

Phytotoxic potential of common nettle (*Urtica dioica* L.) on germination and early growth of cereals and vegetables

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ABSTRACT

This study was done to evaluate the phytotoxic potential of weed *Urtica dioica* L. (common nettle) on germination and early seedling growth of cereals (wheat, barley and oat) and vegetables (lettuce, spinach and radish). The seeds of test plants were grown in *U. dioica* leaf leachate-amended soil, the leachates influenced the germination and seedlings growth of all tested plants. The *U. dioica* leachate were rich in total phenolic and flavonoids as well as had significant antioxidant activity. Based on vigour index, vegetables were very sensitive to *U. dioica* leachate, while cereals were tolerant (except barley). Thus *U. dioica* negatively affects the germination and growth and may cause problems in cultivation of these plants, hence, the *U. dioica* should be removed from the crop fields to avoid its harmful effects on crops and vegetables.

Key words: Barley, cereals, common nettle, flavonoids, germination, lettuce, oat, phenolics, phytotoxicity, radish, seedling growth, spinach, stinging nettle, *Urtica dioica* L., vegetables, wheat.

INTRODUCTION

Many live or dead plant species may produce chemicals which interferes with the growth of other plants. These natural toxins exert adverse effects which persists over long periods (7). Various secondary metabolites (alkaloids, phenols, terpenes and glycosides) produced by plants influences the plant to plant interactions and communities (21). These chemicals disrupt the plant growth and development through changes in cell wall structure, preventing the cell division, seed germination, some enzyme function and nutrients uptake (34,38). The phytochemicals released into the environment inhibits the germination and growth of neighbouring plants by altering their metabolism (9). In agroecosystems, chemicals released from weeds are detrimental to the growth of crops. Besides, the weeds are also allelopathic to crop plants and decreases their pest and disease resistance (13,21). The allelopathy is difficult phenomenon to study, because it is difficult to separate the effects of allelopathy from competition (5). Therefore, the field assessment of allelopathic interference remains challenging owing to the methodological difficulties associated with investigations of allelopathy (11).

Urtica dioica L. (Stinging nettle, common nettle) is a perennial herb with extensive rhizomes and stolons, plant height in 1.5 - 2 m, stems and leaves are covered with stinging hairs (Figure 1). This plant species occurs in ruderal sites, disturbed sites and in crop fields rich in nitrogen (35). *U. dioica* contain large amount of chlorogenic acid, different

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flavonoids and anthocyanins with antiradical, antimicrobial and antifungal effects (12,27). Its extract is inhibitory to microorganisms and fungi (22,30), however, its phytotoxic effects on other higher plants is scarce in literature.



Figure 1. *Urtica dioica* L. in natural habitat during the fruiting stage

The soil eutrophication and increased soil nitrogen concentration has led to the infestation of *U. dioica* in crop fields. During the fields ploughing, aboveground parts of *U. dioica* get incorporated in soil and after irrigations the water-soluble chemicals are released in soil. This study aimed to determine the water-soluble chemicals in *U. dioica* (total phenolics, flavonoids) and their harmful effects on cereals and vegetables. The antioxidant activity of *U. dioica* water leachates was also determined.

MATERIALS AND METHODS

The cereals [wheat (*Triticum aestivum* L. cultivar KG 56), barley (*Hordeum vulgare* L. cultivar NS 565) and oat (*Avena sativa* L. cultivar LH 7770)] and vegetable species [lettuce (*Lactuca sativa* L.), spinach (*Spinacia oleracea* L.) and radish (*Raphanus sativus* L.)] were used as test crops. These species were selected due to their intensive cultivation and their seeds were obtained from local agricultural pharmacy. The seeds were surface sterilized with 70% ethanol (v/v) for 30 s followed by soaking in 4% sodium hypochlorite solution (v/v) for 20 min. Finally, seeds were rinsed several times with sterile distilled water.

The *U. dioica* (50 g) and soil were collected in July 2017 near Kragujevac, Central Serbia (44° 17' 951" N, 20° 92' 566" E). Altitude: 210 m; highest point: 895 m and the lowest 130 m above sea level. The average annual temperature is 11.2 °C and annual precipitation is 713 mm (26). The soil was dried at room temperature, sieved and stored in paper bags until the use.

