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# Effect of altitude and soil factors on morphological and phytochemical traits of *Mentha pulegium* L. in different habitats of Kerman Province, Iran

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**Abstract**

To investigate the effect of environmental and soil factors on morphological and qualitative characteristics of Pennyroyal or *M. pulegium*, research was conducted in five habitats of the species in 2022 in Kerman Province of Iran. Plant samples were collected from random quadrats ($1\times1m^2$) during the flowering stage in natural habitats of Kerman, Kohbanan, Raver, Zarand, and Chatrood. The collected data were subjected to one-way analysis of variance (ANOVA) and means were compared among locations. The results indicated significant diversity among the sites. Altitude significantly influenced essential oil yield and composition. The highest essential oil percentage (0.198%) was observed at Kohbanan (highest altitude), which was 35% higher than Chatrood (lowest altitude, 0.112%). However, Ravar and Kohbanan sites had the highest overall essential oil yields (2.77% and 2.133%, respectively). The greatest chlorophyll content (1.26) was recorded at Kohbanan, which was 44% higher than at Ravar. While soil properties like nutrient content and texture were important, altitude had a more pronounced effect on plant quality characteristics. This study demonstrates that altitude is a critical ecological factor influencing the growth and chemical composition of *Mentha pulegium*. While soil characteristics contribute to plant development, environmental conditions at varying altitudes have a greater impact on essential oil content, chlorophyll levels, and anthocyanin production. These insights are valuable for optimizing Pennyroyal cultivation for medicinal or industrial purposes.

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## Interdiction

Medicinal plants containing essential oils have garnered significant attention for their therapeutic potential and industrial applications. These plants are rich in bioactive compounds such as thymol, carvacrol, eugenol, cinnamaldehyde, and linalool, which exhibit antimicrobial and antioxidant properties (Sharifi-Rad et al., 2017). Essential oils derived from aromatic plants are widely utilized as flavoring agents in the food, pharmaceutical, perfumery, and cosmetics industries. These concentrated extracts impart distinctive flavors and fragrances to products. Thymol and carvacrol, for instance, are primary constituents of essential oils from *Origanum vulgare* (Pennyroyal), *Thymus vulgaris* (Thyme), *Satureja montana* (Savory), and *Trachyspermum ammi* (Ajowain) (Hui et al., 2017).

*M. pulegium* L., a perennial herb of the Lamiaceae family, is characterized by square, pubescent stems (up to 40 cm tall), opposite oval leaves (2–3 cm long), and lilac-mauve flowers clustered in whorls. Its fruit consists of four brown nutlets (Amtaghri et al., 2024). The essential oil of *M. pulegium* is dominated by monoterpenes, with composition varying by chemotype and region (Casiglia et al., 2017). Pulegone, the major constituent, is particularly abundant in highland varieties, alongside piperitenone, 1,8-cineole, and piperitone (Khalilipour & Dejam, 2014). Oil content fluctuates with growth stage, peaking at 9.3% during flowering (Azimi & Asgarpanah, 2020).

Despite its traditional use for treating coughs, gastrointestinal disorders, and anxiety, *M. pulegium*'s toxicity—linked to pulegone-induced hepatotoxicity—limits its medicinal application (Anderson et al., 1996). Safer alternatives like peppermint, chamomile, and rosemary are often preferred (Azimi & Asgarpanah, 2020). Plant height decreases with elevation, while leaves become thicker and larger (Kofidis et al., 2003). Higher altitudes elevate thymol/carvacrol content but reduce linalool, altering bioactivity (Ibrahim et al., 2022). Phenolics, flavonoids, and antioxidant enzymes accumulate more at elevated sites, enhancing medicinal potential. Soil properties (pH, nitrogen, organic carbon) and slope aspect critically affect secondary metabolite production. High soil nitrogen boosts yield but may compromise medicinal quality by prioritizing vegetative growth over metabolite synthesis (Yang et al., 2024). Acidic soils at higher altitudes restrict nutrient availability but can enhance photosynthetic efficiency and secondary metabolites (Pan et al., 2023). *Espeletia schulizi* essential oil showed no compositional variation across flowering stages but differed significantly with harvest altitude (Ibañez & Usubillaga, 2006). While *M. pulegium* demonstrates adaptability to environmental stressors, further research is needed to elucidate: 1. Interactions between soil composition and altitude on yield and chemical profiles. 2. Cultivation strategies to maximize beneficial compounds while mitigating toxicity risks.

