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Allelopathic Effects of *Lepidium sativum* Aqueous Extract on Germination and Seedling Growth of *Phalaris minor*: A Dose-Response Study

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Abstract

This investigation analyses the allelopathic effects of aqueous extract of Lepidium sativum (garden cress) against the annual weed Phalaris minor (red rescue grass). This weed is a known problem in wheat crops. The application of Lepidium extracts with different concentrations of 0 to 80 percent was made over P. minor seeds in the laboratory and the growth parameters including the root and shoot length, fresh weights, germination percentage, mean emergence time, germination index and time to 50 percent emergence were analyzed. The lepidium extract was analyzed using HPLC and quercetin, gallic acid, vanillic acid, and p-coumaric acid were identified as the phenolic compounds responsible for allelopathic effects. The conclusion drawn is that there were significant concentration-dependent effects of Lepidium sativum extract on Phalaris minor. Root length was inhibited at 80 percent by 47 percent of the control, whereas shoot length exhibited a potential hormetic response and increased by 25.6 percent at 20 percent. There were variable responses of fresh weights and germination parameters across the extract treatments. A biphasic dose-response model identified an EC50 value of 12.4 percent for root length. The study showed that Lepidium extracts possess significant allelopathic potential against P. minor and can be helpful in sustainable weed management. However, the complex dose-dependent responses, including the hormetic effects, should be considered carefully for practical applications. The study contributes to the research on plant-derived allelochemicals with the potential for eco-friendly weed control while highlighting the importance of dose-response relationships in allelopathic interactions. Further field studies are required to validate the findings under real-world conditions. It also calls for broader ecological studies to recognize the potential adverse implications of artificial allelopathic procedures on the environment.

Keywords: *Allelopathy, hormesis, phenolic, weed, agriculture, management*

1. Introduction

Allelopathy the chemical interaction between plants, is another process that has attracted widespread attention as a potential for sustainable agriculture [28]. It is defined as 'an experiential process in which plants compete or cooperate by releasing allelochemicals, secondary metabolites into the environment that alter growth, survival or reproduction of neighboring plants [5, 26]. In general, allelopathy provides promising applications in weed management, crop protection, and also soil health improvement [2, 26]. Production and efficacy of allelochemicals are affected by different abiotic and biotic factors, including light, temperature, water availability, soil characteristics, and plant species [29]. Allelopathy provides an alternative strategy to reduce its use for environmental sustainability in agriculture [5, 26].

Allelochemicals are naturally released secondary metabolites (having a chemical composition different from primary metabolites) synthesized by organisms that possess ecological roles beyond primary metabolism [18, 20]. They are released into the surrounding environment through a variety of mechanisms and aid in plant defense, interference, and nutrient dynamics [20]. As a biochemical interaction between plants mediated by these chemicals, it can be stimulatory, inhibitory, or both [23]. Working in symbiosis with the crop, allelopathy in a crop production system can influence production from other crops, suppress plantings after a mono-crop, and likely lead to weed suppression as well [9].

Lepidium sativum, or garden cress, is a fast-germinating edible herb, [30]. *Lepidium sativum* (cress) has significant allelopathic effects on surrounding plants. Its seed exudates have highly active allelochemicals and affect cell growth and organ morphology in receiver plants, especially by regulating cell expansion [19]. Lepidimoide, a major allelochemical of cress seed mucilage, was isolated which induced shoot expansion and root inhibition of some plant species (e.g. *Lolium multiflorum*, Maize and Tall Wheatgrass) [16]. *Lepidium sativum* itself is also affected by allelopathic effects from other plants.

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The germination and seedling growth of *L. sativum* were significantly inhibited by *Lantana camara* leaf extracts [15]. These results highlight the complex allelopathic interactions of *L. sativum* and its potential as a useful plant in agriculture.

