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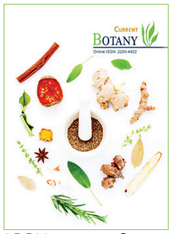
Research Article



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Allelopathic potential of *Lathyrus aphaca* L. on seedling growth of *Triticum aestivum* L.

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ABSTRACT

The allelopathic substances released into the environment from plants inhibit various physiological processes. These allelopathic secondary plant metabolites attract or repel and stimulate or poison other organisms. In the present investigation, the allelopathic potential of a common weed, *Lathyrus aphaca*, was studied on the seedling growth of wheat (*Triticum aestivum* L. cv. Dara). The seedling growth was monitored under the different dilutions of *L. aphaca* extract with water. Control seedlings were grown in distilled water only, i.e., without extract. It was found that the growth and biomass accumulation were significantly suppressed. The aqueous extract of *L. aphaca* was analyzed through LCMS and revealed the presence of several allelochemicals, *viz*. 2,4,6-tetrahydroxychalcone, 3-iodo-benzoic acid, p-coumaric acid, kynurenic acid, fumaric acid, sebacic acid, caffeic acid, and ferulic acid, proving the allelopathic potential of the weed.

KEYWORDS: Allelochemicals, Weeds, Growth, Aqueous extracts, Metabolites

INTRODUCTION

Plant allelochemicals are the organic compounds produced as plant secondary metabolites and play an important role in plant-plant, plant-microorganism, and plant-insect interaction, beneficially and detrimentally. These may inhibit the developmental process in the early stages of other plants growing nearby and act as an important ecological factor that affects the plant dominance succession, climax vegetation, and crop productivity. It is the natural process that plants release a wide variety of primary and secondary metabolites into the environment, mostly from the roots, and this is the way that a plant interacts with other plants both positively and negatively (Kong *et al.*, 2019). These are the signaling chemicals of plants through which it senses and communicate with other organisms in the environment. The defensive mechanism governed by the plant's secondary metabolites or allelochemicals is exerting a negative effect on the growth of other plants. The allelochemicals can suppress cell differentiation, growth, and development of plants and affect many physiological processes, including photosynthesis and respiration, by inhibiting enzyme activity. They may be responsible for the change in micro- and ultra-structure of cells, inhibition of cell division and cell elongation, change in permeability of the cell membrane, and imbalance in the antioxidant system and plant growth regulator system. Allelopathic compounds involve short-chain fatty acids, phenolics, flavonoids, terpenoids, amino acids, peptides, alkaloids, cyanohydrins, etc. (Cheng & Cheng, 2015). Many weed plants produce allelochemicals to compete with the crop plants. Lathyrus aphaca is a leguminous weed that grows dominantly with wheat crop and competes with it. It is a problematic weed of the world. It has a trailing or scrambling annual broad leaf and medium height. The seeds are germinated, and the plant completes its life cycle from October-November to April, and after their death, it spreads their seed in the soil for the next cycle. L. aphaca can withstand under various abiotic stresses and can grow even in saline soil at 0-150 mm plant level of salinity (Aamir *et al.*, 2019). The wheat crop grows in the same season as the weed. L. aphaca showed the symptoms of stress in the early stages that are responsible for the reduction of yield and the quality of the produce (Figures 1a & b). The productivity of wheat in Balrampur district of Uttar Pradesh is 2209 kg/ha and about 291 tons. The soil is loamy in the district, and the major crops are rice, wheat, maize, pigeon pea, and sugarcane (ICAR, 2013). The wheat crop production has fallen short due to the low rainfall and as well as biotic stresses (Ujala, 2024).