Pennyroyal's bioactive properties underscore its value in medicine and food preservation. Environmental factors like altitude and soil conditions critically shape its growth and chemistry, necessitating targeted studies to optimize cultivation for safe, high-quality yields.

## Materials and methods

We investigated the morphological, phytochemical, and physiological characteristics of *M.pulegium* in 2022 in five sites. In each habitat, seven samples were collected in quadrats (1x1) square meters from the soil surface. Each *M.pulegium* site belonged to a habitat and these habitats included Kohbanan (2090m), Raver (1175m), Zarand (1650m), Kerman (1756) and Chatrood (1923m) located in Kerman Province (Table 1). The amount of yearly rainfall in Kerman is 84.6 mm, Kohbanan 158.1 mm, Chatrood 101.9 mm, Zarand 107.16 mm, and Ravar 147.6 mm. Sampling was implemented in each habitat during the flowering stage on June 30. Samples were identified in Razavi Khorasan Province

Agricultural and Natural Resources Research Institute. Traits such as plant height, stem diameter, number of flowers per plant, inflorescence length, leaf length and width, flower length and width, dry matter yield, essential oil percentage, essential oil yield, amount of chlorophyll (a, b and total), amount Carotenoids and anthocyanin of leaves and flowers were evaluated.

**Table1.** Geographical coordinates, altitude, and soil properties in *M.pulegiumn* sites.

| Site | Altitude masl | Geographical coordinates | Precipitation mm | EC (dSm - 1) | pH | N (%) | P (ppm) | K (ppm) | Sand (%) | Silt (%) | Clay (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Ravar | 1175 | 56°31°15 | 147.6 | 0.61 | 7.52 | 0.05 | 2.4 | 260 | 25 | 31 | 44 |
| Zarand | 1650 | 57°30°8 | 107.16 | 0.52 | 7.61 | 0.11 | 4.4 | 241 | 42 | 36 | 22 |
| Chatrood | 1923 | 56°75°35 | 101.9 | 0.76 | 7.73 | 0.24 | 3.4 | 120 | 52 | 34 | 14 |
| Kohbnan | 2090 | 56°42°15 | 158.1 | 0.9 | 7.57 | 0.05 | 5.8 | 163 | 74 | 26 | 10 |
| Kerman | 1756 | 55°32°11 | 84.6 | 0.75 | 7.41 | 1.02 | 4.5 | 411 | 20 | 44 | 36 |

To measure the dry weight of the plants, after removal from the soil surface, they were placed in plastic bags and transported to the laboratory. The fresh weight was measured immediately upon arrival. The samples were then dried in the shade under open air conditions until reaching a constant weight, at which point the dry weight was determined.

The extraction of essential oil from plant material was performed using steam distillation. In this process, plant material is placed in a distillation apparatus where steam is passed through it. The steam volatilizes the essential oils, and the resulting vapor mixture is condensed back into liquid form. The essential oil is then separated from the hydrosol through density differences. This method is widely employed for extracting essential oils from aromatic plants due to its efficiency and scalability.

For essential oil quantification, three samples of young branches from each experimental condition were selected. A standardized 50 g sample was prepared for each replicate, and essential oil extraction was conducted using water-based distillation in a Clevenger apparatus for a duration of three hours (Cartwright, 2016). The essential oil yield was calculated as the percentage of extracted oil relative to the dry matter weight, expressed mathematically as (weight of extracted oil/dry weight of plant material) × 100. This measurement provides a standardized metric for comparing oil production across different samples and conditions. Spectrophotometric methods are commonly used to measure the amount of chlorophyll-a, chlorophyll-b, and total chlorophyll in a plant sample. The following formulas are typically employed for the estimation of these photosynthetic pigments:

$$Chlorophyll\ a\ (mg\ /g\ FW) = (12.7 \times A663) - (2.59 \times A645)$$

$$Chlorophyll\ b\ (mg\ /gFW) = (22.9 \times A645) - (4.7 \times A663)$$

$$Chlorophyll\ a + b\ (mg/\ gFW) = (8.2 \times A663) + (20.2 \times A645)$$

where: A663 and A645 refer to the absorbance of the sample at 663 nm and 645 nm, respectively.