Phalaris minor is a yield-reducing competitive weed in wheat crops, and its control is greatly affected by the extensive use of herbicides in weed management [6, 32]. Weeds like *P.minor* generally suffer highly competitive exclusions through the globalization of trade and international travel because, without a doubt, through competition and predation, they lose their habitat [17]. Most weeds are still managed extensively using herbicides as a tool, especially since weeds like *P. minor* are already showing resistance to multiple herbicide classes including ALS inhibitors, ACCase inhibitors, and Photosystem II inhibitors [27]. Now that populations have already evolved to some level of resistance, allelopathic management using plant-derived extracts has shown a potential to control its growth [19]. Such wide-ranging approaches may help us to control such species but, at the same time, reduce future evolution to resistance.

Phenolic compounds could play a remarkable role in seed germination and seedling growth, for instance, phenolic leaf-water extract of *Plectranthusamboinicus* and *Ocimum basilicum* could restrict the growth of common weeds *Phalaris minor* and *Anagalis arvensis* associated with *Pisum sativum* and significantly induce the growth and yield of the pea crop [13], however, aqueous extract of alfalfa showed allelopathic effects on *Phalaris minor* and gefarnate elicited substantially less germination of *Lepidium sativum*, but Glycyrrhiza glabra at lower concentration conferred relief for the germination of *Lepidium sativum*[11]. Moreover, the effect of phenolic herbicides on the germinability and morphological changes of the roots of *Lepidium sativum* was studied referring to this species as a good test species for toxicity assessment as it has a high germinability and good repeatability [4]. This highlights the diversity of plant-plant interactions [21], hence, the involvement of the phenolic compounds cannot be overlooked. However, phenolic effects on plant-plant interactions are dependent on the type of the compound, the concentration, and the target species. Therefore, further studies are needed to determine the mechanism and application of plant-derived phenolic compounds in plant-plant interactions.

The term hormesis was first used by Southam and Ehrlich in 1943: Exposure to toxins in small amounts causes beneficial stimulation whereas the same toxins in large amounts cause toxic inhibition-a biphasic dose-response phenomenon [25]. Hormesis is an adaptive, stress-responsive mechanism in diverse organisms, in the presence of different chemical and environmental stressors [8]. Hormetic responses are directly linked to acclimation, and phenotypic plasticity is linked to the evolution of organisms, in adapting to various environmental changes [8]. Therefore, the current study was planned to describe the effect of phenolic compounds of *Lepedium sativam* on *Phalaris minor.*

Although it is previously known that garden cress (*Lepidium sativum*) extracts are phytotoxic, few studies have been done on aqueous extracts, especially the impact on *P. minor*. We are exploring the potential to use *Lepidium sativum* (garden cress) as a natural alternative to the synthetic herbicide, controlling *Phalaris minor* through the application of its phytotoxic effects. The focus will be on exploring the phytotoxic effect of *Lepidium sativum* (garden cress aqueous extract) on the growth and germination of *Phalaris minor* and will be studied in this work.

It is hypothesized that a higher concentration of *L. sativum* aqueous extract will induce a phytoxic effect on *Phalaris minor*. Current research was planned to explore the effect of Phenolic compounds of *Lepedium sativam* on the emergence and seedling growth of *Phalaris minor* as a bio-herbicide.

2. Material and Methods

2.1. Experimental site

This study was conducted in the Weed Science Laboratory, Department of Agronomy, University of Agriculture Faisalabad in the CRD management study. The three replications were used. In the lab, the garden cress allelopathic potential on the winter vegetable *Phalaris minor* (Dumbi sittee) was assessed. For separating dry samples these samples were cut into two-centimeter pieces. After separating the chopped sample put in different soaking tanks for allelopathic water extract. It calculates in a 1:80 ratio. After taken out it runs through the cotton fabrics to take out the water from the given sample which is divided into different parts. As per the therapy the extracts dilute with 0, 0.25, 0.5, 10, 20, 40,and 80%. In this experiment waters of *P. minor* were treated with *L. sativum*. In this experiment for the given experiment 3 replications of (garden cress extract) 10 seeds each were applied on the Dumbi sittee in petri dishes and wrap. There are seven treatments given; T_1Distilled water (control), T_2 2.5%, T_3 5%, T_4 10%, T_5 20%, T_6 40%, and $T_7$80%.

