Allelopathic effects of Stem Aqueous Extract of Lepidium didymum L. against Lens culinaris and Melilotus alba

MO Shadab

shadabalig89@gmail.com

Allelopathy and Plant Taxonomy Laboratory, Department of Botany, Aligarh Muslim University, Aligarh
https://orcid.org/0000-0002-5747-8676

Uzma Parveen

Aligarh Muslim University Department of Botany

Quratul Ain

Aligarh Muslim University Department of Botany

Nazish Akhtar

Aligarh Muslim University Department of Botany

Mumayyaza Khan

Aligarh Muslim University Department of Botany

M.B. Siddiqui

Aligarh Muslim University Department of Botany

Research Article

Keywords: Allelochemicals, Germination percentage, Scanning Electron Microscope, GC-MS, Proline content

Posted Date: March 22nd, 2024

DOI: https://doi.org/10.21203/rs.3.rs-3954673/v1

Allelopathic effects of Stem Aqueous Extract of *Lepidium didymum* L. against *Lens culinaris* and *Melilotus alba*

Mo Shadab[1]*, Uzma Parveen[1], Quratul Ain[1], Nazish Akhtar[1], Mumayyaza Khan[1], M.B. Siddiqui[1]*

1. Allelopathy and plant taxonomy laboratory, Department of Botany, Faculty of Life Science, Aligarh Muslim University, Aligarh, U.P., India, 202002

Corresponding zaman.amu@gmail.com,

Abstract

Allelopathy involves plants releasing allelochemicals, inhibiting nearby plants' growth and influencing their physiological and morphological characteristics. Donor plants with allelopathic capabilities dominate, impacting the survival of other plant species. The effect of *Lepidium didymum* stem aqueous extracts (SAE) on *Lens culinaris* and *Melilotus alba* was investigated in this study. The experiment involved extracting compounds from the stems of *Lepidium didymum* and applying varying concentrations (0.5, 1, 2, and 4%) of these stem extracts to *test plant* seeds and seedlings. Multiple parameters, including germination percentage, growth parameters, and biochemical activities, were measured and compared against the control group. Germination percentage, seedling growth, seedling length, fresh and dry biomass, chlorophyll, and carotenoid content decreased notably, especially at higher concentrations (4%), while proline content increased in a concentration-dependent manner. In 2021–2022, the experiment was carried out in the allelopathy laboratory department of Botany Aligarh Muslim University, Aligarh. Scanning electron microscopy revealed ultrastructural abnormalities in the roots of the treated plants as compared to the control. Using GCMS analysis, 32 volatile chemicals were found in the methanolic extract of the stem. The compounds found in the major amounts are benzyl nitrile (291.19%), lauric acid (7.86%), benzene acetic acid (6.44%), 11-bromoundecanoic acid (7.36%), palmitic acid (4.85%), dodecanoic acid (4.80%), menthol, trans-1,3, cis-1,4 (3.28%), 11-bromoundecanoic acid (7.36%), and linolenic acid (8.64%). These compounds may be responsible for imparting an allelopathic effect on the test species, *Lens culinaris* and *Melilotus alba*. Our study revealed that the stem of *L. didymum* possesses a high concentration of water-soluble allelochemicals, which are thought to have reduced test plants' growth and can be used as potential weedicides.

Keywords: Allelochemicals, Germination percentage, Scanning Electron Microscope, GC-MS, Proline content

Introduction

Crop plants continuously face competition from weeds, which reduces their output significantly. As a result, weeds have long been recognised as significant plant pests (Zimdahl 2018). Competition between weeds and crops always exists in the natural world; weeds rob crops of their nutrients, water, light, and space and drastically lower their production (Sidhu *et al.* 2018). Allelopathic weeds have been extensively documented in the literature; they significantly reduce crop yields by affecting crop plants from emergence through maturity (Zohaib *et al.* 2016). According to Abouziena and Haggas (2016), the losses brought on by weeds much outweigh those brought on by any type of agronomic pest, including insects, disease, nematodes, rodents, etc. To manage excessive weed growth, eradication becomes crucial. Since the inception of crop cultivation, humans have continually devised strategies to eliminate weeds from their growing areas, employing different methods based on weed type and location. Manual weeding remains a prevalent method to control weeds in crops, but labour scarcity and high costs hinder its widespread adoption. As a result, synthetic chemical herbicides are generally preferred in crop fields. Synthetic herbicide use alters soil composition, contaminates the environment and food, fosters herbicide-resistant super weeds, depletes soil organic matter, and leaves herbicide residues in food, posing severe risks to human health and food safety (Scavo *et al*. 2019, Li *et al*. 2021). Allelopathy is a viable and efficient method for preventing environmental contamination and herbicide resistance in weed control, which can help address these issues. Allelopathy involves plant-produced chemicals affecting nearby plants, either positively or negatively, altering their growth and development. These compounds, called allelochemicals, are released into the environment, impacting neighbouring plant growth (Hierro and Callaway, 2021). Derived from plant primary metabolism, these chemicals, termed secondary metabolites (Bacchedi 2020), hinder plant growth and interact metabolically. They offer bioherbicidal potential, with selective effects varying among plants due to their distinct synthesis. These compounds could be engineered so that their application could lead to the ecologically safe control of weeds. Utilising *Lepidium didymum* L. from Brassicaceae in our study, despite its limited economic value, proves vital. As a troublesome agricultural weed, its ecological role in biodiversity sustenance underscores the importance of studying non-commercial species' allelopathic impact on resident plant communities. In light

of this, the current study was carried out in order to: (a) ascertain the phytotoxic effects of *L. didymum's* stem aqueous extracts (SAE) on the growth and germination of its co-occurring crop, *Lens culinaris* (lentil), and weed *Melilotus alba;* (b) use gas chromatography-mass spectrometry (GC-MS) to identify the allelochemicals present in *L. didymum*.

