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Assessment of Biochemical Diversity in Herbal Extracts and Their Effects on the Germination, Growth, and Metabolism of *Colchicum kotschyi* Boiss. from various regions in Iran

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ABSTRACT

This study explored the effects of herbal extracts, derived from naturally occurring field herbs, on the germination, growth, and specific metabolic changes of the significant crop plant *Colchicum kotschyi* Boiss. The research was conducted in two main sections. First, morphological diversity across various populations of *Colchicum* was evaluated based on stem length, stamen count, petal number, petal length and breadth. Biochemical profiling using LC-MS identified the presence of non-enzymatic antioxidants in the plant extract, including ascorbic acid, caffeic acid, gallic acid, and rosmarinic acid, from seven distinct geographic locations. In the second part, a factorial experiment with a randomized complete block design and three replications was implemented to test the effects of *Colchicum* extract at concentrations of 0%, 50%, and 100% on the germination of wheat, barley, camelina, and weed vetch. Multiple germination and seedling growth parameters were measured, including germination percentage, sprout count, stem and root length, leaf and root number, biomass, and seedling indices. Results showed that 100% extract concentration completely inhibited germination in all plant species across both regions, while control treatments showed maximum germination rates (up to 100%). The highest root length index (24.48) was observed in control wheat and barley seeds, with a dramatic reduction in treated seeds. Principal component analysis revealed that two independent components explained 89.78% of variation. Cluster analysis grouped traits into three and genotypes into two major clusters. Regional differences had minimal influence on germination traits. The findings from both analyses unequivocally confirm the absence of kaempferol in *Colchicum* petals. Meanwhile, due to the observed effects of ascorbic acid, caffeic acid, gallic acid, and rosmarinic acid on growth and germination, aqueous extracts from the Ilam and Mishkhas regions were chosen for further study. In conclusion, 100% *Colchicum* extract strongly suppresses seed germination, suggesting its potential use as a natural bioherbicide for weed control.

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1. Introduction

Colchicum (*Colchicum kotschyi* Boiss) is a genus of perennial flowering plants containing around 160 species which grow from bulb-like corms (Noghondar *et al*., 2011). It is a member of the botanical family Colchicaceae, and is native to West Asia, Europe, parts of the Mediterranean coast, down the East African coast to South Africa and the Western Cape (Gulsoy-Toplan *et al*., 2018). Species of *Colchicum*, commonly referred to as "Sorenjân," are significant medicinal plants that have been used across various cultures to treat conditions such as osteoarthritis, gout, cancer, inflammation, jaundice, and sexual impotence. The pharmaceutical properties of *Colchicum* primarily come from its corms and seeds (Davoodi *et al*., 2021). Due to the high morphological uniformity and limited diagnostic characteristics among species, the taxonomy of the *Colchicum* genus has long been a subject of debate in identifying specific species (Persson *et al*., 2011). Despite advancements in molecular systematics, morphological characteristics continue to play a crucial role in phylogenetic studies (Patwardhan *et al*., 2014).

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Modern agriculture is in a perpetual struggle against weeds, which can significantly diminish crop yields and quality by competing for vital resources such as water, nutrients, and light (Rys *et al*., 2022). Weeds can also inflict substantial financial damage and act as secondary hosts for certain pests (Madden *et al*., 2021). Consequently, synthetic herbicides are extensively employed to combat weeds (Besati *et al*., 2024). However, the continuous use of these herbicides can have detrimental effects on human health and the environment, leading to soil and groundwater contamination, the accumulation of toxic residues in agricultural products, and the development of herbicide resistance in many weed species (Heap, 2021; Jabran *et al*., 2015). To mitigate the reliance on herbicides, integrated strategies are essential for enhancing weed management. There has been a notable surge in interest in employing biological methods to protect plants from weeds, as they are environmentally benign and cost-effective (Barratt *et al*., 2018; MacLaren *et al*., 2020).

Every year, dozens of tons of corms from various *Colchicum* species, including *C. jesdianum*, are harvested by locals and exported out of the country through various means (Sevindik *et al*., 2025). This excessive harvesting, often sold at low prices, leads to a decline in genetic diversity and destruction of plant habitats, putting these species at risk of extinction. This issue is especially prevalent in developing countries, where many medicinal and aromatic plant species face overharvesting. To address this, several strategies for conserving endangered plant species have been proposed (Rajeswara Rao, 2015). Some recommend transferring wild species to cultivation systems, while others believe that sustainable harvesting is the primary conservation strategy for most wild species. This approach supports local economies and provides long-term benefits for harvesters (Mykhailenko *et al*., 2025).

In recent decades, agricultural production has heavily relied on chemical inputs, leading to significant environmental issues (Clark and Tilman, 2017). One solution to address this problem is the adoption of sustainable agriculture principles. Sustainable agriculture involves an integrated system based on ecological principles that utilizes crop residues, manure, and organic fertilizers instead of external inputs like chemical fertilizers and pesticides. This approach not only ensures biological pest control but also increases soil nutrient storage, controls weeds, and enhances biodiversity (Altieri *et al*., 2012). Species within the *Colchicum* genus and other members of the Colchicaceae family contain important bioactive compounds, including alkaloids (especially tropolone and isoquinoline alkaloids), phenolic compounds, tannins, flavonoids, and carbohydrates. The concentration of these compounds varies with the growth cycle and season (Gulsoy-Toplan *et al*., 2018). These compounds are emitted from various plant parts -leaves, flowers, seeds, stems, and roots- as volatiles, foliage leachates, root exudates, or through decomposed plant residues, which are transformed and transported in the environment to reach other plants (Mahmoodzadeh and Mahmoodzadeh, 2014). The objective of the research was to assess the impact of herbal extracts with varying germination potentials, derived from herbs that grow naturally in fields among cultivated crops and can be utilized as natural herbicides, on the germination, growth, and selected metabolic changes of an important crop plant, *C. kotschyi* Boiss.

2. Materials and methods

2.1. Plant materials and extraction methods

To explore the morphological and biochemical diversity of the *Colchicum* and assess the impact of its extract on the germination properties of various cultivated and wild plants, petals were collected from *Colchicum* in seven regions of Ilam Province, Iran (Asmanabad, Eyvan, Mishkhas, Malekshahi, Badrah, and Darehshahr). The geographical characteristics of these regions are presented in Table 1.