2.2. Data collection

During laboratory experiments, different parameters given below were observed by using standard procedures:

2.2.1. Germination percentage %

For 12 days of the observation, the number of seeds cultivated was measured every day. When a seedling reached a length of 1-2 cm, it was considered to have germinated. The germination % was calculated using the following formula.

$$\mathbf{GP} = \frac{\text{Germinated Seeds at final count}}{\text{Total Seeds}} \times 100$$

2.2.2. Time to 50% germination (T50)

The following formula, developed by Cool Bear et al. [7] was used to calculate the time to 50% emergence (T50):

$$\mathbf{T_{50}} = ti + \frac{\left(\frac{N}{2} - ni\right)(tj - ti)}{(nj - ni)}$$

Where N is the number of seeds that germinate, and nj and ni are the cumulative number of seeds that emerge at times tj and ti, where ni<N/2 <nj.

2.2.3. Mean germination time (MGT)

Ellis and Reborts,[12] equation was used to get the mean emergence time (MET):

$$\mathbf{MGT} = \frac{\sum Dn}{\sum n}$$

where D is the number of days measured from the start of germination and n is the number of seeds that emerged on Day.

2.2.4. Germination index (GI)

The Association of Official Seed Analysis's formula [3] was used to compute the germination index.

$$\mathbf{GI} = \frac{\mathbf{N1}}{\mathbf{D1}} + \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots + \frac{\mathbf{NL}}{\mathbf{DL}}$$

Where D_1 is the day to first count, N_1 is the no. of germinated seeds onthe first day, N_L is the no. of germinated seeds at the final count and D_L are days to final count.

2.2.5. Root length (mm)

After 12 days, all of the seedlings from each replication were removed, and the length of each root was measured using a scale in millimeters starting from the place where the root and shoot joined together. The length of the root was then calculated by separating the root from that connecting point. Next, the average root length was calculated.

2.2.6. Shoot length (mm)

After 12 days, all of the seedlings from each replication were removed, and the length of each shoot was measured using a scale in millimeters starting from the point where the shoot and root joined and continuing outward from that point to determine the shoot's length. Then the shoot's average length was determined.

2.2.7. Fresh weight of shoot (mg)

Every seedling's shoot was divided, and each replication's weights for every treatment were recorded independently. By dividing the total weight by the total number of shoots, each plant's weight was determined in milligrams.

2.2.8. Fresh weight of root (mg)

Every sprout's roots were cut out, and each replication's mass was measured independently for every treatment. The weight of every plant was then calculated.

2.2.9. Phenolic content

The seed extract of *Lepidium sativum* was analyzed for its phenolic content by HPLC (Gradient, Reverse phase made in Shimadzu Japan; detector SPD-10AV pump LC -10AT). Garden Crees in the form of powder (10 g) were taken in 90% of methanol. They were taken in a beaker and kept covered with aluminum foil for eight days. It was dried for eight days and five milligrams of it was taken out for phenolic analysis. Quercetin, gallic acid, vanillic acid and P-conmeric acid are found in the seed extract of *Lepidium sativum*.

2.3. Statistical analysis

ANOVA (commonly referred to as Fisher's Analysis of Variance) was used to statistically analyze the data and the Least Significant Difference Test (LSD) was utilized to differentiate the treatment means from each other.

2.4. Measurement of dose-response relationship

The dose-response relationship of the allelopathic compound on weed growth was mathematically modeled through an automated curve-fitting procedure. To this aim, the Dr-Fit software (Di Veroli, 2015) was used. Dr-Fit belongs to a set of robust approaches to the modeling of complicated dose-response curves which can be multiphasic (i.e., with one or more transitions between linearity and curvature). Experimental data on plant growth are fitted to candidate models representing monophasic and/or multiphasic formulations of the relationship between exposure to a phytotoxic compound and plant growth. The fitting procedure is based on an optimization by maximum likelihood and it is controlled by a BIC-based model-selection procedure. The optimization is carried out using a trust-region-reflective non-linear optimization algorithm. Fitting parameters are weighted by the standard deviation of replicate measurements, which is a way of performing the correct treatment of heteroscedasticity in dose-response data. The baseline response is a variable parameter with a fixed value (set to unity), as it is the minimum response (in control conditions).The best BIC score was a biphasic function, with an initial stimulatory phase characterized by a positive interaction between the two species at low allelochemical concentrations and a subsequent inhibition phase at high doses.