Materials and Methods

Collection of plant material

Mature *Lepidium didymum* plants were collected in November 2021 from waste and agricultural land in the Aligarh district. After separating the stems and giving them a thorough wash with tap water to get rid of any dirt, dust, or pathogens, the plant material was shade-dried. Additionally, a grinder was used to grind the dried stems into a fine powder. After that, the stem powder was preserved for later use in airtight polythene bags.

Collection of test plant seeds

The IARI in New Delhi provided vigrous, viable, and standardised seeds of the test plant, *L. culinaris*, while the ICAR-Directorate of Weed Research in Jabalpur provided the seeds of *M. alba*.

Preparation of stem aqueous extract

Four grammes of powdered *Lepidium didymum* stem were steeped in one hundred millilitres of double-distilled water. The combination was left overnight at room temperature (22-25 ºC) to allow the chemical components to dissolve in the stem powder. The combination was filtered using muslin cloth and Whatman No. 1 filter paper twice in a 24-hour period, with the filtrate being designated as 4%. The combination was further diluted after filtering to get an aqueous extract with a range of 0.5, 1, 2, and 4%, respectively.

Experimental design

After being cleaned with distilled water, the obtained seeds were surface sterilised for two minutes at 25 °C using 0.1% mercuric chloride. Petri dishes that had been autoclaved were filled with 10% chlorax and ethanol for five minutes. Whatman No. 1 filter paper was used to line a single layer of the Petri dishes. Five millilitres of the test extract. In each petri dish, 4%, 2%, 1%, and 0.5% of each concentration were added. Petri dishes treated with distilled water were used as a control. In each prepared petri dish, ten seeds of each kind were added. Five

copies of the sets featuring. The previously mentioned dosages were kept at room temperature (22–25 ^{0}C) in the lab for 10 days without being disturbed in November 2021. The measurements of germination percentage, fresh and dry biomass, shoot length, and root length were made after 10 days. Plant specimens were dried in an oven at 80 degrees Celsius for 24 hours. The dried biomass was then determined by weighing the samples using an electric balance. In November 2022, the experiment was conducted again to examine the test plant's morphological and biochemical characteristics and the phytochemical analysis of *L. didymum* stem.

Determination of chlorophyll and carotenoid

The leaf sample was homogenised in 10 millilitres of 80% acetone solution and centrifuged at 12,000 rpm for 20 minutes. A spectrophotometer was used to evaluate the total chlorophyll content using a spectrophotometer at 663 nm and 645 nm (Arnon 1949). The absorbance of carotenoids was determined to be 480 nm (Duxbury and Yentsch, 1956).

Total mg chlorophyll Kg^{-1} leaf sample = 20.2(A_{645}) + 18.02 (A_{663}) × $\frac{V}{1000 \times W}$ mg

Carotenoid $Kg^{-}1$ leaf sample = $\frac{7.6\ (A480) - 1.49\ (A510)}{d \times 1000 \times W} \times V$

Estimation of Proline content

The procedure outlined by Bates *et al.* (1973) was used to determine the proline content of the test plant leaves. A solution comprising 0.2 g of fresh leaves and 3% aqueous sulfosalicylic acid was mixed together. Blend 2 millilitres of ninhydrin reagent and 2 millilitres of extract in glacial acetic acid in a hot water bath set to 100 degrees Celsius. After 30 minutes of chilling, add 4 millilitres of toluene. The absorbance of toluene with a chemical group at 520 nm was measured with a spectrophotometer. Using l-proline, a standard curve was constructed to calculate the amount of proline.

Scanning electron microscopy

Utilising SEM, root tips and epidermal cells were investigated. 7-day-old plants taken and their fresh roots were fixed for two hours in a solution of 2% glutaraldehyde buffer, 0.1 M sodium cacodylate buffer, and paraformaldehyde. After that, the sample material was properly dehydrated in a succession of ethanol grades (that is, 50%, 60%, 70%, 80%, and 90%), as well

as absolute ethanol. After applying Gold-Palladium coating to the root samples for ten minutes, they were examined with a JEOL JSM-JSM 6510 electron scanning microscope (Japan).

