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Effect of aqueous extract of *Trianthema portulacastrum* on growth and physiological attributes of a potential weed, wild onion (*Asphodelus tenuifolius*)

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Abstract
Weeds are the earliest and most severe limiting issue in crop production. Among weed control methods, the chemical method is a commonly used one, which provides better control of weeds, but induces resistance in weeds. To overcome these issues, allelopathy is the most effective and biocontrol method that is used for controlling weeds. This study was conducted to determine the allelopathic potential of *Trianthema portulacastrum* on the growth of the wild onion (*Asphodelus tenuifolius*) by the application of shoot and root aqueous extracts along with a synthetic herbicide. Different morphological, anatomical, and physiological traits were calculated. The results showed that the extracts of the shoot and root of *T. portulacastrum* significantly depressed all morphological and physiological parameters, except for total soluble sugars, total soluble proteins, CAT, proline, and potassium ions. Anatomical parameters, such as adaxial stomatal density, were significantly decreased in the extract treatment as in the synthetic herbicide treatment. The extract of allelochemical intervention in physiological processes caused growth reduction in wild onion. Overall, the shoot extract in combination with the synthetic herbicide showed the inhibitory action. The shoot extract of *Trianthema portulacastrum* could be used as a bio-herbicide to control the wild onion weed.

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Introduction

Weeds cause severe damage to forestry, agriculture, and human creativity. Weeds affect the growth and developmental stages of different crops and ultimately decrease crop yield (Elahi et al., 2022). Weeds damage the crops in various ways, such as promoting the growth of plant pathogens, affecting the reproductive and dispersal ability (Marwat et al., 2013; Horvath et al., 2023). Weeds also affect different growth factors essential for plants, such as water, space, light, and nutrients (Kaur et al., 2018). Widespread weed infestation in agricultural fields might cause a loss of food for the human population. However, weed eradication increases cultivation cost. Therefore, farmers suffer losses of a portion of their input in agricultural yields (Dangwal et al., 2010; Kubiak et al.,

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2022).

Weed management is important to ensure the quality and quantity of crop yield. Different approaches, such as mechanical, physical, and chemical, are most commonly used to control the weeds. These methods are not reliable due to their high cost and low crop yields (Paul et al., 2024). However, the biological control methods are currently used for the eradication of weeds in agricultural lands (Panghal et al., 2018; Raza et al., 2025). Currently, different herbicides are used to restrict weed development in crop fields (El-Abbassi et al., 2017; Paul et al., 2024). Consequently, extensive application of these synthetic herbicides causes serious issues to the environment and health (Vikkey et al., 2017; Ahmad et al., 2024). These issues have been overcome with allelopathic plants that exclude the use of synthetic herbicides to enhance crop yield (Al-Samarrai et al., 2012; Khamare et al., 2022).

Different strategies have been adopted to control the weeds in agricultural fields. These include the exploration of safe substitutes for synthetic herbicides to overcome the adverse impacts of synthetic herbicides (Nath et al., 2024). Courtesy has been given to allelopathy investigation for weed control due to growing emphasis on agriculture and environmental security to overcome ecological contamination issues (Jabran et al., 2015; Ain et al., 2023). Allelopathy is the interfering process in which plants or plant parts (dead) discharge allelochemicals that interrupt the physiological and developmental traits of other plants to control the weeds (Rob et al., 2020).

Wild onion (*Asphodelus tenuifolius*) belongs to the Liliaceae family. It is an erect annual, monocot herb that is grown in different regions such as India, Pakistan, northern Japan, Korea, and China (Hassan et al., 2023). Wild onion is abundantly found as a weed in wheat and chickpea crops (Mehriya et al., 2021). Different plants are being employed as potential allelochemicals in controlling different types of weeds (Khamare et al., 2022). Identification of these plants with allelochemical potential offers better weed control to reduce the usage of synthetic herbicides in crops (Hossen et al., 2023).

Considering the importance of allelopathic weed control, the current study was conducted with a major objective to determine the allelopathic potential of *T. portulacastrum* on wild onion by shoot and root extracts treatments, together with a synthetic herbicide (Tribenuron methyl + Metsulfuron methyl).

