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Journal of Advanced Research in Natural and Applied Sciences

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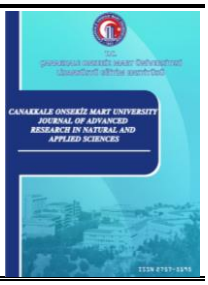

e-ISSN: 2757-5195 2024, Vol. 10, Issue 1, Pages: 150-160 doi.org/10.28979/jarnas.1364216

In vitro Allelopathic Potential of Leaf Water Extracts of *Plantago lanceolata* and *P. major* on the Germination of Some Crops

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Article History

Received: 23 Sep 2023

Accepted: 22 Dec 2023

Published: 15 Mar 2024

Research Article

Abstract − Alongside increasing productivity in plant production, reducing crop losses has become a major focus for the struggle of today's man against hunger. Allelopathy, an ecological phenomenon in which organisms interfere with each other, can be a useful strategy in agricultural systems, especially for weed management. In this study, the allelopathic effects of leaf water extracts from two weed species, *Plantago lanceolata* and *P. major*, on the seed germination of some crop plants, including wheat, sunflower, lentil, bean, carrot, radish, and purslane were investigated. *In vitro* germination assays were conducted to determine the effects of different dilutions of the stock extract on the germination rate. The results of the study showed that as the concentration of the extracts from both weeds increased, germination rates decreased in all crop plants. As to the seeds that were exposed to *P. lanceolata* extract, the ones with the most inhibited germination were carrot (11.84-100%), purslane (18.53-100%), radish (6.17-98.84%), lentil (13.77-95.56%), sunflower (17.57-94.98%), and wheat (30.12-78.31%). The seeds of beans (28.69-39.15%), on the other hand, were the least affected. Similarly, for seeds exposed to *P. major* extract, the ones with the most inhibited germination were carrot (4.67-100%), lentil (65.46-99.55%), purslane (25.09-99.24%), radish (48.69-95.51%), sunflower (43.68-93.16%), and beans (20.70-66.80%), while wheat (12.35-60.62%) seeds were the least affected. If purslane and radish are considered as weeds, our findings suggest that higher concentrations of *P. lanceolata* and *P. major* extracts can be effective bioherbicides for controlling these weeds.

Keywords − *Plantago, allelopathy, leaf extract, seed germination, Petri dish assay*

1. Introduction

The United Nations Population Division has reported that the world population reached 8 billion as of November 15, 2022, and it is projected to reach 8.5 billion by the year 2030, 9.7 billion by 2050, and 10.4 billion by 2100 [1]. Our planet will need to sustain this continuously growing population. The foundation of the essential food supply necessary for human life and existence is formed by food nutrition. Agriculture, which has been ongoing from the past until today, is the primary source of food [2], and plants constitute more than 80% of humanity's primary food sources. Furthermore, plants are indirectly related to human nutrition as they serve as the main food source for livestock [3].

In addition to efforts to increase productivity in plant-based products and obtain more crops from less land, minimizing crop losses caused by various factors has been a major challenge in combating hunger for contemporary society. There are several factors in plant production that negatively affect yield and quality, leading to crop losses. One of these factors is weed infestation, which accounts for approximately 34% of crop

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losses [4], and if left uncontrolled, can result in crop losses of up to 100% [5].

Allelopathy, an ecological phenomenon in which organisms influence each other, can be a beneficial strategy, especially in weed control, within agricultural systems [6]. Allelopathy involves the ability of plants to release certain chemical compounds (allelochemicals) that can either benefit or harm other plants, plant pathogens, and herbivorous insects. Unlike synthetic agricultural pesticides, allelochemicals can easily degrade in nature, thereby minimizing environmental damage. Additionally, allelopathy helps reduce costs in agricultural production. As the importance of effective utilization of allelopathy in agriculture is recognized, research in this field is increasing, allowing for the discovery of new and more potent allelochemicals [7,8]. In this context, this study aims to explore the allelopathic potential of two weed species, *Plantago lanceolata* and *P. major*, with a focus on the former, which has not yet been identified as exhibiting allelopathic traits.

