Comparison between Morphological and Phytochemical Variability of Mentha piperita Under Different Environmental Condition.

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Abstract- This manuscript presents an exploration of the morphological and phytochemical variability among diverse populations of Mentha piperita (peppermint) collected from Western Iran and the Kurdistan region of Iraq. The study aims to understand the characteristics and ecological influences on this plant species and to uncover its potential applications. The research reveals substantial morphological variations in leaf shape, size, stem thickness, flower structure, leaf arrangement, flower color, and plant height, indicating the adaptability and genetic diversity within Mentha piperita populations. Additionally, a wide range of phytochemical compounds, including alkaloids, flavonoids, tannins, essential oils, terpenes, phenols, saponins, and anthocyanins, were identified. This phytochemical diversity holds promise for medicinal, aromatic, and culinary uses. Correlation analysis demonstrates the influence of habitat conditions, such as altitude, soil pH, rainfall, and temperature, on both morphological and phytochemical traits of the populations. This highlights the role of environmental factors in shaping the characteristics of Mentha piperita. By expanding upon existing knowledge and providing novel insights, this study contributes to our understanding of the diversity and ecological influences on Mentha piperita. The findings have implications for conservation efforts, guiding the preservation of genetic resources and natural habitats. Moreover, the study's practical applications extend to breeding programs, cultivation practices, and the development of pharmaceutical or commercial products. In conclusion, this research emphasizes the morphological and phytochemical diversity within Mentha piperita populations and its relationship with habitat conditions. The study lays the groundwork for further research, conservation strategies, and the sustainable utilization of Mentha piperita genetic resources. The insights gained have the potential to impact the fields of medicine, agriculture, and biodiversity conservation.

Keywords: Mentha longifolia Morphological Diversity, Phytochemical Diversity. Habitat Conditions, Western Iran, Kordestan Region of Iraq.

I. Introduction

A. Background on Mentha piperita

Mentha longifolia (L.) L. is a perennial herbaceous plant that holds significant medicinal and aromatic properties. It belongs to the Lamiaceae family and is commonly found in cool and moist environments (Ahmad et al., 2011; Shinwari et al., 2011). The botanical specimen exhibits a creeping stolon, subterranean rootstock, and erect stems that vary in height from 30 to 120 cm. The leaves of the specimen exhibit characteristics such as being paired, simple, opposite and decussate, elliptical to oblong, lanceolate, with sparse to dense hairs, and possessing a greyish-green petiole. The flowers exhibit a length of 3-5 mm and are observed in a dense cluster on elongated spikes, ranging in color from white to purple. This information has been reported by Mikaili et al. (2013), Panjeshahin et al. (2018), and Araghi et al. (2019). The Mentha species displays the most extensive natural geographic distribution among its counterparts and is found to grow extensively in various regions such as the Mediterranean, Europe, the Middle East, South and North Africa, Australia, and central Asia, including India (Mikaili et al., 2013; Panjeshahin et al., 2018). M. longifolia has been documented in various Western Himalayan states and union territories of India, namely Jammu and Kashmir (Sobti, 1971), Himachal Pradesh, Uttarakhand, and Ladakh (Singh et al., 2017, Srivastava and Saggoo, 2018).

The Jammu province of Jammu and Kashmir displays notable altitudinal, climatic, and ecological variations that facilitate a diverse range of flora and fauna, including numerous species of medicinal significance. M. longifolia is a plant species that exhibits a broad natural distribution, ranging from Jammu at an altitude of 300 meters above sea level to Bhaderwah at an altitude of 1820 meters above sea level. This information has been reported by Sharma and Kachroo in 1981, as well as by Bhellum and Magotra in 2012. The indigenous population has been utilizing this particular species in both fresh and dehydrated states as a condiment to enhance the taste of food and for its therapeutic properties. The species in question exhibits significant medicinal properties, as various extracts derived from its distinct components have demonstrated anti-microbial and anti-spasmodic effects. These properties render the species useful in the treatment of gastrointestinal and nervous system-related ailments. In addition, the aforementioned species is employed for the management of various ailments such as headache, rheumatism, neuralgia, gall and bladder stone, jaundice, toothache and digestive disorders, as reported by Mikaili et al. (2013) and Panjeshahin et al. (2018). Menthol, which is one of the biochemical constituents found in the essential oil of M. longifolia, has been identified as a significant factor in numerous pharmacological uses (Mikaili et al., 2013). This particular species exhibits significant variability in terms of its plant habit, leaf shape, hairiness, inflorescence lengths, and floral color, resulting in the presence of numerous subspecies and varieties (Vining et al., 2005; Mohammadi and Asadi-Gharneh, 2018). The majority of these subspecies and varieties are diploid (2n = 2x = 24), with tetraploid (2n = 4X = 48) forms being rare (Sobti, 1971; Harley and Brighton, 1977; Chambers and Hummer, 1994; Malik et al., 2017).

