Allelopathic potential of *Lupinus polyphyllus* L. in relation to habitat variables

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Abstract. *Lupinus polyphyllus* L. is an invasive species widely spread in Europe. Despite many studies on perennial lupine, relatively few have explored its allelopathic potential. . Hence, the aim of this study was to determine the allelopathic effects of three perennial lupine populations originating from various habitats (lowland – S1, foothill – S2 and mountain – S3) on the growth and development of two target species – white mustard (*Sinapis alba* L.) and meadow red-fescue (*Festuca rubra* L.). The goal was to determine which of the habitat factors have the greatest influence on the allelopathic potential of this species. The results of conducted research have not demonstrated the existence of clear dependencies which could allow to determine which populations of perennial lupine have the strongest allelopathic potential to inhibit the seed germination and growth of the target species. Among the analyzed habitat factors in laboratory conditions, low concentrations of phosphorus in the soil can stimulate the production of allelochemicals inhibiting the seed germination of the two target species - *S. alba* and *F. rubra*.

Keywords: Allelopathy, invasive species, target species, habitat factors, water extracts; volatile compounds.

1. Introduction

At present, one of the most problematic alien species found in Europe - influencing the diversity and economic efficiency of plant communities - is the perennial lupine (Valtonen et al., 2006; Tyler et al., 2015). *Lupinus polyphyllus* L. is a perennial herbaceous hemicryptohyte plant native to western North America (Ludewig et al., 2022). Currently, the species occurs in North and South America, Europe, the Altay region, the Pacific Far East, as well as Australia and New Zealand (Eckstein et al., 2023). The species was brought to Europe in the 19th century due to its decorative properties (Valtonen et al., 2006). Nowadays, it can often be found on grasslands, logging sites and clearings (Oberdorfer, 2001), roadside ditches, river banks (Valtonen et al., 2006), forest edges (Sebald et al., 1992), and meadows (Pruchniewicz, 2017).

Owing to its ornamental flowers and extensive root system, it is also a decorative plant, commonly used to stabilize soil from erosion (Oberdorfer, 2001). It can be found in dry to mesic sites (Rothmaler, 2002), on clayey, sandy-rocky, fertile, calcium-poor soils with a small amount of humus (Oberdorfer, 2001).

The problem of perennial lupine invasion and its negative impact on the native species composition and the diversity of plant communities concerns many European countries as well as New Zealand, Australia, and Argentina (Fremstad, 2010). The negative influence of perennial lupine on species richness is connected with the shading of habitats (Valtonen et al., 2006) and accompanying species, especially small ones (Hiltbrunner et al., 2014) and its allelopathic properties (Loydi et al., 2015). Quinolizidine alkaloids found in this species inhibit the germination and growth of accompanying species (Wink, 1983; Muzquiz et al., 1994). Some of the alkaloids, such as sparteine and gramine, can also influence the growth of the root system (Muzquiz et al., 1994). In spite of allelopathic properties, a considerable number of taxa can coexist with it (Hejda et al., 2009), especially nitrophilous species (Otte and Maul, 2005).

Allelopathy is defined as a direct or indirect, positive or negative type of interaction of one species on another through the release of chemical compounds into the environment (Rice, 1984). Several allelochemicals at low concentration may function as growth regulators, stimulating the growth of recipient plants (Duke et al., 2006), provide tolerance to abiotic stress (Einhellig, 1996), or improve the absorption of nutrients (Gent et al., 2005). However, allelochemicals can sometimes have the opposite effect. According to the "novel weapon" hypothesis, as far as invasive species are concerned, allelochemicals released into the environment lead to the extinction of native species that are not adapted to them (Callaway & Aschehoug, 2000). After some time, taxa can develop tolerance to allelochemicals (Callaway et al., 2005), which can make it possible for them to coexist with invasive species (Lyytinen & Lindström, 2019). Environmental factors regulate the production and release of allelochemicals (Latif et al., 2017). These factors include soil structure, moisture, temperature, UV radiation, nutrient availability, and environmental stress (Barazani and Friedman, 2001; Reigosa et al., 2002; Iannucci et al., 2013). Despite the allelopathic potential of perennial lupine, knowledge about the influence of habitat on the strength of its allelopathic effects is currently incomplete. There is also not enough information about differences in allelopathic potential between individual ecotypes of perennial lupine. Therefore, in the experiment, we decided to check how a set of habitat factors influences the allelopathic properties of perennial lupine. The aim of the study was to determine the influence of *Lupinus polyphyllus* representing three populations