2. Materials and Methods

2.1. Plant Material and Preparation of Extracts

The leaves of *Plantago lanceolata* and *P. major* were collected from vacant lots in the Değirmenaltı neighborhood of Süleymanpaşa district, Tekirdağ province, Türkiye, in April and May of 2022, prior to flowering. After washing the leaves with tap water, they were left to dry in a dimly lit room at room temperature, protected from dust, for several weeks. Following the drying process, the leaves were manually crushed into a powdered form [9,10]. From this material, 25 g was soaked in 250 ml of distilled water at room temperature and in darkness for 24 hours. The mixture was first filtered through a cheesecloth and then filtered twice through Whatman filter paper No. 1 [11]. The filtrate was stored in a refrigerator. Another 250 ml of distilled water was added to the residue, and it was left to soak for 24 hours at room temperature in darkness. The same procedure was followed to obtain the second filtrate, and the two filtrates were combined and stored at 4 °C as a stock extract (%5) for later use. In the experiments, concentrations of 100% (50 mg ml^{-1}), 75% (37.5 mg ml^{-1}), 50% (25 mg ml^{-1}), 25% (12.5 mg ml^{-1}), and 1% (0.5 mg ml^{-1}) of the stock extract were used.

2.2. Plant Material and Preparation of Extracts

Wheat, sunflower, lentil, bean, carrot, radish, and purslane were employed as test crops, all of which underwent allelopathy testing for *P. lanceolata*; additionally, excluding purslane, they were also tested for *P. major* allelopathy for the first time in this study. The commercial seeds of wheat, sunflower, carrot, radish, and purslane from several different brands were purchased from a local seed sales company in Tekirdağ, Türkiye. Lentil and bean seeds were obtained from a grocery store as food-grade products. Seeds that were broken, deformed, or had different colors were visually inspected and removed. Empty and underdeveloped seeds were discarded by floating in water. To eliminate the possibility of fungal or bacterial toxins affecting germination, the intact seeds were subjected to surface sterilization for two minutes with a solution of 10:1 distilled water to commercial bleach (sodium hypochlorite) (with agitation). After surface sterilization, the seeds were washed three times with distilled water. The Petri dishes, filter papers, glassware, and forceps used in the germination experiments were wrapped in aluminum foil and sterilized in an autoclave at 121 °C for 15 minutes [12]. The seeds were germinated between two filter papers in Petri dishes with a diameter of 12 cm. Depending on the seed size, a homogeneous placement of 20 (bean), 40 (wheat, sunflower, lentil), 60 (radish), 150 (carrot), and 300 (purslane) seeds was made in each Petri dish. Before sowing the seeds into the Petri dishes, the filter paper placed at the bottom of the dish was moistened with 3 ml of extract (%100, %75, %50, %25, and %1) for experimental seeds and 3 ml of distilled water for control seeds. After placing the seeds, they were covered

with filter paper, and 9 ml of the corresponding concentration of extract was added to each experimental group, while the same amount of distilled water was added to the control group. The Petri dishes were sealed and incubated at 22-24 °C in an incubator for 72 hours for wheat, sunflower, lentil, bean, radish, and purslane. Carrot seeds were allowed to germinate for 144 hours due to their late germination characteristic. At the end of the germination period, seeds with a radicle length larger than 2 mm were considered germinated [13]. The germination experiments were repeated three times.

2.3. Germination Rate and Effect Compared to Control (%)

The germination rate (%) was calculated by multiplying the ratio of the number of germinated seeds to the total number of seeds at the end of the germination period by 100 [12]. The results of the three repetitions were given as "mean ± standard error." The effect (%) of different concentrations of the extract compared to the control was calculated using the following formula [14].

$$\text{Effect (\%) compared to control} = \frac{(\text{Germination rate of the extract concentration} - \text{Germination rate of the control}) \times 100}{\text{Germination rate of the control}}$$

2.4. Statistical Analysis

The obtained findings were calculated as mean ± standard error (S.E.M.). Statistical analysis was performed using GraphPad Prism software program (GraphPad Prism Version 9, San Diego, CA, USA), and it included one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. A p value of less than 0.05 was considered statistically significant.

