SS, BN, ZG; validation, VJ, ID; formal Analysis, SS, BN; investigation, VJ, SS, BN, ID, ZG, JNŽ, SD; data curation, VJ, SS; writing – original draft preparation, SD, SS, VJ, JNŽ; writing – review & editing, SD, VJ, JNŽ, ID; visualization, SS, VJ, SD, JNŽ, ID; supervision, VJ; funding acquisition, VJ. All authors have read and agreed to publish the presented version of the manuscript.

Conflict of interest disclosure: The authors declare no conflict of interest.

Data availability statement: The dataset underlying the reported findings is available here: https://www.serbiosoc.org.rs/NewUploads/Uploads/Jovanovic%20et%20al_Dataset.pdf

REFERENCES

1. Chauhan BS. Grand challenges in weed management. Front Agron. 2020;1:3. https://doi.org/10.3389/fagro.2019.00003
2. Khamare Y, Chen J, Marble SC. Allelopathy and its application as a weed management tool: A review. Front Plant Sci. 2022;13:1034649. https://doi.org/10.3389/fpls.2022.1034649
3. Quasem JR, Foy CL. Weed allelopathy, its ecological impacts and future prospects. J Crop Prod. 2001;4(2):43–119. https://doi.org/10.1300/J144v04n02_02
4. Hierro JL, Callaway RM. The Ecological importance of allelopathy. Annu Rev Ecol Evol Syst. 2021;52(1):25–45. https://doi.org/10.1146/annurev-ecolsys-051120-030619
5. Sahrir MAS, Yusoff N, Azizan KA. Allelopathy activity under laboratory, greenhouse and field conditions: A review. AIMS Agric Food. 2022;8(1):78–104. https://doi.org/10.3934/agrfood.2023004
6. Holm L, Doll J, Holm E, Pancho J V, Herberger JP. World weeds: natural histories and distribution. John Wiley & Sons; 1997.
7. Vetter J. Poison hemlock (*Conium maculatum* L.). Food Chem Toxicol. 2004;42(9):1373–82. https://doi.org/10.1016/j.fct.2004.04.009
8. Meier P, Hotti H, Rischer H. Elicitation of furanocoumarins in poison hemlock (*Conium maculatum* L.) cell culture. Plant Cell Tissue Organ Cult. 2015;123(3):443–53. https://doi.org/10.1007/s11240-015-0847-7
9. Ahmad M, Alam SM. *Conium maculatum*: A review. J Pharmacogn Phytochem. 2018;7(5):621–9.
10. Nestorović M, Konstantinović B. Overview of the weed flora in the Serbia. Contemp Agric. 2011;60(1):215.
11. Chen H, Singh H, Bhardwaj N, Bhardwaj SK, Khatri M, Kim KH, Peng W. An exploration on the toxicity mechanisms of phytotoxins and their potential utilities. Crit Rev Environ Sci Technol. 2022;52(3):395–435. https://doi.org/10.1080/10643389.2020.1823172
12. Zandavar H, Babazad A. Secondary Metabolites: Alkaloids and flavonoids in medicinal plants. IntechOpen. 2023;1–28. https://doi.org/10.5772/intechopen.108030
13. Green BT, Welch KD, Panter KE, Lee ST. Plant toxins that affect nicotinic acetylcholine receptors: a review. Chem. Res. Toxicol. 2013; 26(8):1129-1138. https://doi.org/10.1021/tx400166f
14. Mutlu S, Atici Ö, Esim N, Mete E. Essential oils of catmint (*Nepeta meyeri* Benth.) induce oxidative stress in early seedlings of various weed species. Acta Physiol Plant. 2011;33(3):943–51. https://doi.org/10.1007/s11738-010-0626-3
15. Ma J, Xing G, Yang W, Ma L, Gao M, Wang Y, Han Y. Inhibitory effects of leachate from *Eupatorium adenophorum* on germination and growth of *Amaranthus retroflexus* and *Chenopodium glaucum*. Acta Ecol Sin. 2012;32(1):50–6. http://dx.doi.org/10.1016/j.chnaes.2011.12.004