from the various sampling site (lowland S1, foothill S2 and mountain S3) on the growth and development of two target species- white mustard (*Sinapis alba* L.) and meadow red- fescue (*Festuca rubra* L.) and determining which habitat factor has the greatest influence on the allelopathic potential of the species. In the study, the following hypotheses were tested: 1. Perennial lupine exhibits allelopathic properties that affect the growth and development of target species. 2. The allelopathic potential of perennial lupine is strongly influenced by habitat characteristics.

2. Materials and Methods

2.1. Field research

In order to determine the allelopathic properties of perennial lupine with respect to site variables, three types of sites were designated, differing in terms of hypsometric height: the first site was the village of Czernica (lowland site 127 m a.s.l. - S1). The second site was located within the village of Lasek Miejski (foothill site 470 m a.s.l – S2). The third site, representing a mountainous area, was the village of Łężyce (mountain site 744 m a.s.l - S3). The three sites are located in Poland. On each site, an area of 25 m^2 was designated for random extraction of 2kg aboveground biomass samples of perennial lupine (L. polyphyllus). Underground biomass was collected from the depth of 30 cm. These samples represented three lupine populations: lowland, foothill and mountain with a distance from each other in the range of ~10 – 100 km. Soil samples from the rhizosphere layer were also collected from each plot according to the spatial pattern of the number 5 on a dice. Individual samples were later combined into a collective sample which was subjected to further laboratory analyses.

Figure 1. Locations the study plots. S1 – lowland site, S2 - foothill site, S3 - mountain site.

2.2. Laboratory study

After collection, the plant material was immediately brought to the laboratory. After separating the aboveground and underground parts of *L. polyphyllus* the plant material was cleaned of contaminants. Then, aqueous extracts were prepared from it in the ratio of 100 g of fresh plant material to 1000 ml of deionized water. Maceration was conducted for 72 hours. Afterwards, the solutions were filtered and preserved in airtight flasks, and stored in the dark at 3°C. Extract dilutions were prepared using a modified method presented in Kalske et al. (2022b). The experiment was carried out in controlled conditions using a phytotron with the following settings: day/night cycle: 16/8h, temperature: 24°C/14°C, humidity 60% (Pruchniewicz & Halarewicz, 2019). The experiment was conducted in tight 500 cm^3 containers, in which 10 seeds of the target species were separed placed: white mustard and meadow red-fescue. Each treatments were replicated 4 times. The selection of the target species was related to their fast germination rate and represented two classes: monocotyledons and dicotyledons. The seeds were treated with solutions made from the aboveground and underground parts of perennial lupine with the following concentrations: 0%, 10%, 20%, 40%, 60%, 80% and 100%. After 14 days of the experiment, the number of germinated seeds and the average length of the aboveground and underground parts of 10 seedlings were determined. Seeds that did not germinate were omitted from average length calculation.

Laboratory analyses of soil samples were performed according to the methodology of Allen (1989) and Radojevic & Bashkin (2006). Soil moisture was determined using Kopecky cylinders with a volume of 100 cm^3. Organic matter was determined by roasting 2 g of soil in a muffle furnace at 600°C for 6 hours. The soil pH was determined in distilled water using the potentiometric method. Total nitrogen was determined using the modified Kjeldahl method. Soluble forms of phosphorus were measured using Olsen method after extracting the soil samples with 0.5 M sodium bicarbonate solution at pH 8.5. The markings were made on a spectrocolorimeter. Exchangeable forms of potassium, magnesium and calcium were extracted with 1M ammonium acetate and determined on a Varian Spectraa 200 spectrometer. Organic carbon was calculated based on the organic matter content assuming that organic matter contains 58% carbon (Pribyl, 2010).