Table 1. Geographical characteristics of the study areas in Ilam Province

Location	Altitude	Direction	Slope (%)	Latitude (N)	Longitude (E)
Asmanabad	1321	N	9-16	33° 49' 5.96"	46° 26' 10.66"
Eyvan	1323	E	11-19	33° 45' 10.17"	46° 21' 12.26"
Malekshahi	1427	SE	9-16	33° 22' 47.40"	46° 36' 53.85"
Mishkhas	1544	S	19-28	33° 28' 34.47"	46° 36' 51.97"
Badrah	905	SE	9-16	33° 18' 1.93"	47° 9' 23.05"
Darehshahr	1050	NW	38-48	33° 5' 51.36"	47° 20' 6.48"

North: N, East: E, Southeast: SE, South: S, Northwest: NW

2.2. Measurement of morphological characteristics

To investigate the morphological diversity of *Colchicum*, 10 samples from each region were selected completely randomly with proper intervals (Fig. 1). Some traits (petal number, petal length, petal width, stem length and number of stamens) were measured 0.5 m^{-2} in each region (Quadrat 1.0×1.0 m).

Figure 1. Different samples of the flower of *Colchicum* from six regions of Ilam province: (a) Darehshahr, (b) Mishkhas, (c) Asmanabad, (d) Badrah, (e) Malekshahi, and (f) Eyvan

2.3. Physicochemical characteristics of the soil in the region

To determine the physicochemical properties of the soil of each region, soil was collected from a depth of 30 cm. The samples were air-dried, then sieved through a 2 mm mesh and transported to the university's soil laboratory in Ilam for analysis. Key parameters such as acidity (pH), soil salinity (Ec), organic carbon (Oc), nitrogen (N), and plant-available phosphorus (Pav) were measured.

2.4. Colchicum extract

Harvest and thoroughly clean the colchicine-rich bulbs or corms, then dry them with care. Once dried, grind them into a fine powder to maximize surface area. Soak the powder in alcohol or hydroalcoholic solutions, typically with periodic agitation, for several hours to several days. Afterward, filter the mixture to separate the liquid extract from the plant residues. Finally, evaporate the filtrate under reduced pressure to concentrate the extract (Bayrak *et al*., 2019).

2.5. Effect of the extract on germination

This experiment aimed to assess the impact of *Colchicum* extract at various concentrations on the germination of three crops: wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), camelina (*Camelina sativa* L.), and the weed Vetch (*Vicia villosa* Roth L.). Seeds for the experiment were obtained from the gene bank of Ilam University. Prior to starting the experiment, seed vigor was assessed. The seeds were disinfected with a $HCLO_4$ 5% solution for 5 minutes, rinsed three times with distilled water, and then placed into Petri dishes. Eighteen seeds from each cultivar were evenly distributed across 8 cm diameter Petri dishes, with 10 seeds positioned on a filter paper layer in each dish. The germination test was conducted as a factorial experiment using a completely randomized design with three replicates. The experiment, involving *Colchicum* extract, was carried out separately in two regions, Ilam and Mishkhas, with three treatment concentrations: control, 50%, and 100%. In the control treatment, 6 ml of distilled water was added, while the extract was used for the treatment groups. The samples were incubated for 10 days in an incubator (Memmert IN/IF 55, Hettich, North America) at temperatures ranging from 19-21°C. The number of germinated seeds was recorded daily. Germination was assessed based on a 2 mm root emergence criterion (Fig. 2).

a

b

Figure 2. The effect of the extract at three different levels on the germination of wheat and barley (a) and the effect of the extract at three different levels on the germination of camelina and V. villosa (b) are reported as follows.

2.6. Morphological measurements

After 10 days, various plant traits were measured, including the number of roots, number of leaves, root length, stem length, leaf length, fresh weight of stems, fresh weight of roots, and fresh weight of leaves, using a Ruler. The separate parts (roots, stems, and leaves) were then placed in foil and dried in an oven (model Binder German) at 60°C for 24 hours. The dry weight of the samples was subsequently measured with an accurate scale. For each Petri dish, the measurements were averaged from ten sprouts. The germination percentage was calculated using the Equation 1.

(1) $$Germination\ percentage = \left(\frac{N_i}{N}\right) \times 100$$

where N_i is the number of germinated seeds on the i^{th} day, and N is the total number of seeds.

2.7. Liquid chromatography–mass spectrometry (LC-MS) analysis

Washing conditions for HPLC were established through a review of relevant literature and analytical methods, as well as preliminary tests with analytical standard solutions. For this purpose, water and methanol with 0.1% formic acid were chosen as the mobile phase to adjust the pH. The washing flow was optimized using an isocratic method (fixed percentage) at a flow rate of 0.3 mL min^{-1}. An AC18 chromatography column (C18, 5µ, 4.6×120 mm) was employed for separating the measured compounds. The final HPLC conditions are detailed in Table 2.

Table 2. Method of LC analysis

Percentage	Solvent	Mobile phase type
70%	Methanol (0.1% Formic acid)	A
30%	Water (0.1% Formic acid)	B

After separating the compounds using the HPLC system, a triple quadrupole mass spectrometer equipped with an electrospray ionization chamber was employed to identify and quantify the analytes. The analysis was conducted in negative mode, with the can and capillary voltages set to 25 V and 4000 V, respectively. The collision energy was set to 30^5 electron volts.

2.8. Statistical analysis

Descriptive statistics, including mean, median, variance, coefficient of variation, and trait correlations, were analyzed with Minitab software. Chromatograms and mass spectra of compounds identified in the aqueous extract of the *Colchicum* were generated using MestReNova software. Data on the extract's effects on plants were also organized in Excel. Analysis of variance and mean comparisons were conducted using SAS 9.1 software, with mean comparisons performed using the LSD test at a 1% probability level. Figures for mean comparisons were created using Excel.

3. Results and discussion

3.1. Physicochemical properties

The characteristics of these soils, including soil acidity, salinity, organic carbon, and available nitrogen and phosphorus, are presented in Table 3.

Table 3. Characteristics of soils in the collection areas of *Colchicum*

Sample	Acidity (pH)	E.C. (dS m^{-1})	O.C. (%)	N (%)	P (Pav)
Eyvan	8.09	0.261	1.59	0.137	9.06
Mishkhas	7.83	0.138	0.91	0.079	7.76
Badrah	7.80	0.172	2.18	0.188	6.11
Malekshahi	7.72	0.294	2.24	0.193	6.22
Asmanabad	7.60	0.256	3.66	0.316	6.83
Darehshahr	7.52	2.25	0.76	0.065	6.29

O.C.: Organic Carbon

3.2. Correlation results between the morphological characteristics of Colchicum

The correlation results between the morphological characteristics of the *Colchicum* are presented in Table 4. The data analysis results showed that the highest correlation exists between the characteristics of stem length and petal length, with a value of 0.3964, and the lowest correlation is between the characteristics of stem length and the number of stamens, with a value of 0.0103 (Table 4). This Table shows the correlation coefficients between different morphological characteristics of the *Colchicum*. The highest correlation is observed between stem length and petal length (0.3964), while the lowest correlation is between stem length and the number of Stamens (-0.0103).

