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Table: 2, Figs : 3

Allelopathic influence of *Urtica dioica* L. weed on seed germination and seedling growth of *Glycine max* (L.) Merr. and *Linum usitatissimum* (L.)

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ABSTRACT

We investigated the allelopathic effects of *Urtica dioica* (stinging nettle) leaf and root aqueous extracts concentrations (1 %, 3 %, 5 % and 7 %) on germination, shoot length and root length of *Glycine max* (soybean) and *Linum usitatissimum* (flax). Findings indicated a concentration-dependent inhibitory response in both crops, with notable interspecies differences. Leaf extract results showed that *G. max* germination was highly sensitive to lower concentrations, exhibiting up to 80 % inhibition at 1 % and 3 %, while *L. usitatissimum* exhibited greater inhibition of 81.79 % at 7 %. The inhibitory effects on shoot and root lengths were more pronounced at higher concentrations, with *L. usitatissimum* root length showing drastic inhibition (95.50 %) at 7 %. Root extract at lower concentrations were less inhibitory in *G. max* but showed severe inhibition at 7 %, while, in *L. usitatissimum* there was mild stimulation at lower concentrations. These concentration-dependent interactions highlight *U. dioica*'s potential as a bio-herbicide, selectively impacting different crop species and growth parameters. The study underscores the necessity of understanding allelopathic interactions in sustainable agriculture and suggests future investigations to incorporate positive controls, evaluate physicochemical parameters (osmotic potential and pH) and explore the specific allelochemicals present in *U. dioica* for eco-friendly weed management strategies.

Keywords: Allelopathy, Extracts, *Glycine max,* Leaves, *Linum usitatissimum,* Linseed, Roots, Seed germination, Seedling growth, Soybean, *Urtica dioica.*

INTRODUCTION

Allelopathy, a significant ecological phenomenon, involves the release of chemical compounds by certain plants, affecting the growth and development of neighbouring plant species (23). These allelochemicals, often secondary metabolites, have both inhibitory and stimulatory effects on other plants, shaping the dynamics of plant communities (12). Over the past decades, the study of allelopathy has gained increasing attention due to its implications for plant-plant interactions (11), biodiversity (7,29), and agricultural practices (5,18). Allelochemicals, including phenolics, terpenoids, and alkaloids, are typically low-molecular-weight compounds found in plant leaves, roots, rhizomes and seeds. They are released into the environment through volatilization, leaching, root exudation and the decomposition of plant residues, where they may influence seed germination and seedling growth of nearby plants (15,21).

Urtica dioica L. (stinging nettle, Urticaceae family) grow 1-3 m height and have stinging hairs (8) (Figure 1). This plant releases allelochemicals from its roots, leaves and decaying plant material. These chemicals inhibit the germination and growth of

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Figure 1. (a) Population cluster of *Urtica dioica* L. plants in natural habitat and (b) single plant.

neighbouring plants, often reducing seedling vigour and root elongation in sensitive species (9,10,19). Due to its widespread prevalence and competitive nature, its allelopathic effects on crops are studied within agro-ecosystems. This study aimed to assess the allelopathic influence of *U. dioica* on two crops: *Glycine max* (L.) Merr. (soybean) and *Linum usitatissimum* L. (flax). *G. max* (Fabaceae family) is cultivated for its high oil content and nutritional value, serving as a key source of protein and essential elements for both human consumption and animal feed (17). *L. usitatissimum* (Linaceae family) is valued for its fibre and oil-rich seeds, which are important for industrial and culinary applications (20). This study provided insights into the potential of this weed to influence seed germination and seedling growth of crops with broader implications for crop management and agricultural productivity.