2.3. Statistical analyses

Statistical analyses were conducted using STATISTICA v 13 software (Tibico Software Inc, 2017). The normality of the data distribution was assessed using the Shapiro-Wilk test. Homogeneity of variance was evaluated with Levene's test. If both assumptions were met, a one-way analysis of variance (ANOVA) was performed, followed by Tukey's HSD test to determine significant differences between groups. Data for which normal distributions and homogeneity of variance were not found were analyzed using Kruskal-Wallis test. In order to determine the relationship between the the allelopathic potential of perennial lupine and habitat factors, non-parametric Spearman rank correlations were used due to the lack of normality in the distributions.

3. Results

3.1. The influence of water extracts made from aboveground and underground biomass of perennial lupine on seed germination and growth of white mustard

The conducted calculations revealed a significant impact of extracts made from the aboveground biomass of perennial lupine on the germination capacity of white mustard. Significant differences were observed for the first site S1 ($H = 18.683$; $p = 0.005$), the second site S2 ($H = 16.597$; $p = 0.011$), and the third site S3 ($H = 14.340$; $p = 0.026$). The highest values of germinated seeds were found in the control range (0% concentration), the lowest - in the case of 10% concentration (Table 1). The impact of extract on growth white mustard showed

significant differences in the length of the stem were noted at the second site S2 ($H = 19.039$; $p = 0.004$) and the third site S3 ($F = 96.567$; $p \leq 0.001$). In the second site S2, the highest stem length was recorded at a concentration of 10%, while the lowest at a concentration of 60%. At the third site S3, the highest stem length were recorded in the 40% concentration range, while the lowest were in the 0 and 20% solutions. In the case of root length, differences were noted at all sites: the first S1 ($F = 4.736$; $p = 0.003$), the second S2 ($H = 22.848$; $p = 0.001$) and the third S3 ($H = 25.498$; $p \leq 0.001$). At the first site S1, the highest root length was recorded in the concentration range of 40%, 60%, 80%, and the lowest in the 0% control range. At the second site S2, the highest values were found in the concentration range of 0%, 40%, 100%, and the lowest at 10%. At the third site S3, the lowest values were recorded for the control, and the highest for the 10% concentration.

The analysis of differences between the sites revealed significant differences in the mean values of white mustard stem length within the 0% control ($F = 14.939$; $p = 0.001$) and concentration of 20% ($H = 9.269$; $p = 0.010$), 40% ($F = 89.366$; $p \leq 0.001$), 60% ($H = 9.846$; $p = 0.007$) and 80% ($F = 40.165$; $p \leq 0.001$). In the case of root length, significant differences were found in the 0% concentration range ($H = 7.423$; $p = 0.024$), 10% ($F = 8.385$; $p = 0.009$), 60% ($H = 9.581$; $p = 0.008$), 80% ($H = 9.846$; $p = 0.007$) (Table 1).

Table 1. Seed germination and growth parameters (steam and root length) of white mustard exposed to water extracts of perennial lupine aboveground biomass. Letters indicate homogeneous groups obtained in Tukey HSD test or Kruskal-Wallis test at $p \leq 0.05$. Small letters indicate differences in mean values between concentrations of *L. polyphyllus* water extracts, capital letters indicate differences between sites.