3. Results and Discussion

When the effects of *P. lanceolata* extract concentrations of 1%, 25%, 50%, 75%, and 100% on the germination of our test plant seeds were examined, it was determined that as the extract concentration increased, the germination rates of the seeds decreased significantly in a statistically significant manner (p<0.001 for all concentrations). However, the application of a 1% concentration did not show a statistically significant increase in germination rate in wheat seeds and a decrease in germination rate in radish seeds (Figure 1).

The extent to which different concentrations of *P. lanceolata* extract affected the germination of the test plant seeds compared to the control is shown in Table 1. As can also be seen from the relevant table, the seeds that were most inhibited from germinating by *P. lanceolata* extract were, in order, carrots, purslane, radishes, lentils, sunflowers, and wheat seeds. Bean seeds, on the other hand, were the least affected in terms of germination by *P. lanceolata* leaf extract.

When the effects of *P. major* extract concentrations of 1%, 25%, 50%, 75%, and 100% on the germination of the test plant seeds were evaluated, it was determined that as the extract concentration increased, the germination rates of the seeds decreased significantly in a statistically significant manner. However, it was observed that the 1% concentration of *P. major* extract increased the germination rate of wheat, lentil, bean, radish, and purslane seeds; although the increase was not statistically significant for wheat, beans, radishes, and purslane, it was significant for lentils (p<0.001) (Figure 2).

The extent to which different concentrations of *P. major* leaf extract affected the germination of plant seeds compared to the control is shown in Table 2. From this table, it can be seen that the seeds most inhibited from germinating by *P. major* leaf extract were, in order, carrots, lentils, purslane, radishes, sunflowers, and bean seeds. Wheat seeds, on the other hand, were the least affected in terms of germination by *P. major* leaf extract.

In recent years, there has been an increase in studies highlighting the role of allelopathy in intercropping, crop-weed, and weed-weed relationships, as well as research on the potential use of allelopathic plants in weed control. These studies have gained considerable popularity [15,16]. In this study, we investigated the potential allelopathic effects of leaf water extracts of *Plantago lanceolata* and *P. major* on the germination of seeds of crops such as wheat, sunflower, lentil, bean, and carrot, in the context of the crop-weed relationship. Additionally, we explored the possible allelopathic effects on the germination of weed seeds, specifically radish and purslane, in the context of the weed-weed relationship. To date, studies have unveiled the allelopathic effects of *Plantago major*, *P. lagopus*, *P. squarrosa*, *P. virginica*, and *P. psyllium*. Notably, investigations into the impact of *P. major* have primarily focused on the germination of purslane as a crop. Meanwhile, the allelopathic effects of *P. major* and other *Plantago* species on the germination of various weed seeds have been explored in these studies [17-20]. There has been no experimental study regarding the allelopathic properties of *P. lanceolata*, making our study the first in this regard. Nevertheless, there is only one article reporting observations made by Professor Knut Schmidtke (Dresden, Germany), an agricultural professor and co-author of the relevant article, stating that there was a significant decrease in yield when wheat (*Triticum aestivum*) was grown in a field previously inhabited by *P. lanceolata* [21].

The most common biological experiments used to determine the effects of allelochemicals and reveal the allelopathic potentials of various plant extracts are seed germination and seedling growth and development studies (including root and shoot lengths). These experiments are typically conducted using the Petri dish method in their simplest form. In seed germination studies, the germination rate is often calculated [22]. In this study as well, the allelopathic potential was determined through seed germination experiments using the Petri dish method, and the results of seed germination were presented as germination rates.