In the present investigation wheat is grown under *L. aphaca* whole plant extract in the laboratory condition and seedling growth was assessed. The whole plant aqueous and methanolic

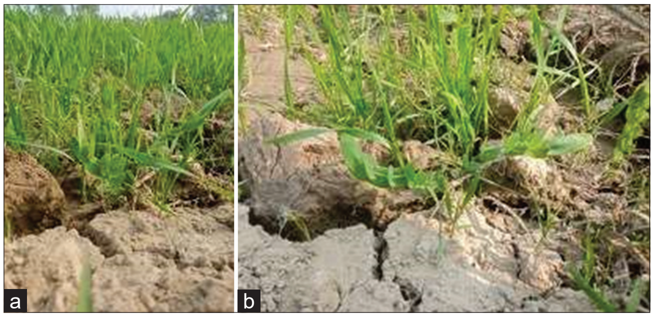

Figure 1: a and b Growth of weeds *L. aphaca* in the fields with the *T. aestivum* crop in Balrampur (U.P.)

extract was analyzed through LCMS/MS to identify the allelochemicals present in the extract. The climate is sub-humid.

MATERIALS AND METHODS

Plant Material

Seeds of *Triticum aestivum* L. var Dara were collected from the local market of Balrampur and the plants of *Lathyrus aphaca* L. were taken from the field (27.43157 N, 82.19218 E) nearby area of Balrampur. The work was conducted in the month of November and December 2022 and repeated in 2023. The geographical area of district is about 3349 sq. km. The soil is a mixture of clay, sand and loam and climate is sub-tropical.

Experiment Design

Seedling plants of wheat were grown in the Petrie plates in the extracts of *L. aphaca*. For the extract preparation 1 g fresh leaves of *L. aphaca* were crushed and grind finely in 100 mL distilled water with the help of mortar pastel to make a fine paste. Filtered the extract with Whatman filter paper No. 1 and considered this extract as 100%. Using this extract makes the dilutions 5, 10, 25, 50 and 100% with water (V/V). The soaked seeds were placed at equal distance in pre sterilized Petrie-plates. The seeds were irrigated with distilled water as control and in the rest of the Petrie-plates different dilution of extracts was given respectively and accordingly. Growth was observed at regular intervals. The shoot-root length was measured with the scales and the fresh weight and the dry weight was taken on the digital weighing balance.

Extraction Procedure

The leaves of *L. aphaca* were procured from the local area, washed and shade dried at room temperature and then ground to make a fine powder. The whole plant (70 g) was extracted with water in Soxhlet for three days. The extract was filtered through Whatman no. 44 filter paper and concentrated in a rotatory evaporator at 45 °C. The dried methanolic extract was used in dilutions for further assessing the inhibition of plant growth respectively. The aqueous extracts of *L. aphaca* were subjected to LCMS analysis.

Biochemical Analysis

Total proline was estimated by the standard protocol (Chinard, 1952). About 0.5 g of plant material was homogenized in 10 mL of 3% aqueous sulphosalicylic acid and the extract was filtered through Whatman No. 2 filter paper. About 2 mL of filtrate was taken in a test tube. After that 2 mL of glacial acetic acid and 2 mL acid ninhydrin was added. The mixture was heated in the boiling water bath for 1h. The reaction was terminated by placing the tube in an ice bath. In the reaction mixture 4mL of toluene was added and the mixture stirred well for 20-30 sec. The toluene layer was separated and warmed to room temperature. The red colour intensity was measured at 520 nm. For the preparation of the standard curve a series of standard with pure proline was run in a similar way. The amount of proline in the test sample was determined from the standard curve.

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

The effect of *L. aphaca* plant extract was observed in the present investigation on seed germination and seedling growth of wheat. *L. aphaca* is the common weed of the field where the wheat crop is grown. The results showed that there is a negative interaction exists between the two crops. The growth of wheat was suppressed under the extract of weed.

The fresh and dry weight of the plant was highly suppressed, immediately after the five days after germination. Seedlings under stress accumulate less biomass as compared to the control because of the inhibition of the root due to the presence of extracts (Figures 2a & b).

The shoot length was highly suppressed in the wheat plant under all extract dilutions. The shoot length started to decrease in the 5% extract, significantly and it increased as the dilution was decrease *i.e.*, 10, 25, 50, and 100%. The 100% extract showed maximum suppression of the shoot length as compared to the distilled water grown control. Hence, the extract showed a detrimental effect on early shoot growth of wheat (Figures 2c & 3).

