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Impact of Climatic and Geographical Conditions on Morphological Diversity in Harmel (*Peganum harmala*) Ecotypes: A Study from Western Iran

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ABSTRACT

Given the significance of the harmel plant (*Peganum harmala*) in various pharmaceutical and health industries, along with its extensive geographical distribution across certain regions of the country, this experiment was designed to assess the impact of climatic and geographical conditions on morphological traits. The study evaluated the genetic diversity of different harmel ecotypes collected from various locations in western Iran. Future research will investigate the effects of climatic factors and environmental conditions across four habitats: three located in Asadabad (Hamedan) Mousi-Abad Village 1, Mousi-Abad Village 2, and Najaf-Abad Village 3 and one in Kangavar (Kermanshah), specifically Kharkhane Village, situated in western Iran. The study aims to assess how these varying habitats influence the morphological traits of harmel plants. harmel samples were collected and identified simultaneously in early July 2023. The results of the analysis of variance indicated that habitat significantly influenced various traits at the 1% probability level, including chlorophyll index, height, number of flowers per plant, number of leaves, number of branches, fresh weight of a single stem, total fresh weight of the bush, and dry weight of a single stem. Additionally, a significant effect was observed at the 5% probability level for stem diameter. Ecotype No. 4 (from Mousi-Abad Village 2) exhibited a higher total dry weight yield compared to the other ecotypes studied. Principal component analysis identified key components related to seed yield and plant height, while cluster analysis successfully differentiated the harmel growing habitats across the two provinces. This research contributes significantly to our understanding of harmel ecotypes' morphological diversity and adaptability to different habitats in Iran. The findings highlight the importance of environmental factors in shaping plant traits and provide a foundation for future studies aimed at optimizing harmel cultivation practices. By integrating traditional knowledge with modern statistical methods, it is possible to enhance the sustainability and productivity of this valuable medicinal plant.

Keywords: Cluster analysis, Genetic diversity, Habitat, Morphology, Medicinal plant, Principal Components Analysis

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The medicinal plant *Peganum harmala* L. commonly known as African rue, belongs to the Zygophyllaceae family. It is one of the notable medicinal plants in Iran, possessing a wide range of physiological and therapeutic properties due to the active medicinal compounds found in its reproductive and vegetative organs. The significance of this plant species in traditional medicine and the pharmaceutical industry underscores its economic importance for development and production. There are several reasons for the expansion and cultivation of *Peganum harmala*, including its value as a vegetation cover, its critical role in restoring local ecosystems, and the presence of important medicinal compounds [1]. The leaves of the *P. harmala* plant are light green with various irregular divisions and feature relatively large flowers that have five narrow sepals and five white petals. This plant possesses woody stems with numerous branches and produces fruit in the form of a round, three-part capsule [2]. In general, we can be able to take basic steps regarding the sustainability of rangeland use provided that the Indigenous and local knowledge of the people living in the same place is used to identify the medicinal plants of each section and region. Therefore, this can provide important basic information to various scientists in this research field [3]. Despite the fact that today a significant part of the drugs for treating patients are of chemical origin, currently, about 30% of pharmaceutical products are of plant origin [4], the importance of recognizing, researching and studying medicinal plants is very useful. Therefore, rangelands as natural resources, if managed properly, can be effective on the economy of the users because rangelands are the ecological origin of many plants, including medicinal plants [2]. In a report by Kakaei et al. [5], in the study of the oilseed rape plant, using the statistical method of cluster analysis, the genotypes studied were grouped using the UPGMA method and the dendrogram cut was performed with discriminant function analysis. In the study of chickpea genotypes, researchers used cluster analysis to detect the relationship between the studied chickpea genotypes and introduced it as a valuable statistical method for grouping genotypes [6]. Bakhtiar et al. [7] in the study of diversity in agro-morphological traits of fenugreek medicinal plants stated that two species *Trigonella filipes* and *Trigonella spruneriana* can be selected as suitable species for use in agricultural and pharmaceutical systems. Identifying diverse traits in plants, understanding their functions, and examining their mutual effects can enhance research programs. However, it is also crucial to study the relationships between traits separately [8]. The use of multivariate statistical methods is highly effective in detecting the genetic potential of traits in relation to breeding goals. These methods allow for the simultaneous analysis of multiple variables, facilitating the exploration of their interrelationships. They are particularly valuable for identifying key traits that influence yield and determining the relative contribution of each trait to overall productivity [6]. In a study Kakaei & Mazahery Laqab [9], multivariate statistical methods such as cluster analysis, principal component analysis, and factor analysis were used to study the genetic diversity of alfalfa ecotypes, and these methods were considered reliable methods for distinguishing the varieties and traits under study. The harmel plant contains important alkaloids in its various parts including roots, seeds, leaves, stems, and flowers [10] including harmaline, harmine, harmane, harmalol, harmol, tetrahydroharmine and opioids [11, 12 and 13] as well as peganine, isopeganine, dopamine, gamma-aminobutyric acid, hydroxytryptamine, benzodiazepine, imidazoline and aromatic aldehydes including benzaldehyde and methoxybenzaldehyde [14, 11 and 15]. In another study [16], they stated that they used the multivariate statistical method of principal component analysis in studying the morphological and micromorphological traits of flowers and fruits of the genus Sanguisorba, and they also stated that the results of principal component analysis led to the formation of two groups and complete separation of the evaluated species and subspecies.

