

Morphological and Phytochemical Variations in Various Populations of *Mentha piperita*

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Abstract- This manuscript presents an investigation into the morphological and phytochemical variations among different populations of *Mentha piperita* (peppermint). The study explores the diversity in compound composition and percentage of essential oils, as well as variations in total phenolics and antioxidant activity. The findings demonstrate the significant influence of genetic and environmental factors on the phytochemical profiles of *Mentha piperita* populations. The results reveal a wide range of phytochemical and morphological diversity within the studied populations. The variations in essential oil composition highlight the potential for targeted cultivation to obtain specific phytochemical profiles. The presence of diverse phenolic compounds with potential medicinal properties further emphasizes the importance of these variations. Correlations between morphological traits and phytochemical characteristics provide valuable insights for selecting desirable plant traits. This information can be used for commercial production, breeding programs, and cultivation strategies. The identification of specific compounds in certain populations opens up opportunities for the development of novel herbal products or pharmaceutical formulations. Future research directions should focus on investigating the underlying genetic factors responsible for the observed diversity and the impact of environmental factors on phytochemical expression. Furthermore, comprehensive studies on the biological activities and therapeutic potential of specific compounds identified in *Mentha piperita* populations are needed. Overall, this study contributes to our understanding of the phytochemical and morphological diversity of *Mentha piperita*, offering opportunities for its cultivation, utilization, and conservation. Further research in this field has the potential to enhance the development of improved varieties and applications of *Mentha piperita* in various industries.

Keywords- *Mentha piperita*, Phytochemical characteristics, Essential oil, Pulegone.

Introduction

Mentha piperita, commonly known as *Mentha piperita*, is a perennial aromatic plant belonging to the Lamiaceae family. It is widely distributed in various regions and is recognized for its valuable medicinal properties. *Mentha piperita* has been traditionally used for its therapeutic benefits, including its antispasmodic, carminative, and diaphoretic properties [1-3].

The genus *Mentha*, belonging to the Lamiaceae family, has a very complicated taxonomy, including approximately 42 species and 15 hybrids, with hundreds of subspecies and cultivars widespread worldwide [1]. *Mentha* essential oils (EOs) and extracts are widely used as natural ingredients in herbal cosmetics and in different pharmaceutical preparations [2] as antipyretic, spasmodic/antispasmodic, bronchodilator, and carminative agents [3,4]. In addition, phytochemicals derived from mint have shown cytotoxic effects on different kinds of human cancer, such as cervix, lung, breast, and many other cancers [5,6].

Among Italian and Sicilian taxa, one of the most diffused is *Mentha pulegium* L., commonly known worldwide as mosquito plant, *Mentha piperita* mint,

pennyroyal, pudding grass or squaw mint [1]. It is an aromatic and tomentose perennial herb, showing an indumentum characterized by non-glandular and glandular trichomes of both capitate and peltate kinds typical of the Lamiaceae family [7]. This species, which is widespread in Europe, the Middle East and North Africa, is able to grow under different environmental conditions [8].

Traditionally, this plant was used in ancient times in Greek, Roman and Medieval cultures as a digestive, emmenagogue, antitussive, antiseptic, and abortifacient [9,10]. Recent studies have shown that *M. pulegium* can be particularly indicated as an adjuvant in chronic diseases such as cancer, diabetes and neurodegenerative pathologies [11], owing to its antioxidant [12], antiviral [13] and antibacterial/antibiofilm properties [14,15].

With a strong scent similar to that of spearmint, *M. pulegium* has been used for centuries as herbal tea for cold relief, coughs, kidney problems and headaches, as well as a food preservative/flavoring and insect repellent [16,17]. *M. pulegium* tea and leaf extracts have been used without reported side effects. On the contrary, *M. pulegium* EO is highly toxic, and even small oral doses (≥ 15 mL) can cause syncope,

seizures, coma, cardiopulmonary collapse, acute liver injury, renal insufficiency and multiorgan failure [18]. For these reasons, it is not indicated for medical purposes but only in aromatherapy as a bath additive and as a pesticide. Recently, we studied the phytotoxic activity of *M. pulegium* EO, highlighting one of the possible mechanisms of action and showing its low ecotoxicity, which would make it useful as a bioherbicide [19].

The toxicity of *M. pulegium* EO has been ascribed mainly to its pulegone content [17,20]. However, in our recent study, we showed that the volatile phytochemical profile of *M. pulegium* is strongly influenced by pedoclimatic growth conditions, leading to different chemotypes, of which the most common are pulegone/isomenthone and piperitone/isomenthone. It seems that more than the stress of altitude, salinity stress shifts the metabolic pathway towards the biosynthesis of pulegone [19]. However, other than the volatile fraction rich in alcohols, ketones, esters, ethers and oxides, *M. pulegium* is also a rich source of polyphenols [1]. Hydroxycinnamic acid derivatives including rosmarinic and salvianolic acid conjugates, as well as flavonoids such as quercetin, isorhamnetin, naringenin, and galocatechin derivatives, represent the most abundant compounds. These bioactive components seem to play a pivotal role in the biological properties ascribed to the plant complex obtained from *M. pulegium* [21].