Preparation of plant material for GCMS analysis

The methanolic extract was made using the procedures outlined by Arora and Pandey (2014), with modifications. Ten grammes of dried stems were weighed and finely ground in an electrical grinder. To prepare the extract, the powder was submerged in 100 millilitres of HPLC-grade methanol for the whole night. Whatman No. 1 filter paper was used to filter the supernatant after the filtrate was centrifuged for 30 minutes at 24 °C at 4000 g. To create a concentrated methanolic extract, the resulting filtrate was let to evaporate at 40°C for 48 hours. To prevent photoreaction, the extract was stored at 4°C in a glass bottle covered in black cloth until it was needed. With a built-in programmed headspace auto sampler and auto-injector, the GCMS-QP-2010 Plus with Thermal Desorption System TD, Shimadzu, Japan, was used to chemically assess the methanolic extract of the *Lepidium* stem. Helium was employed as a carrier gas at a flow rate of 3 mL/min at the volume of 1 mL injection, and DB-1/RTX-MS (30 m) was utilised as the capillary column. Following the sample's injection into the column, which was initially fixed at 100°C for two minutes, the temperature of the column was increased to 170°C with a heating ramp of 10°C per minute, and then it was raised again to 215°C with a heating ramp of 5°C per minute for eight minutes. After that, a heating ramp of 10°C per minute was used for 15 minutes to reach the ultimate temperature of 240°C. At 250°C, the injections were performed in split mode (30:1). The injector and detector maintained their respective temperatures of 250°C and 260°C. The sample was subjected to an operating pressure of 76.2 kPa for a duration of 70 minutes. For a flame ionisation detector (FID), a nominal starting flow of 3.1 mL/min and a temperature of 230°C were established. The scan range (m/z) of 40,650 atomic mass units (AMU) under electron impact (EI) ionisation (70 eV) was chosen as the MS parameters. The chemical composition of the samples was evaluated by comparing their mass weights and retention times to GC-acquired reference samples, as well as by utilising mass spectra from the Wiley libraries and the National Institute of Standards and Technology (NIST) database.

Statistical analysis

Statistical analysis was performed using the mean values of the replicates. Using SPSS version 20.0 statistical software, a one-way ANOVA was used to analyse the data (SPSS, SAS Institute,

USA). The mean values at 0.05% (P≤0.01) were compared using Duncan's multiple range test (DMRT) after analysis of variance. Graphs were designed using Origin Pro (2020) software.

Results

Germination and seedling growth

The test plants' fresh biomass, dry biomass, root length, shoot length, and germination percentage were all considerably decreased by the *L. didymum* stem aqueous extract (SAE). A concentration-dependent decline in the percentage of seed germination was noted in the test species (*Melilotus alba* and *Lens culinaris*) treated with extracts compared to control (control<0.5%<1%<2%<4%). At 2% and 4% concentrations, there was a noticeable decline in the percentage of germination. It was lowered by 63.33% and 46.66% at 4% and 2% concentrations for *L. culinaris* and by 71.42% and 50% for *M. alba*, respectively (Fig. 1a). The results of *L. culinaris* and *M. alba* seedling length suggest that allelopathic chemicals have a deleterious impact on seedling development. At the highest concentration of 4%, the shoot and root length were dramatically decreased. When compared to the control, the largest reduction in seedling length was found to be 4%, and the least was found to be 0.5%. For *L. culinaris* and *M. alba*, the response to SAE at 4% was 64.16% and 68.79% for shoot length, and 88.41% and 97.25% for root length (Fig. 1bc). Fresh biomass and dry biomass of both species were considerably impacted by SAE; at 4% extract concentration, the percentage loss of fresh biomass in *L. culinaris* and *M. alba* was observed to be 68.39% and 82.39%, respectively, while the percentage reduction of dry biomass was 78.72% and 81.25% (Fig. 1de).