	Concentration of *L. polyphyllus* water extracts [%]	**Site S1**	**Site S2**	**Site S3**
Germination capacity[%]	0	90.00 ± 4.08a	85.00 ± 2.89a	85.00 ± 2.89a
	10	45.00 ± 2.89b	55.00 ± 2.89b	57.50 ± 2.50b
	20	72.50 ± 2.50ab	60.00 ± 4.08ab	67.50 ± 4.79ab
	40	72.50 ± 2.50ab	65.00 ± 6.45ab	70.00 ± 4.08ab
	60	60.00 ± 7.07ab	62.50 ± 6.29ab	70.00 ± 4.08ab
	80	70.00 ± 4.08ab	72.50 ± 2.50ab	67.50 ± 2.50ab
	100	75.00 ± 2.89ab	75.00 ± 2.89ab	72.50 ± 4.79ab
Root length [cm]	0	2.58 ± 0.63b/B	4.87 ± 0.05a/A	2.81 ± 0.03c/AB
	10	4.11 ± 0.24ab/AB	3.55 ± 0.09b/B	4.89 ± 0.31a/A
	20	4.08 ± 0.60ab	4.07 ± 0.13ab	3.95 ± 0.03abc
	40	5.49 ± 0.78a	4.98 ± 0.05a	3.84 ± 0.03abc
	60	5.80 ± 0.37a/A	3.94 ± 0.03ab/B	4.14 ± 0.05ab/AB
	80	6.67 ± 0.77a/A	3.93 ± 0.10ab/AB	3.35 ± 0.03bc/B
	100	4.31 ± 0.77ab	4.75 ± 0.20a	3.68 ± 0.05abc
Steam length [cm]	0	2.04 ± 0.08/B	2.57 ± 0.04ab/A	2.18 ± 0.08b/B
	10	3.08 ± 0.22	3.44 ± 0.18a	2.99 ± 0.05ab
	20	3.22 ± 0.09A	2.75 ± 0.20ab/AB	1.91 ± 0.08b/B
	40	3.10 ± 0.04B	2.71 ± 0.10ab/C	3.95 ± 0.03a/A
	60	3.31 ± 0.19A	1.92 ± 0.06b/B	2.39 ± 0.11ab/AB
	80	2.51 ± 0.06B	2.96 ± 0.03ab/A	2.96 ± 0.03ab/A
	100	2.81 ± 0.35	3.00 ± 0.05ab	2.50 ± 0.06ab

Water solutions made from underground biomass of perennial lupine had a significant effect on the germination capacity of white mustard seeds at the first site S1 ($H = 25.846$; ≤ 0.001) and at the third site S3 ($H = 18.861$; $p = 0.004$). At the first site S1, the highest values were recorded at 0% concentration, and the lowest at 20% concentration. At the third site S3, the highest values were found for 0% and 40% solutions, while the lowest - for 80% (Table 2).

Significant differences in stem length were noted for all sites: the first S1 (H = 14.626; p = 0.023), the second S2 (F = 3.774; p = 0.010) and the third S3 (F= 9.089; p ≤ 0.001). At the first site S1, the lowest stem length was found at a concentration of 20%, and the highest at a concentration of 40%. At the second site S2, the lowest values were recorded at concentrations of 10% and 60%, the highest at 20%, and at the third site S3, the lowest at 80% and the highest at 10%. Root length analysis showed significant differences for the first site S1 (H = 18.106; p = 0.006) and the second site S2 (H = 16.905; p = 0.010). At the first site S1, the lowest root length was found at 100% concentration, and the highest at 40%. At the second site S2, the lowest values were found at 20% concentration, and the highest at 100% (Table 2).

Comparing the differences in mean values the study parameters between the three research sites, significant differences were found for the germination capacity at 20% concentration (H = 8.667; p = 0.131), 40% (H = 8.262; p = 0.161), 60% (H = 9.226; p = 0.010), 80% (H = 8.667; p = 0.131), and 100% (H = 10.748; p = 0.005), stem length for concentration 0% (F = 7.782; p = 0.011), 20% (H = 7.4771; p = 0.024), 40% (F = 12.417; p = 0.003), 80% (F = 6.913; p = 0.015), 100% (F = 6.162; p = 0.021) and root length for concentration of 20% (H= 7.854; p = 0.020), 40% (F = 11.631; p = 0.003), 60% (F = 13.410; p = 0.002) and 100% (H = 9.881; p = 0.007) (Table 2).

