UDC 581.1:581.9:632.954:574.5 DOI: https://doi.org/10.33730/2077-4893.3.2025.340783

ASSESSMENT OF THE ALLELOPATHIC AND PHYTOTOXIC EFFECTS OF *AMBROSIA ARTEMISIIFOLIA* L. RESIDUES ON SEED GERMINATION OF KEY AGRICULTURAL CROPS

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Ambrosia artemisiifolia L. — один із найагресивніших інвазійних видів бур'янів, що становить серйозну загрозу біорізноманіттю, стабільності агроекосистем та здоров'ю населення через високу алергенність пилку. Її потужні алелопатичні властивості зумовлюють пригнічення росту аборигенних видів і культурних рослин, що призводить до зниження врожайності та порушення трофічних зв'язків. Метою дослідження було кількісне оцінювання алелопатичного впливу водних екстрактів різних морфологічних органів рослини (корені, стебла, листки, суцвіття) на енергію проростання (GE) та лабораторну схожість (G) трьох культурних видів: Triticum aestivum L., Phaseolus vulgaris L. і Zea mays L. У лабораторних біотестах використано чотири методи екстракції: 24-годинна мацерація, 1-годинна екстракція, термічна обробка (водяна баня) та екстракція з сухої біомаси у концентраціях 1–10%. Алелопатичну активність виражено в умовних кумаринових одиницях (CCU). Встановлено дозозалежне та органоспецифічне інгібування: найбільш виражений ефект мали 24-годинні екстракти суцвіть і коренів. T. aestivum L. проявила найвищу чутливість (зниження GE і G до 15% за впливу 5–10% екстрактів), P. vulgaris L. займала проміжну позицію щодо чутливості, тоді як Z. mays L. демонструвала толерантність (CCU⩽4–5 у більшості варіантів). Статистичний аналіз (ANOVA) підтвердив значущий вплив органа-донора та способу екстракції ($p<0,05$). Висока фітотоксичність інфлоресценцій і коренів, ймовірно, зумовлена накопиченням сесквітерпенів, флавоноїдів та поліацетиленів, що підтверджує гіпотезу «нової біохімічної зброї» і свідчать про потенційно важливу роль алелопатії у конкурентних перевагах A. artemisiifolia L. Практичне значення полягає у необхідності систематичного фітомоніторингу, врахування алелопатичного ефекту під час планування сівозмін та застосування T. aestivum L. як біоіндикатора. Подальші дослідження слід спрямувати на ідентифікацію активних сполук, оцінку їхньої стійкості у ґрунті та вплив на ґрунтову мікробіоту, що дасть можливість інтегрувати отримані дані в екологічно безпечні стратегії контролю інвазій.

Ключові слова: *інвазійні види, вторинні метаболіти, умовні кумаринові одиниці, екстракційні методи, стійкість культур, біомоніторинг, біоконтроль бур'янів, сесквітерпеноїди, фітоінгібітори, агроекологічний ризик.*

INTRODUCTION

In modern agro-landscapes, uncontrolled spread of invasive plant species represents a serious challenge to crop productivity, agroecosystem resilience, and the conservation of agrobiodiversity. One of the most aggressive alien species in Ukraine and throughout Europe is *Ambrosia artemisiifolia* L. (common ragweed), an annual invasive plant from the *Asteraceae* family, native to North America. Its high fecundity, ecological plasticity, and the capacity to synthesize biologically active compounds have facilitated its rapid colonization of croplands, roadsides, abandoned fields, and urbanized habitats [1–4].

Ecological success of *A. artemisiifolia* L. is driven not only by its reproductive strategies but also by allelopathy — a phenomenon involving the release of phytotoxic secondary

metabolites that suppress seed germination, seedling development, and root elongation in surrounding plant species [5; 6]. Among these allelochemicals, sesquiterpenoids and phenolic acids have been identified as major contributors to its phytotoxicity, particularly those derived from root and inflorescence tissues [1; 7–9].

In line with the «Novel Weapons Hypothesis», which posits that invasive species gain competitive advantages by producing biochemical agents unfamiliar to native or cultivated flora [10], *A. artemisiifolia* L. has demonstrated allelopathic effects across a range of crop species. Recent studies have shown that aqueous and organic extracts of this species can inhibit the germination and growth of *Triticum aestivum* L., *Hordeum vulgare* L., *Trifolium repens* L., and *Zea mays* L., with the degree of inhibition varying by concentration, extraction method, and target species [11–13].

For instance, Zeng et al. [14] reported that sesquiterpenoids at 200 μg/mL reduced wheat root growth by over 50%, while aqueous extracts at dilution ratios of 1:4 to 1:8 markedly inhibited barley and clover seed germination [11]. Moreover, [15] observed differential species sensitivity: carrot and basil exhibited pronounced inhibition, while soybean was affected minimally. In some cases, legumes such as alfalfa (*Medicago sativa* L.) even showed neutral or stimulatory responses under low extract concentrations [16]. Such crop-specific effects underscore the importance of selecting appropriate test species in allelopathic bioassays.

Despite these findings, the organ-specific allelopathic effects of *A. artemisiifolia* L., particularly during early developmental stages such as seed germination and laboratory viability, remain underexplored. Moreover, there is a lack of comparative studies addressing how different extraction protocols — including soaking, heat-based extraction, and infusions from dried biomass modulate the phytotoxicity of plant residues. These methodological gaps limit our capacity to predict allelopathic risks across different cropping systems and constrain the development of integrated weed management strategies based on ecological principles.

The aim of the study is to quantify the allelopathic potential of aqueous extracts obtained from different morphological parts of *Ambrosia artemisiifolia* L. (roots, stems, leaves, and inflorescences) prepared using four distinct extraction methods (24-hour soaking, 1-hour soaking, water bath treatment, and dry biomass infusion). We assessed their phytotoxic effects on germination energy and laboratory viability of *Phaseolus vulgaris* L., *Triticum aestivum* L., and *Zea mays* L. — three economically and ecologically significant crops in temperate agroecosystems.

ANALYSIS OF RECENT RESEARCH

In recent years, the allelopathic potential of *Ambrosia artemisiifolia* L. has attracted increasing scientific attention due to its growing impact on crop productivity and ecological balance. As a widespread invasive species, *A. artemisiifolia* L. demonstrates a pronoun0 ced ability to influence both natural and cultivated phytocoenoses chemically, primarily through the release of phytotoxic secondary metabolites.