Materials and Methods

Plant collection and solution preparation

Trianthema portulacastrum samples were collected from wheat fields in District Layyah and washed with distilled water. Plant parts (root and shoot) were isolated, dried well, powdered finely, and maintained in airtight glass jars. The allelopathic aqueous extract was obtained by soaking 10 g of dried mass of root and shoot individually in 100 mL distilled water (10% w/v) for a day and a night. After filtration, the extracts were used to form final levels of 30%, 60%, and 100% of each of the root and shoot aqueous extracts.

Pot experiment

Asphodelus tenuifolius seeds were taken at the maturity phase from wheat fields in Choubara, District Layyah (30.9058 N & 71.4774 E). The pots were frequently irrigated to keep a constant moisture content of the soil. After seed germination, thinning was done to maintain 5 plants in each pot. The pots were arranged in two groups; one group of pots was irrigated with 30%, 60%, and 100% root extract, while the other group was irrigated with the above-mentioned concentrations of shoot aqueous extract. Distilled water was used as a positive control, and as a negative control, a commonly used herbicide. The current experiment was conducted in a completely randomized design (CRD) with three replicates. After three weeks of treatment, plants were harvested and their morpho-physiological and anatomical traits were studied.

Morphological parameters

Morphological characteristics such as shoot and root lengths, dry weights of shoots and roots, leaf area (cm^2), and number of leaves (per plant) were determined. Plants were gently uprooted, washed with distilled water to remove soil, and the root length was measured from the collar region to the root tip using a measuring scale, while the shoot length was taken from the base to the tip of the uppermost leaf. The total number of fully expanded leaves per plant was counted manually. Leaf area was determined by tracing leaf outlines on a graph paper and calculating the total area. For dry

weight determination, shoots and roots were separated and oven-dried at 70 ± 2 °C for 48 h until a constant weight was achieved, after which they were weighed using an electronic balance. Mean values for each morphological parameter were calculated from five plants per pot for all treatments.

Physiological and anatomical parameters

The following physio-biochemical traits were examined at the adult stage**:**

Photosynthetic pigments

The chlorophyll content was quantified from 100 mg of fresh leaf tissue in accordance with the method established by Arnon (1949). The leaf tissue was homogenized in 80% (v/v) acetone, and the final volume of the extract was adjusted to 5 mL. The homogenate was subjected to centrifugation at 10,000 rpm for 10 minutes, and the supernatant was utilized for pigment estimation. Absorbance measurements were taken at 645 nm and 663 nm using a UV-visible spectrophotometer. The concentrations of chlorophyll a and chlorophyll b were calculated using the following equations:

$$\mathbf{Chl.\,a}\left(\frac{\mathbf{mg}}{\mathbf{g}}\boldsymbol{FW}\right) = (\mathbf{12.7} \times \boldsymbol{A}\,\mathbf{663} - \mathbf{2.69} \times \boldsymbol{A}\,\mathbf{645} \times \frac{\boldsymbol{V}}{\mathbf{1000}} \times \boldsymbol{W})$$

$$\mathbf{Chl.\ b}\left(\frac{\mathbf{mg}}{\mathbf{g}}\boldsymbol{FW}\right) = (\mathbf{22.9} \times \boldsymbol{A}\,\mathbf{645} - \mathbf{4.68} \times \boldsymbol{A}\,\mathbf{663} \times \frac{\boldsymbol{V}}{\mathbf{1000}} \times \boldsymbol{W})$$

Total soluble proteins (TSP)

Total soluble protein (TSP) content was estimated following the method of Bradford (1976). Fresh leaf tissue (0.25 g) was homogenized in 5 mL of 0.05 M K_3PO_4 buffer (pH 7.8) using a chilled mortar and pestle. The homogenate was centrifuged at 12,000 × *g* for 10 minutes at 4 °C, and 100 µL of the resulting supernatant was mixed with 5 mL of the Bradford reagent. The mixture was vortexed for 10 seconds, and absorbance was recorded at 595 nm using a UV-visible spectrophotometer (U2020, IRMECO). Protein concentration was calculated from a standard curve prepared with bovine serum albumin (BSA).

