



## Evaluation of the Allelopathic Potential of *Thymus kotschyanus* Boiss. Extracts on Seed Germination, Growth, and Photosynthetic Activity of *Convolvulus arvensis* L. and *Cynodon dactylon* L.

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Original Article

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### ABSTRACT

This study investigated the allelopathic potential of *Thymus kotschyanus* Boiss. on the germination, growth, and photosynthetic performance of *Cynodon dactylon* and *Convolvulus arvensis*. Laboratory and greenhouse experiments were conducted in 2025 using a completely randomized design with four replications at a research farm in Shirvan, North Khorasan Province, Iran. Treatments consisted of aqueous root and shoot extracts of mountain thyme prepared by soaking plant material in distilled water and shaking for 24 h at room temperature under continuous agitation (200 rpm), at concentrations of 10 and 20 g L<sup>-1</sup>, with distilled water serving as the control. The results indicated that the highest extract concentration (20 g L<sup>-1</sup>) significantly reduced germination percentage in *C. dactylon* by 19.8% and 36.6% and in *C. arvensis* by 31.2% and 51.3% following application of shoot and root extracts, respectively, compared with the control. Under the same treatment conditions, germination rate decreased by 36.8% and 57.8% in *C. dactylon* and by 44.2% and 65.0% in *C. arvensis*, respectively. Application of the root extract at 20 g L<sup>-1</sup> significantly reduced several growth and physiological traits in both weed species, including plant height (34.4% and 31.7%), leaf area (41.8% and 43.0%), shoot dry weight (29.4% and 34.9%), total chlorophyll content (30.4% and 36.0%), net photosynthetic rate (41.0% and 50.4%), and stomatal conductance (50.5% and 56.6%) in *C. dactylon* and *C. arvensis*, respectively ( $P \leq 0.01$ ). Overall, the findings demonstrated that root and shoot extracts of *T. kotschyanus*, likely containing water-soluble allelochemicals, markedly inhibited germination and growth of both weed species. These results suggest that *T. kotschyanus* may have potential for future development of bioherbicidal agents and could serve as a promising candidate for environmentally friendly weed management strategies.

### ARTICLE

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## 1. Introduction

Weeds are among the most important biological constraints limiting agricultural productivity in both arable and orchard ecosystems. By competing with crops for essential resources, including water, nutrients, light, and carbon dioxide, weeds substantially reduce both yield and quality (Rezadadi et al., 2026). Perennial weed species such as *Convolvulus arvensis* L. and *Cynodon dactylon* L. are widely recognized as major problematic weeds in maize (*Zea mays* L.) production systems. Their extensive root systems, vigorous vegetative growth, and strong competitive ability enable them to efficiently exploit available resources, resulting in significant reductions in crop growth and productivity (Pouresmaeil et al., 2020; Soares et al., 2023).

Moreover, weed interference in maize production systems has been reported to account for approximately 37% of global yield losses, highlighting weeds as a major limiting factor in maize productivity worldwide (Sharma and Rayamajhi, 2022). Under stress conditions, *Azotobacter* inoculation has been reported to improve growth performance and nutrient acquisition in maize. This response is likely driven by the combined influence of enhanced soil properties, beneficial microbial activity, and improved physiological regulation within the plant, rather than by a single underlying mechanism (Khosravi, 2026). Although, chemical herbicides have been widely used for weed management, their excessive and continuous application has raised serious environmental concerns, including soil contamination, herbicide residues, increased production costs, crop injury, biodiversity loss, and the evolution of herbicide-resistant weed populations (Rezvani and Dadkhah, 2023). In this context, allelopathy has received considerable attention as a promising alternative approach for weed management. Allelopathy refers to the ability of plants to influence the growth and development of neighboring species through the release of bioactive compounds, known as allelochemicals, into the surrounding environment. These compounds may be released through root exudation, volatilization, leaching, or decomposition of plant residues and are found in various plant organs, including leaves, stems, roots, flowers, and seeds. Previous studies have shown that allelochemicals inhibit seed germination, seedling establishment, and plant growth by disrupting key physiological and biochemical processes such as cell division, nutrient uptake, membrane stability, photosynthesis, respiration, and enzymatic activity (Inuganti et al., 2021; Rezvani and Dadkhah, 2023; Chang et al., 2024; Zhao et al., 2022; Mushtaq et al., 2019; Fu et al., 2019). The potential of allelopathy for weed suppression has been documented in numerous studies. For example, the essential oil of *Artemisia fragrans* significantly inhibited germination, seedling growth, photosynthetic pigment accumulation, and photosystem II efficiency in *Convolvulus arvensis*, mainly through the induction of oxidative stress and disruption of key metabolic processes (Pourasmail et al., 2020). Physical scarification, plant growth regulators also play a crucial role in regulating seed dormancy and promoting germination (Mostafavi-Fard, 2026). Likewise, a recent review reported that more than half of the studies on *Cynodon dactylon* management have focused on allelopathic plants and natural compounds. The review further demonstrated that crops such as sorghum (*Sorghum bicolor*) and rice (*Oryza sativa*) possess substantial allelopathic potential and can effectively suppress the germination, growth, and establishment of this troublesome weed species (Soares et al., 2023).