The spectrophotometer was adjusted using an acetone/ethanol mixture, and the calculations for chlorophyll concentrations were made after the absorbances were read at these specific wavelengths (Arnon, 1949). The method of Cakmak and Marschner (1988)) was used for the measurement of anthocyanins in the plant samples.

The collected data were subjected to one way analysis of variance (ANOVA) and the means were compared among sites using LSD test. SAS statistical program version 9.4 was used for data analysis. Before analyzing the data, they were tested and confirmed for normality.

## Results

### *Effect of altitude on morphological traits*

Analysis of variance showed that the effect of site on plant height, stem diameter, number of flowers per plant, leaf width, flower length, and width, and dry matter yield was significant ($p<0.01$). The effect of site on inflorescence length and leaf length was also significant ($p<0.05$) (Table 2).

The higher and lower values of plant height (40.33 and 29.22 cm) were obtained from plants collected from Kohbanan and Raver sites, respectively. The height of plants at the Kuhbanan site was about 24% higher compared to the Raver site (Table 3).

The higher and lower values of stem diameter (2.26 and 0.8 mm) were observed in the Zarand and Ravar, respectively. The highest number of flowers per plant was obtained in the Kohbanan region, but it was not significantly different from the Zarand and Ravar sites (Table 3). The lowest number of flowers in plants was observed in the Ravar site. At higher altitudes, the number of flowers in plants increased. In the Chetrood site (12.34 cm), the length of inflorescence in plants was significantly higher in comparison with Zarand, Raver, and Kohbanan (Table 3). The length of inflorescence in plants differed significantly among the Chetrood, Zarand, Raver, and Kohbanan sites. The lowest length of inflorescence was observed in the Raver site. Leaf length was 28% higher in the Kohbanan site compared to the Raver site (Table 3). The leaf length in the Zarand site was 60% higher than in the Ravar site (Table 3). Chatrood site produced significantly more Floret length compared to other sites (Table 3). Raver, Zarand, and Kerman sites similarly had the lowest floret width. The plants collected from the Kerman area had the lowest floret width and the rest of the sites produced significantly more floret width compared to Kerman (Table 3).

**Table 2.** Analysis of variance of morphological traits of *M.pulegium*

| S.O.V | df | MS | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Plant height | Stem diameter | Number of flowers | Inflorescence length | Leaf length | Floret length | Essential oil(%) |
| Site | 4 | 50.09** | 0.86** | 2.59** | 11.48* | 0.28* | 0.16** | 0.001* |
| Error | 10 | 2.83 | 0.01 | 0.38 | 2.43 | 0.05 | 0.01 | 0.0003 |
| C.V(%) | - | 5.09 | 6.72 | 12.32 | 13.35 | 10.32 | 8.32 | 12.32 |