To properly investigate the potential of phytotoxic material, it is important to select more than three levels of concentrations, which are close to the natural agroecosystems (1).

To simulate phytotoxic interference, the use of water leachate is particularly relevant to assess the joint action of mixtures of metabolites rather than a single metabolite of interest (9,10,14). Previously, water extracts were used to simulate leaf leachates that could potentially be important in chemically-mediated field interactions (10,23). The collected plants were soaked in 900 ml distilled water for 72 h and then filtered through Whatman No. 1 filter paper. To test toxicity of water leachates of *U. dioica*, the leachates of 5%, 10%, 20%, 40% and 80% concentrations were prepared.

I. Germination bioassay

To study germination, bioassay was done with seeds sown in 9-cm Petri dishes lined with filter paper (Whatman No. 2). Ten ml of leachate or distilled water (Control) were added per Petri dish as per treatments. All Petri dishes were kept in growth chamber [22 ± 2 °C at 60% RH, 16 h photoperiod]. Germination was recorded daily till 5-days.

For test cereals and vegetables, 3-germination parameters: [Final Germination Percentage (FGP), Rate of Germination (RG) and Mean Time to Germination (MTG)] were determined as per Espanani *et al.* (8) and Fernandez *et al.* (11).

(i). Germination percentage (GP) was calculated as under:

$$GP = \frac{\text{Total seeds germination after 5 days}}{\text{Total number of planted seeds}} \times 100$$

(ii). Mean Time to Germination (MTG) was calculated as under:

$$MTG = \frac{\sum n_i \times t_i}{\sum n_i}$$

Where, n_i : Number of newly germinated seeds in the time i and t_i : Time from the start of experiment to the observation (in days) (29).

(iii). Rate of Germination (RG) was estimated by using modified Timson's index of germination velocity:

$$\text{Germination velocity} = \sum G / t$$

Where, G : Seed germination (%) at one-day interval, t : Total germination period.

The germination parameters were determined by daily counting the number of germinated seeds in 5 days period. The emergence of at least 2 mm long radicle was considered as evidence of germination.

II. Soil bioassay

To examine the phytotoxic behaviour of soil amended with water-soluble leachates from *U. dioica*, this study was designed as per Al Hamdi *et al.* (1). Briefly, 90 g dry soil was placed in 9 cm Petri-dishes; 15 seeds were sown on the soil surface and 40 ml leachate was added per Petri dish as per treatments. Seeds germinated in soil irrigated with only distilled water were used as control. The root and stem elongation and fresh weight of seedling grown in the soil were determined on 10th day.

Seedling vigour index: It was calculated as per Kharb *et al.* (16) as under:

(i). Seedling Length Vigour Index (SLVI) = Mean shoot length + Mean root length x FGP

(ii). Seedling Weight Vigour Index (SWVI) = Mean seedling weight x FGP

Where, FGP: Final germination (%).

III. Secondary metabolites content

The obtained water leachate dilutions of 5, 10, 20, 40 and 80 % were used to evaluate the secondary metabolites content and antioxidant activity.

(i). **Determination of total phenolic contents:** We determined the total soluble phenolic compound in *U. dioica* leachates with Folin-Ciocalteu's reagent using a spectrophotometric method with gallic acid as standard (31). The absorbance was determined at $\lambda_{\max} = 765$ nm. The samples were prepared in triplicate and the mean value of absorbance was obtained. The same procedure was used for the gallic acid and the calibration curve was prepared. The total phenolic content was expressed in terms of gallic acid equivalent (mg GAE/g dry weight).

(ii). **Determination of flavonoids:** The total flavonoid contents were determined spectrophotometrically (28). The absorbance was determined at $\lambda_{\max} = 415$ nm. The samples were prepared in triplicate and the mean value of absorbance was obtained. The same procedure was repeated for the rutin and the calibration curve was prepared. The total flavonoid content was expressed in terms of rutin acid equivalent (mg RUE/g dry weight).