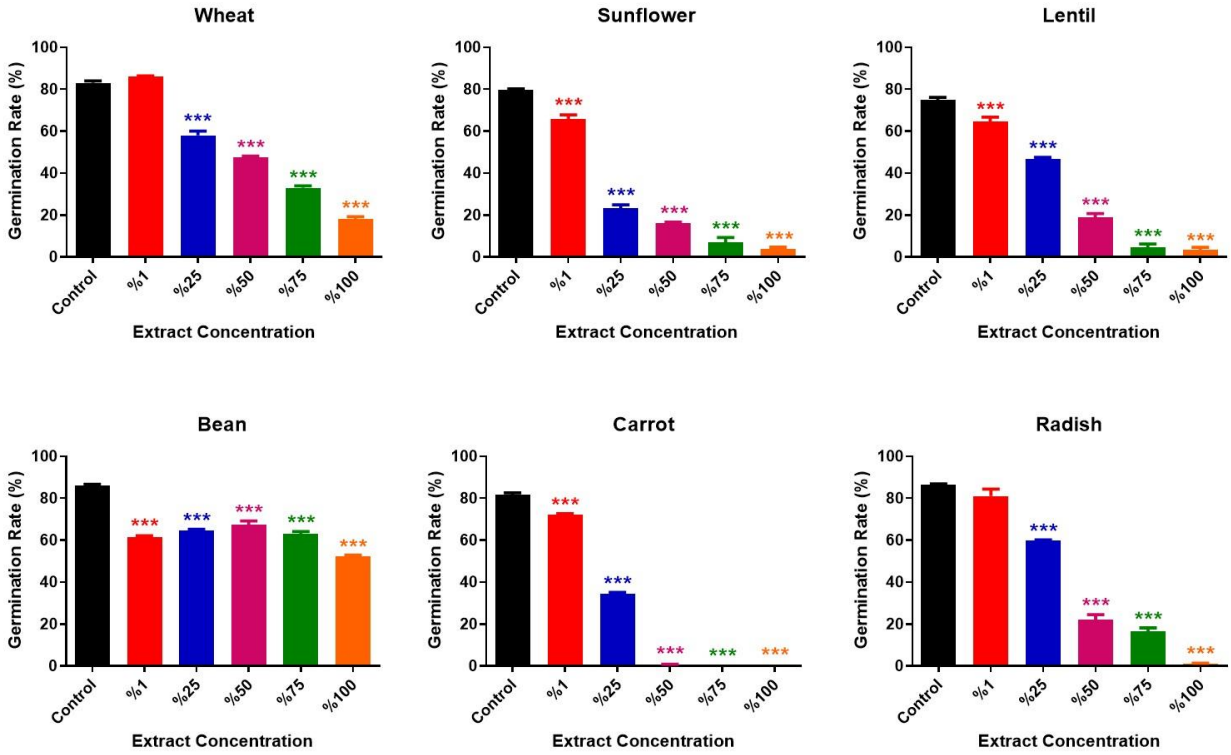

Figure 1. The effect of different concentrations (1%, 25%, 50%, 75%, and 100%) of *P. lanceolata* leaf water extract on the germination of wheat, sunflower, lentil, bean, carrot, radish, and purslane seeds. $^{***}p<0.001$; statistical significance compared to the control group

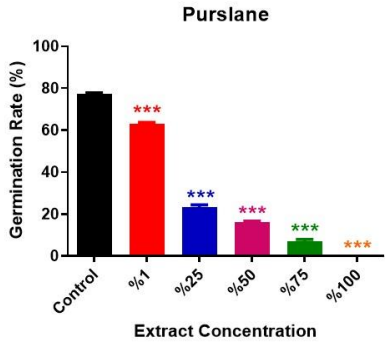

Figure 1. (Continued)

In an allelopathic study, one of the most important aspects is the selection of target species from both monocotyledonous and dicotyledonous plants to determine the potential selectivity of allelochemicals [22]. Accordingly, in the present study, both monocotyledonous (wheat) and dicotyledonous (sunflower, lentil, bean, carrot, radish, purslane) species were used as test plants.