Considering the importance of the Harmel plant in various pharmaceutical and health industries, as well as its extensive geographical distribution in certain regions of the country, this experiment was designed to assess the effects of climatic and geographical conditions on morphological traits. The study evaluated the genetic diversity of different Harmel ecotypes collected from various regions in western Iran, specifically in Hamedan province (three samples) and Kermanshah province (one sample), using various statistical methods.

MATERIAL AND METHODS

Plant Materials

To investigate the morphological characteristics of the harmel plant across four habitats, an experiment was conducted using a Randomized Complete Block Design with three blocks in 2023. This study examined morphological traits related to four habitats: three in Asadabad (Mousi-Abad Village 1, Mousi-Abad Village 2, and Najaf-Abad Village 3) and one in Kangavar (Karkhaneh Village), located in western Iran (Table 1). Harmel plant samples were collected and identified simultaneously in early July 2023. The samples were randomly collected in their habitats. The following traits were evaluated for the identified ecotypes of harmel in these habitats: chlorophyll index, stem height, number of flowers per plant, number of leaves, number of branches per stem, number of nodes, number of branches per compartment, total fresh weight of the compartment, dry weight of a single stem, fresh weight of a single stem, total dry weight of the compartment, and stem diameter. All measurement methods for the mentioned traits were done with standard methods.

Statistical Studies

SPSS version 26 software was used to perform simple statistical analyses such as analysis of variance and comparison of means, and multivariate statistical analyses such as cluster analysis and principal component analysis.

Table 1 Location of the habitat of the studied harmel samples

Row	Habitat name	City	State
1	Mousi-Abad village 1	Asad-Abad	Hamedan
2	Mousi-Abad village 2	Asad-Abad	Hamedan
3	Najaf-Abad village	Asad-Abad	Hamedan
4	Karkhaneh village	Kangavar	Kermanshah

The samples collected are from Hamedan and Kermanshah provinces. These two provinces are from the western provinces of Iran and have a moderate-cold climate. These two provinces are geographically located next to each other.