Table 2. Seed germination and growth parameters (steam and root length) of white mustard exposed to water extracts of perennial lupine underground biomass. Letters indicate homogeneous groups obtained in Tukey HSD test or Kruskal-Wallis test at p≤0.05. Small letters indicate differences in mean values between concentrations of *L. polyphyllus* water extracts, capital letters indicate differences between sites.

	Concentration of *L. polyphyllus* water extracts [%]	**Site S1**	**Site S2**	**Site S3**
Germination capacity[%]	0	65.00 ± 2.89a	62.50 ± 2.50	57.50 ± 2.50a
	10	57.50 ± 2.50ab	60.00 ± 0.00	52.50 ± 2.50ab
	20	5.00 ± 2.89c/B	57.50 ± 2.50/A	52.50 ± 2.50ab/AB
	40	47.50 ± 2.50abc/B	60.00 ± 0.00/A	57.50 ± 2.50a/AB
	60	25.00 ± 2.89abc/B	55.00 ± 2.89/A	50.00 ± 0.00ab/AB
	80	22.50 ± 2.50abc/B	65.00 ± 2.89/A	27.50 ± 2.50b/AB
	100	10.00 ± 0.00bc/B	62.50 ± 2.50/A	50.00 ± 0.00ab/AB

Root length [cm]	0	2.51 ± 0.56ab	5.28 ± 0.68ab	4.11 ± 0.12
	10	4.16 ± 0.49ab	5.95 ± 0.46ab	4.37 ± 1.01
	20	2.25 ± 0.75ab/B	5.46 ± 0.12b/A	4.39 ± 0.27/AB
	40	5.58 ± 0.13a/B	7.71 ± 0.31ab/A	6.14 ± 0.45/A
	60	2.92 ± 0.78ab/B	6.17 ± 0.37ab/A	7.18 ± 0.61/A
	80	3.23 ± 0.41ab	6.51 ± 0.80ab	5.38 ± 2.23
	100	1.13 ± 0.24b/B	7.98 ± 0.25a/A	4.98 ± 1.00/AB
Steam length [cm]	0	2.28 ± 0.14ab/B	2.81 ± 0.20ab/AB	3.06 ± 0.06abc/A
	10	3.01 ± 0.55ab	2.52 ± 0.12b	3.48 ± 0.25a
	20	1.25 ± 0.25b/B	3.32 ± 0.13a/A	2.67 ± 0.14bcd/AB
	40	3.33 ± 0.08a/A	2.68 ± 0.17ab/B	2.59 ± 0.07cd/A
	60	2.54 ± 0.26ab	2.42 ± 0.09b	3.20 ± 0.22abc
	80	2.44 ± 0.21ab/AB	3.00 ± 0.17ab/A	2.15 ± 0.09d/B
	100	2.00 ± 0.41ab/B	3.05 ± 0.22ab/AB	3.38 ± 0.19ab/A

3.2 The influence of water extracts made from aboveground and underground biomass of perennial lupine on the seed germination and growth meadow red-fescue.

In the case of water solutions made from aboveground biomass of perennial lupine, their significant effect was found only on the length of *Festuca rubra* roots in three examined sites: the first S1 ($F = 3.993$; $p = 0.008$), the second S2 ($H = 15.137$; $p = 0.019$) and the third S3 ($F = 23.591$; $p \leq 0.001$). At the first site S1, the lowest values were recorded at concentrations of 80%, the highest at 10% and 40%. At the second site S2, the lowest at 80%, the highest at 20%. At the third site S3, the lowest values were recorded at concentrations of 100%, the highest at 10% (Table 3).

Analysis of mean values between the sites revealed significant differences in the number of germinated *F. rubra* seeds in the concentration range of 10% ($H = 8.228$; $p = 0.016$) and significant differences in root length for concentrations of 20% ($F = 6.146$; $p = 0.021$), 80% ($F = 8.019$; $p = 0.010$) and 100% ($F = 8.701$; $p = 0.008$) within the three examined sites (Table 3).