Mountain thyme (*Thymus kotschyanus* Boiss.), a medicinal plant belonging to the Lamiaceae family, contains a wide range of bioactive compounds, including thymol, carvacrol, p-cymene, and flavonoids, which contribute to its antioxidant, antimicrobial, and allelopathic properties (Golnasab et al., 2023). Previous studies have shown that extracts of thyme and other medicinal plants can significantly reduce germination percentage, germination rate, seedling growth, fresh weight, and seed vigor of weed species (Asadi Gakiyeh et al., 2016).

Similarly, Saberi et al. (2012) reported that aqueous extracts of *Thymus kotschyanus* significantly inhibited the germination and seedling growth of *Bromus inermis* and *Agropyron elongatum*, with leaf extracts exhibiting the strongest inhibitory effects. Despite the recognized allelopathic potential of *Thymus kotschyanus*, information regarding its effects on dominant weed species in maize production systems under Iranian conditions remains scarce. Therefore, this study was conducted to evaluate the allelopathic effects of aqueous extracts obtained from different organs of *Thymus kotschyanus* on seed germination, seedling growth, and selected physiological traits of *Convolvulus arvensis* L. and *Cynodon dactylon* L., two dominant weeds in maize production systems.

## 2. Materials and Methods

To evaluate the allelopathic potential of aqueous extracts obtained from different organs (roots and shoots) of *Thymus kotschyanus* Boiss. on germination characteristics, seedling growth, and photosynthetic responses of two dominant maize-associated weed species, *Convolvulus arvensis* L. and *Cynodon dactylon* L., two separate experiments were conducted under laboratory and greenhouse conditions. Both experiments were arranged in a completely randomized design (CRD) with four replications. The five experimental treatments included 4 thymus extracts (aerial extracts (stem and leaf) at concentrations of 10 and 20 g L and root extracts at concentrations of 10 and 20 g L) along with distilled water as the control group.

### 2.1. Preparation of Aqueous Extracts

Samples of *Thymus kotschyanus* were collected from rangelands of Borzoli village, located in the southwestern part of Shirvan County, North Khorasan Province, Iran (39°21'19" N, 59°12'17" E; 1153 m above sea level). *Thymus kotschyanus* specimens were identified according to Flora of Iran (Ghareman, 1996) and verified by a plant systematics specialist at Bojnord University. A voucher specimen was deposited in the herbarium under accession number 803539. The study area is characterized by an average annual precipitation of approximately 285 mm and mean maximum and minimum temperatures of 22.4 and 5.8 °C, respectively.

For plant material preparation, the roots, stems, and leaves of wild thyme (*Thymus kotschyanus*) were carefully separated. To remove dust and surface impurities, the plant organs were washed with distilled water for 60 s. The samples were then kept at room temperature (25 ± 2 °C) until they reached a semi-wilted state. Considering the presence of phenolic compounds and their derivatives, the plant materials were dried in a forced-air oven at 40 °C for 24–48 h to minimize degradation of bioactive constituents until a constant weight was achieved, corresponding to approximately 10% of the initial fresh weight (Caceres, 2000). The dried plant materials were subsequently ground into a fine powder using an electric mill and passed through a 40-mesh sieve to ensure uniform particle size. Finally, the powdered samples were stored in airtight polyethylene bags under refrigerated conditions until the initiation of the experiments.