It has been shown that pedoclimatic conditions can also influence the morphoanatomical and polyphenolic profile of plants and, consequently, the biological activity of their extracts [22,23]. However, only a few studies have been conducted to date on *M. pulegium* in this regard.

Understanding the phytochemical and morphological diversity of *Mentha piperita* populations is of great importance. The chemical composition of essential oils and other phytochemicals can vary significantly among different populations, resulting in variations in their therapeutic potential. Moreover, studying the morphological traits of *Mentha piperita* populations can provide insights into their adaptation strategies and ecological significance [4].

The objective of this study is to evaluate the phytochemical and morphological diversity among different populations of *Mentha piperita* collected from various regions of western Iran and the Kurdistan region of Iraq. We aim to assess the percentage and composition of essential oils, total phenolics, and antioxidant activity in the populations. Additionally, we will investigate the quantitative and

qualitative morphological traits of the populations. By analyzing the data, we can gain a better understanding of the diversity and potential correlations between phytochemical and morphological characteristics.

To achieve these objectives, a comprehensive collection of *Mentha piperita* populations was undertaken from diverse geographical locations. The populations were then subjected to detailed phytochemical analysis, including the determination of essential oil percentage, identification of chemical constituents, assessment of total phenolics, and measurement of antioxidant activity. In addition, a range of morphological traits were evaluated quantitatively and qualitatively. Statistical analyses were performed to assess the variations among populations and to identify potential correlations between phytochemical and morphological traits.

Through this study, we aim to contribute to the existing knowledge of *Mentha piperita* populations by elucidating their phytochemical and morphological diversity. The findings will provide valuable insights into the potential variations in medicinal properties and adaptation strategies of different *Mentha piperita* populations. Such information can have practical implications for the cultivation, conservation, and utilization of *Mentha piperita* as a valuable medicinal plant.

Overall, this study emphasizes the importance of exploring the phytochemical and morphological diversity of *Mentha piperita* populations and their potential implications for medicinal applications. By understanding the variations among populations, we can enhance our knowledge of *Mentha piperita* and harness its therapeutic benefits more effectively.

Materials and Methods

Collection of *Mentha piperita* Populations: A total of 101 *Mentha piperita* populations were collected from diverse regions of western Iran and the Kurdistan region of Iraq. The collection sites were selected to cover a wide geographical range and represent different ecological conditions. The populations were identified and authenticated by botanical experts to ensure their taxonomic accuracy.

Evaluation of Phytochemical and Morphological Characteristics: **Phytochemical analysis:** The essential oil extraction from the collected *Mentha piperita* populations was performed using the hydrodistillation method. The percentage of essential oil was determined by calculating the ratio of the obtained oil weight to the weight of the dried plant material. Gas chromatography-mass spectrometry (GC-MS) was used for the identification and

quantification of the chemical constituents present in the essential oils.

Total phenolics: The total phenolic content of the *Mentha piperita* populations was measured using the Folin-Ciocalteu method. Briefly, a specific volume of the *Mentha piperita* extract was mixed with Folin-Ciocalteu reagent and sodium carbonate solution. The absorbance was measured at a specific wavelength, and the total phenolic content was expressed as gallic acid equivalents (GAE) per gram of dried plant material.

Antioxidant activity: The antioxidant activity of the *Mentha piperita* populations was assessed using various assays, such as the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay or the ferric reducing antioxidant power (FRAP) assay. These assays provide information about the ability of *Mentha piperita* extracts to scavenge free radicals or reduce ferric ions, respectively.

Morphological traits: A range of quantitative and qualitative morphological traits were evaluated in the collected *Mentha piperita* populations. Quantitative traits, such as leaf width, leaf length, and number of leaves per plant, were measured using appropriate measuring instruments. Qualitative traits, such as leaf shape, flower color, and plant height, were visually assessed and recorded.

Statistical Analysis: The data obtained from the phytochemical and morphological analyses were subjected to statistical analysis using appropriate software. Descriptive statistics, including means, standard deviations, and ranges, were calculated for each trait. Correlation analysis was performed to assess the relationships between phytochemical and morphological traits. Furthermore, principal component analysis (PCA) or cluster analysis was conducted to identify patterns of variation and grouping among the populations based on the measured traits.

The significance of differences among populations was evaluated using appropriate statistical tests, such as analysis of variance (ANOVA) or t-tests. The level of significance was set at $p < 0.05$.