The length of root also showed same pattern as shoot growth and its growth also suppressed in the extract, however, the root inhibition is more severe as compared to the suppression of shoot because it is under the direct influence of the extract. Maximum inhibition was observed in the 100% extract (Figures 2d & 3). The branching of the rootlet appeared normal as compared to the distilled water grown control. It was found that free proline contents were increased in the seedlings grow at higher concentrations of extracts (Figure 2e). This indicates that the level of stress increased due to the presence of the allelochemicals in the extracts (Figure 3).

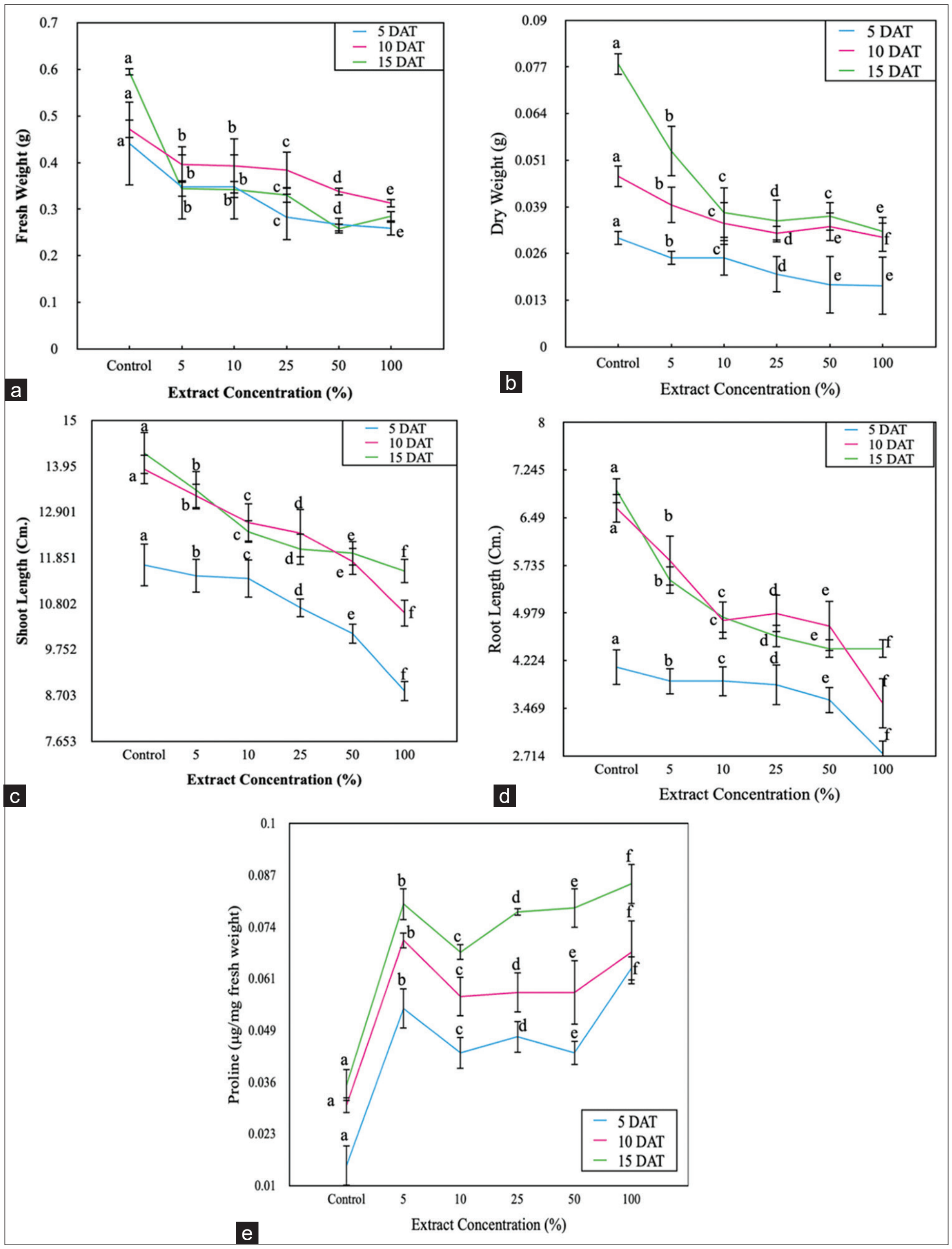

Figure 2: Growth analysis of *T. aestivum* seedlings under different dilutions of *L. aphaca* plant extracts a, b) fresh and dry weight of seedling plant, c, d) shoot and root growth and e) Total free proline. Data represents mean±SE, Values not followed by same letter are significantly different at $p \leq 0.05$. ANOVA with Duncan Multiple Range Test (DMRT)

In the LCMS analysis of the aqueous extract of *L. aphaca* whole plant, 8 different chemical substances were identified. Some of them are phenolic compounds that may be responsible for the allelopathic effect upon the seedling growth of *T. aestivum*. The