Table 4. Correlation between morphological characteristics of the *Colchicum*

Characteristics	Number of stamen	Stem length	Petal width	Petal length	Number of Petals
Number of stamens	1				
Stem length	-0.0103	1			
Petal width	0.1386	0.1325	1		
Petal length	0.0571	0.3964	0.0681	1	
Number of petals	0.1671	0.1481	0.0586	0.0530	1

The results of the cluster analysis indicated that the areas were divided into three clusters. The first cluster includes the regions of Asmanabad, Darehshahr, and Eyvan. The second cluster consists of Badrah, and the third cluster includes Mishkhas and Malekshahi (Fig. 3).

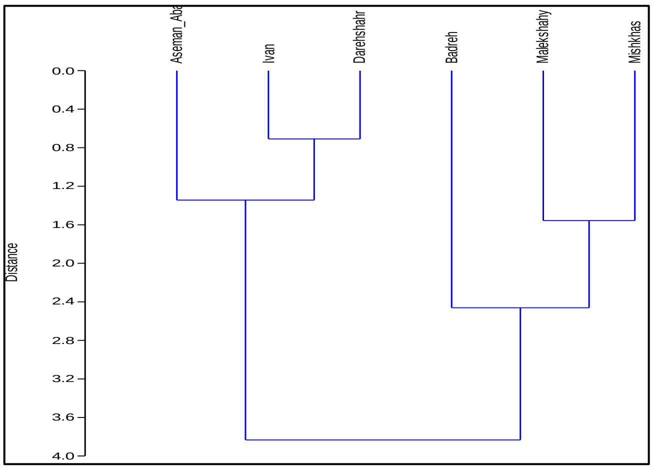

Figure 3. Cluster analysis of the regions for collecting *Colchicum*

3.3. Biological activity

After preparing the plant extracts using the Soxhlet method, LC/MS analysis was employed to identify the biological activity in the aqueous extract of the *Colchicum*. The identification of biological activity in the aqueous extract was conducted only in the regions of Asmanabad and Darehshahr. Fig. 4 and 5 lists the names of these chemical compounds along with their molecular weights. The identification of these compounds was achieved by comparing the retention times and mass spectra obtained with previous references. As shown in Fig. 4 and 5, some compounds may have two or three molecular weights, which is due to the breakdown of the molecule at different times, resulting in different fragments with different molecular weights.

The LC-MS/MS analysis is another method used for identifying biological activity by utilizing standards prepared from ascorbic acid, caffeic acid, gallic acid, rosmarinic acid, and kaempferol. This analysis involved studying the mass spectra, comparing, and examining these parameters with standard compounds. The relative percentage of each constituent compound was determined based on the area under the curve in the chromatogram spectra. The chromatogram and mass spectrum of the identified compounds in the aqueous extract prepared from *Colchicum* in the Darehshahr and Asemanabad regions are shown in Fig. 6.

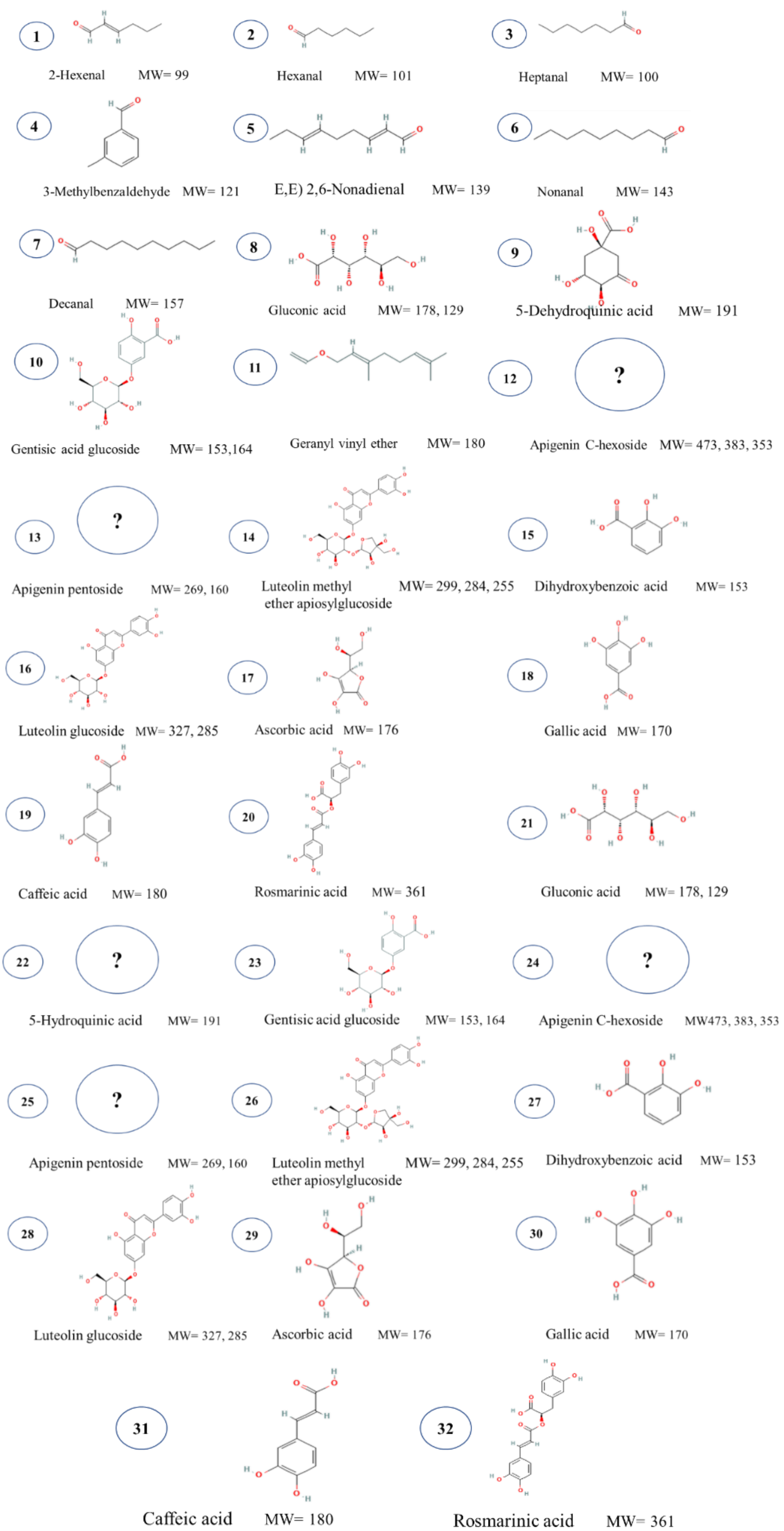

Figure 4. Biological activity was identified in the aqueous extract of *Colchicum* from Asmanabad using LC-MS/MS analysis.