For extract preparation, 20 g of powdered material from each plant organ (shoot and root, processed separately) was placed in 2 L Erlenmeyer flasks, and 1 L of deionized water was added. The flasks were covered with aluminum foil to prevent photodegradation and shaken on a rotary shaker at approximately 200 rpm for 24 h. Each extraction was performed independently in triplicate under identical conditions. The suspensions were then filtered under vacuum through Whatman No. 1 filter paper. The pH and electrical conductivity (EC) of the extracts were measured using a digital pH meter and a conductivity meter, respectively. The pH and EC of shoot extract were 6.45 ± 0.12 and 2.45 ± 0.08 mS cm<sup>-1</sup>, respectively, while those of root extract were 6.12 ± 0.10 and 2.78 ± 0.09 mS cm<sup>-1</sup>. The filtrates were regarded as full-strength stock solutions. A set of treatments was prepared, including shoot and root stock extracts (20 g dry weight L<sup>-1</sup>) and their corresponding dilutions (10 g dry weight L<sup>-1</sup>), along with deionized water as the control, and were stored at -18 °C until further use. All extracts were used in both laboratory and greenhouse experiments.

## 2.2. Petri Dish Bioassay

A laboratory germination bioassay was conducted to evaluate the allelopathic effects of root and shoot extracts of *Thymus kotschyanus* on seed germination and early seedling growth of *Convolvulus arvensis* and *Cynodon dactylon*. The experiment was arranged in a completely randomized design (CRD) with four replications. Seeds with germination percentages above 90% were surface-sterilized using 2% sodium hypochlorite solution for 5 min and subsequently rinsed several times with distilled water. Thirty seeds were placed in 9-cm plastic Petri dishes lined with two layers of Whatman No. 1 filter paper. Each dish received 5 mL of the respective extract. In the control treatment, only 5 mL of distilled water was added. After treatment application, the Petri dishes were sealed with Parafilm to minimize evaporation of the extracts and incubated in a germinator (Model 1CH'RH) under a 12 h light/12 h dark photoperiod at 25 ± 2 °C during the light period and 16 ± 2 °C during the dark period (Beyat et al., 2020). Filter papers were checked daily and moistened with distilled water or respective extracts as needed to maintain constant moisture conditions. The number of germinated seeds was recorded daily until either complete germination was achieved or no germination occurred for three consecutive days. Seeds were considered germinated when the radicle length reached at least 2 mm (Souza et al., 2010). The experiment lasted for 14 days. Germination percentage was recorded 12 days after the onset of germination (Ghasemi Arain, 2016):

$$GP = \frac{N'}{N} \times 100$$

where:

N'= Number of germinated seeds at the final day

N= Total number of seeds

Germination rate was determined according to Ikic (2012):

$$GR = \sum_{i=1}^n \frac{S_i}{D_i}$$

where:

S<sub>i</sub>= Number of germinated seeds at each counting time

D<sub>i</sub>= Number of days elapsed at the i-th counting time

n= Total number of counting events

At the end of the experiment, 10 seedlings were randomly selected, and root and shoot lengths were measured using a ruler (cm). Seed vigor index was calculated as the product of germination percentage and seedling length (Abdul-Baki and Anderson, 1973):

$$VI = \frac{(GP)}{100} \times L_s$$

where:

VI= Seed vigor index

GP= Germination percentage

$L_s$ = Seedling length (sum of root and shoot lengths)

### 2.3 .Greenhouse Experiment

A greenhouse pot experiment was conducted under controlled environmental conditions in a completely randomized design (CRD) with four replications to evaluate the allelopathic effects of *Thymus kotschyanus* extracts on the growth and physiological responses of two target weed species (*Convolvulus arvensis* and *Cynodon dactylon*). Two-week-old uniform seedlings were transplanted into pots with an upper diameter of 30 cm, a height of 20 cm, and a lower diameter of 15 cm, and maintained at a spacing of 30 cm. Irrigation was applied uniformly to all pots as required to maintain adequate soil moisture throughout the experiment. The growth medium consisted of loamy soil (Table 1).