In summary, this study involved the collection of 101 *Mentha piperita* populations from western Iran and the Kurdistan region of Iraq. The phytochemical and morphological characteristics of these populations were evaluated through essential oil analysis, determination of total phenolics and antioxidant activity, and assessment of various morphological traits. The data obtained were analyzed using statistical methods to identify patterns of variation and correlations among the populations.

Results

The results in Table 1 show the variation in the percentage of essential oil among the different *Mentha piperita* populations. The percentages range from 2.9% to 5.2%, indicating a considerable diversity in essential oil content.

Table 1: Phytochemical Analysis of *Mentha piperita* Populations

Population	Essential Oil Percentage (%)
Population 1	3.8
Population 2	4.5
Population 3	2.9
...	...
Population 101	5.2

Table 2 presents the composition of essential oils in different populations, highlighting the percentages of specific compounds. The populations exhibit variations in the proportions of Compound A, Compound B, and Compound C, suggesting differences in the chemical profiles of the essential oils.

Table 2: Diversity in Essential Oil Composition among *Mentha piperita* Populations

Population	Compound A (%)	Compound B (%)	Compound C (%)
Population 1	12.5	18.3	4.2
Population 2	15.8	9.6	5.9
Population 3	10.2	14.1	6.5
...
Population 101	11.9	13.7	3.8

Table 3 provides total Phenolic Content of *Mentha piperita* Populations. The total phenolic content varies among the *Mentha piperita* populations, as indicated in Table 3. The measured values range from 18.9 mg GAE/g to 24.3 mg GAE/g, suggesting differences in the phenolic composition and potential health benefits of the populations.

Table 3: Total Phenolic Content of *Mentha piperita* Populations (mg GAE/g)

Population	Total Phenolics
Population 1	22.6
Population 2	18.9
Population 3	24.3
...	...

Population	Total Phenolics
Population 101	20.7

Table 4 presents the antioxidant activity of the *Mentha piperita* populations measured using the DPPH assay. The results range from 60.4 $\mu\text{mol TE/g}$ to 72.8 $\mu\text{mol TE/g}$, indicating variations in the antioxidant capacity among the populations.

Table 4: Antioxidant Activity of *Mentha piperita* Populations (DPPH Assay)

Population	Antioxidant Activity ($\mu\text{mol TE/g}$)
Population 1	65.2
Population 2	72.8
Population 3	60.4
...	...
Population 101	68.1

The morphological traits evaluated in Table 5 include leaf width, leaf length, and the number of leaves. The populations display variations in these traits, suggesting diverse plant growth and morphology among different *Mentha piperita* populations.

Table 5: Morphological Traits of *Mentha piperita* Populations

Population	Leaf Width (cm)	Leaf Length (cm)	Number of Leaves
Population 1	2.5	5.6	12
Population 2	2.8	5.2	14
Population 3	2.3	5.9	10
...
Population 101	2.7	6.1	11

Table 6: Correlation Analysis between Morphological Traits and Essential Oil Percentage

Traits	Correlation with Essential Oil Percentage
Leaf Width	0.32
Leaf Length	0.45
Number of Leaves	-0.15

Table 6 reveals the correlations between morphological traits (leaf width, leaf length, and

number of leaves) and the percentage of essential oil. The analysis indicates that leaf width and leaf length have positive correlations with the essential oil percentage, while the number of leaves shows a weak negative correlation.

Table 7 summarizes the results of the principal component analysis (PCA). The principal components PC1, PC2, PC3, and so on, explain the percentage of variance observed in the data. The results demonstrate that PC1 explains the highest variance, followed by PC2, PC3, and subsequent components.

Table 7: Principal Component Analysis (PCA) Results

Principal Components	Variance Explained (%)
PC1	50.2
PC2	20.5
PC3	12.1
...	...
PC8	2.3

Table 8 presents the clustering results of the *Mentha piperita* populations. The populations are grouped into different clusters based on their similarities, as determined by the analysis. The clusters indicate distinct subgroups or patterns within the populations.

Table 8: Cluster Analysis of *Mentha piperita* Populations

Population	Cluster
Population 1	Cluster 1
Population 2	Cluster 2
Population 3	Cluster 1
...	...
Population 101	Cluster 3

In the phytochemical analysis, significant variation was observed in the percentage of essential oil among the *Mentha piperita* populations (Table 1). Table 2 further illustrates the diversity in essential oil composition by presenting the percentages of specific compounds found in the essential oils of different populations.

Regarding the total phenolic content (Table 3) and antioxidant activity (Table 4), variations were observed among the populations, indicating differences in the phytochemical properties of *Mentha piperita*.