III. Antioxidant activity

The ability of the plant leachates to scavenge 1,1-dyphenyl-2-picrylhydrazyl (DPPH) free radicals was assessed by the standard method (33), adopted with suitable modifications (19). Dilutions of leachates were made to obtain 10 folds serial concentrations (from 1 to 0.97 $\mu\text{g/ml}$) in methanol. Diluted solutions (1 ml each) were mixed with 1 ml of DPPH methanolic solution. After 30 min in darkness at ambient temperature, the absorbance was recorded at 517 nm. Inhibition (%) was calculated as under:

$$\text{Inhibition (\%)} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100.$$

The IC_{50} values were estimated from the inhibition (%) versus concentration sigmoidal curve, using a nonlinear regression analysis.

IV. Statistical analysis

The experiment was conducted in completely randomized design with three replicates. Data were subjected to analysis of variance (ANOVA) by SPSS v.13 for Windows, and presented as mean \pm SE of three measurement. Tukey's multiple range tests were calculated for the significant data at $p < 0.05$.

RESULTS AND DISCUSSION

Total phenolics content: Phenolic compounds exist in both natural and managed ecosystems, where they cause many ecological and economic problems (21). To determine the phytotoxicity of *U. dioica*, we determined the contents of total phenolics and flavonoids separately in different water leachate dilutions prepared for seed treatment. The total phenolic content of the *U. dioica* leachates are presented in Table 1. Total phenolic content determined in the leachate of highest concentration (80%) were 317.91 mg GAE/g DW, the concentration decreased proportionately with the concentration of water leachate.

Flavonoid concentration: The above ground parts of *U. dioica* possess significant contents of total phenolics including the flavonoids (Table 1), with highest concentration of 36.58 g RUE/g DW. The phenolic constituents possess allelopathic properties (36,39).

Both simple and more complex phenolics, including flavonoids, are released from the decomposing plant tissues as leachates and through the microbial degradation and transformation. Flavonoids, as biologically active metabolites, interact with many diverse targets in subcellular locations to elicit various activities (37).

Table 1. The total phenolic content, flavonoid concentration and antioxidant activity of different concentrations of *U. dioica* leachates

Leachate concentration (%)	Total phenolic content (mg GAE/g DW)	Flavonoid concentration (mg RUE/g DW)	Antioxidant activity IC ₅₀ (µg/ml)
5	34.97 ± 0.58 ^a	8.47 ± 0.67	758.42 ± 1.08
10	59.64 ± 0.71	13.00 ± 0.91	416.64 ± 2.11
20	109.50 ± 0.92	19.87 ± 0.18	276.85 ± 1.91
40	184.36 ± 0.90	22.62 ± 0.70	145.93 ± 1.43
80	317.91 ± 0.33	36.58 ± 0.57	96.31 ± 0.94

^aEach value represents the mean value of three measurements ± S.D.

Antioxidant activity: The different leachates of *U. dioica* significantly decreased the radical scavenging activity, expressed as inhibition concentration (IC₅₀), depending on the leachate concentrations (Table 1). The best DPPH scavenging activity was observed in 80% leachate (IC₅₀ = 96.31 µg/ml). Similar to the total phenolic content and flavonoid concentrations, the antioxidant activity of *U. dioica* leachates was dose dependent.

Many studies in recent years demonstrated that high content of phenolic compounds contribute to the antioxidant activities of plants (15,32). Basically, the extracts with high antioxidant capacity and high content of phenolic compounds may demonstrate negative effects and have phytotoxic activity. This agrees with Ali *et al.* (2), who demonstrated that the *Thymus numidicus* extracts with high antioxidant capacity and high polyphenolic content possessed the allelopathic activity. Since content of total phenolics and flavonoids were determined in aqueous medium, these secondary metabolites from *U. dioica* have phytotoxic activities in crop fields, where precipitation and irrigation are common. However, further studies are needed to compare this evidence under the natural environment.

The seeds germination and the roots development in seedlings are most sensitive stages of plant growth, influenced by the environmental changes. In field conditions, emergence often takes place in stressful conditions (25). Furthermore, the chemicals released from the plant parts in the soil affects the germination, seedlings growth and successful establishment of neighbouring plants (5).