16. Vrbničanin S, Karadžić B, Dajić-Stevanović Z. Adventivne i invazivne korovske vrste na području Srbije. Acta Biol Jugoslav.-serija G: Acta Herbol. 2004;13(1):1-12.
17. Costea M, Weaver SE, Tardif FJ. The biology of Canadian weeds. 130. *Amaranthus retroflexus* L., *A. powellii* S. Watson and *A. hybridus* L. Can J Plant Sci. 2004;84(2):631–68. https://doi.org/10.4141/P02-183
18. Assad R, Reshi ZA, Jan S, Rashid I. Biology of amaranths. Bot Rev. 2017;(83):382–436. https://doi.org/10.1007/s12229-017-9194-1
19. Božić D. *Amaranthus retroflexus* L. - štir obični. Acta Herbol. 2018;27(1):5-19. https://doi.org/10.5937/ActaHerb1801005B
20. Kordali S, Tazegul A, Cakir A. Phytotoxic effects of *Nepeta meyeri* Benth. extracts and essential oil on seed germinations and seedling growths of four weed species. Rec Nat Prod. 2015;9(3):404–18.
21. Slavnić Ž. Prilog flori našeg Podunavlja. Glasnik Biološke Sekcije. 1953;4-6:145-77.
22. Choi B, Song DY, Kim CG, Song BH, Woo SH, Lee C. Allelopathic effects of common ragweed (*Ambrosia artemisiifolia* var. *elatior*) on the germination and seedling growth of crops and weeds. J Weed Sci. 2010;30(1):34–42. https://doi.org/10.5660/KJWS.2010.30.1.034
23. Lehoczky E, Szabó R, Nelima MO, Nagy P, Béres I. Examination of common ragweed's (*Ambrosia artemisiifolia* L.) allelopathic effect on some weed species. Commun Agric Appl Biol Sci. 2010;75(2):107–11.
24. Ognjenovic J, Milcic-Matic N, Smiljanic K, Vuckovic O, Burazer L, Popovic N, Stanic-Vucinic D, Cirkovic Velickovic T. Immunoproteomic characterization of *Ambrosia artemisiifolia* pollen allergens in canine atopic dermatitis. Vet Immunol Immunopathol. 2013;155:38–47. https://doi.org/10.1016/j.vetimm.2013.06.005
25. Božić D. *Ambrosia artemisiifolia* L. – ambrozija pelenasta. Acta Herbol. 2018;27(2):79–95.
26. Lepší M, Lepší P, Koutecký P, Lučanová M, Koutecká E, Kaplan Z. *Stellaria ruderalis*, a new species in the *Stellaria media* group from central Europe. Preslia. 2019;91(4):391–420. https://doi.org/10.23855/preslia.2019.391
27. Rice EL. Allelopathy — An overview. In: Cooper-Driver GA, Swain T, Conn EE, editors. Chemically mediated interactions between plants and other organisms. Recent Advances in Phytochemistry (19). Boston, Massachusetts, SAD: Springer; 1985. p. 81–105. https://doi.org/10.1007/978-1-4757-9658-2_4
28. Cheng F, Cheng Z. Research progress on the use of plant allelopathy in agriculture and the physiological and ecological mechanisms of allelopathy. Front Plant Sci. 2015;(6):1–16. https://doi.org/10.3389/fpls.2015.01020
29. López TA, Cid MS, Bianchini ML. Biochemistry of hemlock (*Conium maculatum* L.) alkaloids and their acute and chronic toxicity in livestock. A review. Toxicon. 1999;37(6):841–65. https://doi.org/10.1016/S0041-0101(98)00204-9
30. Al-Snafi AE. Pharmacology and toxicology of *Conium maculatum* - A review. Pharm Chem J. 2016;(3):136–42.
31. Corsi G, Biasci D. Secretory structures and localization of alkaloids in *Conium maculatum* L. (Apiaceae). Ann Bot. 1998;81(1):157–62. https://doi.org/10.1006/anbo.1997.0547
32. Di Napoli M, Varcamonti M, Basile A, Bruno M, Maggi F, Zanfardino A. Anti-*Pseudomonas aeruginosa* activity of hemlock (*Conium maculatum*, Apiaceae) essential oil. Nat Prod Res. 2019;33(23):3436–40. https://doi.org/10.1080/14786419.2018.1477151