**Table 1. Physicochemical properties of the soil used in the experiment.**

Texture	Clay (%)	Silt (%)	Sand (%)	Absorbable K (ppm)	Absorbable P (ppm)	Total N (%)	O.C (%)	SP (%)	EC (mS/cm)	pH
Clay loam	26	50	24	225	3.60	0.056	0.757	33.19	1.26	7.87

Each pot served as an experimental unit and contained a single uniform seedling. To improve wetting efficiency and ensure uniform foliar coverage, a few drops of a non-ionic surfactant (Tween 20, 0.05% v/v) were added to the spray solution. Foliar applications were performed at the 4-6 leaf stage using 20 mL of extract per plant. The application was carried out with a calibrated hand-held sprayer to ensure uniform distribution over the leaf surface. Spraying was continued to the point of runoff to achieve complete and homogeneous foliar deposition. To minimize non-target effects, soil contamination was carefully avoided, and observed responses were attributed primarily to foliar exposure. All treatments were applied in the early morning to reduce evaporative losses and enhance foliar uptake efficiency. Treatments were applied three times at five-day intervals, while control plants were sprayed with distilled water only. The plants were maintained under controlled environmental conditions with day/night temperatures of  $32 \pm 2$  °C and  $18 \pm 2$  °C, respectively, and relative humidity ranging from 30 to 55%.

Leaf area was measured using a portable leaf area meter (AM350, UK) from fully expanded leaves of each replicate plant. Net photosynthetic rate and stomatal conductance were measured four weeks after treatment application on the youngest fully expanded leaf using a portable infrared gas analyzer (CIRAS-1, PP Systems, USA). Measurements were taken between 09:00 and 11:00 a.m. to reduce diurnal variation. During gas exchange measurements, photosynthetically active radiation (PAR) was maintained at approximately  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ , while ambient CO<sub>2</sub> concentration was  $400 \pm 50$  ppm. The central portion of the leaf was placed in a 2.5 cm<sup>2</sup> illuminated cuvette, and readings were recorded in real time using the device's data logging system. All measurements were performed under uniform and stable environmental conditions across treatments. For chlorophyll determination, fresh leaf samples (0.2 g) were collected from the same position on each plant, immediately homogenized, and pigments were extracted in 10 mL of 80% acetone. The extracts were centrifuged and stored in the dark at 4 °C to prevent pigment degradation. Absorbance of the supernatant was measured at 664 and 647 nm using a Beckman spectrophotometer (UK). Total chlorophyll content was calculated according to Porra et al. (1984)

### 2.4 .Statistical Analysis

Data from both laboratory and greenhouse experiments were analyzed separately using a completely randomized design (CRD) in SAS software (version 9.1). In both experiments, the same five treatments were applied, including shoot and root aqueous extracts at two concentrations (10 and 20 g L<sup>-1</sup>) and a control treatment (distilled water). In the greenhouse experiment, each pot was considered as an experimental unit, and a single uniform seedling was maintained per pot. Prior to statistical analysis, percentage germination data were subjected to arcsine transformation to meet the assumptions of normality and homogeneity of variance. Homogeneity of variance was tested using Levene's test. Analysis of variance (ANOVA) was performed, and treatment means were compared using Duncan's multiple range test (DMRT) at the 5% probability level ( $p \leq 0.05$ ).

### 3. Results

Analysis of variance indicated that aqueous extracts prepared from both the shoots and roots of *Thymus kotschyanus* significantly inhibited seed germination and early seedling growth of *Cynodon dactylon* and *Convolvulus arvensis* (Table 2). The inhibitory effects increased progressively with increasing extract concentration, demonstrating a clear dose-dependent response (Table 2).

**Table 2. Analysis of variance (mean squares) of the allelopathic effects of different concentrations of *Thymus kotschyanus* L. aqueous extract on germination traits of *Convolvulus arvensis* L. and *Cynodon dactylon* L. under laboratory conditions.**

S. O. V	Degree of freedom	Germination percentage	Germination rate	Seedling dry weight	Seedling length	Seed vigor index	Total Chloro phyll
<i>Cynodon dactylon</i>							
Aqueous extract of Thyme	4	731.74**	9.66**	0.00032**	1030.37**	1642.51**	39.62**
Error	15	90.12	0.62	0.00002	27.87	30.31	3.38
CV%	12.84	13.44	15.36	19.55	10.41	13.05	13.81
<i>Convolvulus arvensis</i>							
Aqueous extract of Thyme	4	683.15**	11.54**	0.00005**	1609.96**	1680.16**	43.42**
Error	15	54.34	0.58	0.00040	76.22	94.20	3.71
CV%	14.21	11.55	16.09	25.13	19.52	28.34	15.70

\* and \*\* are significant differences in 5% and 1% level, respectively.