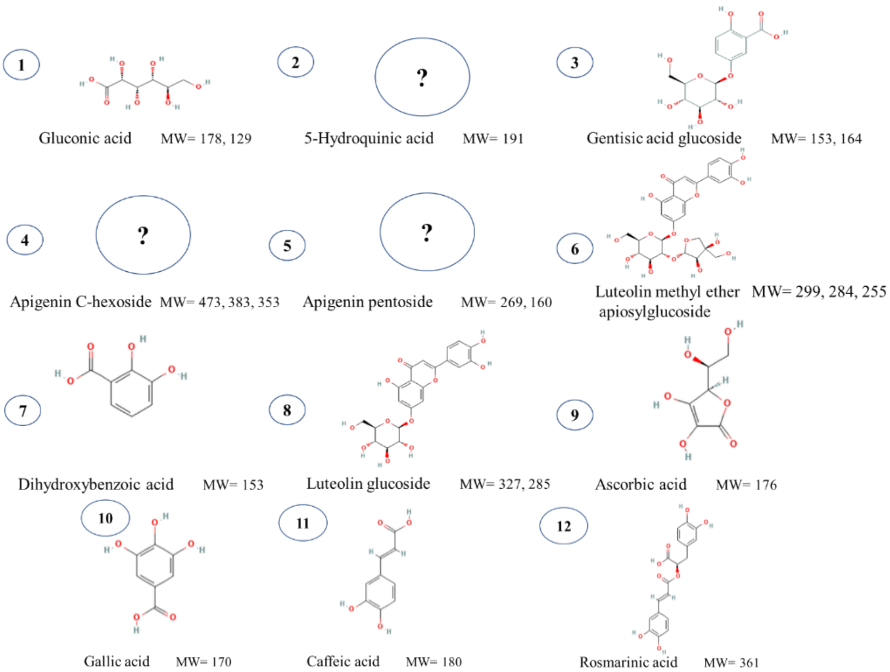

Figure 5. Biological activity identified in the aqueous extract of *Colchicum* from Darehshahr using LC/MS analysis.

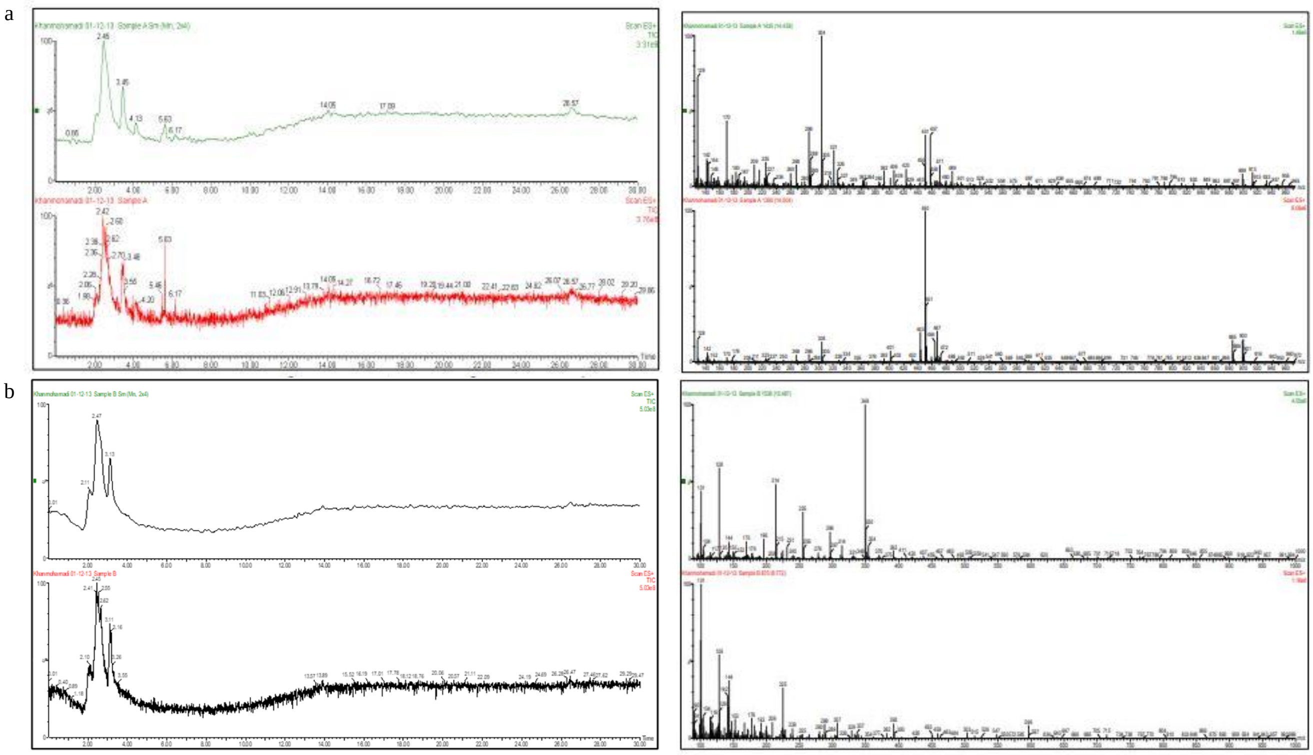

Figure 6. Chromatogram and mass spectrum (a), chromatogram and mass spectrum of the aqueous extract of *Colchicum* from Asmanabad region (b).

The results obtained from both analyses clearly indicate the absence of kaempferol in the petals of the *Colchicum*. On the other hand, considering the effects of ascorbic acid, caffeic acid, gallic acid, and rosmarinic acid on growth and germination, the aqueous extracts prepared from the Ilam and Mishkhas regions were selected. The impact of these extracts on all wheat characteristics, including germination and plantlet growth indices, was investigated. Table 5 shows the concentrations of biological activity identified in the aqueous extract of the *Colchicum* from different regions using LC-MS/MS analysis. The concentrations are expressed in micrograms per liter (μg L^{-1}). The standard deviation for each compound across the different regions is also provided.