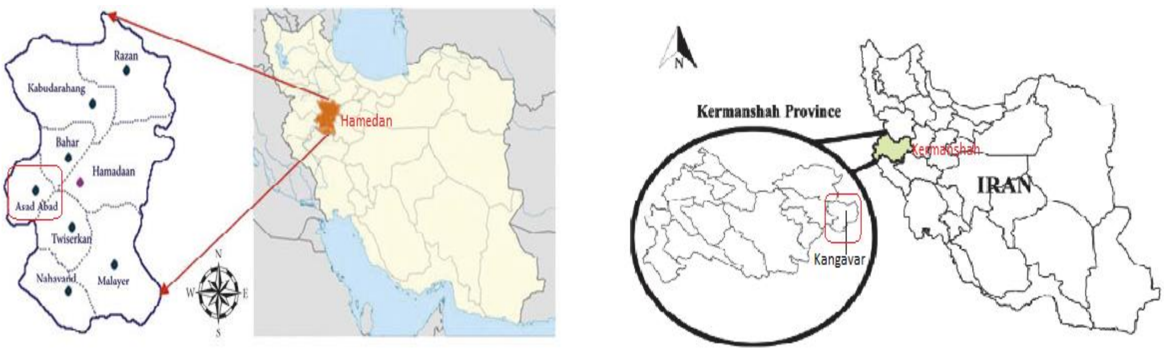

Fig. 1 Location map of the geographical area (study area) for the collection of harmel samples in some areas of Hamedan and Kermanshah provinces.

Table 2 Traits studied in harmel ecotypes

Row	Traits name	Row	Traits name	Row	Traits name
X1	Chlorophyll Index	X5	Number of Branches per Stem	X9	Dry Weight of Single Stem
X2	Stem Height	X6	Number of Nodes	X10	Fresh Weight of Single Stem
X3	Number of Flowers per Plant	X7	Number of Branches per Plant	X11	Dry Weight of the Whole Plant
X4	Number of Leaves	X8	The Weight of the Entire Plant	X12	Stem Diameter

The traits studied in this research are desirable traits that are used in most researches.

RESULTS

Analysis of Variance and Comparison of Mean Traits

The results of the analysis of variance indicated that habitat had significant effects at the 1% probability level (P ≤ 1%) on the chlorophyll index, height, number of flowers per plant, number of leaves, number of branches, fresh weight of a single stem, total fresh weight of the bush, and dry weight of a single stem. Additionally, a significant effect was observed at the 5% probability level (P ≤ 5%) for stem diameter (Table 3). Table 4 presents the comparison of the average values of the evaluated traits across the four harmel ecotypes. As shown in the table, ecotype number 4 (from Mousi-Abad Village 2) exhibited a higher total dry weight yield than the other ecotypes studied. In the biplot space (Figure 4), ecotype number 4 is positioned distinctly from the other ecotypes, This ecotype also demonstrated a high level of chlorophyll, which enhances photosynthesis and metabolic processes in the plant, resulting in superior performance. According to the same table, ecotype number 4 had the highest values for traits such as number of leaves, number of nodes, and dry weight of a single stem, while ecotype number 1 (from Mousi-Abad Village sample 1) had the lowest values for these traits.

Table 3 Variance analysis of studied traits in different harmel ecotypes

S.O.V.	df	Mean square of traits						
		Number of branches per plant	Number of nodes	Number of branches per stem	Number of leaves	Number of flowers per plant	Stem height	Chlorophyll index
Replication	2	0.001	3.583	1.083	11.083	27.583	1.750	67.943
Variety	3	166 ns	2.222 ns	3.333 **	1014.556 **	706.972 **	65.861 **	1241.714 **
Error	6	0.001	1.139	0.417	16.639	23.472	2.528	53.144
(C.V)		0.04	0.1	0.2	0.45	0.69	0.22	0.65

*, ** and ns: Significant at P< 0.05, P< 0.01 and insignificant respectively.

Continuation of Table 3 Variance analysis of studied traits in different harmel ecotypes

S.O.V.	df	Mean square of traits				
		Stem diameter	Fresh weight of single stem	Dry weight of the whole plant	Dry weight of single stem	Dry weight of the whole plant
Replication	2	0.583	1.683	0.106	0.682	0.001
Variety	3	1.417 *	112.87 **	2929.907 **	31.65 **	2690.679 ns
Error	6	0.250	6.115	0.160	1.618	0.0001
(C.V)		0.19	0.21	0.18	0.77	0.35

*, ** and ns: Significant at P< 0.05, P< 0.01 and insignificant respectively.

