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# Effect of Altitude, Environment and Soil Parameters on Morphological Characteristics and Yield of Essential Oil in *Elsholtzia communis*

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## ABSTRACT

*Elsholtzia communis* an aromatic herb found cultivated that has been consumed as a vegetable spice and been used as traditional medicine for the treatment of various ailments like cold, cough, headache, fever and menstrual disorder since time immemorial by many tribes of the North-Eastern states of India. The aim of this study was to know the morphological growth and yield of essential oil variations impacted by the altitude, climate characteristics and soil variations. For which 50 population of plants were selected in each of the four different altitude sites ranging from 551 m to 1433 m msl that was considered. Morphological differences between the sites of selected parameters were analyzed using One-way ANOVA performed in R platform at 1% ($p < 0.01$) and 5% ($p < 0.05$) confidence interval and the morphological growth of the plant showed significant differences in term of p-value $< 0.05$. Further correlation coefficient test was done between essential oil yield and environmental parameters that revealed the positive correlations against its essential oil yield. Yield of 2.3±0.12% was found highest in lower altitude of Lengpui site against 1.39±0.26% in higher altitude of Hmuifang. Henceforth, it can be acknowledged from the results that *E. communis* can be further taken up for enhanced cultivation practices provided the requirement of enough irrigation along with the soil amendments for the particular altitudinal based requirements which will lead to the higher yielding aspects of essential oil of *Elsholtzia communis*.

**Keywords** Altitude, Correlation, *Elsholtzia*, Essential oil, Morphology.

## INTRODUCTION

To comprehend natural variation and how organisms react to their environment, it is essential to understand the origins of phenotypic variation in living things. Variation within a plant species is fairly typical within a geographic range. Size, shape, color, behavior, and physiological variations may be caused by heritable variances (genotype differences = ecotypes) between subpopulations at different locations, by present-day environmental variations across sites (phenotypic

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plasticity), or by both (Nazir *et al.* 2017). According to several studies altitude influences the distribution of species richness. As a result, it is becoming more widely acknowledged that altitude gradients have numerous abilities that make them better suited for determining the root cause(s) of spatial variation in variety (Rawal *et al.* 2018).

Environmental variables and climatic conditions have an impact on plant growth as well as the formation of secondary metabolites and their components in a particular habitat. Changes in the production of secondary metabolites are caused by abrupt changes in environmental conditions, particularly moisture, temperature, and altitude. In other words, stress makes the plant respond to it and create secondary metabolites at an ideal level, whereas severe stress weakens and endangers plant life. Local patterns should be identified and modelled in this regard, and the intended medicinal plant must be grown based on the knowledge gathered. A suitable pattern for providing cultivating conditions in farms and producing essential oils (EO) of high quality can be found in the natural habitat specification with high production capacity (Layeghhaghighi *et al.* 2017).

A study on *Thymus kotschyanus* Bioss. and Hohen harvesting in full blossom from the southern slopes of Alborz at six altitudes (1800-2800 m) found that the elevations level greatly impacted the percentage of essential oil and its primary components (Azimi *et al.* 2014).

Clearly, from a study it has been stated that the climate condition has an impact on the higher EO output. The four bioregions had higher EO yields and of higher quality when there was more yearly rainfall. Higher EO yields with lower quality were obtained by richer in minerals and organic matter soils. A higher altitude encouraged getting EO of higher quality. The populations had a larger EO yield farther south and at lower latitudes (Fernández-Sestelo and Carrillo 2020).

Essential Oils (EOs) with a plant origin are a significant by product of industry based on agriculture. Plant EOs are used in a variety of industries, primarily those pertaining to food, agriculture, cosmetics, beverages, perfumes and health (Moghaddam and Mehdizadeh 2017).

The characterization and identification of the main EO components, which are of great interest to many, particularly the cosmetics and pharmaceutical sectors, came about as a result of extensive phytochemical investigation. EOs may comprise of 20–100 distinct plant secondary metabolites out of various chemical classes (Carson and Hammer 2011). At least 2000 plant species have yielded about 3000 EOs, out of which 300 are significant from a commercial standpoint (Dijlani and Dicko 2012). The majority of the time plants store them in their trichomes (glandular hairs), glands, oil ducts, and resin ducts (Berger 2007).

EOs and their components are currently receiving more attention, particularly for their extensive antimicrobial activity, and which can provide alternatives to conventional functional additives, for instance, to lengthen the shelf life of food goods and ensure consumers' microbiological safety (Sánchez-González *et al.* 2011).