* and ** are significant at the level of 5% and 1%, respectively

**Table 3.** Comparison of mean of morphological traits of *M.pulegiumn*

| Site/ Altitude | Plant height (cm) | Stem diameter (mm) | Flowers Number | Inflorescence Length (cm) | Leaf length (cm) | Floret length (cm) | Total DM ($g\ m^{-1}$) |
|---|---|---|---|---|---|---|---|
| Ravar (1175m) | $29.33^{c}$ | $0.90^{d}$ | $3.33^{c}$ | $8.33^{b}$ | $1.80^{b}$ | $1.00^{b}$ | $371.55^{b}$ |
| Zarand (1650m) | $35.33^{b}$ | $2.16^{a}$ | $5.00^{ab}$ | $10.00^{b}$ | $2.29^{a}$ | $1.00^{b}$ | $433.33^{b}$ |
| Chatrood (1923m) | $30.67^{c}$ | $1.85^{b}$ | $4.00^{bc}$ | $11.00^{ab}$ | $2.28^{a}$ | $1.49^{a}$ | $416.67^{ab}$ |
| Kohbnan (2090m) | $40.33^{a}$ | $1.39^{c}$ | $5.64^{a}$ | $9.00^{b}$ | $1.81^{b}$ | $1.00^{b}$ | $380.67^{b}$ |
| Kerman (1756m) | $31.66^{bc}$ | $1.06^{d}$ | $4.66^{ab}$ | $13.33^{a}$ | $1.79^{b}$ | $0.89^{c}$ | $309.33^{c}$ |

Means followed by the same letter within a column are not significantly different according to the LSD test.

### Effect of altitude on essential Oil% and Oil yield

The results showed that the essential oil% and essential oil yield were significant ($p<0.01$) (Table 4). The essential oil in plants harvested from the Ravar region (0.186%) was significantly higher than other sites except Chatrood. The essential oil% in the Ravar site increased by 37% compared to the Zarand site (0.116%) (Table 5). Similar to the percentage of essential oil%, the highest yield of essential oil (2.77 $g/m^2$) was observed in the Ravar site. The essential oil% was between 0.116 and 0.186% is supported by the research results. The results indicate that the essential oil% and oil yield can vary significantly among different plant sites, and the Ravar region showed notably higher essential oil% and oil yield compared to other sites (Table 4).

and $p<0.01$) (Table 4). In the Kohbanan region, the highest amount of anthocyanin was observed in flowers and leaves (0.56 and 5.28 mg/ g fresh weight of flowers and leaves) (Table 5). The lowest amount of anthocyanin in flowers and leaves (0.23 and 2.25 mg/ g fresh weight of flowers and leaves) was obtained in the Chatrood and Raver sites, respectively. The results showed that the amount of anthocyanin in the leaf was significantly higher than the anthocyanin of the flower by nearly tentimes (Table 5).

Kerman site (1.78 g/m$^2$) produced the lowest essential oil yield. No significant difference was observed between the sites of Kohbanan, Chatrood, and Raver in terms of essential oil yield (Table 5). According to the results (Table 1), the altitude above sea level had important effect on the essential oil%. The Ravar site, which was located at the lowest altitude, produced the highest amount of essential oil%.

**Effect of altitude on photosynthetic pigments**

The results showed a significant effect of habitat on photosynthetic pigments ($p<0.05$

**Table 4.** Analysis of variance of qualitative features of *M.pulegium*

| S.O.V | df | MS | | | | | |
|---|---|---|---|---|---|---|---|
| | | Essential oil | Chlorophyll a | Chlorophyll b | Chlorophyll a+b | Carotenoid | Flower anthocyanins |
| Site | 4 | 0.45* | 0.17** | 0.05** | 0.39** | 0.005* | 0.04** |
| Error | 10 | 0.11 | 0.0006 | 0.002 | 0.0003 | 0.001 | 0.008 |
| C.V(%) | - | 14.36 | 2.25 | 2.37 | 3.39 | 9.38 | 8.03 |