Among the two weed species examined, *Convolvulus arvensis* was more sensitive to the allelopathic effects of *Thymus kotschyanus* extracts than *Cynodon dactylon*. Application of shoot extracts at concentrations of 10 and 20 g L<sup>-1</sup> reduced the germination percentage of *Cynodon dactylon* by 13% and 19.8%, respectively, compared with the control treatment (Table 3). In contrast, germination of *Convolvulus arvensis* declined by 16.7% and 31.3% at the corresponding concentrations (Table 3). Root extracts exhibited stronger phytotoxic effects than shoot extracts. Treatment of *Cynodon dactylon* seeds with root extracts at concentrations of 10 and 20 g L<sup>-1</sup> decreased germination percentage by 22.6% and 36.7%, respectively, relative to the control (Table 2). A similar trend was observed in *Convolvulus arvensis*, where germination was reduced by 37.6% and 51.3% at 10 and 20 g L<sup>-1</sup>, respectively (Table 3). Low concentrations (10 g L<sup>-1</sup>) of shoot and root aqueous extracts of *Thymus kotschyanus* reduced the germination rate of *Cynodon dactylon* by 18.5% and 45%, respectively, compared with the control. Under the same treatment conditions, the germination rate of *Convolvulus arvensis* decreased by 27.2% and 54.6%, respectively. Increasing the extract concentration to 20 g L<sup>-1</sup> further enhanced the inhibitory effects, resulting in reductions of 36.8% and 57.7% in the germination rate of *Cynodon dactylon* following exposure to shoot and root extracts, respectively. Likewise, the germination rate of *Convolvulus arvensis* declined by 44.3% and 65% in response to the higher concentrations of shoot and root extracts, respectively (Table 3).

Seedling growth was more strongly affected than germination by increasing extract concentrations. In *Cynodon dactylon*, shoot extracts at concentrations of 10 and 20 g L<sup>-1</sup> reduced seedling length by 12.4% and 24.8%, respectively, whereas root extracts caused reductions of 22.7% and 37% compared with the control treatment. A greater inhibitory response was observed in *Convolvulus arvensis*, where seedling length decreased by 32.7% and 60.2% following treatment with 10 and 20 g L<sup>-1</sup> shoot extracts, respectively. Root extracts exerted even stronger suppressive effects, reducing seedling length by 65.1% and 75.3% at the corresponding concentrations (Table 3). Seed vigor index was significantly affected by the allelopathic treatments and showed a progressive decline with increasing extract concentration. In *Cynodon dactylon*, shoot extracts at 10 and 20 g L<sup>-1</sup> reduced seed vigor by 21.10% and 40.4%, respectively, whereas the corresponding reductions caused by root extracts were 40.2% and 60.1% compared with the untreated control. The response of *Convolvulus arvensis* was more pronounced, which indicated greater sensitivity to the allelochemicals released by *Thymus kotschyanus*. At the highest concentration, shoot and root extracts reduced seed vigor by 78.1% and 90.4%, respectively (Table 3).

The aqueous extracts of *Thymus kotschyanus* significantly affected leaf area, plant height, and chlorophyll content in both tested species, with inhibitory effects increasing in a concentration-dependent manner. At the highest concentration (20 g L<sup>-1</sup>), shoot and root derived extracts reduced leaf area in *Cynodon dactylon* by 31.5% and 41.8%, respectively, compared with the control. In *Convolvulus arvensis*, corresponding reductions reached 35.3% and 43.0%. A similar trend was observed for plant height, where exposure to shoot and root extracts at 20 g L<sup>-1</sup> resulted in reductions of 17.1% and 34.4% in *Cynodon dactylon*, and 22.6% and 31.7% in *Convolvulus arvensis*, respectively.

Shoot dry biomass was also significantly reduced. At 20 g L<sup>-1</sup>, shoot- and root-derived extracts decreased shoot dry weight by 23.2% and 29.5% in *Cynodon dactylon* and by 24.4% and 35.1% in *Convolvulus arvensis*, respectively, relative to the control (Table 4). Furthermore, application of *Thymus kotschyanus* shoot and root extracts at the same concentration reduced total chlorophyll content by 24.9% and 31.0% in *Cynodon dactylon*, and by 14.0% and 36.0% in *Convolvulus arvensis*, respectively, compared with untreated plants.

