

ALLELOPATHIC EFFECT OF *LATHYRUS SATIVUS* L. ON SEED GERMINATION AND SEEDLING GROWTH OF *LENS CULINARIS* L.

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ABSTRACT

In the present study, lentil (*Lens culinaris*) was chosen as the target plant to study the effects of *Lathyrus sativus* L., which grows as a weed in fields. This weed affects the growth and productivity of plants growing nearby. The effects of different concentrations of *L. sativus* root extract on shoot and root length of *L. esculenta* were assessed at five and fifteen days after germination. The data showed that the shoot and root lengths of the test plants decreased significantly. Biomass accumulation of the germinated seedling also followed the same pattern and decreased with increasing extract concentration. The methanolic and ethanolic extracts of *L. sativus* were prepared and tested on the same plant. The maximum growth inhibition was observed in the methanolic extract. LCMS/MS analysis of the methanolic extract revealed growth inhibitory phenolics.

Keywords: Extract, Growth, *Lathyrus sativus*, *Lens culinaris*, Root length, Shoot

The phytotoxic action of some plants is a common means of defense against other plants competing for food, shelter, and existence. This interaction between plants and microorganisms is called allelopathic interaction, which is both beneficial and harmful. Plants release allelopathic chemicals, which are secondary metabolites that play an important role in the interaction between plants, plant microorganisms, and plant insects, both positively and negatively. They act as important ecological factors affecting plant dominance, succession, climatic vegetation, and plant productivity. Allelopathic interaction is one of the most important factors responsible for the distribution of a species in plant communities (Cheng and Cheng, 2015). *Lathyrus sativus* L. (grass pea) is an annual weed that grows together with other legumes (Wall and Friesen, 1991). It is called a robust legume because it is considered to be particularly resistant to climatic changes and has a hardy, penetrating root system. It is an efficient nitrogen fixer (Lambein *et al.*, 2019). It contains the water-soluble non-protein amino acid β -N-oxalylidiaminopropionic acid (β -ODAP), a neurotoxin that causes neurolathyrism in some populations (Sharma *et al.*, 2000; Polignano *et al.*, 2009). Grass pea is a diploid species ($2n=14$) with 12 metacentric and 2 submetacentric chromosomes (Ghasem *et al.*, 2014). It shows great potential for cultivation in arid areas and is resistant to several diseases common to other legumes (Almeida *et al.*, 2015). The weed exhibits marked tolerance to various herbicides such as trifluralin, bentazone, and imazethapyr (Wall and Friesen, 1991). However, grass pea is sensitive to several plant pathogens such as

Ascochyta lentis var. *lathyri*, which causes late blight of grass pea, produces lathyratoxin A and B characterized by necrotic lesions on the leaf and stem (Masi *et al.*, 2018). Phytotoxicity of *Ageratum conyzoides* L. was also observed in relation to antioxidant defense of grass pea and lentil, with lentil being more affected than grass pea. However, mitosis and meiosis were affected in both plants (Talukdar, 2013). The weed also has potent mutagenic potential towards cadmium sulfide and copper oxide nanoparticles (Gosh *et al.*, 2017). Scientists have attempted to reduce β -ODAP content in grasspea and improve its agronomic properties (Das *et al.*, 2021).

Lentil (*Lens culinaris* L.) is an edible legume believed to have originated in Rome (Cubero *et al.*, 2009). It is a self-pollinating plant that is cultivated worldwide as a legume. The plant is a good source of nitrogenous proteins, carbohydrates and iron in human nutrition. In carbohydrates, it contains sugars and oligosaccharides such as stachyose, ciceritol and raffinose (Faris *et al.*, 2013). Some genetic variation has been observed in currently cultivated species, and yet plant breeders improved monogenic traits through conventional breeding techniques such as selection and recombination (Lasker *et al.*, 2019).

The lentil field is invaded by the *L. sativus* that grows luxuriantly with the crop and compete for the nutrients, shelter, and growth. The morphological features of the lentil crop are similar as with the *L. sativus* and sometimes, it is very difficult to identify crop and weed plants. In Balrampur district, it is a common problem in lentil growing fields and the farmers used chemical herbicides to get rid of this innocuous weed. However, it was earlier observed that *L. sativus* was negatively

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Date of receipt: 23.06.2023, Date of acceptance: 30.12.2023

affected with *Lantana camera* L. leaf aqueous extract (Talukdar, 2013). But in lentil crop field the *L. sativus* is dominant weed that grow luxuriantly and filled the whole space.