Seeds germination: The success of cultivars mainly depends on the germination performance of their seeds. In our study, the studied germination parameters were either inhibited or stimulated with *U. dioica* leachates both in cereals and vegetables, but effects were dependent on the type of plant as well as leachate concentration. The leachate stimulated the seeds germination (%) in *T. aestivum* and *A. sativa* (Figure 2). The 5 % leachate stimulated the germination of wheat. The *A. sativa* germination was significantly stimulated with 5, 40 and 80 % leachates. While the *U. dioica* leachate significantly inhibited the seeds germination of *H. vulgare*. Likewise, the higher concentrations of leachate, reduced the *L. sativa* and *S. oleracea* seeds germination. In *R. sativus*, 10 % *U. dioica* leachate also significantly inhibited the seeds germination.

The leachates concentrations significantly influenced the inhibition and stimulation in seeds germination (Figure 2). The 80% leachate caused maximum inhibition (66.67%) in seeds germination of *L. sativa*, followed by (60% inhibition) in *S. oleracea* seeds germination.

Mean Time to Germination (MTG): The high germination (%) of seeds does not necessarily imply the high seedling emergence (%) in soil and, therefore, seed vigour tests are required (24). The MTG can be described as the length of lag period from the start of imbibition to radicle protrusion (25). For the tested seeds, it ranged from 1.24 to 2.45 for cereals seeds germinated per day (Figure 3) and from 1.25 to 4.61 seeds germinated per day for vegetables (Figure 3). The lower values of MTG in control (without *U. dioica* leachate) indicate the faster seeds germination. Only in *H. vulgare* and *O. sativa* seeds, 20 % leachate slightly hastened their seeds germination.

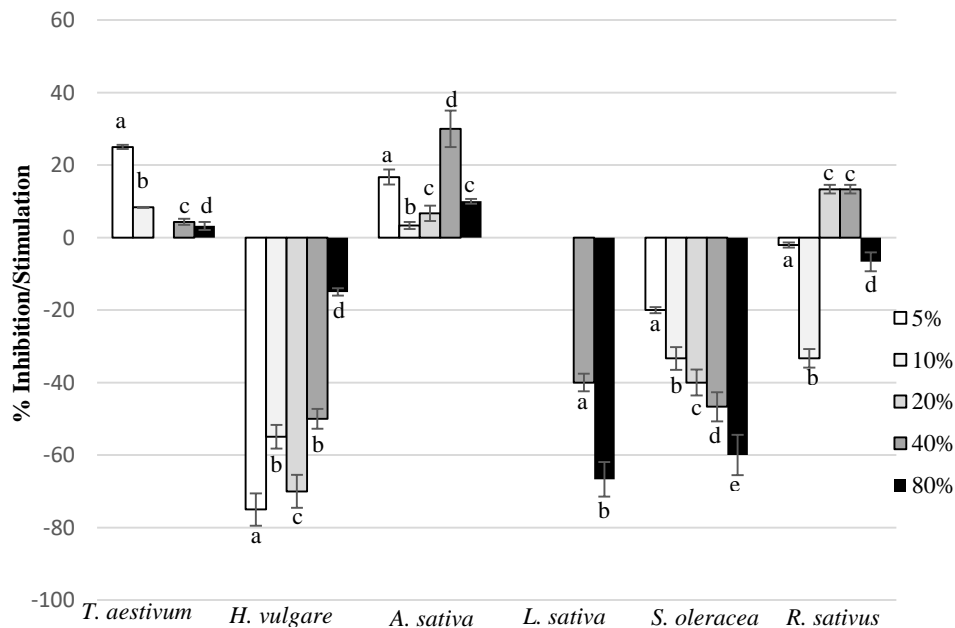


Figure 2. Inhibitory and stimulatory effects of *U. dioica* leachate on seeds germination of test crops. The negative (-) value on the Y-axis indicates inhibition and positive (+) value indicates stimulation over the control. Vertical bars above mean indicate standard error of three replicates. The different letters above means indicate significant difference among treatments for each plant species ($p < 0.05$, according to Tukey's test).

In this experiment, although germination (%) was stimulated with *U. dioica* leachate in some cases, but the radicle emergence was slowed down.