Table 5. Biological activity identified in the aqueous extract of *Colchicum* using LC/MS/MS analysis

Region	Ascorbic acid	Caffeic acid	Gallic acid	Rosmarinic acid	Kaempferol
	(μg L^{-1})				
Eyvan	4146.6	-	-	-	-
Mishkhas	4069.7	45.6	12	-	-
Badrah	3357.5	29.8	-	2.2	-
Darrehshahr (White petals)	3289.3	55	-	5.2	-
Malekshahi	3705.6	8.66	7.6	-	-
Asmanabad	3738.1	40.75	6.11	4.4	-
Ilam	6885.4	-	-	9.65	-
Darehshahr (Purple petals)	2243.2	46.6	-	5.52	-
Standard deviation	1200.4	3817.4	55.16	332.5	110.3

3.4. Correlation between traits

In plant breeding, the correlation between traits is of particular importance because it indicates the degree and type of relationship between two or more traits. Incomplete knowledge of the relationship between different traits and selection for agricultural traits without considering other traits may not yield desired results. The correlation coefficient, as a measure of the relationship between two traits, was introduced by Francis Galton. The results of the correlation coefficients between the studied traits are presented in Table 6.

- Germination percentage: Based on the correlation coefficients of the traits (Table 6), germination percentage had a positive and significant correlation at the 1% level with the traits of leaf length (0.799), stem length (0.958), root length (0.903), number of leaves (0.988), number of roots (0.933), fresh leaf weight (0.965), dry leaf weight (0.945), fresh root weight (0.911), dry root weight (0.805), fresh stem weight (0.977), stem length to root length ratio (0.891), plantlet length (0.951), seed morphological index (0.927), and seed weight index (0.627). However, it had a positive and significant correlation at the 5% level with the trait of dry stem weight (0.493) (Table 6).
- Leaf length: The results of the correlation coefficients between the studied traits showed that leaf length had a positive and significant correlation at the 1% level with the traits of stem length (0.783), root length (0.855), number of leaves (0.762), number of roots (0.809), fresh leaf weight (0.900), dry leaf weight (0.830), fresh root weight (0.937), dry root weight (0.689), fresh stem weight (0.744), stem length to root length ratio (0.819), plantlet length (0.849), and seed morphological index (0.879). However, it had a non-significant correlation with the traits of dry stem weight (0.299) and seed weight index (0.445) (Table 6).
- Stem length: The results of the correlation coefficients between the studied traits showed that stem length had a positive and significant correlation at the 1% level with the traits of root length (0.899), number of leaves (0.933), number of roots (0.950), fresh leaf weight (0.925), dry leaf weight (0.922), fresh root weight (0.915), dry root weight (0.815), fresh stem weight (0.975), stem length to root length ratio (0.854), plantlet length (0.960), seed morphological index (0.924), and seed weight index (0.616). However, it had a positive and significant correlation at the 5% level with the trait of dry stem weight (0.490) (Table 6).
- Root length: Based on the correlation coefficients of the traits, root length had a positive and significant correlation at the 1% level with the traits of number of leaves (0.841), number of roots (0.975), fresh leaf weight (0.910), dry leaf weight (0.869), fresh root weight (0.948), dry root weight (0.860), fresh stem weight (0.839), stem length to root length ratio (0.986), plantlet length (0.981), seed morphological index (0.991), and seed weight index (0.606). However, it had a positive but non-significant correlation with the trait of dry stem weight (0.451) (Table 6).
- Number of leaves: The results of the trait correlations showed that between the number of leaves and the number of roots (0.874), fresh leaf weight (0.953), dry leaf weight (0.940), fresh root weight (0.851), dry root weight (0.741), fresh stem weight (0.971), stem length to root length ratio (0.832), plantlet length (0.8991), and seed morphological index (0.870), there was a positive and significant correlation at the 1% level. With the seed weight index (0.585), there was a positive and significant correlation at the 5% level. However, with the trait of dry stem weight (0.457), there was a positive but non-significant correlation (Table 6).
- Number of roots: The results of the trait correlations showed that the highest and lowest positive and significant correlations were observed between the number of roots and plantlet length (0.992) and seed weight index (0.647), respectively (Table 6).

Table 6. Correlation between studied traits

Variable	GR %	LL	SL	RL	LN	RN	FLW	DLW	FRW	DRW	FSW	DSW	S/R	PL	SMI	SWI
GR %	1															
LL	0.799**	1														
SL	0.958**	0.783**	1													
RL	0.903**	0.855**	0.899**	1												
LN	0.988**	0.762**	0.923**	0.841**	1											
RN	0.933**	0.809**	0.950**	0.975**	0.874**	1										
FLW	0.965**	0.900**	0.925**	0.910**	0.953**	0.915**	1									
DLW	0.945**	0.830**	0.922**	0.869**	0.940**	0.890**	0.977**	1								
FRW	0.911**	0.937**	0.915**	0.948**	0.851**	0.966**	0.919**	0.889**	1							
DRW	0.805**	0.689**	0.815**	0.860**	0.746**	0.844**	0.772**	0.796**	0.842**	1						
FSW	0.977**	0.744**	0.975**	0.839**	0.971**	0.900**	0.931**	0.939**	0.872**	0.801**	1					
DSW	0.493*	0.299ns	0.490*	0.451ns	0.457ns	0.509*	0.474*	0.420ns	0.586*	0.213ns	0.439ns	1				
S/R	0.891**	0.819**	0.854**	0.986**	0.832**	0.962**	0.889**	0.851**	0.949**	0.867**	0.819**	0.428ns	1			
PL	0.951**	0.849**	0.960**	0.981**	0.8991**	0.992**	0.942**	0.915**	0.960**	0.865**	0.919**	0.479*	0.959**	1		
SMI	0.927**	0.879**	0.924**	0.991**	0.870**	0.977**	0.935**	0.893**	0.951**	0.854**	0.875**	0.476*	0.965**	0.991**	1	
SWI	0.627**	0.445ns	0.616**	0.606**	0.585*	0.647**	0.618**	0.567*	0.715**	0.379ns	0.566*	0.981**	0.583*	0.627**	0.628**	1

Note: The correlation coefficients are presented with significance levels. ** and *: Significant at 1% and 5%, respectively, ns: nonsignificant. GR: Germination; LL: Leaf length; SL: Stem length; RL: Root length; LN: Leaf number; RN: Root number; FLW: Fresh leaf weight; DLW: Dry leaf weight; FRW: Fresh root weight; DRW: Dry root weight; FSW: Fresh stem weight; DSW: Dry stem weight; S/R: Stem/Root length ratio; PL: Plantlet length; SMI: Seed morphological index; SWI: Seed weight index.