The aim of the present study was to find out the allelopathic potential of *L. sativus* on seed germination and seedling growth of *Lens culinaris* L., because both plants grow in the same season and sometimes it is very difficult to recognize weeds and crop plants due to the similar morphological characters. The presence of allelochemical also has been worked out with the help of LCMS analysis. This will help to find out possible solution to remove the weed from the field that is a big shortcoming of lentil growing areas.

MATERIALS AND METHODS

The present study was conducted in Advance Plant Physiology Laboratory, MLK PG College, Balrampur in the month of December 2022 and January 2023.

Analysis of growth

The roots of *L. sativus* were collected from a field in close proximity to the lentil plant. 10 g of fresh root pieces were carefully washed and crushed in 100 ml of double distilled water and filtered with Whatman's filter paper no. 1. This extract was used to prepare 20, 40, 60, 80 and 100 ml (v/v) solution with distilled water. Pure distilled water without extract was used as control. The above extract solutions were used to irrigate the seeds of *Lens culinaris* L., which were first sterilized with 1% sodium hypochlorite and distilled water and soaked overnight in distilled water. About 10 seeds were placed in a sterilized Petri dish with a thin layer of cotton and Whatman's filter paper. Three replicates per dilution of extract were taken from each Petri dish and incubated at room temperature (20°C) in culture rack. The appearance of radicle was considered as the first day of germination, which may take one or two days of incubation. Seedling length, fresh weight, and dry weight were measured 5 and 15 days after cotyledon appearance.

Phytochemical extraction and estimation

Fresh roots of *L. sativus* were washed in the field and shade dried at room temperature and then ground into coarse powder. The 100 g of dried powder was extracted with methanol in Soxhlet's apparatus. The extraction was carried out at boiling temperature for 24 hours and dried on the water bath. The extract was purified and washed with petroleum ether and ethanol and finally filtered using Whatman's filter paper no. 44. The extract was analyzed by LCMS performed on a Waters UPLC/MS mass spectrometer model ACQ-TQD #QBB1152. ACCUCORE C18, 150 X 2.1, 2.6 µm

column was used for separation.

Statistical analysis

Data were analyzed using Systat V13.2 software (Systat Software Inc.). Values are presented as means of ten replicates, and the presented means were separated using Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$.

RESULTS AND DISCUSSION

L. sativus is a common weed that grows with other legumes such as lentils, and usually it is very difficult to differentiate from crop plant. However, manual picking and removal can be used effectively to increase production and yield. Certain coumarins, phenolic acids, and other allelochemicals increase in plants exposed to mineral deficiencies, water shortages, abnormal radiation, extreme temperatures, and herbicides at the seedling stage, and the allelochemical production of the weed at the first growth stage is an environmental stress (Pedrol *et al.*, 2006; Cheng and Cheng, 2015; Raza *et al.*, 2019).

In the present study, the effects of *L. sativus* root extract on germination and growth of lentil seedlings were investigated. Seed germination of *L. culinaris* was inhibited, and it was found that *L. sativus* extracts caused (percentage) inhibition compared to the distilled water control (Fig. 1).

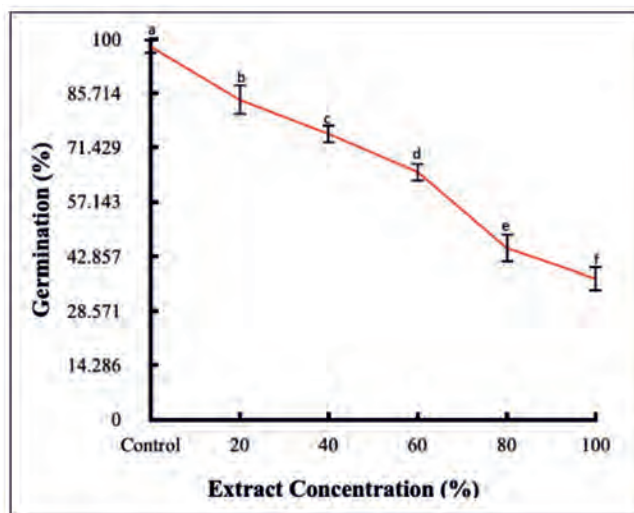


Fig. 1. The graph showing germination percentage of *Lens culinaris* under the extract of *L. sativus* (The values are means $\pm$ SE of five independent replicates. Values denoted by similar letter are not significantly different at $p \leq 0.05$ according to CMRT)