* and ** are significant at the level of 5% and 1%, respectively

**Table 5.** Mean comparison of morphological traits of *M.pulegiumn*.

| Site/ Altitude | Essential Oil (%) | Esse. oil Yield (g m$^{-2}$) | Chlorophyll a (mg g$^{-1}$FW) | Chlorophyll b (mg g$^{-1}$FW) | Chlorophyll a+b (mg g-1FW) | Carotenoid (mg g$^{-1}$FW) | anthocyanin (mg g$^{-1}$ FW) |
|---|---|---|---|---|---|---|---|
| Ravar (1175m) | 0.186$^{a}$ | 2.77$^{a}$ | 0.700$^{d}$ | 0.33$^{c}$ | 1.03$^{d}$ | 0.27$^{c}$ | 0.32$^{b}$ |
| Zarand (1650) | 0.116$^{c}$ | 2.04$^{bc}$ | 1.00$^{c}$ | 0.44$^{b}$ | 1.45$^{c}$ | 0.40$^{a}$ | 0.34$^{b}$ |
| Chatrood (1923) | 0.153$^{ab}$ | 2.56$^{ab}$ | 0.73$^{d}$ | 0.34$^{c}$ | 1.08$^{d}$ | 0.35$^{abc}$ | 0.23$^{c}$ |
| Kohbnan (2090) | 0.141$^{bc}$ | 2.133$^{abc}$ | 1.26$^{a}$ | 0.57$^{a}$ | 1.84$^{a}$ | 0.34$^{ab}$ | 0.56$^{a}$ |
| Kerman (1756) | 0.143$^{bc}$ | 1.78$^{c}$ | 1.15$^{b}$ | 0.57$^{a}$ | 1.72$^{b}$ | 0.32$^{c}$ | 0.32$^{b}$ |

Means followed by the same letter within a column are not significantly different according to the LSD test

**Correlating between altitude, and soil factors and studied traits**

Altitude showed a significant negative correlation with essential oil yield (r = -0.48**) and positive correlations with both flower number (r = 0.75**) and plant height (r = 0.66**). Phosphorus exhibited strong positive correlations with plant height (r = 0.90**) and flower number (r = 0.99**). Clay content demonstrated negative correlations with morphological traits while showing a positive correlation with essential oil percentage (r = 0.56**) (Table 6).

Essential oil yields were negatively correlated with altitude, nitrogen (N), phosphorus (P), potassium (K), and silt content, but positively correlated with rainfall and soil pH. These results indicate that higher essential oil yields are obtained in lower altitude areas with greater rainfall and alkaline soil conditions.

**Table 6.** The degree of correlation between morpho-physiological traits and environmental and soil physico-chemical properties in *M.pulegiumn* sites.

| Character | Altitude | Rainfall | EC | pH | N | P | K | Sand | Silt | Clay |
|---|---|---|---|---|---|---|---|---|---|---|
| Plant height | 0.66** | 0.43 | 0.44 | 0.06 | -0.30 | 0.90** | -0.29 | 0.78** | -0.48* | -0.69** |
| Stem diameter | 0.40 | -0.30 | -0.28 | 0.73** | -0.33 | 0.23 | -0.51* | 0.43 | -0.03 | -0.67** |
| Flowers No | 0.75** | 0.07 | 0.42 | -0.06 | 0.04 | 0.99** | -0.06 | 0.61* | -0.13 | -0.65** |
| Inflorescence | 0.32 | -0.89** | 0.14 | -0.31 | 0.93** | 0.18 | 0.57* | -0.40 | 0.88** | 0.08 |
| Leaf length | 0.18 | -0.42 | -0.42 | 0.80** | -0.29 | -0.13 | -0.49* | 0.21 | 0.09 | -0.47* |
| Floret length | 0.29 | -0.17 | 0.15 | 0.87** | -0.27 | -0.32 | -0.73** | 0.35 | -0.19 | -0.49* |
| DM yield | 0.09 | 0.16 | -0.36 | 0.88** | -0.77** | -0.09 | -0.78** | 0.50* | -0.46* | -0.55* |
| Oil% | **-0.58*** | **0.40** | **0.07** | **-0.13** | **-0.13** | **-0.71**** | **-0.02** | **-0.32** | **-0.26** | **0.56*** |
| Oil yield | **-0.48*** | **0.47*** | **-0.16** | **0.48*** | **-0.63**** | **-0.76**** | **-0.55*** | **0.02** | **-0.54*** | **0.16** |
| Chl. A | 0.61* | 0.04 | 0.49* | -0.45* | 0.32 | 0.95** | 0.29 | 0.35 | 0.05 | -0.34 |
| Chl. B | 0.57* | -0.06 | 0.52* | -0.56* | 0.49* | 0.88** | 0.42 | 0.21 | 0.19 | -0.22 |
| Chl. a+b | 0.61* | 0.01 | 0.50* | -0.48* | 0.37 | 0.93** | 0.32 | 0.32 | 0.09 | -0.32 |
| Carotenoid | 0.54* | -0.37 | -0.17 | 0.48 | -0.12 | 0.51* | -0.28 | 0.42 | 0.11 | -0.67** |
| Anthocyanin | 0.41 | 0.68** | 0.52* | -0.23 | -0.28 | 0.76** | -0.14 | 0.63** | -0.59* | -0.38 |

* and ** are significant at the level of 5% and 1%, respectively.