MATERIALS AND METHODS

Study Area

The plant material for this study was collected from Kataula Forest in Mandi District, Himachal Pradesh, India [Altitude: 1,374 m, Latitude: 31°43'12"N, Longitude: 76°55'12"E (Figure 2)]. The region has annual rainfall: 83.2 cm, temperatures: 6.7°C to 39.6°C. The vegetation in this forest comprises *Cedrus deodara, Pinus roxburghii, Pinus wallichiana, Abies pindrow, Rhododendron arboreum, Rubus ellipticus, Berberis aristata, Solanum nigrum, Quercus incana, Pyrus pashia, Myrica esculenta,* and *Grewia optiva*. These conditions provide a natural environment for *U. dioica.*

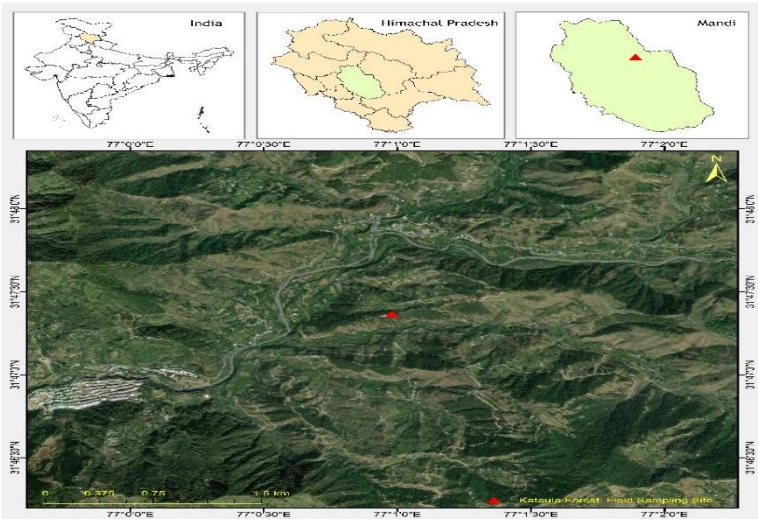

Figure 2. Geographical location of sample collection site at Kataula Forest, Mandi, H.P.

Collection of Plant Materials

Mature leaves of *U. dioica* were collected in January 2024 from the Kataula sub-tehsil forest. The leaves and roots were air-dried in shade for one week to minimize degradation of allelochemicals and were subsequently stored in sealed plastic bags at room temperature (25 °C) for one month. The bioassay experiments were conducted from April 6 to April 18, 2024.

Extract Preparation

Dried root and leaf samples of *U. dioica* were finely ground using a pestle and mortar. To prepare aqueous extracts, 100 g dried leaf or root powder was soaked in 1,000 ml distilled water in 2.0 L glass container. The mixture was stirred vigorously and left to stand for 24 h. After incubation, the solution was filtered through two layers of Whatman No. 1 filter paper. This stock solution was then diluted with distilled water to get concentrations of 1 %, 3 %, 5 % and 7% (w/v). These concentrations were chosen based on preliminary experiments. The extracts were stored in sterile conical flasks at 4 °C to prevent microbial contamination until use.

Experimental Treatments

The laboratory bioassay had 3-factors: (i) Aqueous extracts from leaves and roots of *U. dioica*; (ii) 5-extracts concentrations: Control (0 %), 1 %, 3 %, 5 % and 7% and (iii) Two test crops: *G. max* (soybean) and *L. usitatissimum* (flax). Each treatment was replicated thrice in completely randomized design (CRD).

Germination Test

Seeds of *G. max* and *L. usitatissimum* were first surface-sterilized by immersing in 1 % sodium hypochlorite solution for 20 min to eliminate contaminants. After multiple rinses with distilled water, sterilized seeds were evenly sown on sterile Whatman No. 1

filter paper in petri dishes. Five ml of respective extract solution (1 %, 3 %, 5 %, or 7 %) were added to each petri dish, while control plates received 5 ml distilled water. To maintain adequate moisture levels, 2 ml corresponding extract or distilled water was added every two days. Seed germination was recorded after 7 and 12 days, while shoot and root lengths were measured after 12 days. This method ensured that the observed effects were directly due to the aqueous extracts.