*Elsholtzia* is a genus under the Family Lamiaceae, consisting of 42 species (Chen *et al.* 2022), where *Elsholtzia communis* (Collet. and Hemsl.) Diels is a one rare species of this genus found to be grown and consumed all over the regions in the North-East of India used as spices and as traditional medicine for the treatment of various ailments cold, cough, headache, fever and menstrual disorders (Jamir and Tsurho 2016, Salam *et al.* 2009, Nanda *et al.* 2013, Ishwori *et al.* 2014). A green and lush, erect herb that can attain a height of about 60 cm, with attractive bunch of light green lemony scented leaves and inflorescence owing to its typical essential oil production potential (Nath *et al.* 2021). *Elsholtzia* is generally found to be grown within the wide altitude range of about 400 to 1500 m msl. It is widely distributed and has existed for ages in East Asia, Africa, North America, and Europe (Rana *et al.* 2012). Previous studies have shown that the plants in the *Elsholtzia* genus have potential antiviral, antibacterial, and antioxidant activities China (Guo *et al.* 2012).

The goal of the current study is to understand how environmental factors and soil characteristics along the altitudinal gradient affect growth characteristics and allocation patterns that could enhance EO yields. This study sought to identify the backgrounds that are most beneficial and productive for the growth of *E. communis* in order to establish strategies for cultivation, conservation, and sustainable usage of this particular cultivated species.

## MATERIALS AND METHODS

### Study sites and plant material

This study has been done across four different altitudinal variations ranging from 511 m msl to 1433 m msl for its various studies as indicated in Table 1. in the state of Mizoram, Northeast of India. Aerial parts of the 50 individual plants samples per population have been randomly selected for the study from each *Jhum* field (Shifting cultivation) in selected altitude gradients at their flowering stage for the 2019-2021 (Fig. 1). Further, the samples were dried at room temperature and kept until further examination in paper bags in a dark room. Plant identity was authenticated by preparing the herbarium and identified by Botanical Survey of India, Eastern Regional Center, MoEFCC, Govt of India.

### Soil physico-chemical analysis

Soil samples were collected in triplicate using a soil corer having an inner diameter of 5.2 cm. 5 random soil cores at depth 0–15 cm collected and composited to acquire a true soil sample representative of the site. A single composited soil sample composed of five soil cores from each site collected from each site and therefore, a total of 4 composite samples (1 composited sample × 4 study sites) were collected for the study.

**Table 1.** The study site and microclimatic characteristics of the study sites. Source: Data access viewer, NASA.

| Study site | Altitude (in m) | Coordinates | Annual temperature (in °C) 2019-2020 | Annual rainfall (in mm) 2019-2020 |
|---|---|---|---|---|
| Lengpui (LP) | 511 | N-23º48'34.0" E- 092º37'11.4" | 23.66 | 7.64 |
| Sumsuih (SM) | 855 | N- 23º30'28.1" E- 092º44'39.8" | 22.83 | 8.45 |
| Reiek (RK) | 1260 | N- 23º42'17" E- 092º36'36.09" | 22.34 | 8.75 |
| Hmuifang (HF) | 1433 | N- 23º26'25.8" E- 092º46'11.5" | 22.85 | 8.34 |

Soil moisture content (SMC%) was determined by gravimetric method and pH using digital pH meter electrical conductivity (EC) was determined (Mettler Toledo, Switzerland) with soil-water ratio 1:2.5 w/v based on (Bandyopadhyay *et al.* 2012). Bulk density (BD) was determined based on (Blake and Hartge 1986) using known volume of fresh soil. Soil water holding capacity (WHC) was determined by Keen-Raczkowski box method using a fresh soil sample after removal of gravel (Coutts 1930). Total Organic carbon (TOC) was determined by Walkley and Black (1934) rapid oxidation and titration method (TN) content in the soil samples were determined by Kjeldahl Method of Nitrogen estimation (Baethgen and Alley 1989). For the analysis of soil ex-changeable nutrients air dried soil samples were extracted in Mehlich-I

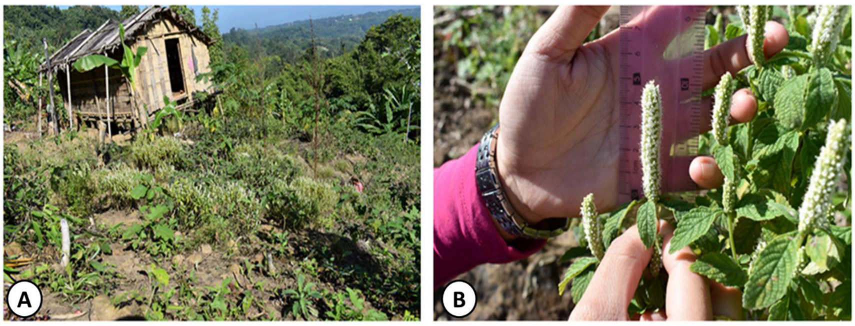


**Fig. 1.** (a) Shifting cultivation in Mizoram, (b) *Elsholtzia* plant at flowering stage.

solution (0.05 M HCl + 0.025 M $H_2SO_4$) and analyzed (K, Na, Ca, Mg) using the inductively attached plasma spectrometer (iCAP6300 series, Thermo scientific) and soil nutrients like Zn and Fe were analyzed using atomic absorption spectroscopy (AAS).