Table 1. Effect of different concentrations of *P. lanceolata* leaf water extract compared to the control (%)

Test Plants	Germination Rates (%)*					
	Control	Extract Concentrations				
		%1	%25	%50	%75	%100
Wheat	83	86	58	47.33	33	18
The effect compared to the control		+3.61	–30.12	–42.98	–60.24	–78.31
Sunflower	79.67	65.67	23.33	16	7	4
The effect compared to the control		–17.57	–70.72	–79.92	–91.21	–94.98
Lentil	75	64.67	46.67	19	4.67	3.33
The effect compared to the control		–13.77	–37.77	–74.67	–93.77	–95.56
Bean	86	61.33	64.67	67.33	63	52.33
The effect compared to the control		–28.69	–24.80	–21.71	–26.74	–39.15
Carrot	81.67	72	34.33	0.67	0	0
The effect compared to the control		–11.84	–57.97	–99.18	–100	–100
Radish	86.33	81	59.67	22	16.33	1
The effect compared to the control		–6.17	–30.88	–74.52	–81.08	–98.84
Purslane	77.33	63	23.33	16	7	0
The effect compared to the control		–18.53	–69.83	–79.31	–90.95	–100

*Germination rates are given as "Mean". Concerning the effect compared to the control, positive (+) values indicate the promotion of germination, while negative (–) values indicate the inhibition of germination.

Most allelochemicals are hydrophilic [23] and can dissolve either completely or partially in water [24,25]. Therefore, in nature, they are usually released from the aboveground parts of plants through dew, rain, or fog droplets, either by dissolution or washing. They can exhibit their effects in this dissolved state and can also acquire allelopathic properties after being modified by soil microorganisms [26]. Consequently, in this study, water extracts were used to simulate naturally dissolved/washed allelochemicals.