A number of studies have confirmed the substantial inhibitory effect of this species on seed germination, root elongation, and early seedling development of major crops. For instance, Liu et al. [1] reported that aqueous extracts suppressed the germination and root growth of *Triticum aestivum* L. and *Brassica napus* L. in a dose-dependent manner. Similarly, Zeng et al. [13; 14] demonstrated that extracts from different plant organs, particularly roots and inflorescences, significantly reduced germination energy and root length in wheat, thereby confirming the organ-specific nature of allelopathic activity.

Increasing attention has been devoted to the biochemical mechanisms underlying allelopathy. The primary allelochemicals identified in *A. artemisiifolia* L. are sesquiterpene lactones, including psilostachyins A, B, and C, isolated from aerial organs and known for their pronounced phytotoxicity [17]. A recent phytochemical investigation identified four novel sesquiterpenes from the whole plant,

all exhibiting allelopathic activity [1]. Inflorescences were found to be especially rich in more than twenty sesquiterpenoids, several of which markedly suppressed wheat root and shoot growth [14]. A comprehensive review by Kovács et al. [18] further systematized current knowledge of Ambrosia sesquiterpenes, emphasizing their structural diversity, phytotoxicity, and potential pharmacological properties.

Besides sesquiterpenes, representatives of the genus *Ambrosia* contain flavonoids (quercetin, luteolin) and polyacetylenes. Flavonoids are known to affect hormonal regulation and photosynthetic processes, whereas polyacetylenes are recognized for their cytotoxicity. However, the specific roles of these compounds in *A. artemisiifolia* L. allelopathy remain insufficiently understood [15].

The extraction method is a key factor determining allelochemical stability. Sesquiterpene lactones are thermolabile and degrade upon heating, while flavonoids display greater thermal stability. This explains the stronger phytotoxicity of cold (24-hour) aqueous extracts compared to extracts prepared from dried biomass or by heating [1; 18].

Allelopathy interacts with other competitive traits of *A. artemisiifolia* L., including rapid growth, an extensive root system, and high seed productivity. Collectively, these characteristics enhance the invasive potential of the species. At the same time, significant gaps remain in the literature: systematic comparisons of extraction methods and detailed organ-specific analyses of phytotoxicity are still lacking.

Maksimović et al. [11] documented a strong allelopathic effect of *A. artemisiifolia* L. on *Hordeum vulgare* L. and *Trifolium repens* L., even at dilutions of 1:4 and 1:8, indicating that sublethal concentrations can cause notable physiological disturbances in cereals and legumes. Similarly, Bonea et al. [12] and Gunda et al. [15] demonstrated that extracts negatively affect early development of *Zea mays* L., reducing seedling biomass, altering root architecture, and delaying shoot emergence, particularly under stress conditions such as nutrient deficiency or drought.

More detailed biochemical studies confirm high variability in secondary metabolite composition depending on plant organ and extraction method. Ding et al. [8] reported considerable differences in the content of sesquiterpene lactones, phenolic acids, and flavonoids between organs, while Šućur et al. [19] identified distinct «allelochemical fingerprints» in roots and leaves, suggesting targeted allelopathic strategies in different ecological contexts. Additionally, Wang et al. [20] proposed the use of Conditional Coumarin Units (CCU) as a standardized index for quantifying allelopathic stress, thereby improving comparability across studies. An et al. [21] highlighted the critical role of species-specific sensitivity: for example, soybean exhibited relative tolerance, while wheat and carrot showed heightened susceptibility. Similarly, Flores-Duarte et al. [22] reported non-linear responses among legumes, with *Medicago sativa* L. occasionally displaying neutral or even stimulatory effects at low extract concentrations.

Taken together, these findings emphasize the multifactorial nature of *A. artemisiifolia* L. allelopathy, driven by extract concentration, donor organ, recipient species physiology, and methodological approaches. Addressing these methodological inconsistencies is essential for accurate risk assessment of *A. artemisiifolia* L. in agroecosystems and for the development of effective management strategies to mitigate its spread.

MATERIALS AND METHODS OF RESEARCH

In this study, seeds of three agriculturally important crops were used as test species: *Phaseolus vulgaris* L. (common bean), *Triticum aestivum* L. (bread wheat), and *Zea mays* L. (maize). The seeds were pre-calibrated by size and weight to ensure sample uniformity, followed by surface sterilization in a 1% potassium permanganate ($KMnO_4$) solution for 5 minutes and subsequent triple rinsing with distilled water.

Samples of *Ambrosia artemisiifolia* L. were collected from agricultural fields, where the soil cover is represented by podzolized cher-

nozem. The choice of this site was determined by its typical agroecological conditions, which may influence the qualitative and quantitative composition of plant secondary metabolites. Plant material was collected during the flowering stage (autumn). Leaves, stems, and inflorescences were air-dried at 25–28°C until a constant weight was achieved, and then fragmented into 0.5–1 cm fractions mechanically. Grinding plant biomass into fractions of 0.5–1 cm provides an optimal balance between the surface area available for solvent contact and the preservation of secondary metabolites. Excessive grinding (to a powder) can lead to the degradation of thermolabile or volatile components (including certain sesquiterpenes and phenolic compounds); oxidation due to intensive contact with oxygen; increased sample heterogeneity resulting from uneven particle distribution. Conversely, excessively large fragments (over 1 cm) reduce the efficiency of bioactive compound extraction due to a smaller contact surface area. Thus, the chosen range of 0.5–1 cm represents a methodologically justified compromise, allowing for maximal preservation of the natural composition of allelochemicals while ensuring sufficient extraction.

To evaluate allelopathic activity, we prepared such four types of aqueous extracts as [23] 24-hour cold macerate: 10 g of dried biomass per 100 mL of distilled water, extracted at ambient temperature for 24 hours; 1-hour extract: identical plant-to-solvent ratio, extracted at room temperature for 1 hour; heated extract (water bath): 10 g of plant material in 100 mL of water, heated at 80°C for 30 minutes; dry extract: prepared under standard laboratory drying conditions. All extracts were filtered through Whatman No. 1 paper without further sterilization. Based on the approach of Maksimović et al. [11], such concentrations as 10% (undiluted), 5% (1:2), 2.5% (1:4), and 1% (1:8) were tested. The use of four approaches (cold maceration, short-term infusion, heating in a water bath, and dry extract) enables us to assess the effect of temperature on the stability of secondary metabolites (sesquiterpenes, flavonoids, polyacetylenes), the role of extraction time in the release of active compounds, the comparison of fresh and dry biomass as a source of allelochemicals and the method-dependent nature of allelopathy, which is particularly important for interpreting the ecological risks of ragweed.