Seed germination is the first step in plant growth where the plant begins to develop. It is the most important process used to evaluate the inhibitory

process in allelopathy (Zhao *et al.*, 2022). The high concentration of the extract has a strong inhibitory effect on seed germination (Radwan *et al.*, 2019), and this effect was also observed in our studies. However, in most cases, low concentration of the extract has a high inhibition rate (Wang *et al.*, 2018; Yan *et al.*, 2018). Five days after germination, shoot and root length were inhibited compared with the control grown with distilled water, and inhibition of root length was greater than that of shoot (Fig. 2 A and B). Fresh weight and dry weight of seedlings also showed a similar pattern of growth inhibition. Dry weight or biomass accumulation was inhibited at all dilutions, but greater inhibition was observed at the 100% extract concentration (Fig. 2 C and D).

The stress exerted by the root extract of weed on *L. culinaris* plant growth from the first to the fifteenth day of germination resulted in a significant reduction in seedling growth (Fig. 3 A, B, C, and D).

Quantitative analysis of the methanolic extract of *L. sativus* was performed by LCMS and detected the presence of various allelochemicals. The mass spectrum analyzed the mass of compounds that appeared in the form of peaks at different m/z ratios (Fig. 4).

These mass spectra were identified from the National Institute of Standards and Technology Chemistry WebBook (NIST) (U.S. Secretary of

Commerce, 2018) (<https://webbook.nist.gov/chemistry/mw-ser/>). Allelopathic chemicals can be considered stressors that affect plant vigor and, in the long term, plant distribution and community structure (Kong *et al.*, 2019). A broad definition of stress in higher plants includes all external influences that reduce dry matter production. Water deficits, mineral deficiencies, temperature extremes, radiation quality and quantity, herbicides, insect feeding, and disease problems are common. LCMS data from the *L. sativus* plant show that a variety of allelochemicals are present (Fig. 5 and Table 1).

These include fumaric acid, succinic acid, aminobenzoic acid, nitrobenzoic acid, chlorvaleric acid, and quinolinic acid. The combined effect of these chemicals may be additive rather than individually (Hartung *et al.*, 1990). All of these compounds inhibit germination and growth of lentil plants and reduce yield (Nehela *et al.*, 2021; Zamir *et al.*, 2023). The presence of allelochemicals such as benzoic acid also has a negative effect on plant growth, and when present in larger amounts, they sometimes completely inhibit seed germination. After germination, root growth is inhibited by these chemicals compared to the control (Fig. 4). It has been previously reported that p-hydroxybenzoic acid inhibits root growth through the accumulation of reactive oxygen species (ROS) in the root tip, which deactivates the meristem and reduces cell length