Bioassays

i) Germination Percentage (G %) was calculated as under (22):

Germination (%) = (No. of seeds germinated/Total no. of seeds sown) x 100

ii) Inhibition or stimulation (%) was calculated as under (2):

$$I = 100 \times (R_2 - R_1)/R_1$$

Where, I: Inhibition or Stimulation (%), R_1: Response of control and R_2: Response of test crop.

Statistical Analysis

The arcsine transformation was applied to seed germination and growth parameters data. Three-way Analysis of Variance (ANOVA) was conducted in R Studio to evaluate the effects of different root and leaf extract concentrations on seed germination and growth metrics. Duncan's Multiple Range Test (DMRT) was subsequently employed to analyze and compare variations across the different extract concentrations. This statistical approach, implemented within R Studio using agricolae package, facilitated the identification of significant differences between treatments, enabling a comprehensive assessment of the impact of varying extract concentrations on test crop performance.

RESULTS AND DISCUSSION

The analysis of variance (ANOVA) results demonstrated significant effects of extracts on seed germination, shoot length, and root length, with varying levels of statistical significance (Table 1). Extract type significantly influenced seed germination and root length ($p \leq 0.001$), whereas, its effect on shoot length was non- significant. Test crop had highly significant impacts on seed germination ($p \leq 0.001$), with a weaker but still significant effect on root length ($p \leq 0.05$), while its influence on shoot length was non- significant. Concentrations exhibited a consistent and highly significant effect ($p \leq 0.001$) across all three parameters, indicating its critical role in influencing these traits. The interactions between extract type and test crop also significantly affected seed germination ($p \leq 0.001$), shoot length ($p \leq 0.01$), and root length ($p \leq 0.001$). Similarly, the interactions between extract type and concentration significantly impacted seed germination ($p \leq 0.001$), shoot length ($p \leq 0.05$), and root length ($p \leq 0.001$). The interactions between test crop and concentration showed significant effects across all parameters ($p \leq 0.001$), emphasizing their combined influence. However, the three-way interactions among extract type, test crop, and concentration exhibited statistically significant effects on seed germination only. These findings underscore the dominant role of concentration and the importance of specific interactions between extract type and test crop, as well as their combined effects with concentration, in determining germination and growth metrices.

Table 1. Analysis of Variance (ANOVA) for extract type, test crop, concentration and their interactions on seed germination and growth parameters.

Source of variances	Degree of Freedom	Prob > F (Probability that the F-statistic is greater than the observed value)			
		Seed Germination	Fresh Weight	Shoot Length	Root Length
Extract type	1	0.0001[a]	0.118	0.436	0.0001[a]
Test crop	1	0.0001[a]	0.0001[a]	0.263	0.044[c]
Concentration	4	0.0001[a]	0.0001[a]	0.0001[a]	0.0001[a]
Extract type x Test crop	1	0.0001[a]	0.144	0.008[b]	0.0001[a]
Extract type x Concentration	4	0.0001[a]	0.232	0.037[c]	0.0001[a]
Test crop x Concentration	4	0.0001[a]	0.0001[a]	0.0001[a]	0.0001[a]
Extract type x Test crop x conc	4	0.0001[a]	0.341	0.051	0.105

Where [a] denotes significant difference at 0.1 %; b at 1 % and [c] at 5 %

Leaf Extract

The analysis of germination and growth parameters for *Glycine max* and *Linum usitatissimum* under varying concentrations of leaf extracts revealed significant differences across treatments (Table 2). Statistical groupings indicate a clear distinction between the control and treatments, with progressively lower performance at increasing concentrations. The control treatment consistently formed a separate group (*a*) for most parameters, demonstrating the highest germination and growth values, whereas higher concentrations grouped together (*b*, *c*, *d*, or *e*), reflecting significant inhibition.

Table 2. Effects of *U. dioica* aqueous leaf and root extracts on germination and seedling growth of test crops.