Seed germination was performed in 90 mm diameter Petri dishes, each lined with two layers of Whatman filter paper moistened with 5 mL of the respective extract. For each treatment, 10 seeds were placed in each dish, with four biological replicates per treatment. The experiments were conducted under controlled laboratory conditions: temperature 22±2°C, photoperiod of 16 h light / 8 h dark, and regulated humidity.

To assess organ-specific allelopathic effects, additional treatments using 10% aqueous extracts derived separately from leaves, stems, inflorescences, roots, and rhizospheric soil of *A. artemisiifolia* L. were included.

The following germination parameters were evaluated: germination energy (%) — assessed on day 3 of incubation; laboratory germination (%) — assessed on day 7.

Data were analyzed statistically using Statistica 10 and Microsoft Excel. Treatment effects were compared using the Student's t-test, with statistical significance set at $p<0.05$. Results are expressed as mean ± standard deviation (SD).

Quantitative assessment of allelopathic impact was conducted using the Conditional Coumarin Unit (CCU) method, adapted from the scale developed by Hrodzynskyi (1979), with further interpretation based on a modified 10-point allelopathic activity scale (Kuzmenko et al.).

Calculation procedure:

- **Step 1:** Determine the percentage of seed germination for each treatment;
- **Step 2:** Convert to CCU using the formula:

$$CCU = 45 - (\text{Germination } (\%) \times \times 0.365); \quad (1)$$

where 100% germination corresponds to 8.5 CCU; 0% to 45 CCU;

- **Step 3:** Convert CCU to a 10-point scale:

$$\text{point} = (CCU - 8.65)\backslash 3.65; \quad (2)$$

Table 1. **Allelopathic activity scale based on Conditional Coumarin Units (CCU)**

Score	CCU Range	Allelopathic Activity Description
0	≤ 8.5	No or very weak effect
1	8.6–12.0	Very weak
2	12.1–15.5	Weak
3	15.6–19.0	Moderately weak
4	19.1–22.5	Moderate
5	22.6–26.0	Moderately strong
6	26.1–29.5	Strong
7	29.6–33.0	Very strong
8	33.1–36.5	Extremely strong
9	36.6–40.0	Near-complete inhibition
10	40.1–45.0	Complete inhibition

- **Step 4:** Interpret allelopathic activity:

0–3 points (0–20.5 CCU): Weak allelopathic activity;

4–6 points (20.6–30.5 CCU): Moderate activity;

7–10 points (30.6–45 CCU): Strong allelopathic activity.

RESULTS AND DISCUSSION

Allelopathy represents a multifaceted ecological mechanism in which plants release bioactive secondary metabolites (allelochemicals) into their environment, influencing the growth and development of neighboring species. These substances enter the soil via leaf and stem litter, rain-induced leaching, fruit decomposition, and root exudation [24; 25]. Their vertical distribution in the soil varies: surface residues primarily affect the upper horizons, while root-derived compounds penetrate deeper, exerting direct influence on seed germination and early seedling development. Although the ecological role of allelopathy is well recognized in natural ecosystems, its dynamics in agricultural settings remain insufficiently characterized.

The present study confirms the high allelopathic potential of *Ambrosia artemisiifolia* L., particularly evident in aqueous extracts derived from root biomass. A 10% concentration of freshly prepared root extract significantly reduced germination energy in *Triticum aestivum* L. and *Phaseolus vulgaris* L., approaching near-zero values. Total germination capacity decreased by 40–60%, depending on the concentration and preparation method. The most pronounced inhibitory effects were observed in extracts prepared using a water bath, where both germination and seedling growth (root and shoot length) were reduced by more than 50% compared to control treatments. These findings corroborate prior studies demonstrating similar phytotoxic effects of *A. artemisiifolia* L. sesquiterpenoids on wheat roots [14; 26].

Among the various extraction types, 24-hour aqueous macerates exhibited the highest phytotoxicity. For *P. vulgaris* L., exposure to a 10% extract resulted in a drastic decline in germination energy (to 15%) and total germination (to 25%). In *T. aestivum* L., suppression was observed even at 5%, while at 10%, germination was nearly inhibited entirely (approximately 5%). By contrast, *Zea mays* L. demonstrated relative tolerance under identical conditions, with germination energy and viability decreasing only to 55% and 65%, respectively.

Short-duration extracts (1-hour maceration) exhibited milder allelopathic effects, yet remained potent for sensitive species. Even at 1% concentration, *T. aestivum* L. exhibited a >60% reduction in germination energy, suggesting pronounced sensitivity. *P. vulgaris* L. displayed moderate sensitivity, with germination energy decreasing by 35% only at the highest concentration tested. *Z. mays* L. again emerged as the most resilient species, exhibiting only gradual reductions in seedling development, consistent with its known resistance to allelochemical interference [11].

Water bath-prepared extracts showed a modified toxicity profile. In *P. vulgaris* L. and *Z. mays* L., the inhibitory effects were moderate. However, *T. aestivum* L. displayed strong suppression even at 5–10% concentrations, possibly due to the release of thermally stable phenolic acids and sesquiterpenes during heating [1; 27]. These compounds likely retain bioactivity despite thermal exposure and may

even become more extractable under such conditions.

The least pronounced allelopathic effects were associated with extracts prepared from dried plant biomass. In most treatments, germination energy and capacity remained within 70–85%, even at the highest concentrations. This suggests partial degradation or volatilization of key allelochemicals during drying or storage, consistent with earlier reports on the instability of certain phytotoxins [8; 12].

Species-specific sensitivity to allelopathic compounds was observed clearly. *T. aestivum* L. consistently exhibited the highest susceptibility, with marked reductions in germination and early growth metrics across all extract types and concentrations. *P. vulgaris* L. showed moderate tolerance, maintaining germination viability at concentrations up to 2.5%. *Z. mays* L., due to its robust morphophysiological characteristics such as thicker seed coats and higher antioxidant capacity proved the least affected [28].

A well-defined dose–response relationship was established. Maximum inhibition (0–5% germination energy) occurred in *T. aestivum* L. under the influence of 10% 24-hour extracts, whereas the minimum inhibitory effect (65–80%) was observed in *Z. mays* L. treated with extracts from dried material. The observed phytotoxicity may stem from the inhibition of key hydrolytic enzymes responsible for mobilizing seed storage reserves during germination [13; 24].