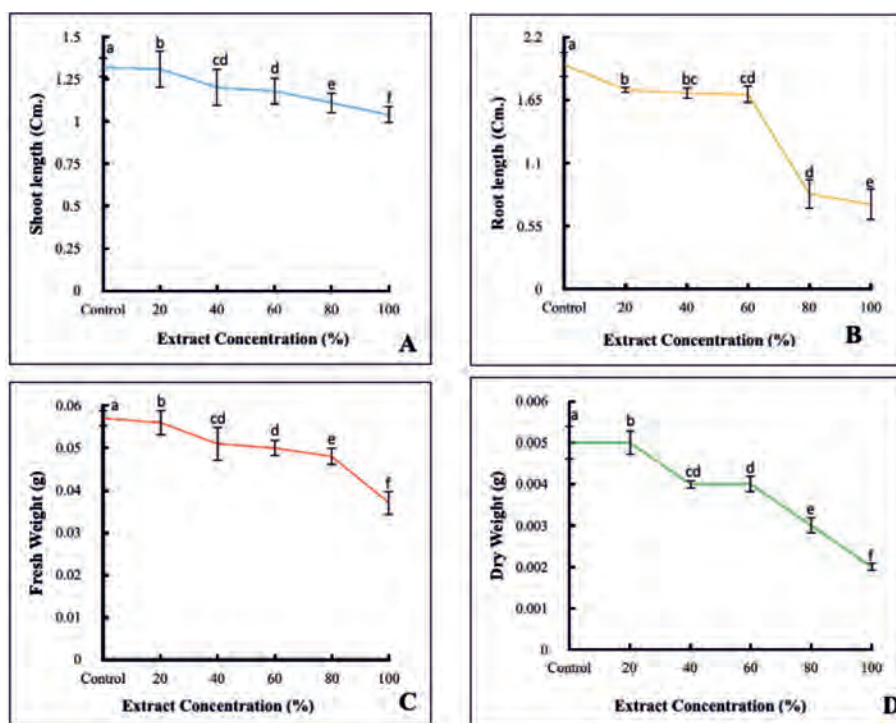


Fig. 2. The graph showing seeding growth of *Lens culinaris* under the extract of *L. sativus* after five days of germination A and B, shoot and root length, C and D, fresh and dry weight (The values are means $\pm$ SE of five independent replicates. Values denoted by similar letter are not significantly different at $p \leq 0.05$ according to DMRT)

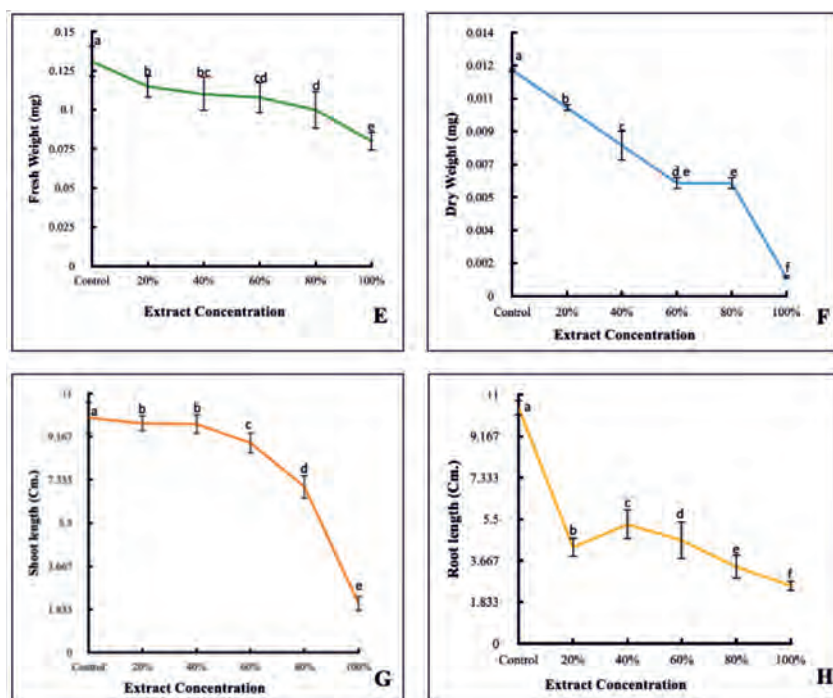


Fig. 3. The graph showing seedling growth of *Lens culinaris* under the extract of *L. sativus* after fifteen days of germination E and F, shoot and root length G and H, fresh and dry weight (The values are means $\pm$ SE of five independent replicates. Values denoted by similar letter are not significantly different at $p \leq 0.05$ according to DMRT)

Table 1. Compounds identified in LCMS of *L. sativus*

S. No.	RT	Name of Compound	Molecular Weight	Formula	Area (%)
1	1.24	N-benzyl-N-Phenethyl-2-fluorophenyl monoamide fumaric Acid	403	C ₂₅ H ₂₂ FNO ₃	0.10
2	1.71	2-(4-nitrophenoxy) ethyl propyl Succinic acid	325	C ₁₅ H ₁₉ NO ₇	0.09
3	2.05	N, O-bis (trifluoroacetyl)-4-Aminobenzoic acid	329	C ₁₁ H ₅ F ₆ NO ₄	1.07
4	2.80	Naphth-2-yl3-chlorophenyl fumaric Acid	352	C ₂₀ H ₁₃ ClO ₄	0.08
5	7.55	Nocodazole	301	C ₁₄ H ₁₁ N ₃ O ₃ S	0.08
6	8.50	Quinolinic acid derivatives	311	C ₁₃ H ₂₁ NO ₄	0.12
7	10.74	3-amino-2,5-dichloro-benzoic acid	206	C ₇ H ₅ Cl ₂ NO ₂	0.11
8	12.71	8-chlorooctyl2-nitrophenyl glutaric acid	399	C ₁₉ H ₂₅ ClNO ₆	0.08
9	13.32	N-(3-methylphenyl)-propylisophthalic acid	297	C ₁₈ H ₁₉ NO ₃	0.07
10	13.79	succinic acid 2-chloroethyl-4-cyanophenyl	281	C ₁₃ H ₁₂ ClNO ₄	0.05
11	15.08	2-Nitrobenzoic acid	239	C ₁₀ H ₁₃ NO ₄	0.11
12	16.98	4-Nitrobenzoic acid	265	C ₁₄ H ₁₉ NO ₄	0.19
13	17.25	4-nitrophenyl 2,3-dichlorophenyl fumaric acid	382	C ₁₅ H ₉ Cl ₂ NO ₆	0.22
14	17.66	N-actyl-N-9H-fluoren-2-yl-actamide	265	C ₁₇ H ₁₅ NO ₂	0.15
15	19.22	2-iodo-1,3,5-trinitro-benzene	339	C ₆ H ₂ I ₃ O ₆	0.10
16	19.70	4-nitrophenyl-5-Chlorovaleric acid	257	C ₁₁ H ₁₂ ClNO ₄	2.10