This hormetic response was expressed mathematically by a two-process model incorporating stimulatory and inhibitory Hill equation terms:

$$E(C) = [1 + (E\infty 1 - 1)/(1 + ((Ec50\_1/c)\text{^}H1] \times$$

$$[1 + (E\infty 2 - 1)/(1 + (c/(EC50\_2\ )\text{^}H2)]$$

Where E(C) is the effect at concentration C, E∞1 and E∞2 are the maximum effects for the stimulatory and inhibitory process, respectively; EC50_1 and EC50_2 are half-maximal effective concentrations; and H1 and H2 are the Hill slopes of each process. That mathematical description in turn yields an analytical framework for assessing the highly nonlinear dose-dependent action of the allelopathic chemical on weed growth, with the advantage that this can be interpolated, extrapolated and relevant toxicological parameters can be derived.

Here, we optimized the fitting parameters using the trust-region-reflective algorithm,weighing the fitting parameters by their standard deviation, andconsidering the baseline as a fitted parameter. This quantitative framework allows us to examine the oftencomplex dose-dependent allelopathic effects on weed growth.

3. Results

The phytotoxic effects of garden cress aqueous extract on *P. minor* were studied across treatments (T_1-T_7) at different concentrations. The measurement of growth parameters across these treatments, revealed significant changes in root length, shoot length, root and shoot fresh weight, mean emergence time (MET), germination percentage, germination index, and time to 50% germination. As shown in Table 2, root length ranged between 62.3 mm (control) and 33.0 mm (80% treatment), reflecting a clear inhibitory effect of higher concentrations of extract on root elongation. Shoot length also ranged between 83.8 mm (20% treatment) and 64.0 mm (2.5% treatment). This suggests that lower concentrations of extract can stimulate shoot length while higher concentrations might inhibit shoot length. Root fresh weight was highest in 80% of treatment and 40% of treatment, with 5.2 mg and 5.1 mg respectively while shoot fresh weight was the highest in 20% of treatment at 8.6 mg but lowest in 40% of treatment at 5.3 mg. This suggests that high concentrations of extract might stimulate root biomass. Mean emergence time ranged between 7.3 days (20% treatment) and 7.8 days (80% treatment), implying that 20% treatment had the shortest mean emergence time and hence the fasted germination rate while the longest emergence time was recorded in the 80% treatment suggesting delayed germination. Treatments with 80% concentration enhanced germination percentage to 95%, as compared to treatment with 2.5% which gave a germination percentage of only 70%. This suggests that a possible stimulatory effect is observed at high concentrations. The germination index ranged between 7.0 (control and 10% treatment) and 4.6 (40% treatment).

Time to 50% emergence ranged between the highest 6.8 days (80% treatment) and the lowest 4.3 days (10% treatment). This implies that a high concentration of extract had an inhibitory effect on germination speed. The HPLC analysis of the aqueous extract of *Lepidium sativum* (garden cress) revealed the presence of four phenolic compounds: quercetin (0.52 ppm), gallic acid (4.82 ppm), vanillic acid (1.94 ppm), and p-coumaric acid (5.93 ppm). P-coumaric acid was found in the highest concentration, followed by gallic acid, vanillic acid, and quercetin (Table. 1)

Table 1: Phenolic compounds detected in aquatic extract of garden cress.