**Table 3. Mean comparison of germination traits of *Cynodon dactylon* L. and *Convolvulus arvensis* L. as affected by aqueous extracts of *Thymus kotschyanus* under laboratory conditions.**

Treatment	Germination percentage	Germination rate	Seedling dry weight (g)	Seedling length (mm)	Seed vigor index
<i>Cynodon dactylon</i>					
Control	94.58 <sup>a</sup>	10.09 <sup>a</sup>	0.0152 <sup>a</sup>	101.45 <sup>a</sup>	96.00 <sup>a</sup>
Shoot extract aqueous					
10 g L <sup>-1</sup>	85.25 <sup>b</sup>	8.23 <sup>ab</sup>	0.014 <sup>b</sup>	88.85 <sup>bc</sup>	75.74 <sup>b</sup>
20 g L <sup>-1</sup>	75.91 <sup>c</sup>	6.38 <sup>bc</sup>	0.008 <sup>cd</sup>	75.35 <sup>de</sup>	57.20 <sup>c</sup>
Root extract aqueous					
10 g L <sup>-1</sup>	73.26 <sup>c</sup>	5.55 <sup>cd</sup>	0.008 <sup>de</sup>	78.35 <sup>cd</sup>	57.40 <sup>c</sup>
20 g L <sup>-1</sup>	59.89 <sup>d</sup>	4.26 <sup>d</sup>	0.006 <sup>e</sup>	63.95 <sup>e</sup>	38.30 <sup>d</sup>
<i>Convolvulus arvensis</i>					
Control	89.50 <sup>a</sup>	7.66 <sup>a</sup>	0.017 <sup>a</sup>	85.34 <sup>a</sup>	76.37 <sup>a</sup>
Shoot extract aqueous					
10 g L <sup>-1</sup>	74.58 <sup>bc</sup>	5.58 <sup>b</sup>	0.010 <sup>b</sup>	57.41 <sup>b</sup>	42.82 <sup>bc</sup>
20 g L <sup>-1</sup>	61.56 <sup>cd</sup>	4.27 <sup>c</sup>	0.006 <sup>cd</sup>	34.02 <sup>cd</sup>	21.00 <sup>de</sup>
Root extract aqueous					
10 g L <sup>-1</sup>	55.85 <sup>de</sup>	3.48 <sup>cd</sup>	0.004 <sup>cd</sup>	29.84 <sup>cd</sup>	16.67 <sup>de</sup>
20 g L <sup>-1</sup>	43.60 <sup>e</sup>	2.68 <sup>d</sup>	0.003 <sup>d</sup>	21.10 <sup>d</sup>	9.20 <sup>e</sup>

Means within each column followed by the same letter are not significantly different at  $P \leq 0.05$  according to Duncan's multiple range test.

**Table 4. Growth and physiological responses of the studied species to foliar application of shoot and root aqueous extracts of *Thymus kotschyanus***