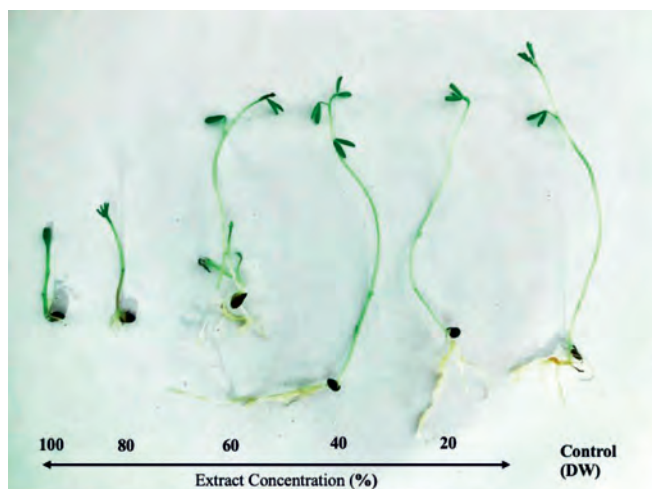


Fig. 4. Seedling growth of *Lens culinaris* under the different concentrations of *L. sativus* root extract after fifteen days of germination. DW= Distilled Water

(Cheng-zhen *et al.*, 2020). When the plant reaches sufficient height and age, this stress becomes more severe and the plant is no longer able to combat the stress caused by allelochemicals, resulting in yield loss.

CONCLUSION

In the present study, a negative allelopathic effect of *L. sativus* on growth of lentil was observed. Growth of lentil was inhibited at the early seedling stage in the presence of *L. sativus* root extract due to various allelochemicals. In fields, removal of this weed by hand is sometimes not possible because the weed is very small and both the weed and crop plant look similar. However, they can be easily removed from the field at the early seedling stage. However, this practice requires an intensive knowledge of the weed and its effects on the crop.

Abbreviations: LCMS-Liquid Chromatography Mass Spectra; UPLC-Ultrahigh Performance Liquid Chromatography; DMRT-Duncan's Multiple Range Test; DW-Distilled Water

Declaration: The authors declare that they do not have any conflict of interest.

Authors' Contribution: Conceptualization and designing of the research work (MA); Execution of field/lab experiments and data collection (AKG, DNS and MS); Analysis of data and interpretation (MA); Preparation of manuscript (MA).

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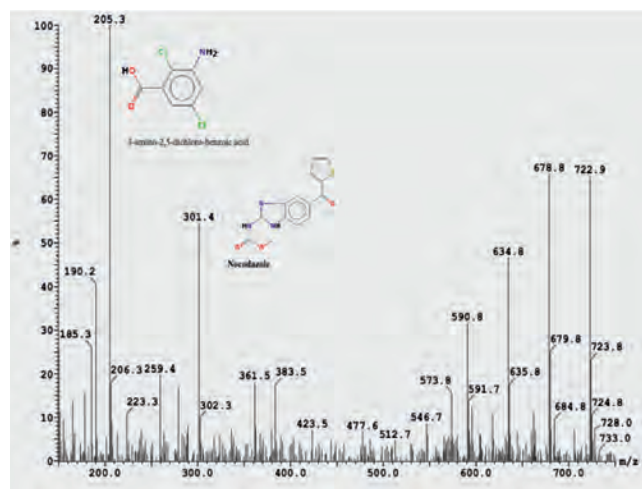


Fig. 5. Liquid chromatography mass spectra of *L. sativus* methanolic root extract

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