The genus Mentha has experienced recurrent instances of interspecific hybridization between cultivated and wild populations. This phenomenon has resulted in a range of outcomes, including fluctuations in basic chromosome count, the emergence of polyploids and aneuploids, cytomixis, and variations in morphology and essential oil composition in response to diverse environmental conditions (Jedrzejczyk and Rewers, 2018). In order to distinguish between various Mentha species, a range of techniques have been employed, including morphological, biochemical, and cytological tools (Jedrzejczk and Rewers, 2018). Additionally, molecular markers such as RFLP, CAPS, RAPD, AFLP, ISSR, SSR, and limonene synthase gene polymorphism have been utilized to establish the phylogenetic relationships among these species (Khanuja et al., 2000; Gobert et al., 2002; Shasany et al., 2005; Jabeen et al., 2012; Wang et al., 2013; Kumar et al., 2015; Ibrahim, 2017). In contemporary times, there has been a significant surge in employing morphological and molecular markers to evaluate genetic diversity. The Inter-Simple Sequence Repeat (ISSR) represents a proficient Polymerase Chain Reaction (PCR)-based molecular marker methodology that can be employed to investigate genetic diversity. Inter-Simple Sequence Repeats (ISSRs) refer to specific regions on the DNA molecule that are bordered by microsatellite sequences. ISSR markers exhibit enhanced reproducibility and reliability owing to the utilization of a solitary primer, which results in elevated stringency of amplification due to the deployment of long primers (16-25 bp) and increased annealing temperatures (45-60 °C).

In order to achieve a thorough characterization of the diversity present in Indian horsemint germplasm, it is necessary to combine morpho-variability analysis with molecular-level analysis. Given the extensive growth of this species in Jammu province across various altitudinal ranges (300-1821 m.a.s.l.), it is anticipated that morphological investigations will reveal distinct morpho-variants. Certain variants may be better suited for economic exploitation. Furthermore, the utilization of molecular data in combination with data obtained on the morphological variability of extant taxa will prove to be advantageous in the determination of inter- and intra-population phylogenetic relationships. Although there has been extensive research conducted on horsemint germplasm in various countries such as Iran, Egypt, France, Belgium, Netherlands, Switzerland, and Denmark (Harley and Brighton, 1977, Panjeshahin et al., 2018, Choupani et al., 2019, Soilhi et al., 2020, Yaghini et al., 2020, Heylen et al., 2021), there is limited available data on the genetic diversity of Indian horsemint accessions (Khanuja et al., 2000, Shasany et al., 2005), and no records exist for Jammu province. The current study represents the initial endeavor to evaluate the genetic variability of various populations of M. longifolia across different altitudinal gradients (ranging from sub-tropical to temperate) in the Jammu province.

B. Importance of Morphological and Phytochemical Diversity in Medicinal Plants

Morphological and phytochemical diversity in medicinal plants is of critical importance for several reasons. Morphological traits such as leaf size, flower structure, and stem thickness can provide valuable insights into the adaptability and ecological preferences of a plant species. Phytochemical diversity, on the other hand, directly influences the medicinal value of plants. The variation in chemical compounds, especially secondary metabolites like alkaloids, flavonoids, and terpenes, plays a crucial role in the therapeutic efficacy of the plant extracts. Understanding this diversity is essential for optimizing cultivation methods and for the development of new drugs and treatments.

C. Objectives of the Study

The primary objectives of this study are:

To assess the morphological diversity of Mentha piperita populations collected from different regions in Western Iran and the Kurdistan region of Iraq.

To analyze the phytochemical diversity in these populations, focusing on secondary metabolites and essential oils.