The results of one-way ANOVA confirmed statistically significant variation in the inhibitory effects of different extract types across crop species. *P. vulgaris* L. showed moderate but significant sensitivity (F=3.30; p=0.048), while *T. aestivum* L. demonstrated highly significant treatment effects (F=11.10; p=0.00035). In contrast, *Z. mays* L. showed no statistically significant response across extract types (F=1.96; p=0.161), reinforcing its role as a relatively allelophytotolerant species.

As visualized in the scatter plot (*Fig. 1*), *T. aestivum* experienced the most severe decline in germination energy across treatments, whereas *Z. mays* L. remained closest to control values. These findings suggest the potential of using *T. aestivum* L. as a sensitive bioindicator species in allelopathic risk assessments of invasive alien plants such as *A. artemisiifolia* L. This aligns with the «novel weapons hypothesis», which posits that non-native species exert chemical interference via allelochemicals novel to the invaded flora, disrupting crop establishment and community dynamics [5].

The obtained results align with earlier research demonstrating the broad-spectrum phytotoxicity of both aqueous and organic extracts of *Ambrosia artemisiifolia* L. against a wide range of cultivated species, including soybean, barley, bean, and maize [12; 28]. These data further corroborate the hypothesis of a universal allelopathic potential inherent to *A. artemisiifolia* L. and underscore its role as a significant biotic threat to agrobiodiversity and crop productivity.

To assess the degree of differential sensitivity among the studied crops, a k-means cluster analysis was conducted using two physiological indicators germination energy (GE) and laboratory germination (G) across treatments involving various aqueous root exudate extracts. The analysis identified three distinct clusters based on the intensity of allelopathic inhibition (*Fig. 2*).

Cluster I grouped variants with minimal phytotoxic impact, predominantly associated with *Zea mays* L., indicating high resilience to the tested extracts. Cluster II included treatments with moderate inhibitory effects, corresponding primarily to *Phaseolus vulgaris* L., which exhibited intermediate sensitivity. Cluster III comprised the most severely affected variants, all linked to *Triticum aestivum* L., reaffirming its status as the most allelosensitive species among the three.

This pattern effectively reflects the species-specific sensitivity gradient and supports the hypothesis that allelopathic inhibition by *A. artemisiifolia* L. root exudates can be quantified using integrative phytotoxicity indices such as Conditional Coumarin Units (CUO), which provide a standardized approach to evaluating allelopathic intensity across treatments.

Furthermore, while the present study focused on root-derived compounds, it is important to note that in natural environments, allelochemicals are also introduced into agroecosystems through the aerial biomass of in-

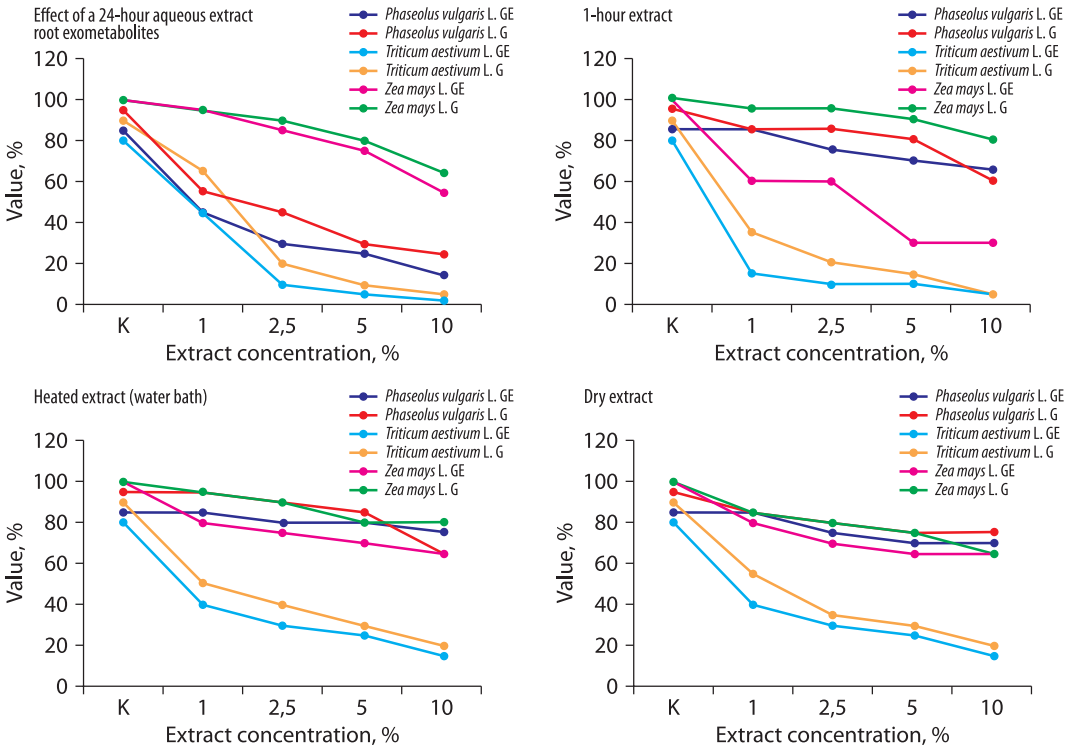

Fig. 1. Effect of 24-hour aqueous extracts from roots of *Ambrosia artemisiifolia* L. on seed germination energy (En) and laboratory viability (Cx) of *Phaseolus vulgaris* L., *Triticum aestivum* L., *Zea mays* L.

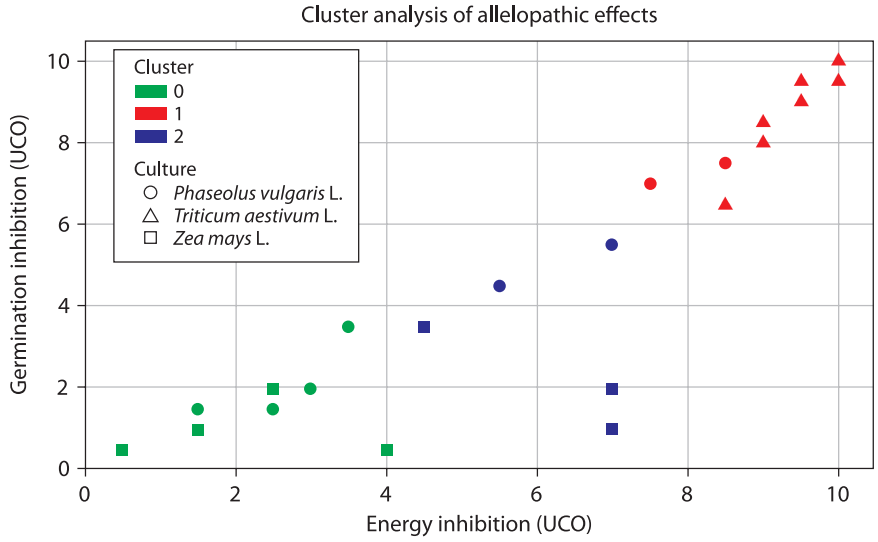

Fig. 2. Clustering of allelopathic effects based on inhibition intensity (CUO — Conditional Coumarin Units)

vasive plants. Decomposing foliage, pollen, and floral remnants contribute significantly to the soil's chemical milieu. Existing literature highlights that aerial parts of *A. artemisiifolia* L. particularly leaves, stems, and inflorescences are rich in a variety of allelopathic compounds, including phenolic acids, sesquiterpenes, flavonoids, and coumarins [1; 7; 8]. These metabolites, many of which possess high thermal and chemical stability, may act synergistically to disrupt germination and early growth processes in competing species.