33. Muro-Medina C V., García-Morales S, Caltempa AO, Tapia-Campos E, León-Morales JM. The potential of piperidine alkaloids-rich extracts from *Prosopis* spp. to combat plant pests and diseases. Int J Biochem Res Rev. 2022;31(1):20–38. https://doi.org/10.9734/ijbcrr%2F2022%2Fv31i130298
34. Scavo A, Restuccia A, Pandino G, Onofri A, Mauromicale G. Allelopathic effects of *Cynara cardunculus* L. leaf aqueous extracts on seed germination of some mediterranean weed species. Ital J Agron. 2018;13(2):119–25. https://doi.org/10.4081/ija.2018.1021
35. Yarnia M, Khorshidi Benam MB, Farajzadeh Memari Tabrizi E. Allelopathic effects of sorghum extracts on *Amaranthus retroflexus* seed germination and growth. J Food, Agric Environ. 2009;7(3–4):770–4.
36. Pannacci E, Masi M, Farneselli M, Tei F. Evaluation of mugwort (*Artemisia vulgaris* L.) aqueous extract as a potential bioherbicide to control *Amaranthus retroflexus* L. in maize. Agric. 2020;10(12):1–13. https://doi.org/10.3390/agriculture10120642
37. Šćepanović M, Sarić-Krsmanović M, Šoštarčić V, Brijačak E, Lakić J, Špirović Trifunović B, Gajić Umiljendić J, Radivojević Lj. Inhibitory effects of Brassicaceae cover crop on *Ambrosia artemisiifolia* germination and early growth. Plants. 2021;10(4). https://doi.org/10.3390/plants10040794
38. Kazinczi G, Béres I, Onofri A, Nádasy E, Takács A, Horváth J, et al. Allelopathic effects of plant extracts on common ragweed (*Ambrosia artemisiifolia* L.). J Plant Dis Proctection. 2008;(21):335–9.
39. Mahmoodzadeh H, Ghasemi M, Zanganeh H. Allelopathic effect of medicinal plant *Cannabis sativa* L. on *Lactuca sativa* L. seed germination. Acta Agric Slov. 2015;105(2):233–9. http://dx.doi.org/10.14720/aas.2015.105.2.06
40. Sbai H, Zribi I, DellaGreca M, Haouala R. Bioguided fractionation and isolation of phytotoxic compounds from *Apium graveolens* L. aerial parts (Apiaceae). South African J Bot. 2017;108:423–30. https://doi.org/10.1016/j.sajb.2016.09.011
41. Nestorović Živković J, Dmitrović S, Jovanović V, Živković S, Božić D, Aničić N, Mišić D. Allelopathic potential of essential oil of *Nepeta rtanjansis*. Allelopath J. 2016;37(2):207–19.
42. Prijović M, Nikolić B, Dragićević I, Nestorović Živković J, Dmitrović S, Giba Z, Jovanović, V. Water emulsion of the essential oil of *Nepeta rtanjensis* Diklić et Milojević: potential use as a bioherbicide. Arch Biol Sci. 2024;76(1):5–14. https://doi.org/10.2298/ABS231107041P
43. Sturm DJ, Kunz C, Gerhards R. Inhibitory effects of cover crop mulch on germination and growth of *Stellaria media* (L.) Vill., *Chenopodium album* L. and *Matricaria chamomilla* L. Crop Prot. 2016;90:125–31. https://doi.org/10.1016/j.cropro.2016.08.032
44. Jovanović V, Galović M, Nikolić B, Dragićević I, Waisi H, Giba Z. The effects of water extracts of *Xeranthemum cylindraceum* Sibth. et Sm. on seed germination of three weed species. In: Kovačević D, editor. Proceedings of the 10th International Scientific Agriculture Symposium "Agrosym 2019"; 2019 Oct 3-6; Jahorina, Bosnia and Herzegovina. East Sarajevo: Faculty of Agriculture: 2019. p. 997–1003.