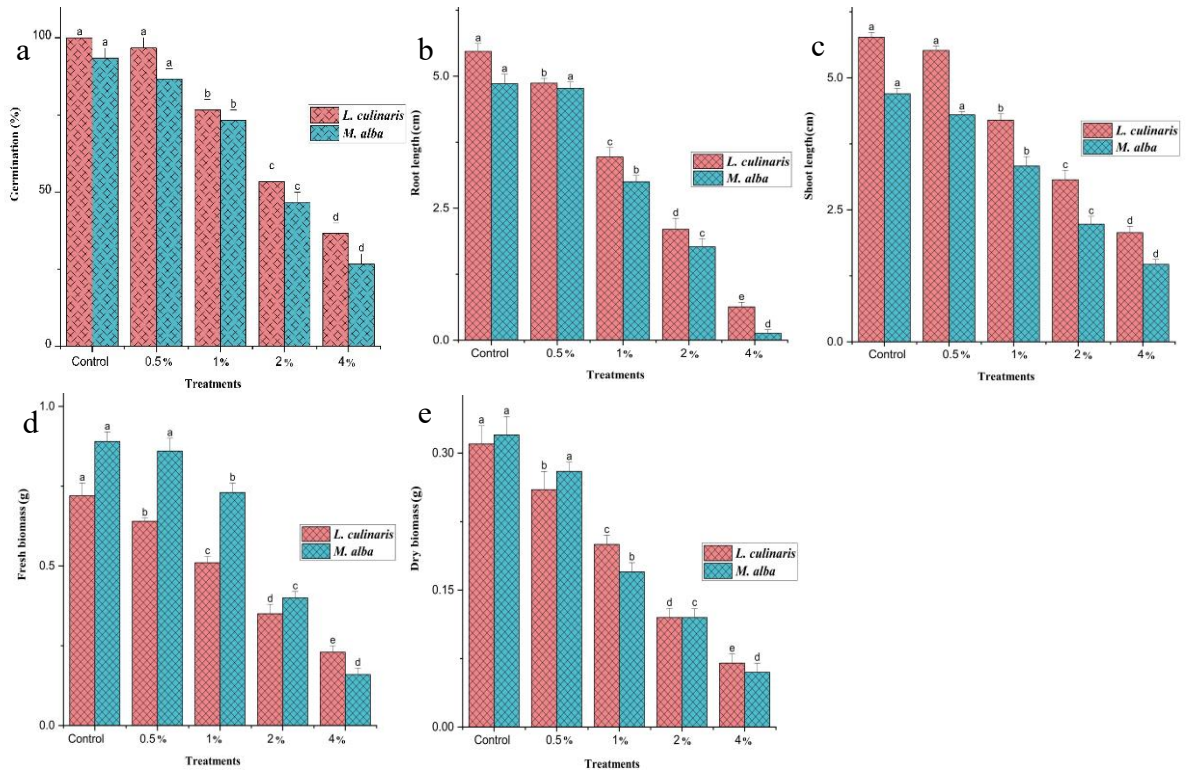

Fig 1 Allelopathic effect of *Lepidium didymum* stem aqueous extract on (a) germination percentage, (b) root length, (c) shoot length, (d) fresh biomass, and (e) dry biomass of *Lens culinaris* and *Melilotus alba*. Using DMRT, different superscript symbols along the bars indicate significant differences between each other at $p < 0.05$.

Estimation of photosynthetic pigments and proline content

The total chlorophyll content and carotenoid content decreased significantly in test plants treated with SAE of *L. didymum* (Fig. 2ab). When compared to the control, the highest reduction in total chlorophyll level was 46.91% and 45.45% in *L. culinaris* and *M. alba* at 4% extract concentration. Whereas a higher concentration 4%, reduced the carotenoid content by 41.83% in *L. culinaris* and 48% in *M. alba* as compared to the control. The free proline content showed an increase in the test plant upon application of SAE. A concentration-dependent increase was observed in the test plants. Maximum proline content was reported in *L. culinaris* (54.20%) and *M. alba* (51.21%) at 4% concentration (Fig. 2c).

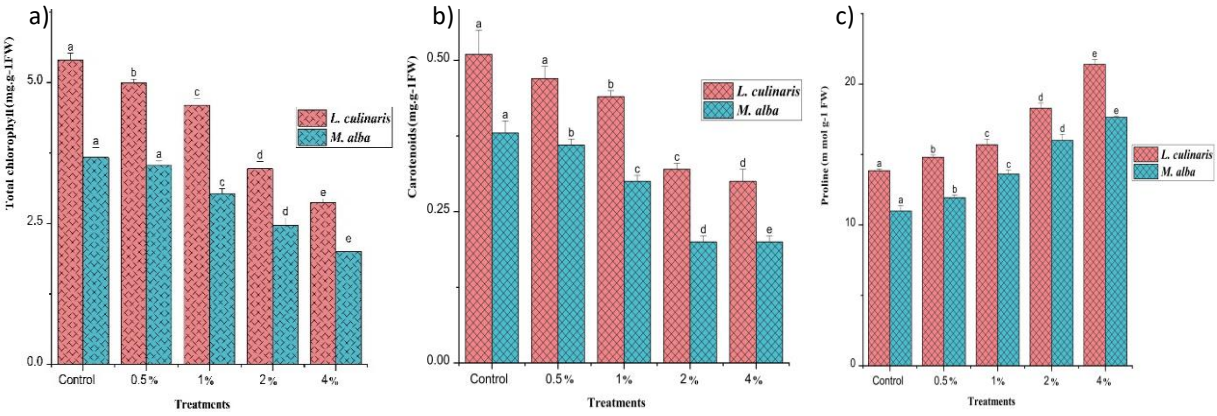

Fig 2 Allelopathic effect of *Lepidium didymum* stem aqueous extract on the content of (a) total chlorophyll, (b) carotenoids, and (c) proline in *Lens culinaris* and *Melilotus alba*. Using DMRT, different superscript symbols along the bars indicate significant differences between each other at $p < 0.05$.

Effect of SAE on root ultarmorphology

The stem extract treatment caused various changes in the apical portion and surface of the roots of both the test plants, as depicted by SEM images (Fig 3). In control, the root tip zone was largely intact. Surface epidermal cells in control had largely uniform size and shape, an organised cell layer, and a smooth surface. The test plants' roots had damaged root tips and root

surface cells, on the other hand, showed signs of disorganisation at 4% concentration, including uneven cell surfaces, flaccid cells, lesions, and cracks.