To this end, the next phase of our investigation will focus on the comparative allelopathic activity of aqueous extracts obtained from different morphological structures of *A. artemisiifolia* L., specifically analyzing leaves, stems, and inflorescences. By dissecting the phytotoxic potential of each plant organ, we aim to determine which biomass sources contribute most significantly to the inhibitory effect, to distinguish the relative roles of vegetative versus generative organs in allelopathic interference, to characterize the composition and concentration of organ-specific allelochemicals, to formulate targeted management strategies to mitigate the invasive impact of *A. artemisiifolia* L. in agricultural systems and to enhance the use of phytotoxicity-based bioindicators for real-time assessment of invasion pressure in agroecosystems.

Preliminary Evaluation of Stem Extract Effects. Preliminary data from ongoing experiments on aqueous stem extracts of *A. artemisiifolia* L. indicate a pronounced inhibitory influence on seed germination in all three crop species: *P. vulgaris* L., *T. aestivum* L., and *Z. mays* L. (*Fig. 3–5*). The inhibition fol-

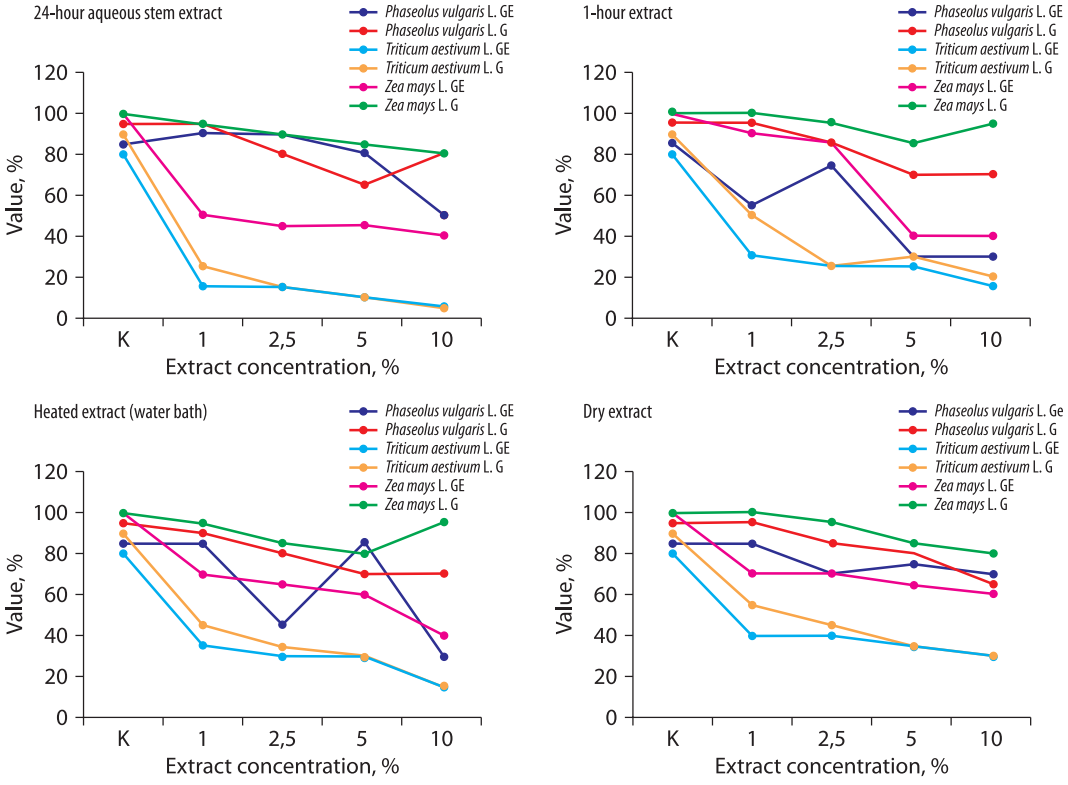

Fig. 3. Effect of aqueous extracts from stems of *Ambrosia artemisiifolia* L. on seed germination energy (En) and laboratory viability (Cx) of *Phaseolus vulgaris* L., *Triticum aestivum* L., *Zea mays* L.