Garden cress		
Sr. No.	**Phenolic compounds**	**Concentration in ppm**
1	Quercetin	0.52
2	Gallic acid	4.82
3	vanillic acid	1.94
4	P-conmeric acid	5.93

These results altogether show that garden cress aqueous extract has considerable effects on the growth parameters of *P. minor*, higher concentration generally inhibits the root length and fresh weight and stimulates the shoot length and fresh weight. The data revealed a complex interactive relationship between concentration and growth response and indicated bio-stimulant and inhibiting effects depending on growth parameters under specific concentrations.

Table2: Phytotoxic effect of garden cress aqueous extract on root length, shoot length, root fresh weight, shoot fresh weight, mean emergence time, germination index, germination percentage, and time to 50% germination of P. minor.

Treatments	Root Length (mm)	Shoot Length (mm)	Root fresh weight (mg)	Shoot fresh weight (mg)	Mean emergence time	Germination index	Germination percentage	Germination index	Time to 50% Emergence
T_1= 0% (Control)	62.3a	66.7bc	3.3d	6.3cd	7.9	7.0a	86.0ab	7.0a	6.1ab
T_2=2.5%	59.1a	64.0c	4.3bc	7.5bc	7.7	5.6c	85.0b	5.6c	5.2c
T_3=5%	62.1a	71.7b	4.1c	8.0ab	7.5	6.7ab	89.3ab	6.7ab	4.6cd
T_4=10%	52.5b	65.6bc	5.0ab	6.3d	7.8	7.0a	91.3ab	7.0a	4.3d
T_5=20%	42.2c	83.8a	4.6abc	8.6a	7.3	7.1a	93.3a	7.1a	4.9cd
T_6=40%	45.3c	65.8bc	5.1a	5.3d	7.4	4.6d	90.0ab	4.6d	5.4bc
T_7= 80%	33.0d	72.5b	5.2a	8.0ab	8.5	5.9bc	89.0ab	5.9bc	6.8a

3.1. Potential hermetic dose range of Garden cress for root length of *P.minor*

The phytotoxic effects of garden cress aqueous extract on the root length of *P. minor* was investigated using a dose-response curve (Fig. 1). The effect of extract concentration on root length showed a biphasic pattern, with an early stimulatory effect at low concentrations and an inhibitory effect at higher doses. A biphasic model provided a good description of this hormetic response, which was a superior model compared to monophasic models for describing the experimental data. A slight root length stimulatory effect of *P. minor* was observed at lower levels of extract concentration (1-5%) with mean values marginally exceeding the control treatment. However, beyond 5%, the inhibitory effect was pronounced with a rapid decline in the root length between 10% and 50% of extract concentration.

The half-maximal effective concentration, EC50, was 12.4 percent, that is, the extract concentration at which root length was reduced to half of the control. This is a value that allows quantification of the extract's ability to inhibit *P. minor* root growth. The steepness of the curve around the EC50 indicates a rather narrow range of concentrations under which the shift from minimum to maximum inhibition occurs. At the highest dose tested (100 percent), the root length was reduced to about 35 percent of the control, meaning that the inhibition was substantial but not total. The dose-response curve seemed to be reaching a plateau at concentrations above 50 percent, suggesting that maximum inhibition was reached within the tested range.

The observed hormesis involving low-dose stimulation and high-dose inhibition is consistent with the existing evidence in the allelopathy and plant ecology literature, which points towards the biphasic nature of the effect. These results yet again emphasize the complexity of plant-plant interactions and, importantly, the fact that non-monotonic responses should be considered when studying phytotoxicity. The results demonstrated garden cress was a potent allelopathic species against *P. minor,* a weed species that has been a problem for centuries in cereal crops. The estimated EC50 and the dose-response relationship would help design applications for weed management. Importantly, any prospective implementation would have to be considered the appropriate dosage.