Rate of Germination (RG): When it comes to rate of germination (RG), similar results were obtained (Figure 4). As the higher RG value shows more rapid germination but *U. dioica* leachates slowed the germination in all tested seeds.

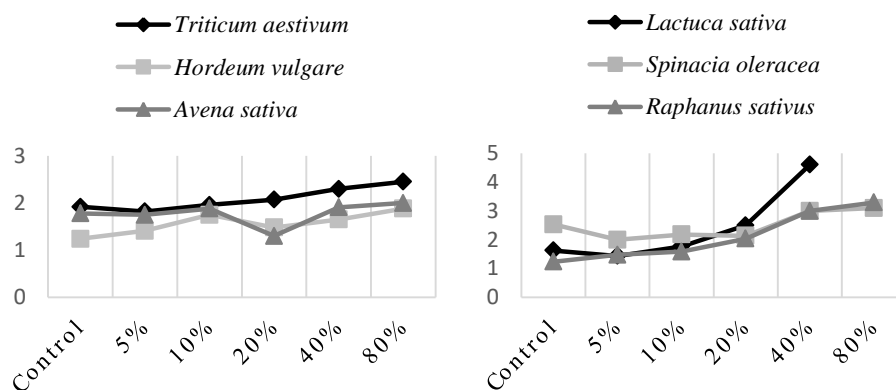


Figure 3. Mean time to germination (MTG) of test cereals and vegetables seeds at different *U. dioica* leachate concentrations

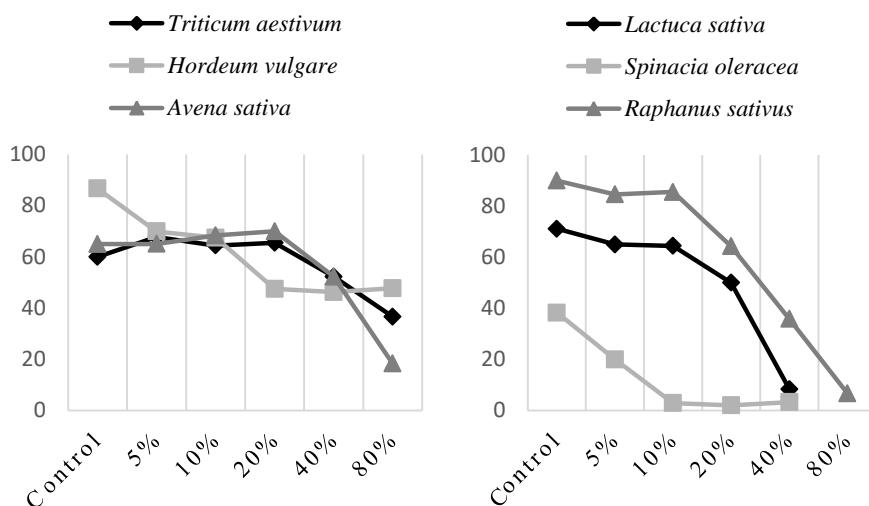


Figure 4. Rate of germination (RG) of test cereals and vegetables seeds at different *U. dioica* leachate concentrations.

Root elongation: Water leachate of *U. dioica* significantly inhibited the root elongation of test cereals and vegetables (Figure 5). Except in *H. vulgare*, where 5% *U. dioica* leachate stimulated the root elongation, in all other tested seeds, root elongation was drastically inhibited at 80% leachate concentration. The negative impact of *U. dioica* leachate on root elongation is shown in Figure 5.