Biplot of two principal components showing the relationship among 14 traits and altitude and soil physico-chemical in *M.pulegiumn*.

**PCA for morpho-physiological traits vs. altitude and soil properties**

Multivariate analysis between altitude and soil properties and plant traits through the principal component analysis (PCA) using two-dimensional ordination diagram is shown in Figure 2. Based on the results of the relationship between the evaluated traits in the two-dimensional space, some factors, such as sandy soil, rainy and alkaline soils have been placed in one group in terms of their impact and relationship with dry matter yield, oil yield, leaf length, and floret lengths. Altitude and other soil factors such as EC, N, P, K, and silty soil are related to flower number and chlorophyll pigments (Figure 1).

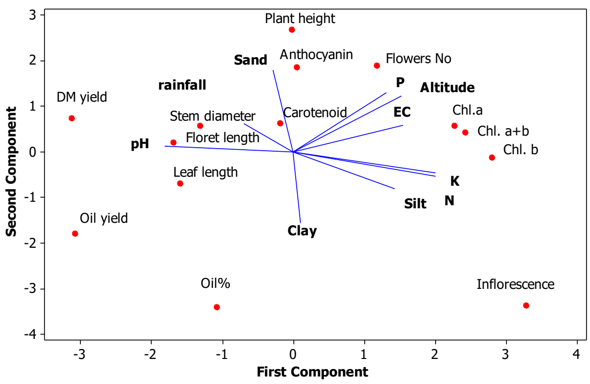


**Figure 1.** Biplot of two principal components showing the relationship among 14 morpho-physiological traits and altitude and soil physico-chemical in *M.pulegiumn* sites.

**Discussion**

The research results provide insights into the impact of altitude on the essential oil content, yield, and composition of medicinal plants. The research suggests that altitude is an important ecological factor that significantly affects essential oil content, yield, and composition. Specifically, the highest percentage and yield of essential oil were obtained from the Raver site, which was located at the lowest altitude above sea level. Additionally, the highest amount of chlorophyll and anthocyanin was observed in Kohbanan site, which had the highest altitude above sea level. This indicates that altitude can have a significant impact on the chemical composition of medicinal plants, including the content of essential oils, chlorophyll, and anthocyanin. The research also suggests that ecological factors, especially altitude, compared to soil characteristics, had a greater effect on plant quality characteristics. Therefore, altitude is an important factor to consider when evaluating the chemical composition and quality of medicinal plants.

Rainfall, altitude, and soil composition can significantly impact the growth of Pennyroyal plants. Rainfall affects soil moisture levels, which can influence the availability of water and nutrients in the soil. Pennyroyal plants require moderate watering and can tolerate dry conditions, but prolonged water stress can negatively impact their growth and yield. Higher altitudes can result in cooler temperatures and more humid conditions, which can affect plant growth and development. Higher altitudes can result in cooler temperatures and more humid conditions, which can affect plant growth and development. Soil composition is also crucial for the growth of Pennyroyal plants. Pennyroyal plants prefer light, well-drained, moderately fertile soil with a neutral pH. The soil pH affects the mineralization of organic matter, solubility of substances, microbiological processes, and plant growth. Humus, which makes up a significant portion of soil organic matter, improves soil structure, water and air regime, and heat retention, and serves as a vital nutrient source for microorganisms and plants. Total nitrogen (N total) is essential for various plant growth processes, and insufficient nitrogen can lead to stunted growth and yellowing of leaves. Plant-available phosphorus (P2O5) is crucial for vital plant functions, and inadequate phosphorus can result in poor growth and curled leaves. Potassium (K2O) is important for optimal plant growth, acting as an activator of photosynthesis, enzymes, and metabolism. Calcium (Ca), magnesium (Mg), and sodium (Na) are also vital for plant nutrition, with calcium aiding in root development, magnesium in photosynthesis, and sodium playing beneficial roles in plant metabolism.