allelochemicals that were identified include 2,4,6-tetrahydroxy chalcone, 3-iodo-benzoic acid, *p*-coumaric acid, kynurenic acid, fumaric acid, sebacic acid, caffeic acid, ferulic acid, etc. (Figure 4 & Table 1).

DISCUSSION

In plant-plant interaction, allelopathy is an important phenomenon which includes both allelopathy or allelobiosis and competition. In allelopathy, the interaction is mostly negative and includes inhibition of growth and yield of neighbouring plants. However, allelobiosis involved in signaling for the recognition of neighboring plants (Kong *et al.*, 2024). Plant secrete allelochemicals in the root exudates upon elicitation through various signaling molecules and inhibit plant growth by disturbing the homeostatic balance of phytohormone concentration (Li *et al.*, 2022).

In the present investigation, wheat seedlings were grown under *L. aphaca* whole plant extract in different dilutions with distilled water. The control was grown under distilled water

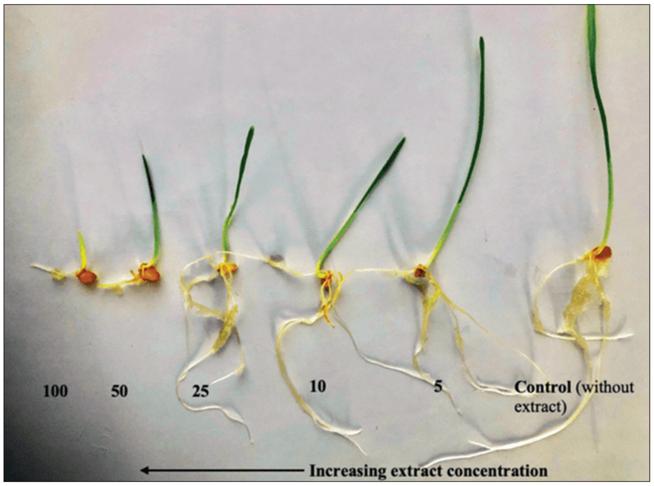

Figure 3: Growth of wheat (*T. aestivum* var. Dara) in the extract of *L. aphaca* extract after 5 days of treatment

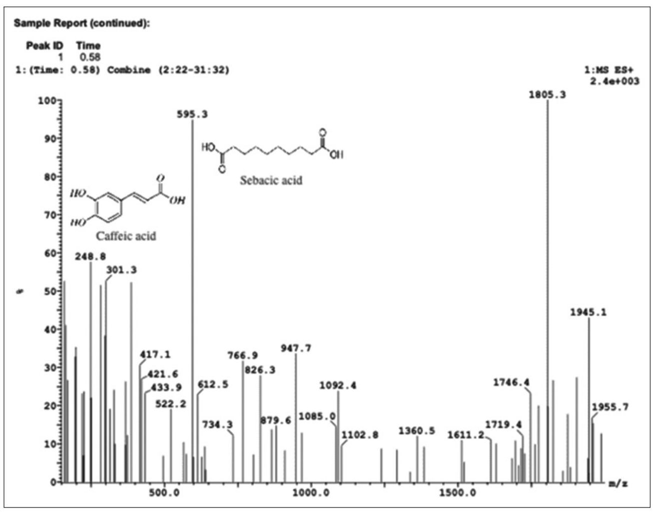

Figure 4: Chromatogram of LCMS analysis of aqueous extract of *L. aphaca* indicating the presence of potent allelochemicals