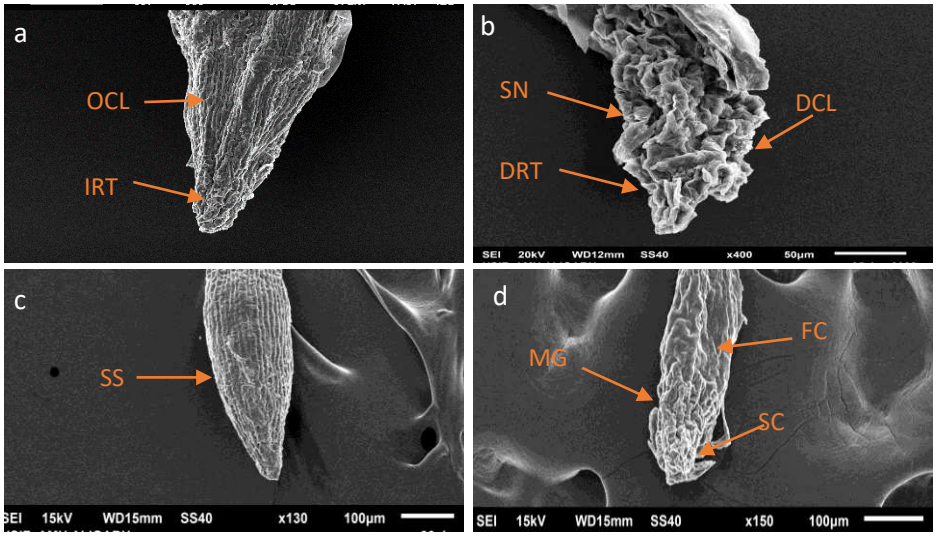

Fig. 3 SEM images showing the effects of *L. didymum* SAE treatment on the root apices of 7 days old seedlings of *L. culinaris* (a & b) and *M. alba* (c & d); Control (a-c), 4% treatment (b-d). IRT: *Intact root tip;* OCL: *Organised cell layers;* DRT: *Damaged root tip;* DCL: *Disorganised cell layers;* SN: *Severe Necrosis;* SS: *Smooth surface;* FC: *Flaccid cells;* SC: *Scraped off cell;* and MG; *Minor Grooves*

GCMS of a methanolic extract of stem of *Lepidium didymum*

Fig. 4 and Table 1 present the chromatogram of the GC-MS spectrum analysis, which displays the peaks of the different compounds from the GC fractions of the methanol extract of *L. didymum* stem. This investigation identified 32 different chemical compounds. The most prevalent chemicals in the methanolic extract were Benzyl nitrile (29.19%), Lauric acid (7.86%), Benzene acetic acid (6.44%), 11-Bromoundecanoic acid (7.36%), Palmitic acid (4.85%), Dodecanoic acid (4.80%), Stigmasta-3,5-diene (4.56%), Menthol, trans-1, 3, cis-1,4 (3.28%), 11-Bromoundecanoic acid (7.36%), and Linolenic acid (8.64%).