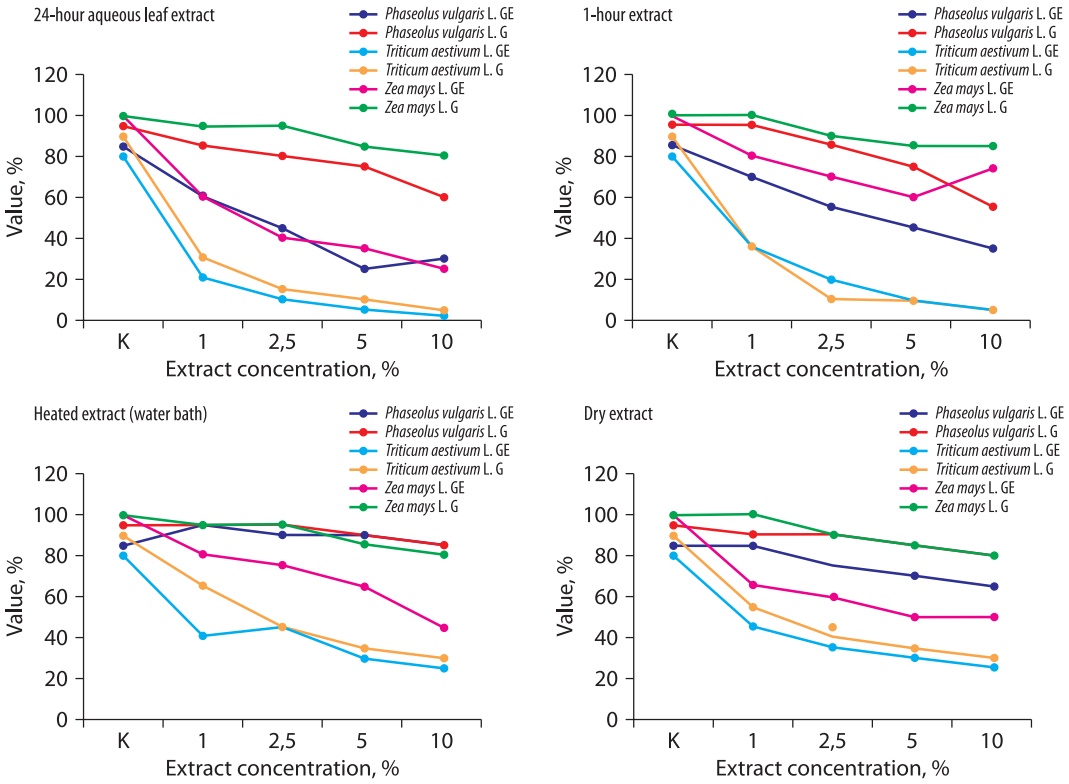

Fig. 4. Effect of aqueous extracts from leaves of *A. artemisiifolia* L. on seed germination energy (En) and laboratory viability (Cx) of *P. vulgaris* L., *T. aestivum* L., *Z. mays* L.

lowed a clear dose-dependent trend across all species. Notably, germination energy and laboratory germination declined progressively with increasing extract concentrations, though the extent of suppression varied among crops.

Interspecific variability was again evident: *T. aestivum* L. exhibited the highest sensitivity to stem-derived allelochemicals, confirming earlier observations with root extracts. *P. vulgaris* L. showed moderate inhibition, while *Z. mays* L. remained the least affected, albeit still showing significant reductions at higher concentrations.

These findings support the hypothesis that allelopathic compounds are distributed throughout the entire plant body of *A. artemisiifolia* L., not solely concentrated in root tissues, and that their phytotoxic potential is consistently expressed across organs.

Allelopathic Effects of Leaf and Inflorescence Extracts of *A. artemisiifolia* L. The aqueous extracts derived from the leaves and inflorescences of *Ambrosia artemisiifolia* L. exhibited a pronounced inhibitory effect on seed germination and germination energy across all tested crops, further confirming the broad allelopathic potential of this species. As in previous experiments with root and stem extracts, the phytotoxic effects were both dose-dependent and species-specific, with distinct differences observed between the types of plant organs and extraction methods.

Triticum aestivum L. once again demonstrated the highest sensitivity. Exposure to 24-hour aqueous extracts from both leaves and inflorescences at concentrations of 10% resulted in near-complete inhibition of germination energy (reduced to 5–10%) and a dramatic drop in total germination (to 10–

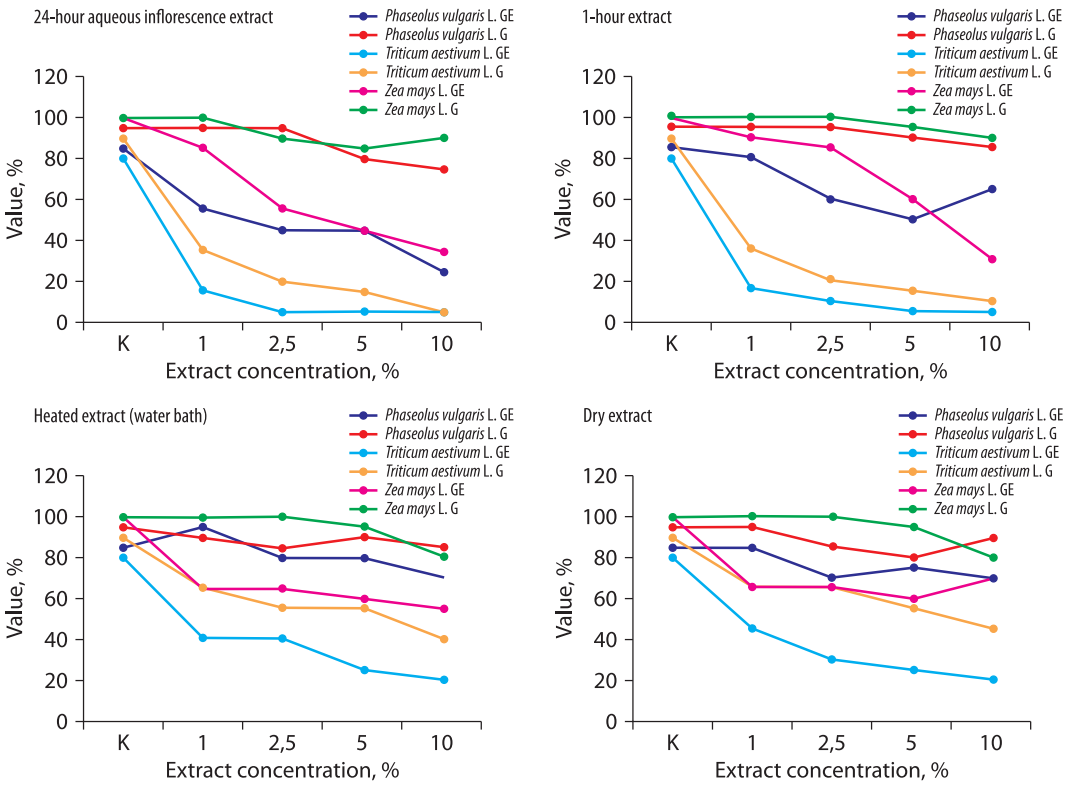

Fig. 5. Effect of aqueous extracts from inflorescences of *Ambrosia artemisiifolia* L. on seed germination energy (En) and laboratory viability (Cx) of *Phaseolus vulgaris* L., *Triticum aestivum* L., *Zea mays* L.

20%). One-hour extracts also caused significant suppression, suggesting a high concentration of fast-releasing allelochemicals in aerial biomass.

Phaseolus vulgaris L. exhibited an intermediate level of sensitivity. Germination energy was reduced to 40–50%, while total germination dropped to 60–70% under the influence of 10% inflorescence extracts. Interestingly, leaf-derived extracts were slightly more phytotoxic than those from inflorescences, possibly due to a higher concentration of phenolic compounds, such as chlorogenic and caffeic acids, typically found in photosynthetically active tissues [1].