Proline

Proline content was estimated following the method of Bates et al. (1973). Fresh leaf tissue (0.25 g) was homogenized in 5 mL of 3% sulfosalicylic acid, and the extract was filtered. To 1 mL of the filtrate, 1 mL of acid ninhydrin and 1 mL of glacial acetic acid were added, and the mixture was incubated at 100 °C for 1 h in a water bath. After heating, the reaction mixture was immediately cooled in an ice bath, followed by the addition of 2 mL toluene. The solution was vortexed, and the upper pink layer was separated and its absorbance recorded at 520 nm using a UV-visible spectrophotometer (U2020, IRMECO). Proline concentration was determined from a standard curve prepared with a known concentration of L-proline.

Total soluble sugars

The soluble sugars were quantified according to the method of Yemm and Willis (1954). Fresh leaf tissue (0.1 g) was homogenized in 5 mL of 0.2% phosphate buffer (pH 6.8). An aliquot of 0.1 mL of the supernatant was mixed with 3 mL of freshly prepared anthrone reagent and vortexed thoroughly. The mixture was incubated in a water bath at 95 °C for 15 minutes and then rapidly cooled under running water. Absorbance was recorded at 625 nm using a UV-visible spectrophotometer (U2020, IRMECO). The concentration of soluble sugars was calculated from a standard curve prepared with a known concentration of glucose.

Determination of K ions

Potassium (K^+) content in leaf tissue was determined using a flame photometer (Jenway, PFP-7). Leaf samples were oven-dried at 70 °C for 48 h before analysis. Potassium concentrations were quantified by comparing sample readings with standard calibration curves prepared from known KCl solutions, and total K^+ content was calculated accordingly.

Determination of stomatal density

Samples were collected from fully expanded leaves, and impressions of the abaxial surface were prepared using a clear nail polish. After drying, the film was carefully peeled with transparent tape and mounted on glass slides. Observations were made under a light microscope, and fields per slide and stomatal density were expressed as the number of stomata per mm^2.

Statistical analysis

Morphological, physiological, and anatomical data were subjected to analysis of variance (ANOVA). Mean comparisons were performed using the Least Significant Difference (LSD) test at a 5% probability level with the help of Statistix software.

Results

Root length, shoot length, root dry weight, and leaf area with application of 30 % root extract of *A. tenuifolius* exhibited a minor decrease, while no alteration was recorded in root dry weight (RDW) and the number of leaves at this treatment. Both extracts showed a significant decrease in all morphological traits. The shoot aqueous extract influence was more apparent compared to the root extract. Analogous results were observed with herbicide treatment (T4). This treatment caused a significant reduction in all morphological traits (**Table 1**).

Table 1: Influence of root and shoot extracts of *T. portulacastrum* on the morphological traits of *A. tenuifolius.*

Treatments	T0	TR1	TR2	TR3	TS1	TS2	TS3	T4	LSD	CV	GM	*F* value
RL (cm)	8 a	7 b	6 c	6 c	7 b	5.6 c	5 d	4 e	0.47	4.44	6.17	62***
RDW (g)	0.35 a	0.35 a	0.28 ab	0.2 bc	0.29 a	0.17 c	0.17 c	0.15 c	0.09	20.99	0.249	8.3***
SL (cm)	29 a	24 b	24 b	22 d	23 c	14 e	12 g	13 f	0.5	1.39	20.62	1464***
SDW (g)	3.5 a	3.2 ab	3 bc	2.3 ef	2.8 cd	2.5 cd	2 fg	1.8 g	0.39	7.99	2.8	18.5***
LA (cm^2/plant)	13 a	12 b	10 d	8 e	11 c	8 e	8 e	7 f	0.44	2.6	9.65	219***
NLP	14 a	14 a	9 bc	9 bc	12 ab	8 c	5 d	5 d	2.52	14.95	9.7	18.1***

Abbreviations: RL-Root length, RDW-Root dry weight, SL-Shoot length, SDW-Shoot dry weight, LA-Leaf area, NLP-Number of leaves per plant, T0-Distilled water treatment, TR1-30% root water extract, TR2-60% root water extract, TR3-100% root water extract, TS1-30% shoot water extract, TS2-60% shoot water extract, TS3-100% shoot water extract, T4-Herbicide, CV-Coefficient of Variation, Grand Mean (GM). *** = Significant at $P \leq 0.001$; ** = Significant at $P \leq 0.01$; * = Significant at $P \leq 0.05$, and NS = Non-significant. Means with the same letters are not significantly different at $P \leq 0.05$.