Table 4 Comparison of the average traits evaluated in four harmel ecotypes

	Traits											
Ecotype	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12
Ecotype 1	56.833	34.66	16	47	5.66	13.33	45	7	6.696	270.33	3.2	140
Most	70.10	37	19	48	6	14	45	7	8.62	271	4.1	140
Least	47.1	33	14	45	5	13	45	7	5.44	270	2.5	140
Range	23	4	5	3	1	1	0.01	0.01	3.18	1	1.6	0.01
Ecotype 2	66	38.33	44.33	68.33	7	14.66	36	5.66	16.76	320.17	7.9	152.81
Most	7.8	39	50	72	8	16	36	6	19.54	320.52	9.3	152.81
Least	58.1	38	38	65	6	14	36	5	13.13	320	6.3	152.81
Range	12.7	1	12	7	2	2	0.01	1	6.41	0.52	3	0.01
Ecotype 3	24.44	43.33	50.33	73.66	8	14	53	6	19.3	318.23	9.36	181.29
Most	27.1	45	57	80	9	15	53	6	21.14	318.7	10.2	181.29
Least	21.8	42	46	68	7	13	53	6	17.91	318	8.5	181.29
Range	5.3	3	11	12	2	2	0.01	0.01	3.23	0.7	1.7	0.01
Ecotype 4	68.7	32.66	43.66	91.66	6	15.33	50	7	19.9	260.71	10.63	109.08
Most	75.1	34	48	95	6	17	50	8	22.07	260.71	12.20	109.08
Least	64.8	31	39	89	6	13	50	6	18.57	260.71	9.7	109.08
Range	10.30	3	9	6	0.01	4	0.01	2	3.5	0.01	2.5	0.01

The Characteristics of the names of the studied ecotypes and traits are based on Table 1 and Table 2, respectively.

Cluster Analysis

To assess genetic diversity and classify the studied ecotypes based on the evaluated traits, cluster analysis using the WARD method was employed. A dendrogram was created to visualize the results, which classified the ecotypes into two distinct groups (Figure 2). The first group comprised the ecotypes from Mousi-abad 1, Najaf-Abad, and Mousi-Abad 2 in Asadabad City, while the second group included the ecotypes from Karkhaneh Village in Kangavar City. The findings indicated that the three ecotypes from Asadabad were clustered together in the first group, whereas the ecotype from Kangavar was placed in a separate cluster. This separation highlights the differences in the evaluated traits across the various habitats and demonstrates the effectiveness of cluster analysis in distinguishing plants grown in these environments.

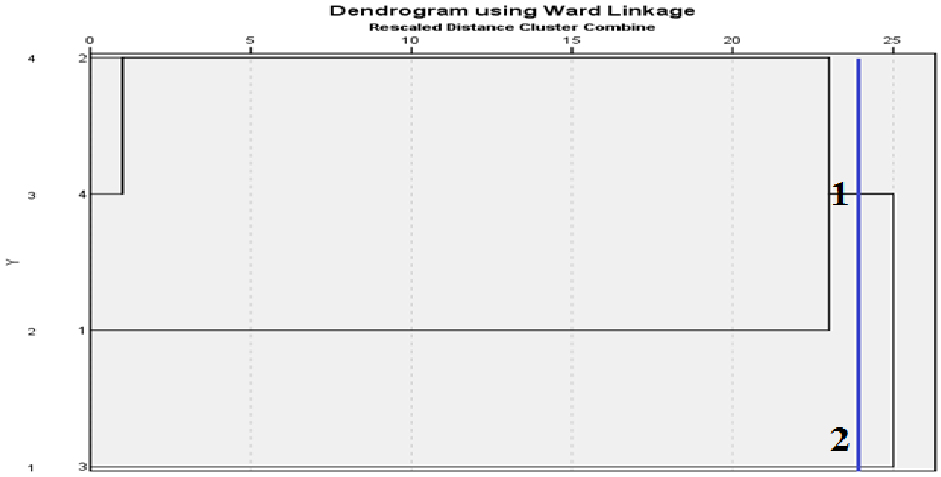

Fig. 2 Dendrogram of cluster analysis by WARD method and Pearson distance matrix square in four harmel ecotypes.