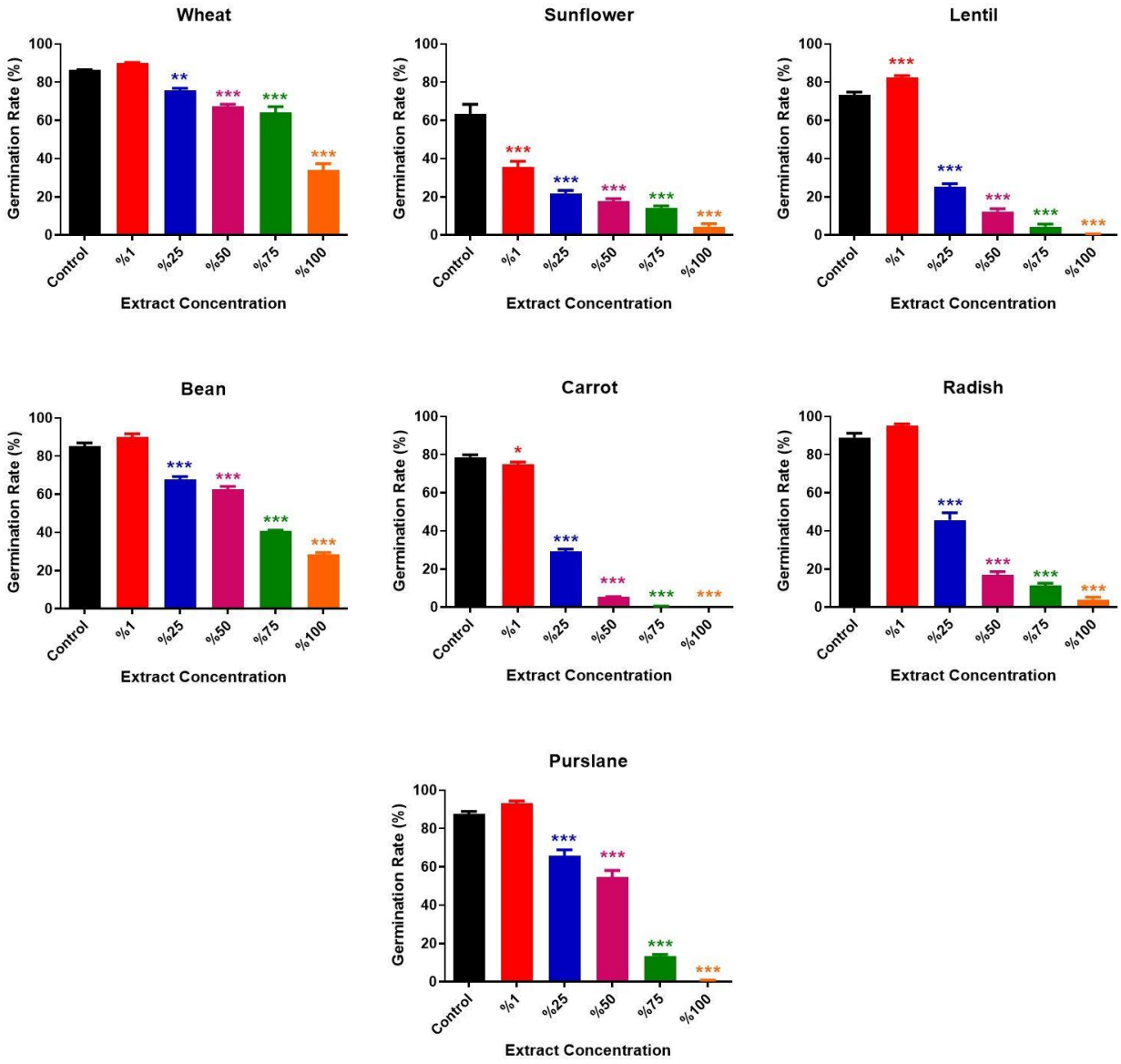

Figure 2. The effect of different concentrations (1%, 25%, 50%, 75%, and 100%) of *P. major* leaf water extract on the germination of wheat, sunflower, lentil, bean, carrot, radish, and purslane seeds. *p<0.05; **p<0.01; ***p<0.001; statistical significance compared to the control group

In the current study, radish and purslane were used as test plants, representing weeds. *Raphanus raphanistrum* ssp. *raphanistrum* (synonym *Raphanus sativus*) (radish) is considered one of the four worst weeds in agricultural fields worldwide [27,28]. It has the ability for vegetative reproduction (forming lateral roots from stem fragments) and can produce a large number of seeds within six weeks after germination (a single plant can produce 10,000 to 242,000 seeds.) [29-31]. Purslane, on the other hand, ranks ninth among the most dangerous weeds globally due to its ability to produce a significant number of seeds within a short period after germination [20,32]. Although commercial seeds of radish and purslane were used in this study, it has been reported that the germination success of seeds from spontaneously growing plants in nature is generally weaker than that of commercial seeds of the same plants [33]. Therefore, it is inevitable that the allelopathic effects observed in the experiments conducted in this study would be even stronger in nature.

Table 2. The effect of different concentrations of *P. major* leaf water extract compared to the control (%)

Test Plants	Germination Rates (%)*					
	Control	Extract Concentrations				
		%1	%25	%50	%75	%100
Wheat	86.33	90	75.67	67.33	64	34
The effect compared to the control		+4.25	–12.35	–22.01	–25.87	–60.62
Sunflower	63.33	35.67	21.67	17.67	14	4.33
The effect compared to the control		–43.68	–65.78	–72.10	–77.89	–93.16
Lentil	73.33	82.33	25.33	12.33	4.33	0.33
The effect compared to the control		+12.27	–65.46	–83.19	–94.10	–99.55
Bean	85.33	90	67.67	62.67	40.67	28.33
The effect compared to the control		+5.47	–20.70	–26.56	–52.34	–66.80
Carrot	78.67	75	29.33	5.33	0.33	0
The effect compared to the control		–4.67	–62.72	–93.23	–99.58	–100
Radish	89	95	45.67	17	11.33	4
The effect compared to the control		+6.74	–48.69	–80.90	–87.27	–95.51
Purslane	87.67	93	65.67	54.67	13.33	0.67
The effect compared to the control		+6.08	–25.09	–37.64	–84.80	–99.24

*Germination rates are given as "Mean". Concerning the effect compared to the control, positive (+) values indicate the promotion of germination, while negative (–) values indicate the inhibition of germination.