Chlorophyll a and b contents reduced with an increase in the extract levels of *T. portulacastrum,* but no change in chlorophyll a content was detected with the application of 30% root extract. The highest reduction was observed with the application of 100% root extract (**Figure 1**).

Application of both *T. portulacastrum* extracts exhibited a prominent effect on the soluble protein content of *A. tenuifolius.* A greater increase was found in this parameter at the diluted extract, while a slight increase was recorded at the concentrated levels. A slight increase in the herbicide treatment was recorded in total soluble proteins (**Figure 1**).

CAT activity was recorded to be increased due to all root extracts of *T. portulacastrum*. However, the maximum rise in the CAT activity was observed at 100% root extract. While, in contrast to the root extract, diluted shoot extract caused a greater increase in CAT activity than the concentrated *T. portulacastrum* shoot extract, as well as at herbicide treatment. Application of the root extract showed an increasing trend in proline levels with an increased level of *T. portulacastrum* root extract. But no change was recorded at 30% root extract. The highest proline level was observed at 100% extract of the root. The proline level increased at all levels of *T. portulacastrum* shoot extract (**Figure 1**).

An increasing trend was documented in the total soluble sugars of *A. tenuifolius* with an increasing level of root allelopathic extract. A similar trend was recorded with the shoot extract treatment, but the diluted shoot extract caused more production than the concentrated extract. A slight increase in total soluble sugars was also found with the herbicide treatment. A maximum increase in total free amino acids was recorded from the concentrated shoot and root extracts, and a minimum increase in this parameter was observed with diluted water extracts. More diluted levels of the shoot and root extracts caused a greater increase in K ions, while the concentrated level caused a slight increase in this trait. So, a maximum increase was observed at 30% root extract level, while a minimum increase was noticed at 100% extract and herbicide treatment (**Figure 1**).

The stomatal density of *A. tenuifolius* exhibited various responses at different extract levels. A slight increase was observed at 30% and 60% levels of the root water extract compared to the control. But adaxial stomatal density decreased in 100% root extract and all shoot extract levels (**Figure 2**).