Cluster analysis is a group of multivariate techniques whose primary purpose is to group people or materials based on their attributes. Then it puts people with similar traits in a cluster with mathematical language." In cluster analysis, people within a cluster have the most similarity and uniformity, and there is maximum difference and non-uniformity between clusters. Therefore, if the grouping is successful, the components or individuals within the cluster will be genetically closer to each other and the distant clusters will be different.

Figure 3 illustrates the dendrogram resulting from cluster analysis using the WARD method and the squared Pearson distance matrix applied to 12 traits of the harmel plant. According to this dendrogram, the traits of number of flowers per plant, number of leaves, number of branches per compartment, stem diameter, dry weight of a single stem, and total dry weight of the compartment are grouped in the first cluster. In contrast, the second cluster comprises traits such as the harmel chlorophyll index, stem height, number of branches per compartment, number of nodes, fresh weight of a single stem, and total fresh weight of the compartment. Within the first cluster, the traits are closely related to one another, while in the second cluster, traits that are also interrelated such as the fresh weight of a single stem and total fresh weight of the compartment are grouped.

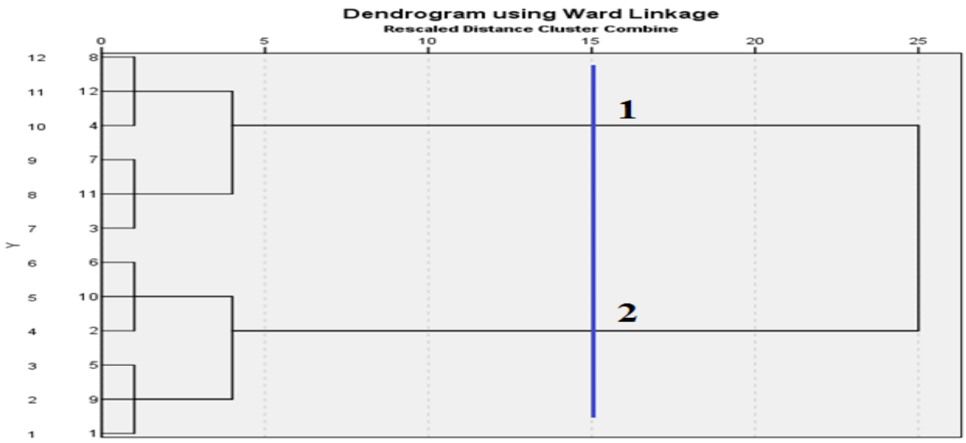

Fig. 3 Dendrogram of cluster analysis by Ward's method and Pearson's distance matrix square in 12 features of harmel plant

Principal Component Analysis

The results in Table 5 indicate that the first three components accounted for 89% of the total data variation. Specifically, Table 6 reveals that the first component alone explained 43% of the total variance. The most significant traits influencing this component were the number of branches per stem (0.382), stem height (0.370), and total dry weight of the bush (0.362). All these traits exhibited a strong positive relationship with the first component, which can therefore be termed the "yield component". Selecting for higher values of this component is likely to result in the identification of ecotypes with greater yields, making a higher level of the first component desirable. According to Table 6, the second component explains 75% of the data variation and has a strong positive relationship with stem diameter (0.322) and stem height (0.240). Conversely, it shows a strong negative relationship with the number of leaves (−0.428), number of nodes (−0.388), and dry weight of a single stem (−0.358). This suggests that increases in stem height and diameter negatively impact the number of leaves, nodes, and single-stem dry weight. Therefore, selecting for higher values in this component may lead to increased stem height while decreasing single stem dry weight, which can be referred to as the "plant height" component. Although this component does not significantly affect yield, its higher values are still desirable. Given that both high values for the first and second components are preferred, the most desirable region in the biplot (Figure 4) corresponds to the first quadrant, which includes the second and third ecotypes.