Extract	*Glycine max*			*Linum usitatissimum*		
	Seed Germination (%)	Shoot Length (cm)	Root Length (cm)	Seed Germination (%)	Shoot Length (cm)	Root Length (cm)
Leaf						
Control	62.50[a]	6.10[a]	4.69[a]	91.66[a]	4.22[a]	6.11[a]
1 %	12.50[d]	2.20[d]	3.10[b]	70.83[cd]	3.56[abc]	2.51[bc]
3 %	12.50[d]	2.17[d]	2.43[bcd]	66.66[d]	2.89[c]	0.71[c]
5 %	16.66[d]	4.27[abc]	1.07[d]	41.66[e]	2.90[bc]	0.36[c]
7 %	37.50[b]	4.02[bcd]	1.69[bcd]	16.69[f]	1.68[d]	0.28[c]
Root						
Control	33.33[b]	5.38[ab]	2.42[bcd]	87.50[ab]	3.52[abc]	6.33[a]
1 %	25.00[c]	2.67[cd]	3.12[b]	91.66[a]	4.03[ab]	6.16[a]
3 %	25.00[c]	2.72[cd]	2.67[bc]	75.00[cd]	3.66[abc]	4.12[ab]
5 %	25.00[c]	2.53[cd]	2.05[bcd]	87.50[ab]	3.10[abc]	1.85[c]
7 %	16.66[d]	2.27[d]	1.38[cd]	79.16[bc]	2.69[cd]	0.75[c]
CD (1%)	7.66	1.87	1.45	8.97	1.14	2.24

Means followed by the same letter within columns of concentration and species are not statistically different.

Table 2 and Figures 3a, 3c, and 3e illustrated the inhibitory or stimulatory effects of *U. dioica* leaf aqueous extract at various concentrations on the germination (%), shoot and root length, respectively, of two test crops: *G. max* and *L. usitatissimum*. The *U. dioica* leaf aqueous extract strongly inhibited the germination of *G. max*, with 80 % inhibition at 1 % and 3 % concentrations. In contrast, *L. usitatissimum* experienced a gradual increase in inhibition, reaching 81.79 % at 7 %. Overall, the germination of *G. max* was more sensitive at lower concentrations, while *L. usitatissimum* was more affected at higher concentrations (Figure 3a). For shoot length, both crops experienced substantial inhibition, with *G. max* peaking at 63.93 % at 1 %, while *L. usitatissimum* showed increasing inhibition, reaching 60.33 % at 7 % (Figure 3c). The root length of both crops was highly sensitive to the extract, with *L. usitatissimum* experiencing extreme inhibition at higher concentrations (95.50 % at 7 %), while *G. max* also showed marked inhibition at 5 % (77.24 %) (Figure 3e). These findings demonstrated concentration-dependent allelopathic effects of *U. dioica* on both crops, with *G. max* displaying higher sensitivity at lower concentrations, particularly in seed germination and shoot length. Conversely, *L. usitatissimum* exhibited pronounced susceptibility at higher concentrations, primarily reflected in root growth inhibition.