The effect of allelochemicals is highly dependent not only on the type of solvent used for extraction [34] but also on the concentration (dose) of the extract [35]. The majority of studies conducted to date have demonstrated that increasing extract concentration significantly reduces seed germination and seedling development in test plants. In some cases, it even completely inhibits them [36-41]. Consistent with the findings of these studies, as well as previous research on other *Plantago* species [17-20], the current study also observed a decrease in the germination rates of all test plants as the concentration of extracts from both weed species increased. Specifically, extracts from *P. lanceolata* at 75% (37.5 mg ml^{-1}) and 100% (50 mg ml^{-1}) concentration inhibited the germination of purslane, carrot, radish, lentil, and sunflower seeds by 90-100%. Similarly, extracts from *P. major* at 75% and 100% concentration inhibited the germination of carrot, lentil, purslane, radish, and sunflower seeds by 80-100%. In the study by Al-Obaidi [20], which supports our findings regarding purslane, the water extract of the aerial parts of *P. major* at 40 mg ml^{-1} inhibited purslane germination by approximately 30.24%, while the lowest concentration (2.5 mg ml^{-1}) resulted in a 4.60% inhibition. However, in our study, a concentration of 75% (37.5 mg ml^{-1}), close to the 40 mg/ml concentration, led to an 84.80% inhibition of germination. It is well-established that the production of allelochemicals varies based on the environment in which plants are grown and the environmental stresses they encounter, including light, nutrients, water and temperature stress, and atmospheric CO_2 [42,43]. As a result, the differences in allelopathic effects on purslane germination observed in our study may be attributed to the diverse environmental conditions under which the *P. major* was grown, along with variations in experimental conditions. Regarding the allelopathic potential of *P. major*, it is noteworthy that the water extract of the aerial parts of *P. major* at concentrations of 10 mg ml^{-1} and 2.5 mg ml^{-1} inhibited the germination of *Bidens pilosa* by approximately 72.41% and 7.41%, respectively [18].

It has been found that allelochemicals can not only negatively affect the growth of certain species but also promote the germination and seedling development of the same or different species when used at different concentrations [44]. In particular, several studies have shown that low-concentration water extracts stimulate germination and growth of various crops and increase their productivity [45,46]. In this study, although the 1% extract of *P. lanceolata* appeared to promote germination in wheat and the 1% extract of *P. major* seemed

to promote germination in wheat, bean, lentil, radish, and purslane, only the promotion of lentil seed germination was statistically significant.

The *Plantago* genus contains various natural products, including iridoids, phenylpropanoid glycosides, flavonoids, tannins, triterpenes, saponins, and sterols, with iridoid glycosides being present in large quantities in *Plantago* species' leaves and above-ground parts [47]. It is known from various studies that iridoid glycosides aucubin and catalpol, found in the leaves and above-ground parts of *Plantago* [48,49], inhibit seed germination and root development [50,51]. The allelopathic effect of alfalfa (*Medicago sativa*) has been attributed to its water-soluble saponins [52]. In the current study, the observed inhibitory effect on germination is likely due to the presence of these iridoids and saponins in the leaves of *P. lanceolata* and *P. major*. It has been reported that *P. major* contains less aucubin in its leaves and above-ground parts compared to *P. lanceolata* [48]. This explains the finding in this study that the inhibitory effect on germination of *P. lanceolata* is stronger compared to *P. major*.

4. Conclusion

In conclusion, based on the data obtained from this study, the presence of *P. lanceolata* and *P. major* should be considered in fields where wheat, sunflower, lentil, bean, carrot, radish, and purslane are cultivated, both before and after sowing, to achieve high yields. Furthermore, the potential use of leaf water extracts of *P. lanceolata* and *P. major* as effective bioherbicides should be evaluated in the control of radish and purslane, which grow as weeds in agricultural fields.

Author Contributions

All the authors equally contributed to this work. This paper is derived from the first author's master's thesis supervised by the second author. They all read and approved the final version of the paper.

Conflicts of Interest

All the authors declare no conflict of interest.

Acknowledgement

We thank Dr. Ebru GÜREL GÜREVİN, Dr. Deniz EROL KUTUCU, and Gülce BAYHUN for their supports.

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