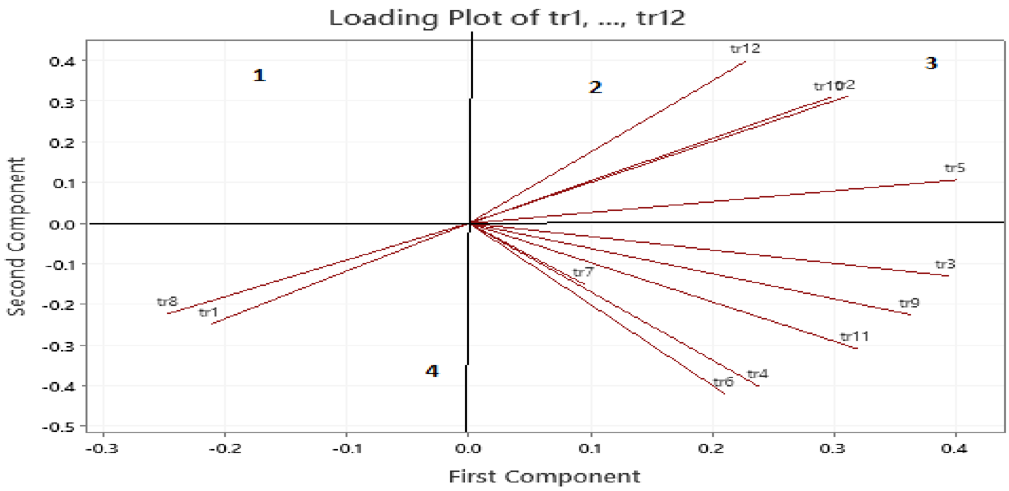

Fig. 4 Biplot of the first component and the second component of the traits studied in four harmel ecotypes (Traits 1 to 12 are based on the traits listed in Table 2)

Table 5 Eigenvalues and cumulative variance of the first, second and third components in four harmel ecotypes

	First component	Second component	Third component
Eigenvalues	5.25	3.81	1.64
Cumulative Variance (%)	43	75	89

Table 6 Special vectors of the first, second and third components in four harmel ecotypes

Traits	First component	Second component	Third component
Chlorophyll index	-0.287	-0.199	0.472
Stem height	0.37	0.240	-0.07
Number of flowers per plant	0.359	-0.271	0.109
Number of leaves	0.159	-0.428	-0.016
Number of branches per stem	0.382	0.105	-0.039
Number of nodes	0.046	-0.388	0.125
Number of branches per plant	0.093	-0.132	-0.718
The weight of the entire plant	-0.287	-0.187	-0.372
Dry weight of single stem	0.308	-0.358	0.045
Fresh weight of single stem	0.353	0.2	0.289
Dry weight of the whole plant	0.263	0.401	-0.003
Stem diameter	0.314	0.322	-0.066