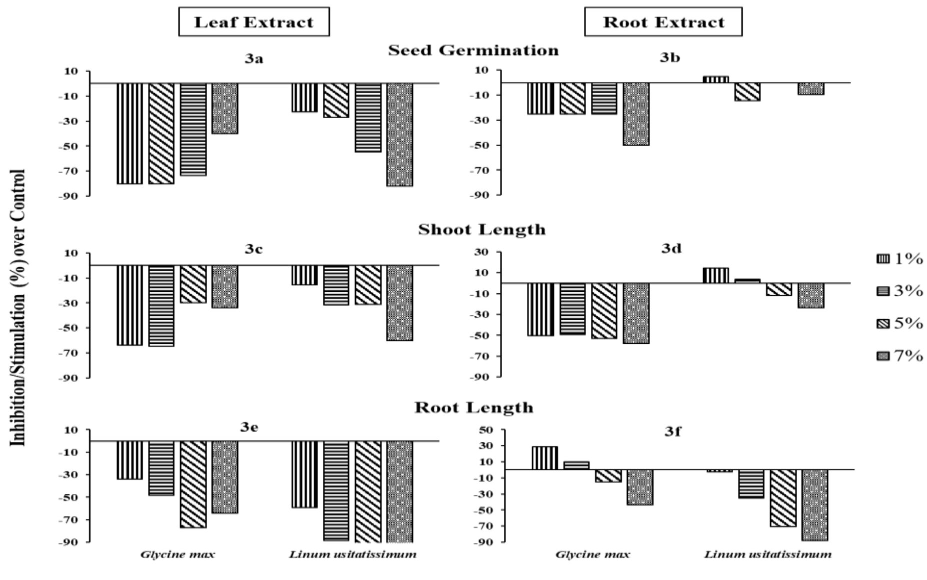

Figure 3. Inhibitory/stimulatory effects of *U. dioica* leaf and root aqueous extracts on seed germination, shoot and root length of test crops under laboratory conditions.

Several studies have documented both stimulatory and inhibitory effects of aqueous plant extracts on the germination and growth parameters of *G. max* and *L. usitatissimum* (14,16,24,25,26,27). The aqueous leaf extract of *U. dioica* exhibited strong inhibitory effects on *G. max*, with an 80 % inhibition of germination at concentrations of 1 % and

3 %. This observation aligns with the findings from (4), which demonstrated that *U. dioica* suppressed seed germination due to hormonal disruptions and the release of allelochemicals that inhibit growth. The findings revealed that *G. max* germination exhibited greater sensitivity to lower concentrations of *U. dioica* extracts, whereas *L. usitatissimum* was inhibited by 81.79 % at 7% extract concentration. This observation underscores the interspecies variability in sensitivity to *U. dioica* extracts, aligning with the findings of (3), who reported the differential tolerance of various crops to allelochemical exposure. Furthermore, the results highlighted the concentration-dependent responses of test crops, providing additional support for the hypothesis (1), which showed that allelopathic interactions are influenced by both extract concentration and plant species. The shoot length and root length of both crops were severely affected, with *G. max* peak shoot length inhibition of 64.48 % at 3 %. On the other hand, *L. usitatissimum* root growth inhibition, was 95.50 % at 7 % concentration. The pronounced impact on root elongation is consistent with (13), which reported that root systems are more sensitive to allelochemicals, thereby limiting nutrients uptake and ultimately affecting overall plant growth.

Root Extract

The aqueous root extract of *U. dioica* had significant allelopathic effects on germination and growth of *G. max* and *L. usitatissimum*, with both inhibitory and stimulatory responses depending on concentration and crop type (Table 2 and Figures 3b, 3d, and 3f). Statistical significance was done using letters to categorize groups with non-significant differences, where groups sharing the same letter indicated comparable performance, while differing letters denoted significant differences (Table 2). The seed germination of *G. max* exhibited consistent low-level inhibition (24.99 %) at 1 %, 3 %, and 5 % concentrations of *U. dioica* root extract, with a marked increase in inhibition (50.02 %) at 7 %. In contrast, *L. usitatissimum* showed mixed responses, displaying mild stimulation in germination at 1 %, no effect at 5 %, and moderate inhibition at 3 % and 7 %. Overall, the extract had a more pronounced inhibitory effects on the germination of *G. max* at higher concentrations, while *L. usitatissimum* exhibited variable responses, with stimulation at lower concentrations and inhibition at higher ones (Figure 3b). Shoot length in *G. max* was consistently inhibited across all concentrations (49.44 % to 57.79 %), while *L. usitatissimum* showed a biphasic response with stimulation at 1 % (14.49 % increase) and inhibition at 7 % (23.58 %) (Figure 3d). Root length in *G. max* exhibited initial stimulation at 1 % (28.97 % increase), but was severely inhibited at 7 % (43.10 %), while *L. usitatissimum* consistently experienced root length inhibition, culminating in a drastic 88.15 % at 7 % (Figure 3f). These patterns indicated that *G. max* was more sensitive to lower concentrations, while *L. usitatissimum* showed greater sensitivity at higher concentrations, especially in root development. The extract's concentration-dependent effects suggested its potential as a natural bio-herbicide, with selective inhibitory impacts on different crops and growth parameters.