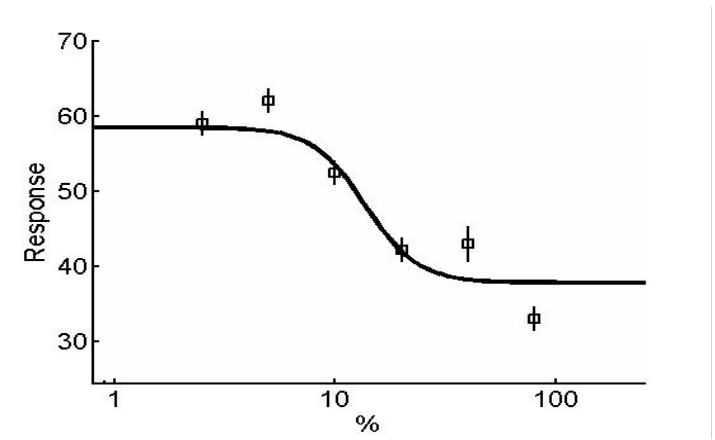

Fig. 1: Dose-Response Curve of Garden cress on Phalaris minor's root length

Table 3: Parameter Estimates and Model Fit Statistics for Biphasic Dose-Response Model (1 Inhibitory + 1 Stimulatory Phase)

Parameters	IC50_1	H_1	Emax_1	IC50_2	H_2	Emax_2	IC50_3	H_3	Emax_3	Scaling	Chi2	GOF	aic	bic	EC50
Biphasic (1 inhib. + 1 stim.)	0	0	0	13.60816	3.638966	1.5	0.025217	4.266875	0.414409	1	101.6984	1.08E-13	215.3968	347.3968	12.4

4. Discussion

The result of this study was bombarded with a sense of the great allelopathic potential of *L. sativum* aqueous extract against *P. minor*, a weed species of wheat crop. These adverse effects on the different growth parameters of *P. minor* could be a representation of the complex nature of allelopathic processes, which can be mediated by multiple allelochemicals. Notably, the response of *P. minor* seeds to the *L. sativum* aqueous extract was a dose-dependent response, as root growth was the most sensitive organ affected by elevated concentrations of extract [1]. This aligned with previous reports on allelopathic interactions among plants. The 'hormetic' effect of the extract on *P. minor* in moderate concentrations (i.e. 20%) could also be an expression of the complexity of the dose-response relationship, which may not always follow a linear relationship[33]. The response surface model fitted to the results represented the biphasic response adequately and yielded EC50 value of root length inhibition, i.e. 12.4%, as an indication of the allelopathic potency of this extract. The phytochemicals isolated in the quench for the crude extract that afforded *L. sativum* aqueous extract were quercetin, gallic acid, vanillic acid, and p-coumaric acid, and these phytochemicals could be causing some of these effects on *P. minor* [31].These findings support previous findings in the phytochemistry of *L. sativum* but more studies are needed to elucidate the mechanism of action of the extract. The effects demonstrated included the reproductive cycle of *P. minor* since the extract inhibited the germination parameters at higher concentrations [14]. Therefore, as demonstrated, *L. sativum* extract could be developed into an herbicide for *P. minor* without any phytotoxicity, with a special focus on wheat cropping systems [22]. Nevertheless, attention should be given to the limitations of this laboratory-scale study and the possible extrapolation of the findings to field conditions. Nonetheless, the soil parameters, nature of the growth medium, and environmental variables may be factors that influence the expression of allelopathy in *L. sativum* and therefore modulate the effects demonstrated in this study. However, the research work will serve as additional information to the growing literature on allelopathy and could be developed into eco-friendly strategies for weed management, which is of utmost importance in sustainable agriculture.

Conclusion

The results show that garden cress aqueous extract inhibits the shoot and root elongation of *Phalaris minor*. Furthermore, there was decreased fresh weight and delayed germination of *P. minor* in garden cress aqueous extract. This suggests its potential use as a herbicide. Further studies are needed on the application of garden cress extract in agriculture and horticulture such as its ability to hinder the growth of unwanted weed species.

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Authors' contributions

B.K. and M.A.A contributed data analysis and experimental work, I.A. wrote the manuscript, and T.R., R.M., and M.A.N. designed the experiment and critically revised it.

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Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare no conflict of interest

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