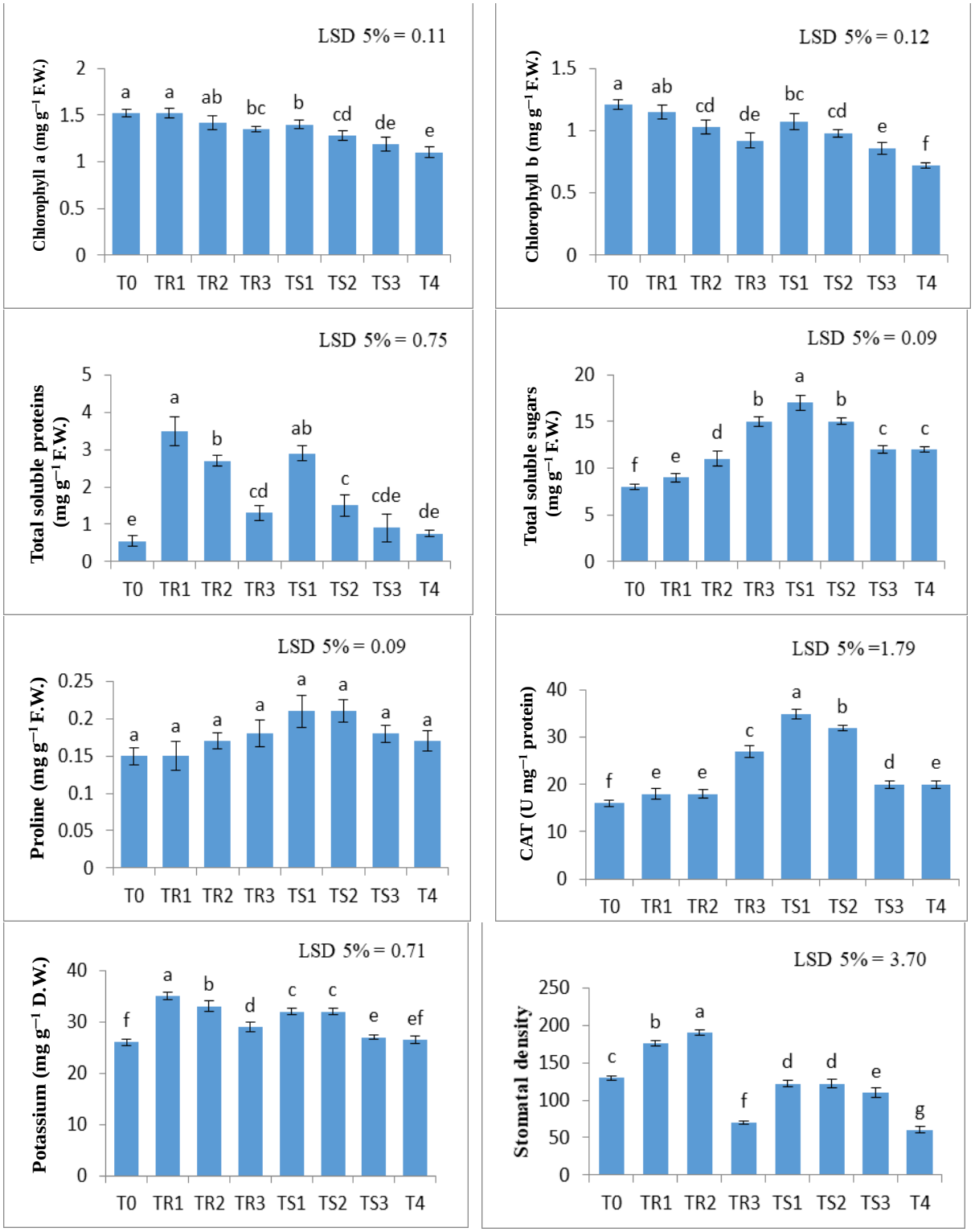


Figure 1: Application of various levels of root and shoot water extracts of *T. portulacastrum*, and herbicide (T0 = Distilled water, TR1= 30% root extract, TR2= 60% root extract, TR3= 100% root extract, TS1= 30% shoot extract, TS2= 60% shoot extract, TS3= 100% shoot extract, and T4= Herbicide) on physiological parameters and stomatal density (stomata/mm^2) of *A. tenuifolius*.