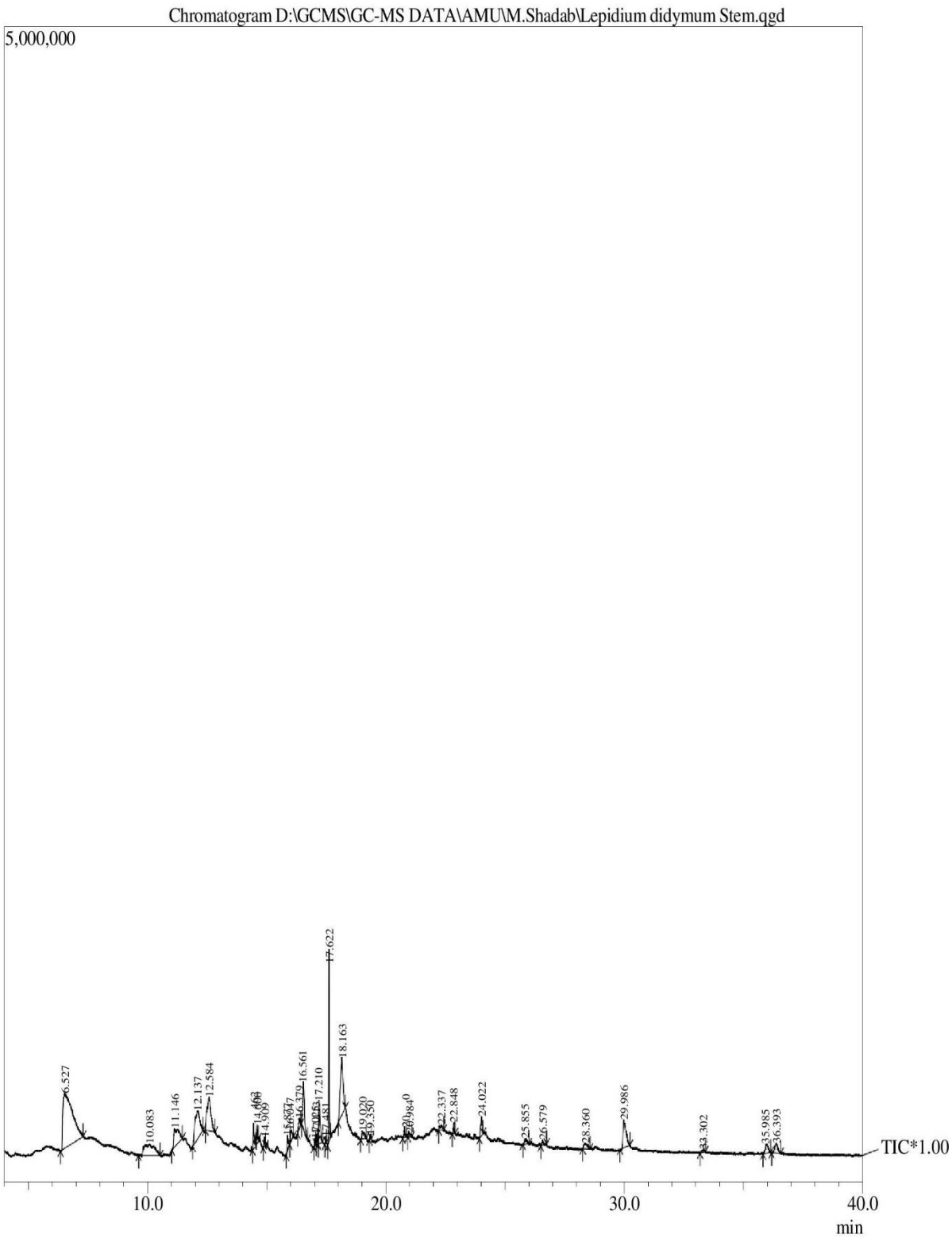

Fig. 4: Chromatogram of stem methanolic extract of *Lepidium didymum*

Discussion

In the present study, the growth of *L. culinaris* and *M. alba* in Petri dishes containing the SAE of *L. didymum* was expressively reduced after 10 days of treatment (Fig. 1). Low germination rates and delayed germination were the results of the stem-aqueous extract treatment. By delaying germination, decreasing the rate of germination, slowing the growth of seedlings, and decreasing the root-to-shoot ratio, the treatments decreased fresh and dry weight. Higher concentrations led to more pronounced inhibitory effects. This study shows that the test species were subjected to a range of allelopathic inhibitory effects depending on the concentration of aqueous extract used. Higher concentrations of 2% and 4% had more potent inhibitory effects. Since phenolics primarily operate through root membranes, the effect is seen on the length of the root. Phenolic acids interact with root cells to generate depolarization, ion efflux, and a reduction in hydraulic conductivity, net nutrient uptake, and water uptake. These processes may affect the shoot length of species as well (Blum, 2005). All of the phenolic acids had inhibitory effects on the growth and germination of seedlings in certain weeds, with varying levels of inhibition seen for different compounds (Tuyen *et al.* 2018).

As seen in several researches, allelochemicals from *N. plumbaginifolia*, extracted with water, reduced the germination and growth of recipient plant (Mushtaq *et al.* 2019). The aqueous extract of *Sonchus oleraceus* has inhibitory effects on emergence and seedling growth of red rice (*Oryza punctata*) (Nadeem *et al.* 2021). These findings supported our study. Both the carotenoid and chlorophyll contents considerably dropped as the concentration of aqueous extracts increased. Because proline is a stress marker, its content rose as the extract's concentration rose. When comparing the test plant to the control, the greatest concentration of proline was found at 4%. Additionally, test plants exposed to allelopathic extract had proline accumulation, according to some studies (Al-Taisan 2014, El-Khatib *et al.* 2016). Either the suppression of chlorophyll formation or the activation of molecules that destroy chlorophyll could be the cause of the significant drop in chlorophyll content seen at all concentrations (Mehal *et al.* 2023). Allelochemicals can hinder oxygen development and photosynthesis through their interactions with components of photosystem II (Leu *et al.* 2002).

Comparing the SAE treatment to the control, the scanning electron microscope (SEM) examination reveals a respectable number of alterations in root ultramorphology. The root tip and epidermal cells of the root were examined under SEM research, which demonstrated damage and disruption of the epidermal cell alignment in the form of irregular root surfaces, lesions, cracks and grooves, necrotic patches, and scraped-off cells (Fig. 4b). However,

as observed in the control treatment, the roots of both test plants have a smooth, nearly normal surface. Related research showing morphological changes in Arabidopsis thaliana along the epidermis upon administration of camphor and menthol (Schulz et al. 2007) confirms our observations. Previously, treatment with allelochemicals has been reported to induce various structural alternations in plant tissues (Gulzar *et al.* 2016, Mushtaq *et al.* 2019).

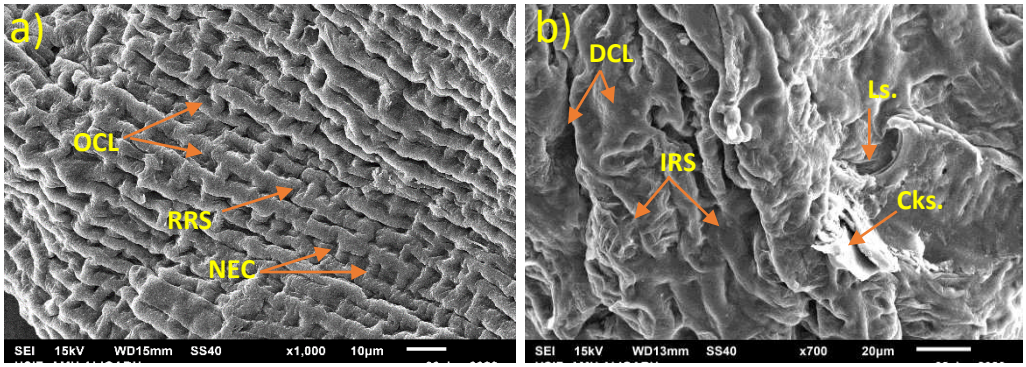

Fig 4 Ultrastructural changes induced by 4% concentration of SAE on surface of *M. alba* root. a Control and b 4%, revealed by scanning electron microscopy. OCL: *organised cell layer*; RRS: *regular root surface*; NEC: *normal epidermal cell*; DCL: *disorganised cell layer*; IRS: *irregular root surface*; Ls: *lesion*s; Cks: *cracks*