Zea mays L., consistent with prior observations, showed the highest tolerance. Even under exposure to 10% 24-hour inflorescence extracts, its germination remained above 75%, and germination energy did not fall below 60%. This further supports the hypothesis of maize's inherent resilience to the chemical stress imposed by ambrosia allelochemicals.

Across all species, extracts from dried biomass showed the weakest allelopathic effects, consistent with previous reports [7; 28], possibly due to the degradation of thermolabile compounds during drying and storage. In contrast, short-duration aqueous extracts from fresh biomass frequently retained higher phytotoxicity, suggesting that volatile or highly reactive secondary metabolites may be crucial contributors to the observed inhibition.

The significantly stronger allelopathic impact of inflorescence-derived extracts, particularly on wheat and beans, aligns with findings by Sun and Roderick [27], who identified

eudesmane-type sesquiterpenes as dominant in reproductive structures of *A. artemisiifolia* L. These compounds are believed to interfere with hormonal regulation and cell division during early germination stages [21].

From a practical standpoint, these results reinforce the notion that aerial biomass, especially during flowering, represents a critical source of allelochemical pollution in agroecosystems. The deposition of inflorescences and leaf litter into the soil via natural senescence or mechanical incorporation (e.g., mowing or ploughing) can introduce a potent mix of phytotoxic substances that negatively affect sensitive crops.

The results of the analysis of variance (ANOVA) confirmed a statistically significant effect of the extraction method of *Ambrosia artemisiifolia* L. inflorescences on seed germination of *Phaseolus vulgaris* L. (F=4.05; p=0.026) and laboratory germination of *Triticum aestivum* L. (F=4.61; p=0.017), indicating a considerable dependence of the allelopathic effect on the type of extraction used. In contrast, for *Zea mays* L., the extraction method showed no significant influence (p>0.86), which confirms this crop's relative tolerance to phytotoxic components released from ragweed inflorescences.

The inhibitory matrix, quantified using Conditional Coumarin Units (CCUs), demonstrated the strongest allelopathic activity in 24-hour extracts, particularly for *T. aestivum* L. and *P. vulgaris* L., where CCU values reached up to 45 points. This corresponds to the highest phytotoxicity level on the proposed inhibition scale. Extracts obtained via water bath treatment or from dried biomass exhibited moderate to weak effects, especially in the case of *Z. mays* L., whose CCU values consistently remained below 4.

The biological assessment further confirmed the strong phytotoxic potential of aqueous inflorescence extracts of *A. artemisiifolia* L., with clear differentiation in impact based on crop species, extract concentration, and extraction method. The most severe inhibition of germination energy was observed in *T. aestivum* L. under 24-hour extract — exposure, with energy values dropping to 5% or complete suppression, and laboratory germination reduced to 5–15% (*Fig. 6*). In *P. vul-*

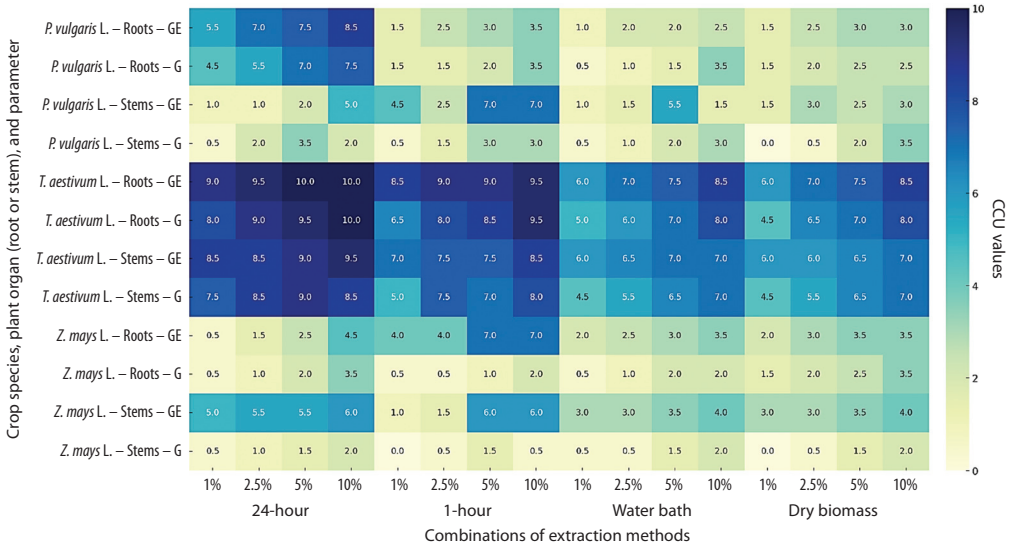

Fig. 6. Inhibitory strength matrix (in conditional coumarin units, CCU)

Notes: The X-axis represents combinations of extraction methods (24-h, 1-h, water bath, dry biomass) and extract concentrations (1%, 2.5%, 5%, 10%). The Y-axis indicates crop species, plant organ (root or stem), and parameter (GE — germination energy, G — germinability). Color intensity represents the mean CCU values: darker shades indicate stronger inhibitory effects.

garis L., a concentration-dependent decline in germination energy to 35–55% was noted, particularly under 24-hour and 1-hour treatments. In contrast, *Z. mays* L. again showed the lowest sensitivity, maintaining germination energy between 60–90% and total germination rates above 80%, even under higher extract concentrations.

The allelopathic index expressed in CCUs reached maximum values in 24-hour extracts: 9.5–10 for *T. aestivum* L., 7.5–8.5 for *P. vulgaris* L., and not more than 4 for *Z. mays* L. Although similar trends were observed for water bath and dried biomass extracts, the inhibition levels were significantly lower, likely due to degradation or transformation of active metabolites during thermal processing or drying.

These results reinforce the hypothesis about the high allelopathic potential of generative organs of *A. artemisiifolia* L., which are known to accumulate sesquiterpenoids, flavonoids, coumarins, and polyacetylenes [7; 21]. The accumulation of such secondary metabolites in flowers and inflorescences supports their role as potent inhibitors of plant development and germination.

The findings are consistent with those of Bonea et al. [12], who reported significant inhibition of *Lactuca sativa* L. and *Z. mays* L. germination under the influence of floral extracts of *A. artemisiifolia* L. In both studies, the inhibitory effect increased with concentration, and sensitivity varied by crop, with cereal species generally more vulnerable.

Further ANOVA results revealed that extract type had a statistically significant effect on germination energy across all species ($p<0.05$), but influenced laboratory germination only in *T. aestivum* L. suggesting that allelochemicals from inflorescences may primarily target early seed physiological processes such as membrane integrity, water uptake, and hormonal signaling, which are crucial during the initial phases of germination [6].