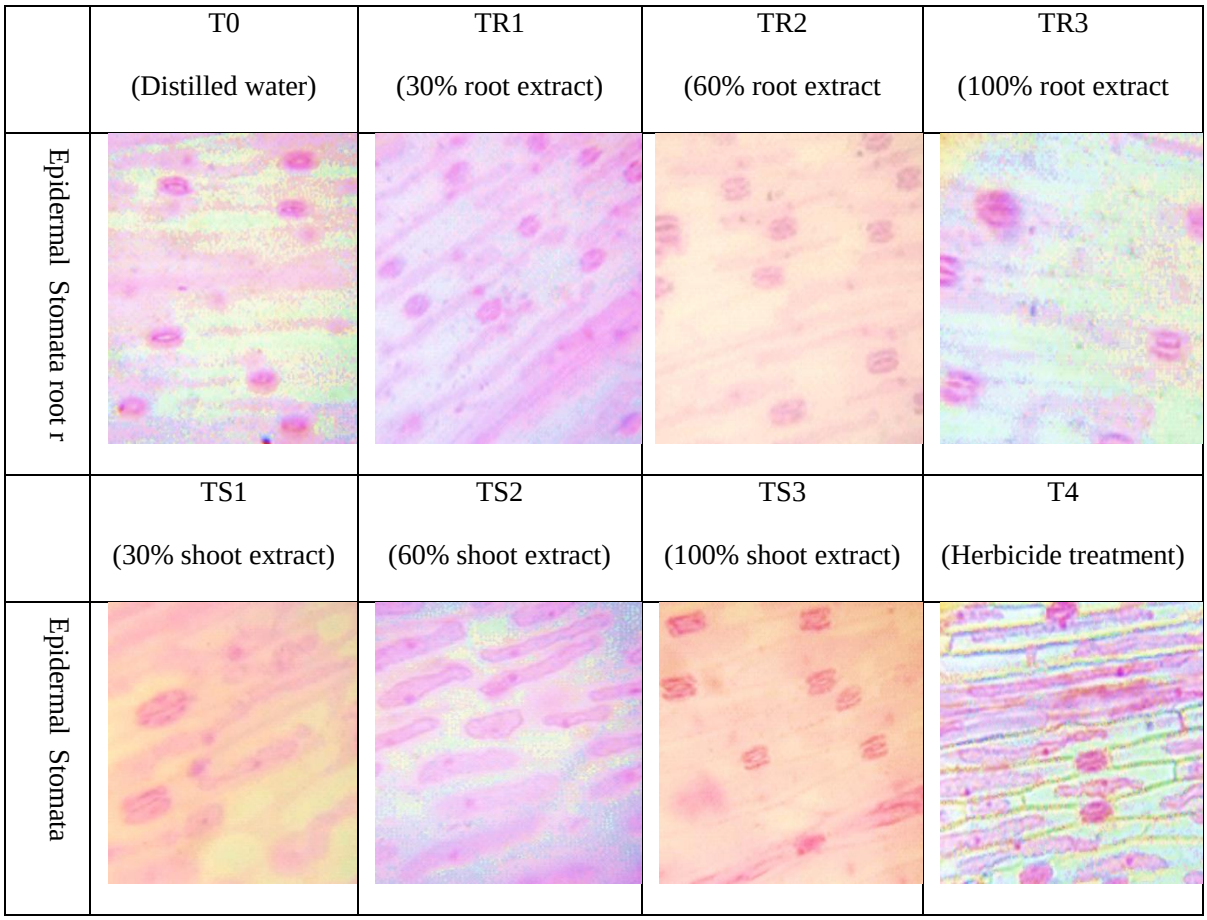


Figure 2: Effect of various levels of root and shoot water extracts of *T. portulacastrum* and herbicide on the number of stomata of *A. tenuifolius*.

Discussion

Different environmental stresses impair plant growth, development, and metabolic processes (Reddy, 2003; Reddy et al., 2004). Weeds are proven to be the most important factor for crop loss in agricultural lands. Different approaches have been adopted to control the weeds, such as mechanical, chemical, and biological techniques. However, biological control is the most efficient technique for weed control (McFadyen, 1998). Recently, allelopathy has become an important substitute for weed control, which is the biotic phenomenon through which an organism secretes biochemical compounds that interfere with germination, seedling growth, development, reproduction, and survival of other organisms (Stamp, 2003; Jamil et al., 2009; Ain et al., 2023). Allelopathy is a cost-effective and eco-friendly weed control method (Khan, 2011). Allelochemicals are biodegradable in a short time, and their persistence in plants or soil systems will not cause problems like pesticides. Therefore, these biochemicals are crucial for novel and eco-friendly pesticide discovery efforts (Kong, 2019).

Different plant species secrete phytotoxins in response to environmental conditions. The sensitivity of different plants is subject to their biochemical and physiological features (Kobayashi, 2004). In the present study, *T. portulacastrum* extracts reduced root and shoot lengths in *A. tenuifolius.* Similar results were reported in the jute plant, where root length considerably decreased under *T. portulacastrum* extract application (Sutradhar et al., 2018). The inhibitory effects of the extract of weeds were also reported on various plant species, such as *Medicago sativa, Triticum aestivum, Trigonella foenum-graecum,* and *Hordeum vulgare* (Mustafa et al., 2019). This reduction is attributed to restricting cell division and disturbing enzyme activities and water uptake inhibition, which decreased cell division. The shoot extract had more severe inhibitory effects compared to the root extract owing to the presence of plenty of allelopathic compounds. Reduced shoot length can cause modifications in the DNA replication, mitochondrial reactions, and modifications in the cell cycles (Khaliq et al., 2013).