### Morphological characterization

Both *ex-situ* and natural standing crops were used in the trials, and photos were obtained. To investigate the numerous morphological characteristics of the species, 50 mature flowering individuals were randomly chosen and tagged from each community and to document the range of floral and vegetative characteristics. The populations' morphological characteristics, including plant height, spread, leaf length, petiole length, number of branches, and flower length at the flowering stage in all the populations, were examined. In order to identify the locations where this species grows best, we employed pearson correlation statistical analysis to evaluate the association between several morphological features along the altitudinal gradient.

### Isolation of essential oils

Samples (250g of dried aerial part of the plant) were separately hydro-distilled with a clevenger apparatus for 6 h (Clevenger 1928, Kumar *et al.* 2012). After distillation the extracted oil samples was measured on dry weight basis (Volume/Weight) of any taken plant parts; hence yield percent was recorded as dry weight basis using formula- V1/W2 × 100, where V1 is the weight if the oil in ml and W2 is the total weight of dried plant used. After being extracted, the essential oil was dried over anhydrous sodium sulphate and stored in sealed amber vials in the refrigerator until examination (Bajalan *et al.* 2018).

### Climatic data

Climatic records such as temperature and rainfall of the study area was collected in the form of secondary data from Source- Data access viewer, NASA. Sites and microclimatic characters of the studied sites are reported in the Table 1 and Fig. 2.

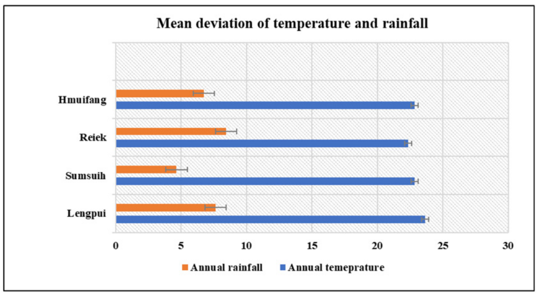


**Fig. 2.** Mean deviation of temperature and rainfall of the study sites.

### Statistical analysis

Descriptive statistics of collected data from the experiment was performed using R statistical software. The parameters such as soil, weather, altitude, yield and morphology were used to perform ANOVA comparison. Differences between the sites of selected parameters were analyzed using One-way ANOVA performed in R platform at 1% ($p < 0.01$) and 5% ($p < 0.05$) confidence interval. Significant differences among selected parameter means ($p < 0.05$) were evaluated through the Tukey HSD post-hoc test. For the calculation of correlation between the altitude, temperature and rainfall against yield, we have used pearson correlation method.

## RESULTS AND DISCUSSION

The 50 population each of *Elsholtzia communis* were selected randomly from the selected *Jhum* cultivated fields. The present study explores the influence of soil, climate and altitudinal variations on morphology and yield of cultivated *Elsholtzia communis* populations in the *jhum* cultivated fields of Mizoram, India.

**Table 2.** Properties of the soil at the study sites showing populations of *E. communis* (n=50).

| Sites | pH | EC (µS/cm) | BD (g/cm³) | SMC (%) | WHC (%) | TOC (%) | TN (%) |
|---|---|---|---|---|---|---|---|
| LP | 7.10±0.26 | 63.60±1.04 | 0.97±0.04 | 28.76±0.96 | 44.02±0.79 | 2.40±0.17 | 0.22±0.02 |
| SM | 6.77±0.25 | 86.40±2.25 | 0.68±0.05 | 45.50±1.21 | 41.89±1.43 | 3.61±0.13 | 0.34±0.03 |
| RK | 6.52±0.06 | 62.07±1.57 | 0.93±0.15 | 41.72±0.48 | 48.81±5.99 | 3.82±0.35 | 0.34±0.03 |
| HF | 6.37±0.12 | 85.63±3.15 | 0.80±0.18 | 49.85±4.52 | 54.42±2.10 | 6.15±0.14 | 0.54±0.02 |