- Fresh leaf weight: Between fresh leaf weight and the traits of dry leaf weight (0.977), fresh root weight (0.919), dry root weight (0.772), fresh stem weight (0.931), stem length to root length ratio (0.889), plantlet length (0.942), seed morphological index (0.935), and seed weight index (0.618), there was a positive and significant correlation at the 5% level. However, with the trait of dry stem weight (0.474), there was a positive and significant correlation at the 5% level (Table 6).
- Dry leaf weight: The results of the correlation table showed that between dry leaf weight and fresh root weight (0.889), dry root weight (0.796), fresh stem weight (0.939), stem length to root length ratio (0.851), plantlet length (0.915), and seed morphological index (0.893), there was a positive and significant correlation at the 1% level. With the seed weight index (0.567), there was a positive and significant correlation at the 5% level. However, with the trait of dry stem weight (0.420), there was a positive but non-significant correlation (Table 6).
- Fresh root weight: The highest positive and significant correlation at the 1% level was observed between fresh root weight and plantlet length (0.960), and the lowest was with the seed weight index (0.715) (Table 6).
- Dry root weight: The results of the correlation table showed that between dry root weight and fresh stem weight (0.801), stem length to root length ratio (0.867), plantlet length (0.865), and seed morphological index (0.854), there was a positive and significant correlation at the 1% level. With the traits of dry stem weight (0.213) and seed weight index (0.379), there was a positive but non-significant correlation (Table 6).
- Fresh stem weight: The results of the trait correlations indicated that the highest positive and significant correlation at the 1% level was between fresh stem weight and plantlet length (0.919), and the lowest was with the seed weight index (0.566). Additionally, there was a positive but non-significant correlation between fresh stem weight and dry stem weight (0.428) (Table 6).
- Dry stem weight: Between dry stem weight and stem length to root length ratio (0.428), there was a positive but non-significant correlation. However, with plantlet length (0.479) and seed morphological index (0.476), there was a positive and significant correlation at the 5% level. With the seed weight index (0.981), there was a significant correlation at the 1% level (Table 6).
- Stem length to root length ratio: Based on the correlation coefficients, there was a positive and significant correlation at the 1% level between stem length to root length ratio and plantlet length (0.959), seed morphological index (0.965), and seed weight index (0.583) (Table 6).
- Plantlet length: The results of the correlations showed that between plantlet length and seed

morphological index (0.991) and seed weight index (0.627), there was a positive and significant correlation at the 1% level (Table 6).

- Seed morphological index: Between this trait and the seed weight index (0.628), there was a positive and significant correlation at the 1% level (Table 6).

3.5. The effect of the extract on germination

The results of the mean comparison for the triple interaction effect of extract concentration × region × genotype on germination percentage showed that the highest germination percentage, with an average of 100%, was observed in all genotypes of wheat, barley, camelina, and cluster vetch in both Ilam and Mishkhas regions under control treatment. The 50% extract concentration in camelina in Ilam, followed by the 50% extract concentrations in wheat in both regions and cluster vetch in Ilam, were ranked next in terms of germination percentage. The lowest germination percentage, with an average of zero, was observed in treatments with 100% extract concentration in the genotypes of wheat in both regions, barley in both regions, camelina in both regions, and cluster vetch in both regions. Statistically, there was no significant difference between these treatments and the 50% extract concentration in barley in both regions, the 50% extract concentration in camelina in Mishkhas, and the 50% extract concentration in cluster vetch in Mishkhas (Fig. 7), which is consistent with the following reports.

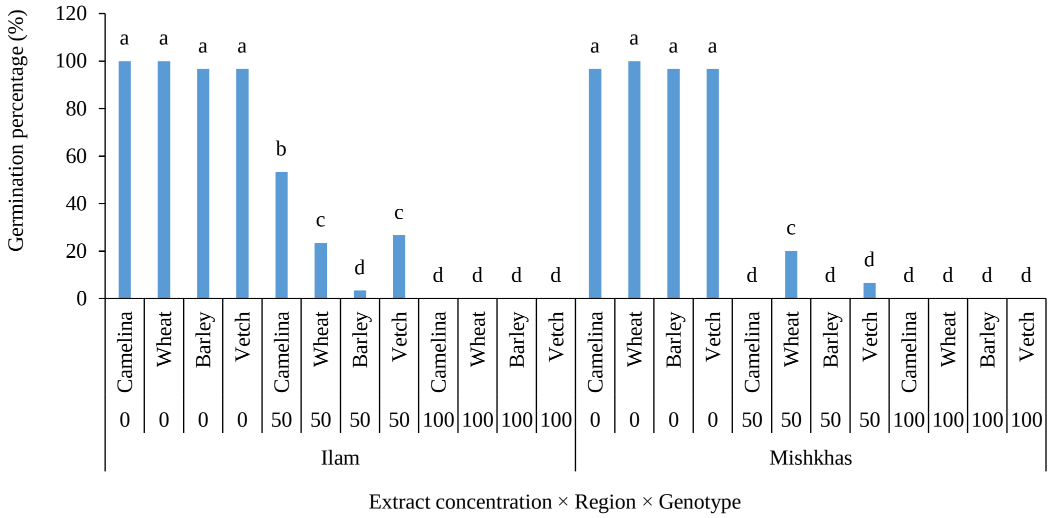

Figure 7. Interaction of extract concentration, region and genotype on different plants germination.

3.6. Principal components analysis for the studied traits

The technique of principal components analysis (PCA) is one of the oldest and most well-known methods for multivariate analysis, serving as a way to reduce data dimensions and explain the overall variability of the data. PCA aimed to identify a few principal components that can be used as diagnostic criteria. These components explain almost 100% of the existing variability between the original variables, allowing them to be used in subsequent analyses without losing much information. The first component accounts for the maximum variability, and subsequently, the last component accounts for the minimum variability. The scree plot of this study showed that only two components (out of 16 possible components) were sufficient to explain the data variability (Fig. 8). This is because, after the second component, the eigenvalues and the percentage of variance sharply decrease, following a straight line. The PCA of this study indicated that two principal and independent components together explained 89.78% of the total variability. The first component explained 81.7% of the total variance, with positive and significant factor loadings for the traits of stem length, root length, number of roots, dry leaf weight, fresh root weight, dry root weight, stem length to root length ratio, plantlet length, seed morphological index, and seed weight index. This component was named the "plantlet length component." The second component explained 8.8% of the total variance, with positive and significant factor loadings for the traits of germination percentage, number of leaves, fresh stem weight, and dry stem weight, and negative and significant factor loadings for the traits of leaf length and fresh leaf weight. This component was named the "dry stem weight component" (Table 7). Given the high percentage of variance explained by the first component (89.17%), most of the differences in the values related to these traits are likely to be found in this component. Therefore, to achieve ideal trait values, efforts should be made to increase these components. Traits with higher factor loadings in the first component can be used for selecting superior genotypes.