The aqueous root extract of *U. dioica* demonstrated a different pattern of allelopathic effects. For instance, *G. max* displayed consistently low-level inhibition in seed germination across the 1 %, 3 %, and 5 % concentrations, with a significant inhibition at 7 % concentration. Similar observations were made by (6), who reported that allelopathic effects exhibit considerable variability not only across different species but also in relation to specific plant parts, such as leaves, stems, roots and seeds. The results portrayed a biphasic response in *L. usitatissimum* regarding its growth metrices-showing mild stimulation at the 1 % concentration and inhibition at higher concentrations. These dynamics underscore the complex interactions that allelochemicals can invoke and corroborate the findings of (28), which suggested that plant responses can be biphasic, alternating between stimulatory and inhibitory effects depending on concentration. These findings also suggested that certain species may exhibit adaptive mechanisms to allelopathic stress, allowing for growth at lower concentrations, before adverse effects become apparent.

This study provided critical insights into the allelopathic potential of *U. dioica* through its effects on the germination and seedling growth of *G. max* and *L. usitatissimum*. Using the controlled bioassays, the study demonstrated clear, concentration-dependent inhibitory effects of both leaf and root aqueous extracts on key growth parameters, highlighting the species-specific sensitivity of test crops. This research lays a foundational framework for exploring *U. dioica* as a natural source of bioactive compounds with potential applications in sustainable weed management. While the focus was on understanding the direct interactions of aqueous extracts with germination and seedling growth, this approach serves as an essential precursor to more complex studies involving soil-mediated interactions and field-based assessments. The absence of positive controls emphasizes the specificity of this study in evaluating *U. dioica* extracts, and future studies are recommended to include established positive controls to enhance comparative analysis. Additionally, exploring parameters such as osmotic potential and pH will provide deeper insights into the interactions between allelochemicals and plant growth. The findings also emphasized the importance of further exploring the chemical composition of *U. dioica* extracts to identify and isolate specific allelochemicals responsible for the observed effects. This study serves as an important step to develop innovative, sustainable weed management strategies.

CONCLUSIONS

The *U. dioica* exhibits significant, concentration-dependent allelopathic effects on *G. max* and *L. usitatissimum*, with distinct species-specific responses. *G. max* was more sensitive to lower concentrations, showing substantial inhibition in germination, while *L. usitatissimum* exhibited increased inhibition at higher concentrations, particularly in root growth. The differential responses suggested that *U. dioica* aqueous extracts, especially from leaves, hold promise as a selective bio-herbicide. These findings highlighted the importance of understanding allelopathic mechanisms in managing crop-weed interactions and encourage further research into the specific compounds in *U. dioica* that drive these effects for sustainable weed management solutions.

DECLARATION OF COMPETING INTERESTS

The authors declare that they have no competing financial interests related to the publication of this manuscript. The research was conducted independently, free from any conflicts of interest that could have biased the findings.

ETHICAL CONSIDERATIONS

This study adhered to established ethical standards for scientific research. All experimental procedures were conducted in accordance with recognized protocols, ensuring the ethical treatment of all biological materials. No endangered or protected species were involved in this research. As this study did not involve human or animal subjects, additional ethical approval was not necessary. The collection and use of plant materials complied with institutional guidelines and relevant local regulations.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration with all authors. All authors finally approved and drafted the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. All authors agree to publish it.

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