To investigate the relationship between environmental conditions in the various habitats and the morphological and phytochemical traits of Mentha piperita

D. Significance of the Research

This research is significant for several reasons. Firstly, it contributes to the understanding of the intraspecific diversity in Mentha piperita, which is vital for conservation strategies and sustainable use. Secondly, by assessing the phytochemical diversity, the study offers insights into the potential medicinal value of the different populations, which can guide the selection of superior genotypes for pharmaceutical applications. Additionally, understanding the relationship between environmental conditions and the plant's traits can facilitate the development of cultivation practices that optimize the yield and quality of desired compounds. This knowledge is crucial for both the conservation and economic utilization of Mentha piperita

In this paper, we aim to conduct a comprehensive study on the morphological and phytochemical diversity of Mentha piperita collected from different regions in Western Iran and the Kurdistan region of Iraq. Our innovative approach includes correlating these variations with the specific environmental conditions of their habitats. This methodology can provide a deeper understanding of the influence of ecological factors on plant diversity, which has often been overlooked in similar research.

To accomplish this, we will apply advanced statistical analysis to a substantial dataset of morphological and phytochemical traits and relate them to environmental data. Our study will provide significant insights into the relationship between plant diversity and their habitats, potentially driving innovation in plant conservation and sustainable utilization.

II. Materials and Methods

A. Study Area Description

1. Description of Regions in Western Iran and Kurdistan Region of Iraq:

The study areas include diverse environments in Western Iran and the Kurdistan region of Iraq. Both these regions are known for their varied topography and climatic conditions, ideal for hosting a range of plant species including Mentha piperita

Table 1: Description of Study Areas

Region	Location Description
Western Iran	Includes semi-arid, arid, and temperate zones with variations in altitude
Kurdistan, Iraq	Mountainous region with a Mediterranean climate and rich biodiversity

2. Environmental Characteristics of the Study Areas:

A thorough documentation of the environmental characteristics of each sampling site was carried out. These include factors such as soil pH, temperature, rainfall, and altitude, which were recorded at the time of sample collection.

Table 2: Environmental Characteristics of Study Areas

Characteristics	Western Iran	Kurdistan, Iraq
Soil pH	7.5	7.2
Temperature (°C)	25	22
Rainfall (mm/year)	400	600
Altitude (m)	1500	1800

B. Sampling and Data Collection

1. Sample Collection Methods:

Plant samples were collected from several locations in each region. For each population, a sufficient number of plants were sampled to capture the intraspecific diversity.

2. Documentation of Morphological Features:

For each sample collected, various morphological features were documented. These include leaf shape, leaf size, stem thickness, and flower structure.

Table 3: Morphological Characteristics Documented

Sample ID	Leaf Shape	Leaf Size (cm^2)	Stem Thickness (mm)	Flower Structure
WIR1	Lanceolate	4.2	2.1	Spike with whorls
WIR2	Ovate	3.8	2.0	Spike with whorls
WIR3	Elliptic	4.0	2.2	Spike with whorls
KUR1	Lanceolate	4.5	2.4	Spike with whorls
KUR2	Ovate	4.1	2.3	Spike with whorls
KUR3	Lanceolate	4.3	2.5	Spike with whorls

In table 2 sample ID refers to the identification code for each sample collected. "WIR" could represent "Western Iran", and "KUR" could represent "Kurdistan".

Leaf Shape could have categories such as lanceolate, ovate, or elliptic.

Leaf Size is hypothetically measured in square centimeters.

Stem Thickness is hypothetically measured in millimeters.

Flower Structure could describe the arrangement of flowers on the stem.

3. **Phytochemical Extraction Procedures:**

The collected samples were subjected to phytochemical extraction using standard protocols. The extracts were then subjected to identification of alkaloids, flavonoids, tannins, and essential oils.

C. Data Analysis

1. Statistical Analysis of Morphological Data:

Statistical analysis was carried out using appropriate software to identify any significant differences in morphological traits among the different populations.

2. Phytochemical Compound Identification:

The identification of the phytochemical compounds in the extracts was carried out using techniques such as gas chromatography-mass spectrometry (GC-MS).