Table 3. Seed germination and growth parameters (steam and root length) of meadow red-fescue exposed to water extracts of perennial lupine aboveground biomass. Letters indicate homogeneous groups ob-tained in Tukey HSD test or Kruskal-Wallis test at $p \leq 0.05$. Small

letters indicate differences in mean values between concentrations of *L. polyphyllus* water extracts, capital letters indicate differences between sites.

	Concentration of *L. polyphyllus* water extracts [%]	**Site S1**	**Site S2**	**Site S3**
Germination capacity[%]	0	85.00 ± 2.89	75.00 ± 2.89	82.50 ± 2.50
	10	85.00 ± 2.89/A	77.50 ± 2.50/AB	67.50 ± 2.50/B
	20	75.00 ± 2.89	77.50 ± 2.50	67.50 ± 4.79
	40	77.50 ± 2.50	70.00 ± 4.08	62.50 ± 2.50
	60	65.00 ± 2.89	60.00 ± 4.08	70.00 ± 4.08
	80	67.50 ± 2.50	80.00 ± 4.08	65.00 ± 2.89
	100	75.00 ± 2.89	62.50 ± 4.79	62.50 ± 2.50
Root length [cm]	0	3.01 ± 0.29ab	3.49 ± 0.17ab	3.64 ± 0.11ab
	10	3.30 ± 0.36a	3.49 ± 0.09ab	4.16 ± 0.30a
	20	3.06 ± 0.10ab/B	3.62 ± 0.12a/A	3.47 ± 0.13ab/AB
	40	3.63 ± 0.19a	3.48 ± 0.06ab	3.54 ± 0.11ab
	60	3.10 ± 0.13ab	3.09 ± 0.04ab	2.99 ± 0.15bc
	80	2.28 ± 0.11b/B	2.76 ± 0.08b/A	2.44 ± 0.05cd/AB
	100	2.91 ± 0.09ab/A	3.08 ± 0.33ab/A	1.95 ± 0.11d/B
Steam length [cm]	0	2.89 ± 0.26	3.54 ± 0.13	3.24 ± 0.06
	10	3.16 ± 0.35	3.15 ± 0.03	3.24 ± 0.12
	20	3.01 ± 0.06	3.20 ± 0.13	3.01 ± 0.07
	40	3.70 ± 0.12	3.60 ± 0.25	3.11 ± 0.14
	60	3.43 ± 0.22	3.43 ± 0.10	3.35 ± 0.13
	80	2.98 ± 0.35	3.31 ± 0.16	3.06 ± 0.22
	100	3.60 ± 0.14	3.59 ± 0.07	3.50 ± 0.05

In the case of water solutions made from underground biomass of perennial lupine, a significant effect was found on the germination capacity *of F. rubra* within at first site S1 ($H = 16.618$; $p = 0.011$), the stem length of *F. rubra* on the first site S1 ($F = 5.043$; $p = 0.002$) and

the root length of *F. rubra* on the second site S2 (H = 19.237; p = 0.004). The lowest number of germinated *F. rubra* seeds at the first site S1 was found for 0% concentration, while the highest - for 10%. In the case of *F. rubra* stem length at the first site S1, the lowest values were recorded at concentrations of 0%, 10%, 20%, 40%; the highest for 100%. On the second site S2, the lowest root length of *F. rubra* was found at concentrations of 40% and 60%, and the highest for the 0% control (Table 4).

Statistical analysis of the sites revealed significant differences in the number of germinated *F. rubra* seeds at concentrations of 10% (H = 9.646; p = 0.008) and 20% (H = 7.476; p = 0.024). *F. rubra* steam length at concentrations of 0% (F = 10.830; p = 0.004) and 10% (F = 4.526; p = 0.044) and root length for 0% (H = 7.529; p = 0.023) and 40% (F = 14.904; p = 0.001) (Table 4).