Increasing altitude leads to shorter plants with larger, thicker, and rounded leaves. The observation that the results obtained in your search for pennyroyal essential oil composition differed, potentially due to increased rainfall at higher altitudes, highlights the complex interplay between environmental conditions and plant chemistry. Here's a breakdown of how these factors can influence the characteristics of essential oils, particularly in pennyroyal. Higher altitudes are often associated with increased rainfall compared to lower elevations in many regions. The increased precipitation at higher altitudes could potentially offset or mitigate the negative effect of altitude on essential oil yield in Pennyroyal plants. Hence, in a scenario where higher altitude corresponds to significantly greater precipitation, it is possible that the negative correlation between altitude and the number flowers in Pennyroyal could be reduced or even reversed. The density of essential oil-producing glandular hairs decreases with higher elevation, while non-glandular hairs increase. These morphological adaptations are likely strategies to cope with the environmental stresses associated with higher altitudes (Kofidis et al., 2003). In Pennyroyal plants, as altitude increases, there is a progressive decrease in the number of umbels per flowering stem, umbellules per umbel, and flowers per umbellule. Higher altitude leads to fewer flowers overall. The reduction in flower numbers at higher altitudes is likely due to harsher environmental conditions, including lower temperatures, thinner air, and reduced oxygen levels. Plants may allocate more resources to survival rather than reproduction. (Kofidis et al., 2003). Essential oil percentage tends to decrease with increasing altitude, the total yield can still be higher in taller plants at higher elevations. The essential oil composition also changes significantly, with some compounds increasing and others decreasing based on the altitude. The overall relationship is complex and varies by plant species and environmental factors (Moghaddam et al., 2023). The content of chlorophyll a and chlorophyll b in plant leaves is strongly correlated with the P content in the soil, with the relationship being statistically significant ($R^2$=0.68-0.76) under cooler weather conditions. The search results demonstrate a clear polynomial relationship between chlorophyll content in plant leaves and the availability of phosphorus in the soil, with carotenoids playing a key role in photoprotection under P deficiency. The chlorophyll-to-carotenoid ratio appears to be the most informative indicator of the plant's photosynthetic performance in relation to soil phosphorus status. (Singh et al 2017).

Mountain tea (*Stachys lavandulifolia Vahl*) showed that the height of the plants differed significantly in different cultivars. These researchers mentioned the environmental conditions and adaptation of these cultivars to the conditions of the region as the reason for the morphological differences (Riazi et al 2011). Nitrogen has a significant effect on the growth of Pennyroyal. Several studies have investigated the impact of nitrogen on the growth and yield of Greek oregano (*Origanum vulgare ssp. hirtum*), a common variety of Pennyroyal. The studies found that nitrogen is a fundamental element for plant nutrition and positively affects the biomass and essential oil yields of Pennyroyal (Moreno et al., 2002).

While the highest altitude above sea level significantly reduced the amount of essential oil. A study on *Prangos ferulacea* (L.) Lindl. Found that the highest altitude significantly reduced the amount of essential oil. The study also found that the essential oil yield was higher at lower altitudes (Azarkish et al 2020). The essential oil content of *Thymus vulgaris* L. can be influenced by nutrition and harvest time (Etehadpour & Tavassolian , 2019). Altitude can affect the morphological diversity and essential oil constituents of *Prangos ferulacea* (L.) Lindl (Azarkish et al., 2020). Nitrogen application can have a positive effect on the biomass and essential oil yields of Greek Pennyroyal (*Origanum vulgare ssp. Hirtum*) (Moreno et al 2002). A study on endemic Yarrow (Achillea eriophora DC.) found that ecological factors regulated essential oil yield, percentage, and compositions. The study suggested that the height above sea level could have an important effect on the percentage of essential oil, and the site located at the lowest altitude above sea level produced the highest percentage of essential oil (Etehadpour & Tavassolian, 2019).