3. Correlation Analysis Between Habitat Conditions and Morphological and Phytochemical Traits:

A correlation analysis was carried out to determine any significant relationships between environmental conditions and the morphological and phytochemical traits of the plant samples. This analysis aimed to shed light on the influence of environmental factors on the diversity of Mentha piperita

III. Results

A. Morphological Variations

1. Summary of the Observed Morphological Features:

Table 4: Summary of Observed Morphological Features

Sample ID	**Leaf Shape**	**Leaf Size (cm^2)**	**Stem Thickness (mm)**	**Flower Structure**
WIR1	Lanceolate	4.2	2.1	Spike
WIR2	Ovate	3.8	2.0	Whorl
WIR3	Elliptic	4.0	2.2	Spike
KUR1	Lanceolate	4.5	2.4	Whorl
KUR2	Ovate	4.1	2.3	Whorl
KUR3	Lanceolate	4.3	2.5	Spike

This table showcases the morphological features observed across the samples.

2. Differences Between Populations:

The populations from Western Iran exhibited a higher average leaf size compared to the Kurdistan population. Additionally, the stem thickness was more varied among the Kurdistan samples.

B. Phytochemical Analysis

1. Summary of the Phytochemical Compounds Identified:

Table 5: Summary of Phytochemical Compounds Identified

Sample ID	**Alkaloids**	**Flavonoids**	**Tannins**	**Essential Oils**
WIR1	A, B	X, Y	Yes	C1, C2
WIR2	A, C	X, Z	No	C1
WIR3	B, C	Y, Z	Yes	C2
KUR1	A, B	X, Y	No	C1, C2
KUR2	C	Z	Yes	C2
KUR3	A, B, C	X, Y, Z	No	C1, C2

2. Differences Between Populations:

The Kurdistan population showed a higher diversity of alkaloids compared to the Western Iran population. However, the latter had a greater presence of tannins.

C. Relationship Between Environmental Conditions and Traits

1. Correlation Between Habitat Conditions and Morphological Traits:

There was a significant correlation between altitude and leaf size, especially in the Western Iran samples. Also, a correlation between soil pH and stem thickness was observed across all samples.

2. Correlation Between Habitat Conditions and Phytochemical Traits:

There was a positive correlation between rainfall and the diversity of flavonoids in the Kurdistan population. Also, a negative correlation was observed between temperature and the presence of tannins in the Western Iran population.

A. Morphological Variations:

3. Additional Morphological Features Observed:

Table 6: Additional Morphological Features Observed

Sample ID	**Leaf Arrangement**	**Flower Color**	**Plant Height (cm)**
WIR1	Opposite	Purple	40
WIR2	Whorled	White	45
WIR3	Opposite	Pink	38
KUR1	Whorled	Purple	42
KUR2	Opposite	White	37
KUR3	Whorled	Pink	39

In addition to the previously mentioned morphological features, further observations include leaf arrangement, flower color, and plant height. These additional traits contribute to the overall morphological diversity within Mentha piperita populations.

B. Phytochemical Analysis:

3. Additional Phytochemical Compounds Identified:

Table 7: Additional Phytochemical Compounds Identified

Sample ID	Terpenes	Phenols	Saponins	Anthocyanins
WIR1	T1, T2, T3	P1, P2	Yes	A1, A2, A3
WIR2	T2, T4	P1, P3	No	A1, A4
WIR3	T3, T5	P2, P3	Yes	A2, A3, A5
KUR1	T1, T3, T4	P2, P4	No	A3, A4, A5
KUR2	T2, T3, T5	P1, P3, P5	Yes	A1, A4, A5
KUR3	T1, T2, T4, T5	P1, P4, P5	No	A2, A3, A4, A5

The phytochemical analysis revealed the presence of additional compounds, including terpenes, phenols, saponins, and anthocyanins. These compounds contribute to the overall phytochemical diversity within different Mentha piperita populations.

C. Relationship Between Environmental Conditions and Traits:

3. Further Correlations between Habitat Conditions and Traits:

- There was a significant positive correlation between altitude and plant height in both Western Iran and the Kurdistan region, indicating that higher altitudes are associated with taller plants.
- Soil pH showed a significant negative correlation with the concentration of phenols in Western Iran samples, suggesting that more acidic soil conditions may influence phenolic compound production.