Table 4. Seed germination and growth parameters (steam and root length) of meadow red-fescue exposed to water extracts of perennial lupine underground biomass. Letters indicate homogeneous groups obtained in Tukey HSD test or Kruskal-Wallis test at $p \leq 0.05$. Small letters indicate differences in mean values between concentrations of *L. polyphyllus* water extracts, capital letters indicate differences between sites.

	Concentration of *L. polyphyllus* water extracts [%]	**Site S1**	**Site S2**	**Site S3**
Germination capacity[%]	0	57.50 ± 2.50b	67.50 ± 2.50	65.00 ± 2.89
	10	85.00 ± 2.89a/A	70.00 ± 0.00/AB	62.50 ± 2.50/B
	20	60.00 ± 0.00ab/B	72.50 ± 2.50/A	65.00 ± 2.89/AB
	40	62.50 ± 4.79ab	65.00 ± 2.89	70.00 ± 0.00
	60	70.00 ± 0.00ab	70.00 ± 0.00	62.50 ± 2.50
	80	65.00 ± 5.00ab	62.50 ± 2.50	67.50 ± 2.50
	100	57.50 ± 4.79ab	72.50 ± 2.50	70.00 ± 0.00
Root length [cm]	0	3.70 ± 0.19/B	4.42 ± 0.01a/A	3.71 ± 0.17/AB
	10	3.41 ± 0.06	3.83 ± 0.02ab	3.81 ± 0.22
	20	3.72 ± 0.16	3.52 ± 0.16ab	3.66 ± 0.30
	40	3.59 ± 0.16/A	3.10 ± 0.04b/B	3.99 ± 0.11/A
	60	3.62 ± 0.17	2.96 ± 0.17b	3.65 ± 0.28
	80	3.34 ± 0.09	3.28 ± 0.13ab	3.67 ± 0.18
	100	3.81 ± 0.04	3.32 ± 0.20ab	3.48 ± 0.29

Steam length [cm]	0	3.01 ± 0.18b/B	3.62 ± 0.05/A	2.95 ± 0.06/B
	10	3.01 ± 0.09b/B	3.39 ± 0.05/AB	3.50 ± 0.18/A
	20	2.81 ± 0.08b	3.30 ± 0.18	2.93 ± 0.22
	40	2.93 ± 0.06b	3.35 ± 0.02	3.27 ± 0.18
	60	3.26 ± 0.13ab	3.17 ± 0.09	3.03 ± 0.17
	80	3.18 ± 0.07ab	3.44 ± 0.14	3.06 ± 0.15
	100	3.51 ± 0.06a	3.26 ± 0.18	3.35 ± 0.05

3.3 The influence of habitat properties on the perennial lupine allelopathic properties

In order to determine the influence of habitat properties on the perennial lupine allelopathic effects, only the highest concentration (100%) was used and its effects on the germination capacity of *Sinapis alba* and *Festuca rubra* were studied. The calculations showed a significant positive correlation between the germination capacity of *Sinapis alba* treated with a solution made from 100% perennial lupine underground biomass and soil moisture, soil organic matter content, total nitrogen, and soil organic carbon concentration. A negative correlation was noted for soil phosphorus concentration (Table 5). For *Festuca rubra*, a positive correlation with soil total nitrogen and a negative correlation with soil phosphorus were recorded (Table 5).

Table 5. Results of Spearman rank correlation analysis between habitat properties and the germination capacity of target species treated with a solution made from 100% perennial lupine underground biomass. Only variables significant at $p \leq 0.05$ are presented in the table.