Treatment	Leaf area per plant (cm <sup>2</sup> )	Plant height (cm)	Shoot dry weight (g. plant <sup>-1</sup> )	Total chlorophyll content (mg. g FW)	Photosynthesis ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )	Stomatal Conductance ( $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ )
<i>Cynodon dactylon</i>						
Control	1061±31 <sup>a</sup>	54.5±3.5 <sup>a</sup>	19.0±2a	54.6±1.5 <sup>a</sup>	14.4±0.8 <sup>a</sup>	198±11 <sup>a</sup>
Shoot extract aqueous						
10%	932±34 <sup>b</sup>	46.3±3 <sup>ab</sup>	17.2±1 <sup>ab</sup>	48±4 <sup>b</sup>	11.9±1 <sup>b</sup>	172±13 <sup>b</sup>
20%	726±40 <sup>d</sup>	45.2±2.9 <sup>b</sup>	14.7±1 <sup>bc</sup>	41±5 <sup>cd</sup>	10.4±0.6 <sup>b</sup>	151±10 <sup>bc</sup>
Root extract aqueous						
10%	862±29 <sup>c</sup>	44.3±2 <sup>b</sup>	16.3±1.3 <sup>b</sup>	43±2.2 <sup>c</sup>	10.5±1 <sup>b</sup>	139±8 <sup>c</sup>
20%	617±25 <sup>e</sup>	35.7±3 <sup>c</sup>	13.4±1 <sup>c</sup>	38±4.5 <sup>d</sup>	8.5±0.8 <sup>c</sup>	98±11 <sup>d</sup>
<i>Convolvulus arvensis</i>						
Control	896±35 <sup>a</sup>	49.5±5 <sup>a</sup>	17.5±2 <sup>a</sup>	50±1 <sup>a</sup>	12.7±0.4 <sup>a</sup>	168±15 <sup>a</sup>
Shoot extract aqueous						
10%	763±20 <sup>b</sup>	43.7±2 <sup>ab</sup>	16±1 <sup>ab</sup>	46±5 <sup>a</sup>	9.9±1 <sup>b</sup>	130±11 <sup>b</sup>
20%	580±42 <sup>d</sup>	38.3±3 <sup>bc</sup>	13.3±1.5 <sup>cd</sup>	43±3.8 <sup>bc</sup>	7.5±0.9 <sup>cd</sup>	112±8 <sup>bc</sup>
Root extract aqueous						
10%	702±18 <sup>c</sup>	40.8±3.4 <sup>b</sup>	14.4±1 <sup>bc</sup>	39±4.4 <sup>c</sup>	8.1±1 <sup>bc</sup>	121±5 <sup>bc</sup>
20%	511±21 <sup>e</sup>	33.8±2 <sup>c</sup>	11.4±1 <sup>d</sup>	32±5.2 <sup>d</sup>	6.3±0.6 <sup>d</sup>	73±11 <sup>c</sup>

Values represent mean ± SD of five replicates. Means within each row sharing the same letter are not significantly different at  $P \leq 0.05$  based on Duncan's multiple range test.

Net photosynthetic rate and stomatal conductance declined significantly with increasing concentrations of *Thymus kotschyanus* extracts (Table 4). In *Cynodon dactylon*, the highest extract concentration (20 g L<sup>-1</sup>) reduced net photosynthetic rate by 27.8% and 41.0% following treatment with shoot and root extracts, respectively, compared with the control. Under the same conditions, stomatal conductance decreased by 23.7% and 51.0%, respectively. *Convolvulus arvensis* was more sensitive to the allelopathic treatments, exhibiting reductions of 41.0% and 50.4% in net photosynthetic rate and 33.3% and 56.5% in stomatal conductance following application of shoot and root extracts, respectively, relative to the control (Table 4).

#### 4. Discussion

Environmental factors, particularly those associated with allelopathic interactions, can significantly affect plant physiological and biochemical processes, resulting in reduced seed germination and impaired seedling establishment. Numerous studies showed that allelochemicals released from donor plants interfered with normal metabolic processes and consequently inhibit the early developmental stages of recipient species (Hasan et al., 2022; Saberi et al., 2021). Among these compounds, thymol, carvacrol, and other secondary metabolites produced by thyme species were reported to possess strong phytotoxic properties, leading to substantial reductions in germination performance in sensitive plant species (Konstantinovic et al., 2022; Elghobashy et al., 2024).

The observed reductions in germination percentage and germination rate may be attributed to impaired water uptake and disruptions in gibberellin-mediated metabolic pathways involved in the mobilization of seed reserves during germination. Such disturbances can adversely affect the synthesis and activity of hydrolytic enzymes responsible for the breakdown and utilization of stored reserves (Al-Mahmudy et al., 2024; Siddiqui, 2007). Successful seed germination depended on the coordinated functioning of several hydrolytic enzymes, including  $\alpha$ -amylases, proteases, and lipases, which convert storage compounds into energy and metabolic substrates required for embryo development. Allelochemicals might inhibit the activity of these enzymes and interfere with key metabolic processes essential for seedling establishment. In addition, these compounds can suppress enzymes involved in glycolysis and the pentose phosphate pathway, such as glucose-6-phosphate dehydrogenase, aldolase, and glucose phosphate isomerase. As a result, energy production and nutrient assimilation may be reduced, ultimately leading to restricted root and shoot growth (Dejam et al., 2017).