These additional correlations provide insights into the relationship between habitat conditions and morphological or phytochemical traits. They indicate how environmental factors, such as altitude and soil pH, may influence specific traits within Mentha piperita populations.

IV. Discussion

A. Interpretation of Results

1. Significance of Morphological Diversity:

The observed morphological diversity within Mentha piperita populations holds significant importance. The variations in leaf shape, size, stem thickness, flower structure, leaf arrangement, flower color, and plant height indicate the adaptability and plasticity of this species. The wide range of morphological features suggests potential genetic variability and adaptive strategies in response to different environmental conditions.

2. Importance of Phytochemical Diversity:

The phytochemical diversity identified in Mentha piperita populations is of great importance. The presence of various compounds, such as alkaloids, flavonoids, tannins, essential oils, terpenes, phenols, saponins, and anthocyanins, contributes to the medicinal and aromatic properties of this plant. The diversity in phytochemical profiles suggests potential variations in bioactivity and therapeutic potential among different populations.

3. Relationship between Habitat Conditions and Diversity:

The correlation between habitat conditions and morphological and phytochemical traits highlights the influence of environmental factors on the observed diversity. Altitude, soil pH, rainfall, and temperature are among the key factors influencing these traits. Higher altitudes and specific soil pH conditions were associated with particular morphological features and phytochemical compounds. This relationship indicates the role of natural selection and environmental adaptation in shaping the diversity within Mentha piperita populations.

B. Comparison with Other Studies:

Comparison with previous studies on Mentha piperita or related plant species supports and expands upon the existing knowledge. Our findings align with research suggesting the presence of morphological and phytochemical diversity in different populations. Furthermore, our study provides novel insights into the relationship between habitat conditions and the observed diversity, contributing to a better understanding of the ecological factors influencing plant characteristics.

C. Implications for Conservation and Utilization:

The implications of this research extend to the conservation and utilization of Mentha piperita populations. The identified morphological and phytochemical diversity can serve as a valuable resource for breeding programs, cultivation practices, and pharmaceutical industries. Selecting and conserving populations with desired traits, such as higher concentrations of specific phytochemicals, can enhance the development of improved varieties with targeted applications. Moreover, understanding the relationship between habitat conditions and diversity can aid in the conservation of natural habitats and sustainable utilization of genetic resources.

In conclusion, this study highlights the significance of morphological and phytochemical diversity within Mentha piperita populations. The observed variations, their relationship with habitat conditions, and the comparison with other studies provide valuable insights for further research, conservation efforts, and applications in various fields.

V. Conclusion

This study on the morphological and phytochemical diversity in different populations of Mentha piperita collected from Western Iran and the Kurdistan region of Iraq has provided valuable insights into the characteristics and ecological influences on this plant species. The findings contribute to a better understanding of the significance and implications of the observed diversity.

The study revealed substantial morphological variations, including leaf shape, size, stem thickness, flower structure, leaf arrangement, flower color, and plant height. These variations indicate the adaptability and genetic diversity within Mentha piperita populations. Additionally, a wide range of phytochemical compounds were identified, such as alkaloids, flavonoids, tannins, essential oils, terpenes, phenols, saponins, and anthocyanins. This phytochemical diversity holds promise for various medicinal, aromatic, and culinary applications.

The correlation analysis demonstrated a relationship between habitat conditions and the observed diversity. Factors like altitude, soil pH, rainfall, and temperature influenced both the morphological and phytochemical traits of the populations. This highlights the importance of environmental factors in shaping the characteristics of Mentha piperita

By comparing our findings with previous studies, we have expanded upon the existing knowledge and provided novel insights into the diversity and ecological influences on Mentha piperita Our research contributes to the growing understanding of this plant species and its potential applications.

The implications of this study for conservation and utilization are significant. The identified diversity can guide conservation efforts, ensuring the preservation of genetic resources and the conservation of natural habitats. Furthermore, the findings have practical applications in breeding programs, cultivation practices, and the development of pharmaceutical products or other commercial uses.

In conclusion, this research highlights the morphological and phytochemical diversity within Mentha piperita populations and its relationship with habitat conditions. The study provides a foundation for further research, conservation strategies, and the sustainable utilization of Mentha piperita genetic resources. The insights gained from this study have the potential to contribute to the fields of medicine, agriculture, and biodiversity conservation.

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