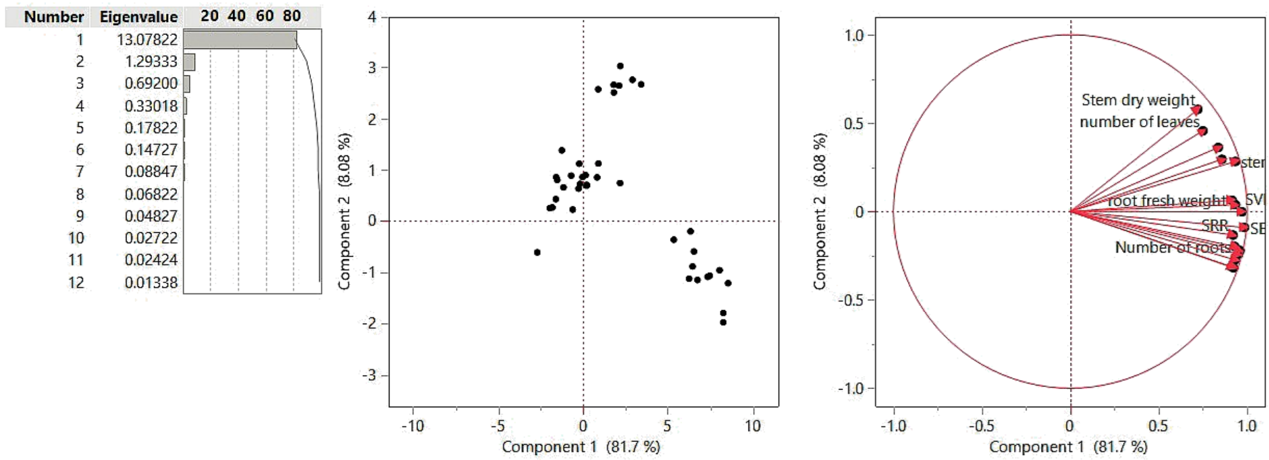

Figure 8. Scree plot of eigenvalues for the principal components

Table 7. Eigenvalues for the evaluated traits using principal components analysis (PCA)

Traits	First component	Second component
Germination percentage	0.23	<u>0.32</u>
Leaf length	0.26	<u>-0.28</u>
Stem length	<u>0.26</u>	0.25
Root length	<u>0.26</u>	-0.21
Leaf number	0.20	<u>0.40</u>
Root number	<u>0.26</u>	-0.17
Fresh leaf weight	0.25	<u>-0.27</u>
Dry leaf weight	<u>0.26</u>	-0.24
Fresh root weight	<u>0.25</u>	-0.05
Dry root weight	<u>0.26</u>	0.03
Fresh stem weight	0.24	<u>0.26</u>
Dry stem weight	0.2	<u>0.50</u>
Stem/Root length ratio	<u>0.24</u>	-0.11
Plantlet length	<u>0.27</u>	-0.08
Seed morphological index	<u>0.26</u>	-0.19
Seed weight index	<u>0.26</u>	-0.002
Eigenvalue	13.08	1.29
Relative variance (%)	81.7	8.8
Cumulative variance (%)	81.7	89.78

Note: The numbers with underlines indicate higher values in the principal components analysis.

3.7. Cluster analysis of the data

In this study, cluster analysis was used to group genotypes and determine their relatedness based on important germination traits. Ward's method, along with Euclidean distance, was employed for the cluster analysis. The analysis, conducted separately for four genotypes and 17 traits, resulted in the formation of two and three distinct intra-cluster and inter-cluster groups, respectively. The formula √(N/2) (where N represents the number of genotypes or traits) was used to cut the dendrogram and determine the number of groups.

The results of this analysis showed that the genotypes of wheat and barley, belonging to the same family, were grouped together, indicating a similar response of these two plants to treatments and the studied traits. Other plants from different families were placed in separate groups. The genotypes of *Camelina sativa* and *Vicia sativa* were clustered together in another group. For the clustering of the studied traits, the analysis revealed that the traits were categorized into three groups. In this case, SER, stem length, dry root weight, dry leaf weight, leaf number, dry stem weight, SSR, and germination percentage were placed in the first cluster; SVI, root number, root length, fresh leaf weight, and leaf length were in the second cluster; and finally, fresh stem weight, SVII, fresh root weight, and total germination number were in the third cluster. The largest cluster, the first one, included eight traits (Fig. 9).

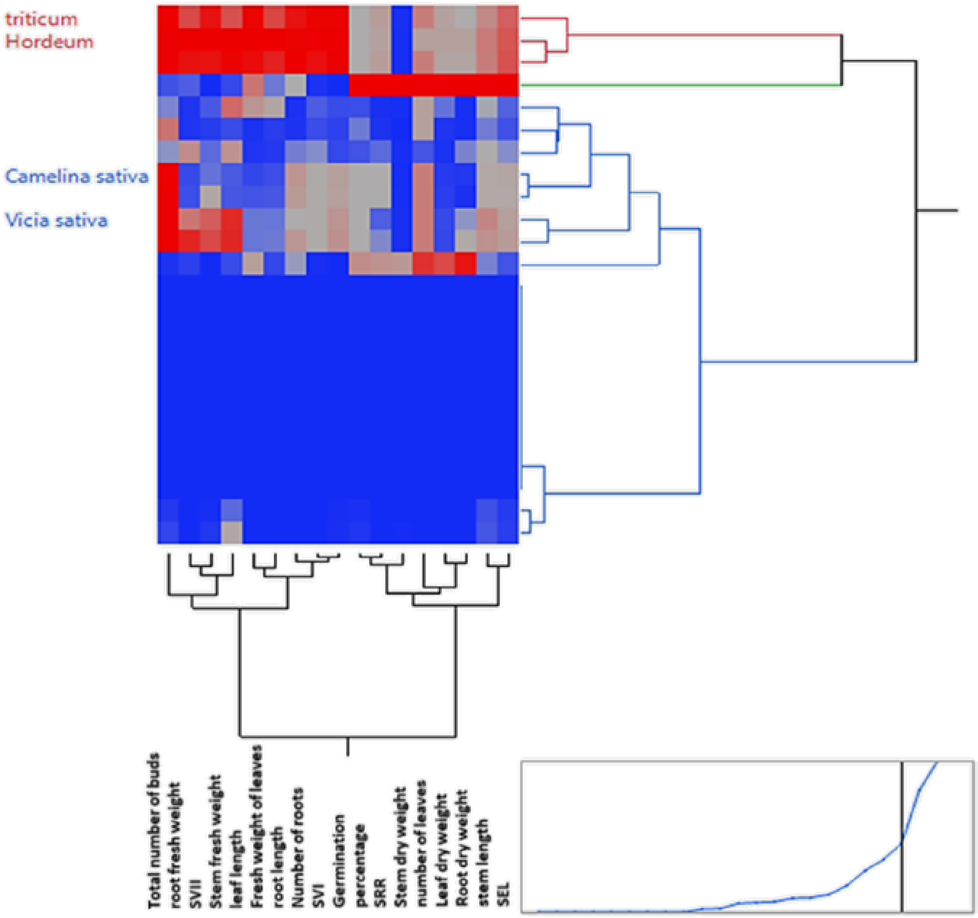

Figure 9. Cluster analysis of the effects of *Colchicum* extract on agricultural plant traits