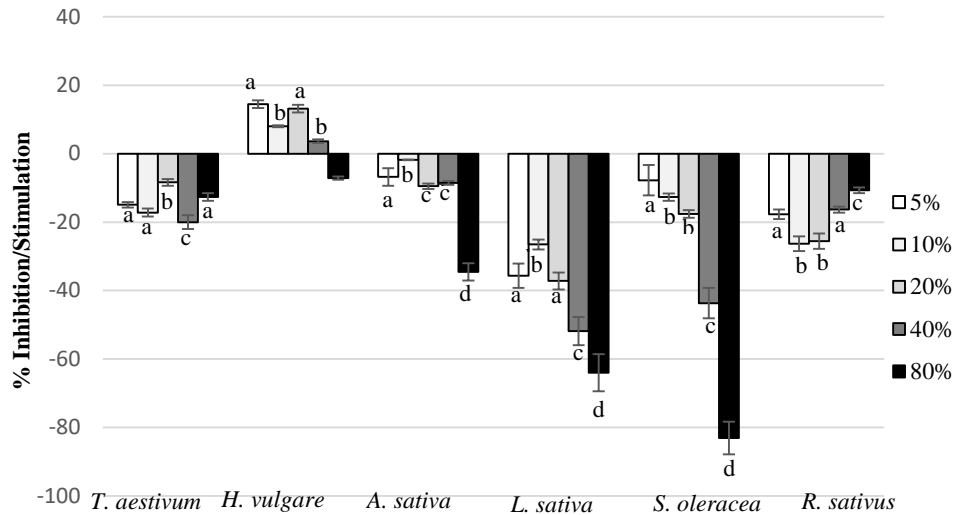


Figure 5. Inhibitory and stimulatory effects of *U. dioica* leachate concentration on root elongation of test crops. The negative (-) value on the Y-axis indicates inhibition and positive (+) value indicates stimulation of elongation over the control. Vertical bars above mean indicate standard error of three replicates. The different letters above means indicate significant difference among treatments for each plant species ($p < 0.05$, according to Tukey's test)

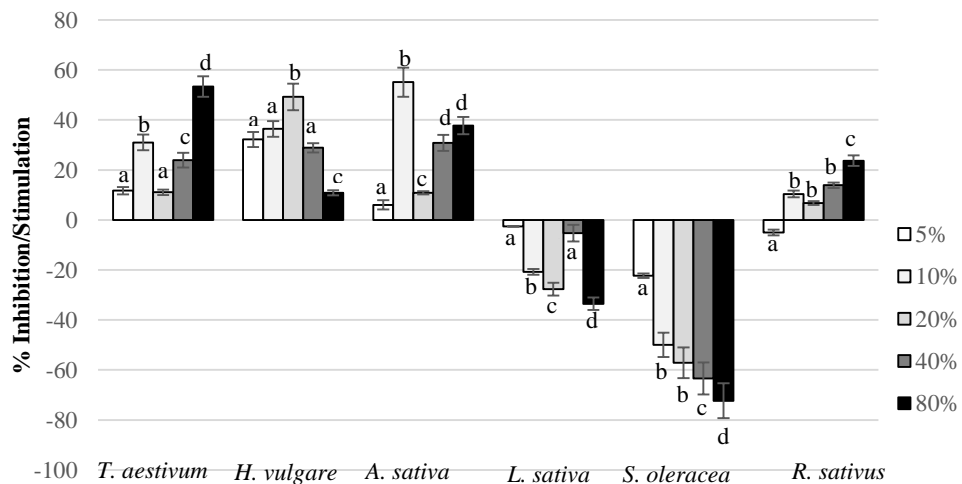


Figure 6. Inhibitory and stimulatory effects of *U. dioica* leachate concentrations on stem elongation of test crops. The negative (-) value in the Y-axis indicates inhibition and positive (+) value indicates stimulation of elongation over the control. Vertical bars above mean indicate standard error of three replicates. The different letters above means indicate significant difference among treatments for each plant species ($p < 0.05$, according to Tukey's test)

Stem elongation: *U. dioica* leachate had variables impacts on stem elongation, depending on the leachate concentration and test species (Figure 6). Generally, vegetables were more sensitive to *U. dioica* presence, where higher leachate concentrations significantly reduced the stem length. In contrast, *U. dioica* leachate stimulated the stem elongation of *T. aestivum*, *H. vulgare* and *A. sativa* at all concentrations. Highest inhibition was in *S. oleracea* and *L. sativa* at 80 % of leachate, but also all other concentration was inhibitory to these plant species.

When plants are exposed to toxins, their growth and development are adversely affected. The readily visible effects are: inhibited or retarded germination, reduced root and shoot elongation, reduced dry weight accumulation and lower production capacity (18). These gross morphological effects may be secondary manifestations of primary events, caused by variety of more specific effects acting at the cellular or molecular level in the receiver plants. Khattak *et al.* (17) reported that lower concentrations of potential allelochemicals may be beneficial to the growth of wheat plants.