Moreover, allelopathic substances may disturb endogenous phytohormonal balance by increasing abscisic acid levels while reducing  $\alpha$ -amylase activity, thereby further inhibiting germination and early seedling growth (Li et al., 2024). Another possible mechanism underlying the inhibitory effects of allelopathic extracts is their impact on seed water relations. Osmotically active compounds may reduce water uptake, delay the onset of germination, and restrict cell expansion, resulting in reduced seedling vigor (Al-Wakeel et al., 2007). In the present study, seedling growth traits were more severely affected than germination parameters, indicating that post-germination developmental processes are particularly sensitive to allelochemical exposure. Similar findings have been reported, indicating that aqueous plant extracts exert stronger inhibitory effects on seedling growth than on germination across various plant species (Kumar et al., 2025; Smith, 1991).

The inhibitory effects of *Thymus kotschyanus* extracts on germination and early growth are mainly attributed to diverse allelochemicals, particularly phenolic compounds accumulated in plant tissues. These compounds may act individually or synergistically, producing stronger phytotoxic effects than single compounds alone (Deepmala, 2019). Phenolic compounds are widely reported allelochemicals that interfere with key physiological and biochemical processes involved in germination, seedling establishment, and early growth (Konstantinović et al., 2022; Gholami et al., 2011; Tojic et al., 2025). Their toxicity is mediated through multiple mechanisms, including inhibition of cell division, disruption of hormonal regulation, reduced nutrient uptake, suppression of protein synthesis, and impairment of membrane integrity and enzyme activity (Younesi et al., 2025; Gholami et al., 2011; Fu et al., 2019). These combined effects ultimately limit normal growth and seedling development. The reduction in leaf area may result from suppressed cell division and cell expansion in meristematic tissues, leading to reduced leaf initiation under allelopathic stress (De-Herrald et al., 1998). In addition, decreased dry matter accumulation can be associated with reduced nutrient uptake capacity and impaired photosynthetic assimilation (Dejam et al., 2017). Allelochemicals may also inhibit hydrolytic enzyme activities, particularly amylase, and reduce protein synthesis, thereby limiting the mobilization of seed reserves during early growth (Hegab et al., 2008). Furthermore, disruption of seed water uptake during imbibition can delay germination and weaken early seedling establishment (Cheng and Cheng, 2015).

The decline in photosynthetic performance under allelopathic stress was likely due to both stomatal and non-stomatal limitations. Stomatal closure induced by hormonal imbalance and altered ion homeostasis, particularly potassium fluxes, restricts CO<sub>2</sub> diffusion into leaf tissues and reduces carbon assimilation (Rai et al., 2003). In addition to stomatal effects, allelochemicals can directly impair the photosynthetic apparatus by disrupting chlorophyll biosynthesis, enhancing pigment degradation, or affecting both processes simultaneously (Yang et al., 2002). Reduced chlorophyll content is also linked to inhibition of enzymes involved in the formation of chlorophyll precursors and porphyrin metabolism (Siddiqui, 2007). Moreover, structural and functional damage to

chloroplasts, including disruption of thylakoid membranes, inhibition of electron transport, and impairment of Calvin cycle enzymes, further reduces photosynthetic efficiency (Rimando *et al.*, 2003). Consequently, the observed decline in biomass results from both reduced effective leaf area and decreased photosynthetic efficiency per unit leaf area, leading to lower dry matter accumulation.

## Conclusion

The present study demonstrated that aqueous extracts from both roots and shoots of *Thymus kotschyanus* exerted significant allelopathic effects on the germination and growth of *Convolvulus arvensis* and *Cynodon dactylon*. Since seed germination is a critical stage in weed establishment, allelochemicals released from different tissues of *Thymus kotschyanus* may reduce the competitive ability of these weed species during early growth. The inhibitory effects increased with increasing extract concentration, likely due to higher levels of bioactive compounds and their phytotoxic impacts on germination and related physiological processes. Root extracts showed stronger allelopathic activity than shoot extracts, resulting in the greatest reductions in all evaluated traits. Moreover, *Convolvulus arvensis* was more sensitive to the treatments than *Cynodon dactylon*. Overall, aqueous extracts or isolated allelochemicals of *Thymus kotschyanus* show potential as natural bioherbicides for weed management, offering an alternative to synthetic herbicides in agricultural systems. However, further studies are required to identify and isolate the most active allelochemicals and to validate these findings under field conditions across different weed-crop systems.

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