From the methanolic extract of the stem of *Lepidium didymum*, 32 volatile phenolic allelochemicals were found (Table 1). The presence of phytochemicals in the stem extract of *L. didymum* indicates that they play a significant role in giving the aforementioned its phytotoxic or allopathic nature. Nevertheless, phenolic acids are the main components of allelopathic plants, even though they are not required for the fundamental activities taking place in plants and are released as secondary metabolites. Allelochemicals with inhibitory effects act as key elements in plant defence mechanisms against weed invasions. There are several organic pesticides that can potentially reduce weed growth in areas that have been manufactured (Farooq *et al.* 2020). According to Tuyen *et al.* (2018), the phenolic acids inhibited the development and germination of seedlings in several weeds, while the degrees of inhibition varied. The research revealed that phenolic allelochemicals can inhibit seed germination and plant root development, hinder biochemical parameters, induce alterations in root ultrastructure, and subsequently disrupt the typical growth and development of the entire plant. Hance allelochemicals as a source of bioherbicides can be highly important for controlling weeds because they present new working principles that show distinct interactions with plants.

Table 1. Volatile components identified from the *Lepidium didymum* stem methanolic extract

Peak	R.Time	Area	Area%	Name
1	6.527	6492129	29.19	Benzyl nitrile
2	10.083	1432818	6.44	Benzene acetic acid
3	11.146	1068556	4.80	Dodecanoic acid
4	12.137	1748710	7.86	Lauric Acid
5	12.584	1636232	7.36	11-Bromoundecanoic acid
6	14.462	355813	1.60	Neophytadiene
7	14.600	136108	0.61	2-Pentadecanone, 6,10,14-trimethyl
8	14.909	113199	0.51	Nonenylsuccinic anhydride
9	15.877	225968	1.02	Dibutyl phthalate
10	16.047	247614	1.11	Diethyl phthalate
11	16.379	201946	0.91	Ethyl (9z,12z)-9,12-octadecadienoate
12	16.561	1077980	4.85	Palmitic Acid, TMS derivative
13	17.025	73608	0.33	Oxalic acid, 6-ethyloct-3-yl heptyl ester
14	17.113	51612	0.23	9,12,15-octadecatrien-1-ol
15	17.210	728609	3.28	Menthol, trans-1, 3, cis-1,4
16	17.481	40425	0.18	Methyl 2-hydroxystearate
17	17.622	1418180	6.38	Phytol
18	18.163	1922661	8.64	Linolenic acid
19	19.020	207297	0.93	1-[(Benzylamino) carbothioyl] proline
20	19.350	39640	0.18	2,6R-Diethyl-3,5S-dimethyl-3,4-dihydro-2H-pyran
21	20.800	132431	0.60	1,2-Benzenedicarboxylic acid
22	20.984	71729	0.32	Palmitic acid, 2-(trimethylsiloxy) ethyl est
23	22.337	152901	0.69	5,8,11-Eicosatrienoic acid, (Z)-, TMS derivative
24	22.848	97294	0.44	Squalene
25	24.022	331953	1.49	2,6,10,14-Hexadecatetraen-1-ol, 3,7,11,15-tetramethyl
26	25.855	146646	0.66	Benzenamine, N, N,3,5-tetramethyl
27	26.579	86613	0.39	Tocopherol, methyl ether
28	28.360	221691	1.00	Tetra decanynoic acid

29	29.986	1013314	4.56	Stigmasta-3,5-diene
30	33.302	72630	0.33	Acetamide, N-methyl-N-[4-[2-acetoxymethyl-1-pyrrolidyl]
31	35.985	332919	1.50	Pentalene, octahydro-2-(1-methylheptadecanate)
32	36.393	364520	1.64	Phytyl decanoate
		22243746	100.00	

Conclusion

Our research indicates that the high content of water-soluble allelochemicals in *Lepidium didymum* stem may have inhibited the growth of the test plants. The *Lepidium didymum* stem's possession of allelochemicals indicates that the plant is allelopathic, and with more study, the chemicals may be used as organic weedicides in the future. This provides compelling proof of its ability to inhibit the growth of weed seeds. The potential for organic farming could be increased by using that knowledge to develop ecologically friendly herbicides for weeds and other undesired plant growth.

Acknowledgement

The authors would like to thank the Council of Scientific and Industrial Research (CSIR), New Delhi, India, for providing financial support for the work. The authors further acknowledge the guidance and support provided by Prof. M. Badruzzaman Siddiqui, Chairman of the Botany Department at Aligarh Muslim University, Aligarh.