Overall, the study highlights that inflorescences of *A. artemisiifolia* L. serve as a potent source of allelopathic compounds capable of significantly suppressing seed energy and germination in sensitive crop species. This supports the «novel weapon hypothesis» [26], which posits that invasive species can outcompete natives through unique allelochemicals to which local flora has not developed resistance mechanisms.

These findings have significant practical implications for the management of *A. artemisiifolia* L. invasions in agricultural systems. The high phytotoxicity of its generative organs, particularly during flowering and seed set stages, poses a potential threat to crop establishment and early development, especially for cereals and legumes. This warrants further attention in terms of monitoring, suppression strategies, and crop rotation planning, especially in regions under high invasion pressure.

Visualization of the data via CCU-based heatmaps confirmed that 24-hour and water bath extracts were the most phytotoxic, particularly to *T. aestivum* L. and *P. vulgaris* L., while 1-hour extracts were less toxic, especially for *Z. mays* L., once again reinforcing interspecies differences in tolerance and vulnerability.

Discussion (continued): Organ-Specific Allelopathic Effects of *Ambrosia artemisiifolia* L. The extended bioassay data, visualized in a CCU-based heatmap, further illustrate the organ-specific allelopathic potential of *A. artemisiifolia* L. In this analysis, the mean inhibitory strength of aqueous extracts prepared from roots and stems was evaluated across four extraction methods (24-hour, 1-hour, water bath, and dry biomass) and four concentrations (1%, 2.5%, 5%, 10%).

Among all tested crops, *Triticum aestivum* L. again exhibited the highest sensitivity, with 24-hour root extracts causing maximal inhibition of both germination energy and viability (up to 10.0 CCU), especially at 5–10% concentrations. Stem extracts, although slightly less potent, still induced significant suppression (up to 9.5 CCU).

Phaseolus vulgaris L. demonstrated moderate sensitivity to root-derived allelochemicals: germination energy was inhibited up to 8.5 CCU under 24-hour extracts at 10% concentration, while 1-hour and dry biomass extracts had only mild effects.

Zea mays L. remained the most tolerant species, with CCU values rarely exceeding 4–5, even under high-concentration root extracts. One-hour and water bath extracts from both roots and stems showed virtually no inhibitory impact on maize germination.

Across all species, root extracts were the most phytotoxic, particularly when produced via 24-hour soaking, followed by stem extracts. Water bath and dry biomass methods yielded substantially lower allelopathic activity, likely due to thermal degradation or reduced extractable compound content.

This organ-specific phytotoxicity was further evident when comparing the effects of extracts from inflorescences and leaves. The heatmap clearly showed that 24-hour inflorescence and leaf extracts caused severe germination suppression in *T. aestivum* L. and *P. vulgaris* L., with CCU values reaching up to 90 — markedly higher than those from roots or stems. This confirms that generative and foliar organs of *A. artemisiifolia* L. contain the highest concentrations of allelochemicals, likely including sesquiterpenes, flavonoids, polyacetylenes, and coumarins [7; 19], which are well-documented for their growth-inhibitory properties.

A comprehensive comparison of morphological parts (roots, stems, leaves, inflorescences) revealed the following hierarchy of phytotoxicity: inflorescences — strongest inhibition, especially in wheat and bean, with complete germination suppression at 5–10% extract concentrations, roots — high toxicity, particularly in *T. aestivum* L., moderate in *P. vulgaris* L., minimal in *Z. mays* L., stems — moderate dose-dependent effects, primarily on wheat, weaker than roots or inflorescences, leaves — lowest phytotoxicity, however, 24-hour high-concentration extracts still caused moderate inhibition in wheat and beans.

All extraction methods showed dose-dependent inhibition, but 24-hour aqueous extraction was consistently the most efficient in releasing water-soluble allelochemicals.

In terms of crop sensitivity, the pattern remained consistent: *T. aestivum* L. — most sensitive; affected by all organ extracts, especially inflorescences and roots; *P. vulgaris* L. — moderately sensitive; strongest inhibition from inflorescence and root extracts at higher concentrations; *Z. mays* L. — most tolerant; minimal suppression across all treatments.

These findings strongly support the «novel biochemical weapons» hypothesis [26], which posits that invasive plants may release allelochemicals unfamiliar to native or cultivated species, thereby suppressing their establishment and growth.

From a phytomonitoring and agroecological risk assessment perspective, this organ- and crop-specific variability in allelopathic activity underlines the importance of targeting inflorescences and roots in control strategies. Moreover, the use of *T. aestivum* L. as a bioindicator species in allelopathy assays appears highly justified, given its high sensitivity and consistent response across treatments.

CONCLUSIONS

This study provides clear evidence of the strong allelopathic potential of *Ambrosia artemisiifolia* L., particularly its inflorescences and roots, which were shown to contain the highest concentrations of phytotoxic compounds. Through a series of bioassays on three economically important crop species (*Triticum aestivum* L., *Phaseolus vulgaris* L., and *Zea mays* L.) we demonstrated a dose-dependent and organ-specific inhibition of seed germination and early seedling development.

Among the tested crops, *T. aestivum* L. was the most sensitive to aqueous extracts, exhibiting near-complete suppression of germination under 24-hour extracts from inflorescences and roots. *P. vulgaris* L. showed intermediate sensitivity, while *Z. mays* L. was the most tolerant across all extraction types and concentrations. The highest allelopathic activity was consistently associated with 24-hour aqueous extracts, confirming their efficiency in releasing bioactive allelochemicals.

These findings substantiate the «novel weapon hypothesis», suggesting that the success of *A. artemisiifolia* L. as an invasive species may be partially attributed to its capacity to interfere chemically with the germination

and establishment of neighboring plants, especially non-adapted crops.

From a practical standpoint, this research highlights the potential agroecological risks associated with the spread of *A. artemisiifolia* L. in cultivated landscapes. The observed phytotoxicity against cereals and legumes underscores the need for monitoring and managing invasive populations, particularly before seed dispersal when inflorescence biomass is highest.

Moreover, the demonstrated crop-specific responses point to the usefulness of bioindicator species such as *T. aestivum* L. for assessing allelopathic stress in agroecosystems. Further investigations should focus on the identification of specific allelochemicals, their persistence in soil, and interactions with native soil microbiota to fully understand the ecological impact of *A. artemisiifolia* L. and explore its possible applications in sustainable weed control strategies.

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Стаття надійшла до редакції журналу 11.07.2025