The results suggest that growing conditions can significantly affect essential oil content in medicinal plant leaves. Various factors influence essential oil production, including light intensity, nutrition, altitude, and nitrogen application. For instance, light intensity has been shown to affect growth, leaf micromorphology, and essential oil production in *Ocimum gratissimum* (Fernandes et al., 2013).

Plants exhibit morphological adaptations to different climatic and soil conditions, including changes in organ dimensions (length and width), pubescence density, organ shape and color, and stem/leaf thickness. Evaluating these adaptations and their relationship with bioactive compound production can help identify superior genotypes (Bano et al., 2019).

Total dry weight in medicinal plants is influenced by multiple factors, including stem diameter, leaf width, flower number per plant, soil nitrogen, and organic carbon content. The Zarand site demonstrated particularly high dry weight values, attributable to its greater stem diameter, leaf width, flower number, and favorable soil nitrogen and organic carbon levels (Riazi et al., 2011).

Anthocyanin content varies considerably among medicinal plants depending on species, altitude, and growing conditions. The Kohbanan site (highest altitude) showed the highest anthocyanin levels, while Chatrood and Raver (lower altitudes) had the lowest. Leaves consistently contained approximately ten times more anthocyanin than flowers. Light exposure plays a crucial role in anthocyanin synthesis, with content varying by species, cultivar, and light conditions. Berries (e.g., strawberries, blueberries, blackberries) are particularly rich sources, containing 100-700 mg anthocyanin per 100 g fresh weight. These findings highlight the need for further research on altitude-anthocyanin relationships across plant species.

Studies on Kerman Thyme revealed significant environmental effects on essential oil quality and quantity, with altitude particularly influencing oil percentage - the lowest elevation site produced the highest oil yield (Ghasemi et al., 2013). Research on altitude effects consistently demonstrates impacts on antioxidant compounds (anthocyanins, carotenoids) in various species (Chanishvili et al., 2007; Spinardi et al., 2019).

*Prangos ferulacea* studies showed altitude-dependent morphological and essential oil variation, suggesting environmental influences on chlorophyll, anthocyanin, and carotenoid content (Azarkish et al., 2020). Wild plant research in Rila Mountain revealed species-specific altitude effects on chlorophyll a/b ratios. Bilberry studies demonstrated increased anthocyanin accumulation at higher altitudes, potentially due to lower daily temperatures (Sotiropoulou & Karamanos, 2010). Herbaceous plant research confirmed altitude-dependent phenol level variations (Amiri, 2008).

This collective research reveals substantial between-site variation in morphological traits, essential oil content, and photosynthetic pigments. Altitude emerges as a key determinant, with the Ravar site (lowest elevation) producing the highest essential oil percentage and yield, demonstrating the profound influence of elevation on phytochemical composition.

## Conclusion

In summary, rainfall, altitude, and soil composition significantly influence Pennyroyal plant growth by affecting soil moisture, temperature, humidity, and soil conditions. Understanding these factors and selecting or breeding Pennyroyal plants adapted to specific environments is crucial for optimizing growth and productivity. The available research demonstrates altitude's substantial impact on essential oil content, yield, and composition, as well as phenolic and anthocyanin levels in medicinal plants.

The findings reveal that altitude serves as a key ecological factor affecting plant chemical composition. The Raver site, situated at the lowest altitude, produced the highest essential oil percentage and yield. In contrast, the Kohbanan site, located at the highest elevation, showed the greatest chlorophyll and anthocyanin content. These results highlight altitude's significant influence on medicinal plants' phytochemical profiles, particularly regarding essential oils, chlorophyll, and anthocyanin concentrations.

The study further indicates that ecological factors, particularly altitude, exert a stronger influence on plant quality characteristics than soil properties alone. Consequently, altitude must be carefully considered when assessing medicinal plants' chemical composition and quality. Based on these findings, the Raver site emerges as superior for essential oil production, while the Yam site is preferable for anthocyanin-rich plants with antioxidant properties. These distinct sites can serve as valuable resources for breeding programs aimed at developing cultivars with desirable agricultural traits.

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