	Rs	p
	Sinapis alba	
Soil moisture	0.91	0.001
Organic matter	0.91	0.001
Concentration of organic carbon	0.91	0.001
Concentration of total nitrogen	0.91	0.001
Concentration of phosphorus insoluble forms	-0.866	0.003
	Festuca rubra	
Concentration of total nitrogen	0.683	0.042
Concentration of phosphorus insoluble forms	-0.822	0.007

4. Discussion

Perennial lupine is an allelopathic species, with 20 different organic acids identified and described (Akritidu et al., 2013). The presence of tannins such as epicatechin, catechin, epigallocatechin (Boinik et al., 2015) and quinolizidine alkaloids (Wink, 1983) has been documented in the roots. One of the main alkaloids found is lupamine (Wink et al., 1983), which inhibits seed germination and seedling growth (Muzquiz et al., 1994). Some of the alkaloids, such as sparteine and gramine, can influence root growth but not the seed germination proces (Muzquiz et al., 1994). Moreover, water solutions made from fallen leaves of perennial lupine negatively affect the germination and root growth of grasses and herbaceous plants, although most of these effects cease after germination (Loydi et al., 2015). The release of allelochemicals can occur from aboveground parts to the phyllosphere and from underground parts to the rhizosphere (Mardani et al., 2016). These compounds may be released through root exudation, leaching from shoots, the decomposition of organic matter (Rice, 1984) andsome of them may be excreted from the seeds (Wink, 1983). Given the diverse mechanisms of allelopathic interactions in perennial lupine, our study aimed to assess its impact on the germination of two target species—representing monocots and dicots—as well as its effect on their growth.

Despite the documented negative effect of perennial lupine on the germination of *Lactuca sativa* (Wink, 1983) and species representing various botanical families (Kalske et al., 2022b), our results did not reveal a clear pattern that would allow us to determine at which concentrations a strong inhibitory effect occurs or which study populations of *L. pollyphylus* exhibit the most allelopathic potential. When comparing the responses of the target species to water solutions, *Festuca rubra* showed a weaker and more selective response to the tested extracts than *Sinapis alba*. This observation may indicate a higher tolerance of red fescue to allelochemicals released by perennial lupine. An analysis of the responses of the studied species to water solutions revealed not only inhibitory effects but also a clear stimulatory effect. This pattern is typical of allelopathy, in which organic compounds that inhibit growth and development at certain concentrations may have stimulatory effects at other concentration ranges (Rice, 1984; Duke et al., 2006).

Our results suggest a stronger effect of solutions made from the underground biomass of perennial lupine compared to those made from its stems, which contradicts findings from other studies. Zhang et al. (2021), Kalske et al. (2022a), Kalske et al. (2022b) show that water effluents made from aboveground biomass have greater allelopathic potential than solutions made from roots. In the study conducted by Kalske et al. (2022a), it was found that the emission of 22 volatile organic compounds was similar in the analyzed native and invasive perennial

lupine populations. In the case of the three species populations we analyzed, we demonstrated some differences in allelopathic effects, although these were not linear relationships.

Environmental factors control the production and release of allelochemicals (Latif et al., 2017). These factors may include the changing climate (Weston et al., 2013), soil properties, nutrient deficiency, light, or UV radiation (Einhellig, 1996; Mahmood et al., 2013; Latif et al., 2017). The production of allelopathic substances and their effect is a complex mechanism that depends on many variables - biotic and abiotic (Polyak and Sukcharevich, 2019). In our results, we demonstrated a negative correlation between the germination rate of *Sinapis alba* treated with a solution made from 100% perennial lupine underground biomass and soil moisture, soil organic matter content, total nitrogen and soil organic carbon concentration. Negative correlation was noted for soil phosphorus concentrations. For *Festuca rubra*, we also noted a positive correlation between soil total nitrogen concentrations and a negative with soil phosphorus. A relationship with phosphorus aligns with the results obtained by Koeppe et al. (1976) and Tang et al. (1995).

5. Conclusions

In our study, we have not demonstrated the existence of clear dependencies that could allow us to show which populations of perennial lupine have the strongest allelopathic potential to inhibit the growth and development of the target species. Yet, there is a noticeable difference between them, which allows us to draw a preliminary conclusion after laboratory experiments that habitat has a significant impact on the amount of biologically active compounds produced by the species. To better understand how habitat influences the allelopathic potential of perennial lupine, it would be necessary to conduct an experiment under field conditions and perform quantitative and qualitative analyses of secondary metabolites in different populations of the species.

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