only i.e., without extract. It was observed that the growth of wheat seedlings was significantly inhibited under the extract of *L. aphaca* as compared to the control (Figures 2a - e). The inhibition of roots was more than shoot growth due to the sensitivity difference between the two organs (Mushtaq *et al.*, 2019). The shoot and root growth were severely reduced as the concentration increased indicating the presence of growth suppressive allelochemicals. The wheat seedlings are under stress and the stress increased as the concentration of extract increases which is further confirmed by free proline contents in the seedlings (Turk & Tawaha, 2003).

The growth analysis confirmed that allelopathic interaction exists between the two plants. In *L. aphaca* plants there present various potent allelochemicals as confirmed by the LCMS analysis (Figure 4, Table 1). These allelochemicals are the phytotoxins responsible for growth reductions in the test plant at the early stage of growth and are allelopathic (Xiao *et al.*, 2024). 2,4,6-trihydroxy chalcone is the precursor of the flavonoid biosynthetic pathway and flavonoids are allelopathic in nature responsible for the inhibition of seedling growth of wheat (Bitencourt *et al.*, 2007). Benzoic acid derivatives are other chemicals that is responsible for irregularly shaped cells arranged in a disorganized manner and disruption of cell organelles in mustard (*Brassica juncea* L.) roots (Kaur *et al.*, 2005).

Table 1: Allelochemicals identified in the aqueous extract of *L. aphaca* plant

S. No.	Allelochemical names	Molecular formula	Molecular weight	RT
1.	2,4,6-trihydroxy-4-methoxy chalcone	$C_{27}H_{44}O_5$	560.9	0.78
2.	3-iodo-benzoic acid	$C_7H_5IO_2$	248.0	1.02
3.	*p*-coumaric acid	$C_9H_8O_3$	164.1	1.70
4.	Kynurenic acid	$C_{22}H_{35}NO_3$	417.6	0.33
5.	Fumaric acid	$C_4H_4O_4$	116.0	1.80
6.	Sebacic acid	$C_{10}H_{18}O_4$	202.2	0.48
7.	Caffeic acid	$C_9H_8O_4$	180.1	2.15
8.	Ferulic acid	$C_{10}H_{10}O_4$	194.1	2.03

The mixture of the allelochemicals like *p*-coumaric acid, ferulic acids and benzoic acid has synergistically strong effect on the seedling growth and as well as inhibition of seedling root elongation. Growth of wheat seedlings are equally affected with all these allelochemicals secreted by the *L. aphaca* plant (Kostina-Bednarz *et al.*, 2023). These allelochemicals modulates the metabolism by disturbing the phytohormone concentrations because many of them are the cofactor of indoleactic acid (IAA)-oxidase promotes the degradation of auxins. However, some polyphenols are showing the stimulation for the increased synthesis of auxin. The Increased concentration of auxin may lead to the increased biosynthesis of ethylene which produces many physiological constrains in the plants. Allelochemicals are also negatively impact on cytoplasmic membrane processes through altering the function of phytohormones by reacting also with receptor sites (Cheng & Cheng, 2015).

CONCLUSIONS

The present manuscript describes the assessment of allelopathic damage caused by weed plants in the wheat field. This knowledge helps us to optimized the conditions for the better and higher yield that can be obtained by early removal of these weeds. The allelochemicals from the plant extracts can be sustainably used as bioherbicides to remove unwanted plants from fields.

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