Authors Contributions

Mo Shadab, Uzma Parveen, and Quratul Ain carried out the experiments and wrote the manuscript. Nazish Akhtar performed statistical analysis. Mumayyaz Khan reviewed and edited. M.B. Siddiqui designed and supervised the work. All the authors read and approved the manuscript.

References

Abouziena HF and Haggag WM. 2016. Weed control in clean agriculture: areview1. *Planta* **34**(2): 377–392.

Al-Taisan AW. 2014. Allelopathic effects of *Heliotropium bacciferum* leaf and roots on *Oryza sativa* and *Teucrium polium*. *Life Science Journal* **11**(8).

Arnon and D I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant physiology* **24**(1): p.1.

Arora N and Pandey-Rai S. 2014. GC–MS analysis of the essential oil of *Celastrus paniculatus* Willd. Seeds and antioxidant, antiinflammatory study of its various solvent extracts. *Indian Crop Production* **61**: 345–351.

Bachheti A, Sharma A, Bachheti, R K, Husen A and Pandey, D P. 2020. Plant allelochemicals and their various applications. *Co-evolution of secondary metabolites*, 441-465. J.-M. Mérillon, K. G. Ramawat (Eds). Springer Nature, Switzerland.

Bates L S, Waldren R A and Teare I D. 1973. Rapid determination of free proline for water-stress studies. *Plant and soil* **39**: 205-207.

Blum U and Gerig TM. 2005. Relationships between phenolic acid concentrations, transpiration, water utilization, leaf area expansion, and uptake of phenolic acids: nutrient culture studies. *Journal of Chemical Ecology* **31**: 1907-1932.

Blum U. 2011. *Plant-plant allelopathic interactions: phenolic acids, cover crops and weed emergence* pp.1-190 Springer, London.

Duxbury A C and Yentsch C S. 1956. Plankton pigment nomographs. *Journal of Marine Research* **15**:(1).

El-Khatib A A, Barakat N A and Nazeir H. 2016. Growth and physiological response of some cultivated species under allelopathic stress of *Calotropis procera* (Aiton) WT. *Applied Science Reports* **14**(3): 237-246.

Gulzar A, Siddiqui, M B and Bi S. (2016). Phenolic acid allelochemicals induced morphological, ultrastructural, and cytological modification on *Cassia sophera* L. and *Allium cepa* L. *Protoplasma* **253**: 1211-1221.

Farooq N, Abbas T, Tanveer A and Jabran K. 2020. Allelopathy for weed management. *Co-evolution of secondary metabolites* pp.505-519 Springer, Switzerland.

Hierro J L and Callaway R M. 2021. The ecological importance of allelopathy. *Annual Review of Ecology, Evolution, and Systematics* **52**: 25-45.

Khanh T D. 2018. Weed suppressing potential and isolation of potent plant growth inhibitors from *Castanea crenata* Sieb. et Zucc. *Molecules* **23**(2): 345.

Leu E, Krieger-Liszkay A, Goussias C and Gross E M. 2002. Polyphenolic allelochemicals from the aquatic angiosperm *Myriophyllum spicatum* inhibit photosystem II. *Plant physiology* **130**(4): 2011-2018.

Mehal K K, Kaur A, Singh H P and Batish D R. 2023. Investigating the phytotoxic potential of *Verbesina encelioides*: Effect on growth and performance of co-occurring weed species. *Protoplasma* **260**(1): 77-87.

Mushtaq W, Ain Q, Siddiqui M B and Hakeem K R.2019. Cytotoxic allelochemicals induce ultrastructural modifications in *Cassia tora* L. and mitotic changes in *Allium cepa* L.: A weed versus weed allelopathy approach. *Protoplasma* **256**: 857-871.

Nadeem M A, Khan B A, Anwar S, Maqbool R, Amin M, Aziz A and Nijabat A.2021. Allelopathic potential of aqueous extracts of sow thistle weed on emergence and seedling growth of red rice. *Pakistan Journal of Weed Science Research* **27**(2): 201.

Nadeem M A, Khan B A, Yaqoob N, Abdullah A I, Irfan M, Ahmad W, and Shakil S. 2023. Allelopathic effect of aqueous extract of wild radish on pea seedling emergence and growth. *Pakistan Journal of Weed Science Research* **29**(1): 46-51.

Scavo A and Mauromicale G. 2021. Crop allelopathy for sustainable weed management in agroecosystems: Knowing the present with a view to the future. *Agronomy* **11**(11): p.2104.

Siddhu G M, Patil B T, Bachkar C B and Handal S B. 2018. Weed management in garlic (*Allium sativum* L.). *Journal of Pharmacognosy and Phytochemistry* **7**(1): 1440 1444.

Tuyen P T, Xuan T D, Tu Anh T T, Mai Van T, Ahmad A, Elzaawely A A, and Khanh T D. 2018. Weed suppressing potential and isolation of potent plant growth inhibitors from *Castanea crenata* Sieb. et Zucc. *Molecules*, **23**(2): 345.

Zimdahl R L, 2018. *Fundamentals of weed science*. Academic press, p 664.

Zohaib A, ABBAS T and TABASSUM T. 2016. Weeds cause losses in field crops through allelopathy. *Notulae Scientia Biologicae* **8**(1): pp.47-56.