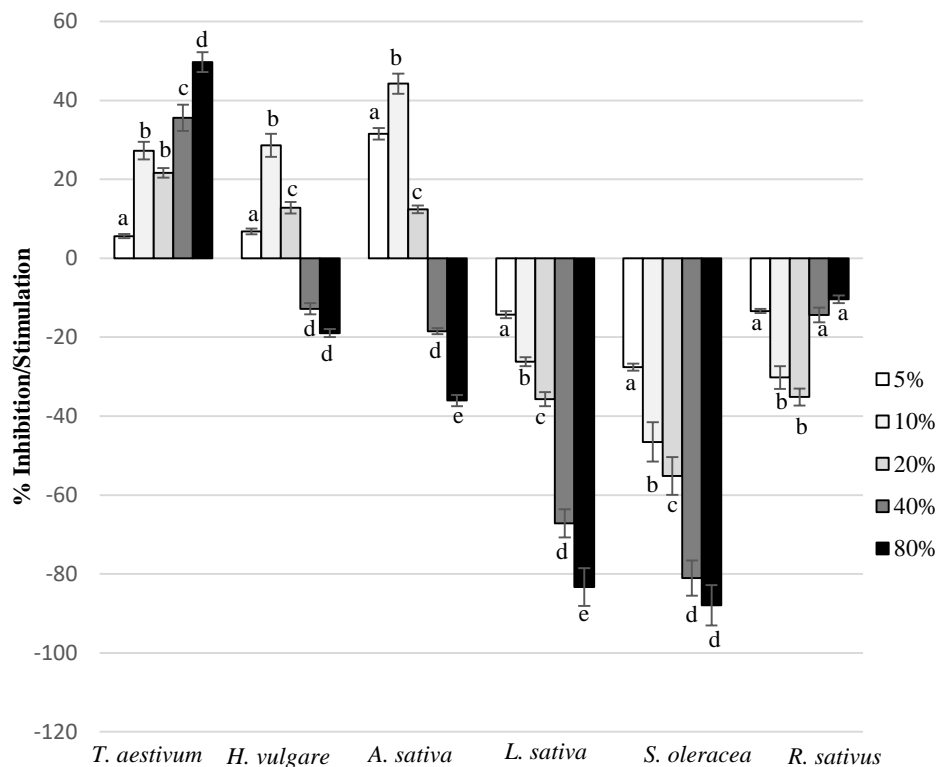


Figure 7. Inhibitory and stimulatory effects of *U. dioica* leachate concentration on fresh weight of test crops. The negative (-) value on the Y-axis indicates inhibition and positive (+) value indicates stimulation of elongation over the control. Vertical bars above mean indicate standard error of three replicates. The different letters above means indicate significant difference among treatments for each plant species ($p < 0.05$, according to Tukey's test).

Fresh weight: Different leachate concentrations have demonstrated significant impacts on the fresh weight of the plants (Figure 7). For *L. sativa*, *S. oleracea* and *R. sativus*, *U. dioica* leachate had significant inhibitory effects. Conversely, stimulatory effects and increase in plants mass has been recorded in *T. aestivum*, *A. sativa* and *H. vulgare*. Li et al. (20) demonstrated that phenolic acids at lower concentrations stimulated the length and dry weight of shoots and roots of strawberry, but these parameters were inhibitory at higher concentrations. When it comes to inhibition/stimulation percent, only lower leachate concentrations caused increase in fresh weight for *A. sativa* and *H. vulgare*, while 40 and 80 % leachate had negative impact and caused inhibition of plant fresh weights in vegetables (Figure 7).

Vigour index: The evaluation of vigour tests for predicting the seed planting value is more important than the standard germination tests (3). Vigour test does not only measure the viable seed (%) in a sample, but also reflects the ability of those seeds to produce normal seedlings under less than optimum or adverse growing conditions, similar to those which may occur in the field (4).