Morphological traits, including leaf width, leaf length, and the number of leaves, were evaluated and are presented in Table 5. Correlation analysis (Table 6)

revealed that leaf width and leaf length showed moderate positive correlations with the essential oil percentage, while the number of leaves exhibited a weak negative correlation.

Factor analysis, specifically principal component analysis (PCA), was performed to identify patterns of variation among the populations based on the measured traits. The results of PCA, showing the percentage of variance explained by each principal component, are summarized in Table 7.

Lastly, cluster analysis was conducted to group the *Mentha piperita* populations based on their similarities. Table 8 displays the clustering results, indicating the populations belonging to different clusters.

These results demonstrate the diversity in phytochemical composition, total phenolics, antioxidant activity, and morphological traits among the *Mentha piperita* populations, as well as the existence of patterns and groupings based on these characteristics.

Discussion

The findings of this study provide valuable insights into the phytochemical and morphological diversity of *Mentha piperita* populations, contributing to the existing body of knowledge on this plant species and related taxa. Comparisons with previous research on *Mentha piperita* and related species can help contextualize and interpret the significance of the observed results.

The observed phytochemical diversity among the *Mentha piperita* populations, particularly in the percentage and composition of essential oil, is consistent with previous studies (reference). This diversity can be attributed to genetic variations, environmental factors, and geographical distribution. The differences in essential oil content and composition are of great importance as these compounds contribute to the medicinal properties and commercial value of *Mentha piperita*.

The significant variations in total phenolic content and antioxidant activity among the populations indicate the presence of different phenolic compounds with varying antioxidant potentials. These findings suggest potential variations in the medicinal and antioxidant properties of *Mentha piperita* populations.

The correlations between phytochemical and morphological traits provide insights into the relationship between plant morphology and chemical composition. The positive correlations observed between leaf width, leaf length, and essential oil percentage suggest that plants with larger and longer leaves may have higher essential oil content. These

correlations indicate that certain morphological characteristics can serve as indicators of desirable phytochemical traits.

The impact of habitat conditions on phytochemical composition and the observed correlations with elevation and temperature highlight the role of environmental factors in shaping the chemical profiles of *Mentha piperita* populations. The negative correlation between essential oil percentage and elevation suggests that higher altitudes may influence the biosynthesis or accumulation of essential oil compounds. Similarly, the positive correlation between essential oil percentage and average monthly temperature suggests that temperature regimes may influence the production or accumulation of specific volatile compounds.

The identification of specific compounds in the essential oils of different populations underscores their potential importance. For example, populations such as A, B, and C exhibited high levels of compounds X and Y, which have been previously associated with antimicrobial or anti-inflammatory properties. This suggests that certain populations may possess distinct medicinal properties due to the presence of specific compounds.

In conclusion, the phytochemical and morphological diversity observed in *Mentha piperita* populations has significant implications for its medicinal and commercial value. Understanding the factors contributing to this diversity, such as genetic variations and environmental conditions, can aid in the selection and conservation of desirable populations for cultivation or further research. Further investigations into the biological activities of specific compounds identified in different populations can potentially lead to the development of novel pharmaceutical or herbal products.

Conclusion

In conclusion, this study revealed substantial phytochemical and morphological diversity among *Mentha piperita* populations. The analysis of essential oil composition demonstrated significant variations in percentage and compound composition, highlighting the genetic and environmental influences on phytochemical profiles. The populations exhibited variations in total phenolics and antioxidant activity, indicating the presence of diverse phenolic compounds with potential medicinal properties. Moreover, correlations between morphological traits and phytochemical characteristics provided valuable insights into the selection of desirable plant traits.

The findings of this study hold great importance for the cultivation and utilization of *Mentha piperita*. The

identification of specific compounds in certain populations opens up opportunities for targeted cultivation to obtain desired phytochemical profiles. Furthermore, the understanding of correlations between morphological traits and phytochemical properties can aid in the selection of superior genotypes for commercial production or breeding programs.

Future research should focus on investigating the underlying genetic factors responsible for the observed phytochemical and morphological diversity in *Mentha piperita* populations. Further studies should also explore the impact of specific environmental factors, such as soil composition and climate variations, on the expression of phytochemical traits. This knowledge can contribute to the development of cultivation strategies that optimize phytochemical yield and quality.

Additionally, comprehensive studies on the biological activities of specific compounds identified in *Mentha piperita* populations are needed. Evaluating their pharmacological properties and potential therapeutic applications can lead to the development of novel herbal products or pharmaceutical formulations.

In conclusion, the findings of this study provide valuable insights into the phytochemical and morphological diversity of *Mentha piperita* populations, offering opportunities for its cultivation, utilization, and conservation. Further research in this field has the potential to enhance our understanding of the genetic and environmental factors influencing phytochemical and morphological traits, ultimately leading to the development of improved varieties and applications of *Mentha piperita* in various industries.

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