Allelochemicals released from weed plants cause a reduction in plant growth and development and the yield of other plants by affecting their physiological activities. Moreover, total soluble proteins, total soluble sugars, proline, and other compatible solutes in cells sustain solute adjustment under stress conditions (Ben Rejeb et al., 2012; Salehi et al., 2016). Decrease in the chlorophyll with allelopathic treatment of *T. portulacastrum* in the current study can be described in the context of an enhanced chlorophyll degradation rate owing to less accessibility of water and minerals. Similar findings were described by Elisante et al. (2013), who showed a decline in chlorophyll content in different weeds under the application of leaf extract of *T. portulacastrum.* Herbicide treatment in our study also reduced the levels of chlorophyll a and b, like the allelopathic treatments. Similar results were reported by Khan et al. (2018), who reported a reduction in the levels of chlorophyll a and b of *Coronopus didymus* with herbicidal treatments. In the present study, the photosynthetic pigments were consistently reduced with increasing concentration of *T. portulacastrum* residues. It is likely that allelochemicals severely affected chlorophyll synthesis, blocked photosynthesis, and reduced plant growth (Ain et al., 2023). In our findings, an increase in soluble sugars was observed in the wild onion. Hence, these findings are similar to those of an investigation of Ozturk et al. (2021), who reported high accumulation of simple sugars such as fructose, glucose, fructan, and trehalose, etc., under stress conditions.

Stress proteins are synthesized in plants during stress conditions (Zhou et al., 2023). In our study, an increase in protein content was recorded by applying the allelopathic stress. Similar findings were reported by Unal and Bayram (2019), who reported an increase in overall protein content, which may have been due to the release of stress proteins. Shinde and Salve (2019) stated that the leaf extract of *Amarantus tricolor* caused an increase in the protein level of wheat. Schmitz et al. (2000) described that the protein synthesis in plants is triggered by stress-induced ABA synthesis. The findings of these studies are consistent with our results, in which allelochemicals caused an enhancement in total soluble proteins. Herbicide treatment also works analogously to allelochemicals and causes an increase in soluble proteins. For example, the Gromster herbicide caused a protein increase in mustard species (Ozen and Onay, 2013).

An increased level of K ions in the present study has shown that K ions are found to be a key factor in the solute adjustment of a plant under stress conditions (Hasanuzzaman et al., 2018). Potassium contributes to plant survival under diverse stress conditions because of the important regulatory role of potassium in various plant physiochemical processes, viz., seed germination, photosynthesis, phloem transport, protein synthesis, anion-cation balance, stomatal regulation, water and solute balance, carbohydrate metabolism, activation of enzymes, and stress resistance (Marschner and Rengel, 2012). K^+ ions are involved in retaining water potential as well as cell turgidity by maintaining ionic balance, so they help cope with stress conditions (Shabala, 2003). Potassium is also involved in plant signaling systems by activating antioxidant defense systems to protect from stress (Hasanuzzaman et al., 2028; Bhardwaj et al., 2025).

In the current study, the stomatal density reduction with the application of shoot extract levels and herbicide treatment can be supported by Shahid et al. (2021), who reported a reduction in the number of stomata in chickpea with the treatment of insecticides. Hameed et al. (2020) also stated a decline in abaxial stomatal density in wheat lines at a higher level of *A. scholaris* leaf extract. Size, density, and opening and closing of stomata adjust the rate of transpiration in plants and are consequently involved in the maintenance of water (Gao et al., 2018). Lower stomatal density causes a reduction in water loss rate, which allows a plant to survive in stress more effectively (Shahbaz et al., 2025). A reduction in stomatal size and number on the adaxial side is more advantageous for the regulation of the transpiration rate (Drake et al., 2019).

Overall, the root and shoot extracts of *T. portulacastrum* negatively affected the growth parameters of *A. tenuifolius*. The extract of allelochemicals intervention in physiological processes caused growth reduction in wild onion. Aqueous extracts of the shoot of *T. portulacastrum* significantly depressed all morphological and physiological parameters except total soluble sugars, total soluble proteins, CAT activity, proline content, and potassium ions. This study will be helpful for future investigation of the shoot extract that could be used as a bio-herbicide to control the wild onion weed.

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Permissions and ethical compliance
This study does not involve human/animal subjects, and thus no ethical approval is required.

Handling of bio-hazardous materials
The authors certify that all experimental materials were handled with great care during collection and experimental procedures. After completion of the study, all materials were properly discarded to minimize/eliminate any types of bio-contamination(s).

Supplementary material
No supplementary material is included with this manuscript.

Conflict of interest
The authors declare no conflict of interest.

Availability of primary data and materials
As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher/Journal management. These are available with the corresponding author (s) and/or with other author(s) as declared by the corresponding author (s) of this manuscript.

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