Table 2. Effects of different *U. dioica* leachate concentrations on the seedling vigour index (based on seedling length and weight of test crops)

leachate conc. (%)	<i>T. aestivum</i>		<i>H. vulgare</i>		<i>A. sativa</i>		<i>L. sativa</i>		<i>S. oleracea</i>		<i>R. sativus</i>	
	Seedling Length Vigour Index	Seedling Weight Vigour Index	Seedling Length Vigour Index	Seedling Weight Vigour Index	Seedling Length Vigour Index	Seedling Weight Vigour Index	Seedling Length Vigour Index	Seedling Weight Vigour Index	Seedling Length Vigour Index	Seedling Weight Vigour Index	Seedling Length Vigour Index	Seedling Weight Vigour Index
5	2389.000	546.000	446.655	86.299	1029.700	303.800	394.000	36.000	101.712	19.597	731.963	148.085
10	2234.961	570.223	793.800	187.200	1036.878	269.702	390.000	31.000	59.994	10.332	458.638	75.194
20	1974.400	503.200	566.800	109.400	878.400	222.600	342.000	27.000	31.192	6.932	853.000	131.000
40	2121.437	607.487	839.583	140.986	1346.613	224.158	201.600	10.800	16.000	2.200	938.000	173.000
80	2507.074	670.745	1248.786	222.674	913.219	133.624	107.656	2.333	3.663	0.466	806.400	144.800

Table 3. Grouping of tested plants based on vigour index (VI) with different concentrations of *U. dioica* leachate

Categories of plants according to vigour index				
Leachate Conc. (%)	Tolerant	Moderately tolerant	Sensitive	Highly sensitive
5	<i>T. aestivum</i>	<i>A. sativa</i>	<i>R. sativus</i>	<i>H. vulgare</i> , <i>L. sativa</i> , <i>S. oleraceae</i>
10	<i>T. aestivum</i>	<i>A. sativa</i>	<i>H. vulgare</i>	<i>L. sativa</i> , <i>S. oleracea</i> , <i>R. sativus</i>
20	<i>T. aestivum</i>	-	<i>H. vulgare</i> , <i>A. sativa</i> , <i>R. sativus</i>	<i>L. sativa</i> , <i>S. oleracea</i>
40	<i>T. aestivum</i>	<i>A. sativa</i>	<i>H. vulgare</i> , <i>R. sativus</i>	<i>L. sativa</i> , <i>S. oleracea</i>
80	<i>T. aestivum</i>	<i>H. vulgare</i>	<i>A. sativa</i> , <i>R. sativus</i>	<i>L. sativa</i> , <i>S. oleracea</i>

The results for seedling length and weight and vigour index of selected plants are shown in Table 2. The test plant species can be divided into following categories of vigour index: Tolerant (VI = 1500-2000); Moderately tolerant (VI = 1000-1499.99); Sensitive (VI = 500-999.99); Highly sensitive = (VI < 500). Based on the results of vigour index related to the concentration of *U. dioica* leachates, categories varied regarding the tested plant

species. Categories of vigour index for tested plants are presented in Table 3. *T. aestivum* is tolerant to all tested leachate concentrations, *A. sativa* was moderately tolerant to all concentrations up to 80 % leachate, while *H. vulgare* was moderately tolerant to the 80 % leachate and sensitive to 5, 10, 20 and 40 % leachate concentrations. In contrast to cereals, vegetables were more sensitive. The *R. sativus*, *L. sativa* and *S. oleracea* were highly sensitive to all *U. dioica* leachate concentrations.

Seedling vigour is critical when competition for growth resources (light, nutrients, air and water) becomes strong. Seedlings with vigorous growth can successfully compete under stress, influencing stand establishment and ultimately grain yield (6). Based on the categories of sensitivity, the most tolerant to *U. dioica* extracts was *T. aestivum*, while *L. sativa* and *S. oleracea* were most sensitive. Generally, cereals were more tolerant than vegetables.

CONCLUSIONS

The *U. dioica* water leachates contained significant quantity of water-soluble phenolics and flavonoids, which inhibited the germination and seedling growth of cereals and vegetables and also had the antioxidant capacity. Since the water leachates of *U. dioica* have significant amount of secondary metabolites with biological activities, the detailed investigations are required to identify the components of secondary metabolites and study their bioactivities on test cereal and vegetables.

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