One of the most significant problems in agriculture is the use of chemical herbicides to control weeds in fields. Due to the increasing costs and environmental pollution associated with these herbicides, the importance of initiating new research to reduce dependency on them becomes evident (Yadegari, 2017). Currently, one of the recognized methods for minimizing the use of chemical toxins is to improve soil fertility, produce high-quality and high-quantity crops, and utilize safe resources such as plant extracts. The results obtained in this study align with those found for *C. kotschyi*, examined in the Mashhad region of Iran, despite the enhanced nitrogen and phosphorus levels in other parameters (Alirezaie *et al*., 2012). Due to the mentioned properties, in the present research, which consisted of two distinct parts, a type of plant extract was used. This dissertation was designed and conducted in two separate parts. After a step-by-step review of each part, the results obtained in each section are described as follows:

In the first part, the morphological diversity and identification of the compounds forming the aqueous extract of *Colchicum* in the Ilam and Mishkhas regions were studied. The descriptive statistical results showed that the least variation coefficient in morphological traits was related to the number of petals (6.15) and the highest variation coefficient was related to the width of *Colchicum* petals (134.10). The results obtained from the correlation table showed that the highest correlation between traits was between stem length and petal length, with a value of 0.3964, and the lowest correlation was between stem length and the number of stamens, with a value of 0.0103. The results of the cluster analysis showed that the areas were divided into three clusters. The first cluster included the areas of Asemanabad, Darehshahr, and Eyvan; the second cluster included Badrah; and the third cluster included the areas of Mishkhas and Malekshahi. The results of the LC-MS/MS analysis showed that the highest amount of gallic acid was present in the Mishkhas extract, and the highest amount of Ascorbic acid was present in the Ilam extract. In the second part, the effect of plant extracts from the Ilam and Mishkhas regions on germination indices and plantlet growth of three genotypes of Camelina, barley, wheat, and clover was studied. Yadegari (2017) reported that the highest germination percentage of alfalfa was observed in the control treatment, and the lowest percentage was obtained with the use of thyme and sage extracts at a concentration of 45 g L^{-1}. The reason for this can be attributed to the presence of certain germination inhibitors in the aqueous extracts of these plants, such as caffeine and vanillin. Additionally, for sainfoin seeds, the highest germination percentage was observed in the control treatment, and the lowest values were obtained with the use of yarrow extract at a concentration of 45 g L^{-1} (Pirzad *et al*., 2012; Yadegari, 2017). Bayat et al. (2020) also reported that increasing the concentration of narcissus bulb and leaf extracts reduced the germination percentage of the seeds of Agropyron and oat plants. Molaei Kordabad et al. (2022) demonstrated that the allelopathic effect of medicinal plants on the germination of *Cuscuta campestris* Yuncker. seeds showed that aqueous extracts of rose and *Humulus lupulus* (15%) reduced the germination of dodder seeds.

The results showed that the effect of the region on all traits, except for the total number of seedlings and germination percentage, was not significant. Therefore, there was no difference between the two regions of Ilam and Mishkhas in terms of leaf length, stem length, root length, number of leaves, number of roots, fresh leaf weight, dry leaf weight, fresh root weight, dry root weight, fresh stem weight, dry stem weight, stem length to root length ratio, plantlet length, seed morphological index, and seed weight index. Furthermore, the control treatments in all four genotypes of wheat, barley, Camelina, and clover in both Ilam and Mishkhas locations had the highest total number of seedlings and germination percentage. Therefore, it can be concluded that the *Colchicum* extract at concentrations of 50% and 100% caused a reduction in germination in all four studied genotypes. In fact, with an increase in extract concentration, their inhibitory effect also increased, which was consistent with the following results. The control treatment in the genotypes of wheat and barley resulted in the highest leaf length, root length, number of leaves, dry leaf weight, plantlet length, and seed morphological and weight indices. Because with an increase in the concentration of the aqueous *Colchicum* extract in these plants, the amount of germination and plantlet growth indices decreased. The compounds present in the aqueous *Colchicum* extract (high concentration of caffeic acid, ascorbic acid, and gallic acid) can reduce plant growth with their toxic and anti-germination effects and generally lead to a decrease in plant performance. Therefore, considering the different conditions of plant cultivation, the use of *Colchicum* extracts at concentrations of 50% and 100% may be an effective method to prevent the germination of wild plants and, at lower concentrations, may improve the germination and growth of agricultural plants in fields. However, it should be noted that the use of high concentrations of *Colchicum* extracts may cause serious damage to agricultural plants. Therefore, when applying these substances for priming agricultural plants, appropriate and principled amounts should be considered.

In general, and considering the results obtained from the present research, it can be said that the aqueous *Colchicum* extract had an inhibitory effect on the germination and plantlet growth of all the studied agricultural and wild plants in this research. Therefore, the use of this type of extract at these two concentrations is not recommended for improving germination and plant growth in future studies. However, it can be used as a strong compound for controlling weeds in the context of organic farming and may also be used in the production of biological herbicides.

4. Conclusion

In summary, this study highlights the significant morphological, biochemical, and genetic diversity among *C. kotschyi* populations in Iran, with notable variation in traits and phytochemical profiles. The identification of key antioxidant compounds and the demonstration of their strong, concentration-dependent inhibitory effects on seed germination underscore the potential of Colchicum extracts as natural bioherbicides. While the results are promising for weed management, especially in organic systems, further research is necessary to validate these findings under field conditions, assess environmental impacts, and optimize application strategies. Future investigations should focus on long-term effects, formulation development, and expanding the range of target species to fully harness the practical applications of Colchicum-based bioherbicides in sustainable agriculture.

Conflict of interests

All authors declare no conflict of interest.

Ethics approval and consent to participate

No humans or animals were used in the present research. The authors have adhered to ethical standards, including avoiding plagiarism, data fabrication, and double publication.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

All authors had an equal role in study design, work, statistical analysis and manuscript writing.

Informed consent

The authors declare not to use any patients in this research.

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