DISCUSSION

Iran is considered one of the most valuable regions in terms of the number of plant species in its natural habitats. One of the potentials for habitat change is climate change, which causes species displacement, and of course, this displacement causes the development of some species. In research in line with the current research, Eisapoor et al. [7], in the study of the effect of habitat on the morphological traits of oregano, reported significant effects of habitat on the studied traits of this medicinal plant. Therefore, the effects of climatic conditions and altitude above sea level are effective on plant characteristics. Genetic diversity is the basis of a breed, which itself originates from natural evolution and is an essential component of stability in biological subsystems. In this study, using a simple statistical method of variance analysis of the diversity between the studied traits for four samples from four growing locations, it was shown that there was a suitable diversity for most traits at a probability level of five percent ($P \leq 0.05$), which indicates that these significant observations indicate the necessary conditions for the selection of traits by plant breeders for use in breeding programs. In a study by Kakaei [10], in the study of different wheat varieties, variance analysis was used to initially detect the presence of genetic diversity between the evaluated traits in bread wheat varieties, and based on this, he concluded that other statistical analyses and data analysis from this study should be performed in future breeding programs. Another result based on the comparison of the mean traits is that ecotype number 4 (sample of Mousi-Abad village 2) has a higher total plant dry weight yield trait than other studied ecotypes. The mentioned trait is an economic and valuable trait that breeding goals should be focused on in terms of ways to increase it. There are several methods for assessing genetic diversity, the most important of which are multivariate statistical methods, including cluster analysis and principal component analysis, and this analysis can simultaneously use information from other individuals and group individuals based on the aforementioned traits based on distance [9]. In this study, cluster analysis divided the studied ecotypes of aspen into two different statistical groups. This division is based on the similarities of these ecotypes in terms of the traits being evaluated. Cluster analysis was also able to separate the habitats where harmel grows in two habitats located in two provinces located in the west of Iran. According to the results, three ecotypes related to the habitats of Asadabad city are located in one cluster (cluster one) and the ecotype related to the factory village of Kangavar city is located in the second cluster. Another important issue is that the medicinal and important plant harmel has a wide distribution in the country of Iran and grows wild and has very few agricultural requirements. Its pharmacological effects are effective in the treatment and prevention of diseases such as diabetes, high blood pressure, Parkinson's, bacterial and fungal infections, and even the production of an effective substance against cancer cells

[1]. Therefore, this goal requires very precise pharmacological research to track the site of action, which can be beneficial under the supervision of relevant experts [17].

The study of the morphological traits of harmel ecotypes in western Iran provides valuable insights into the genetic diversity and adaptability of this important plant species. harmel (*Carya illinoinensis* (Wangenh.) K. Koch) is not only significant for its economic value but also plays a crucial role in various pharmaceutical and health industries due to its rich biochemical composition.

Genetic Diversity and Statistical Analysis

The use of multivariate statistical methods, including cluster analysis and principal component analysis, allowed for a comprehensive evaluation of the genetic diversity among the studied ecotypes. The cluster analysis effectively grouped the ecotypes based on their morphological traits, revealing distinct differences between those from Asadabad and Kangavar. This classification underscores the utility of these statistical techniques in understanding plant diversity and aiding in breeding programs aimed at enhancing yield and adaptability [12].

Implications for Breeding Programs

The identification of key traits associated with yield components, such as stem height and dry weight, provides critical information for breeding strategies. By focusing on traits that exhibit strong positive correlations with yield, researchers can select for ecotypes that are more productive under varying environmental conditions. The study's results indicate that higher values in the identified components are desirable for improving overall yield, which is essential for meeting the demands of both commercial agriculture and ecological sustainability [13].

CONCLUSION

The study on the morphological diversity of harmel ecotypes in western Iran underscores the significant influence of climatic and geographical conditions on various morphological traits. The analysis revealed that habitat variations markedly affected traits such as chlorophyll index, plant height, and dry weight, with ecotype No. 4 from Mousi-Abad Village 2 demonstrating superior yield in terms of total plant dry weight and chlorophyll levels. Furthermore, multivariate statistical methods, including cluster analysis and principal component analysis, effectively distinguished between ecotypes based on their morphological characteristics. The findings highlight the genetic diversity present among the harmel ecotypes, suggesting that environmental factors play a crucial role in shaping these traits. This research contributes valuable insights for future breeding programs aimed at enhancing harmel cultivation, particularly in regions with diverse climatic conditions. It emphasizes the necessity of considering local environmental factors when selecting ecotypes for agricultural development, ultimately supporting sustainable practices in the cultivation of this economically important medicinal plant. In conclusion, it is essential to conduct further research in diverse habitats similar to the current study to better understand the ecological dynamics affecting the harmel plant. Given the excessive and premature harvesting of this species, the potential for future droughts, and the observed lack of regrowth in many local populations, it is crucial to prioritize conservation efforts for these plants. Research aimed at identifying and protecting their natural habitats is vital for ensuring their sustainability and ecological balance. Such initiatives will not only help safeguard the harmel plant but also contribute to broader environmental conservation efforts.

Conflict of Interest

No conflict of interest has been declared by the authors.

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