**Table 3.** Soil CEC and minerals of the soil at the study sites showing populations of *E. communis* (n=50).

| Sites | $K^+$(Meq/ 100 g) | $Na^+$ (Meq/ 100 g) | $Ca^{2+}$ (Meq/ 100 g) | $Mg^{2+}$ (Meq/ 100 g) | $Fe^{2+}$ (ppm) | $Zn^{2+}$ (ppm) |
|---|---|---|---|---|---|---|
| LP | 0.57 | 0.10 | 8.32 | 1.76 | 8.15 | 1.48 |
| SM | 0.53 | 0.10 | 8.32 | 1.60 | 12.07 | 1.40 |
| RK | 0.47 | 0.17 | 6.66 | 2.14 | 4.60 | 0.71 |
| HF | 0.21 | 0.12 | 2.28 | 0.10 | 7.61 | 0.51 |

## Soil and its characteristics

The physico-chemical characteristics of soil samples taken from various altitudinal sites are shown in Table 2. The mean value of pH ranged between 7.10±0.26 and 6.37±0.12 in the lowest altitude site of LP and HF. As the pH is found to be increased in slash and burn type lands than usually it is found slightly acidic in the hill areas (Kong *et al.* 2019). The electric conductivity (EC) of soil samples showed similarity between two sites LP and RK with mean 63.60±1.04 µS/cm and 62.07±1.57µS/cm whereas it showed similarity between the other two sites SM and HF with 86.40±2.25 µS/cm and 85.63±3.15 µS/cm, respectively. All the sites have shown lesser acidic to neutral in nature providing the suitable environment for this species for the growth. Bulk density (BD) in the respective sites was found between 0.68±0.05 g/cm$^3$ in SM to 0.97±0.04 g/cm$^3$ in LP which is found mostly feasible in the shifting cultivated areas in Mizoram (Manpoong and Tripathi 2019). SMC (%) found to have an increasing pattern from LP to HF with 28.76±0.96% and 49.85±4.52%. WHC (%) result did not show much variation among each site with value ranging from 41.89±1.43% in SM to 54.42±2.10 %. High TOC (%) was found in higher altitude of HMF with 6.15±0.14% and the lowest at the lower altitude with 2.40±0.17%. TN (%) is evident of the high effect of soil moisture content (Manpoong

**Table 4.** Site and morphological characteristics of the study sites.

| | Lengpui (LP) | Sumsuih (SM) | Reiek (RK) | Hmuifang (HF) |
|---|---|---|---|---|
| Plant height (cm) | 52.41 ± 3.18 | 32.85 ± 4.65 | 42.26 ± 9.70 | 21.75 ± 2.68 |
| Plant spread (cm$^2$) | 774.03 ±153.89 | 441.81 ±143.72 | 2103.03 ±247.07 | 506.06 ± 55.29 |
| No. of branches | 16.30 ± 1.62 | 10.42 ± 1.18 | 15.92 ± 1.11 | 9.76 ± 1.57 |
| Petiole length (cm) | 1.8± 0.7 | 1.9 ± 0.6 | 1.6± 0.7 | 2.3± 0.6 |
| Leaf length (cm) | 5.38 ± 0.48 | 6.92 ± 0.54 | 4.75 ± 0.54 | 5.42 ± 0.44 |
| Leaf breadth (cm) | 1.96 ± 0.21 | 1.73 ± 0.23 | 1.75 ± 0.28 | 2.34 ± 0.18 |

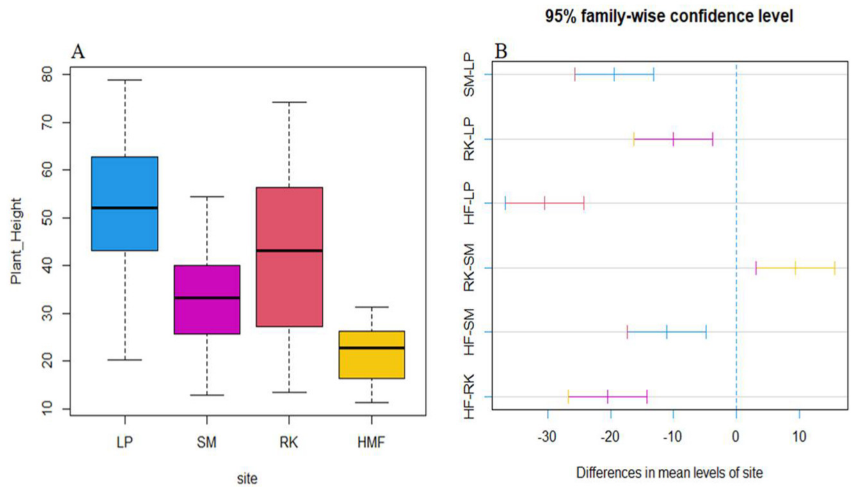


**Fig. 3.** Comparison of plant height between the study sites- A) Boxplot showing the data distribution of sites, B) Comparison of plant height between all possible combinations of sites.

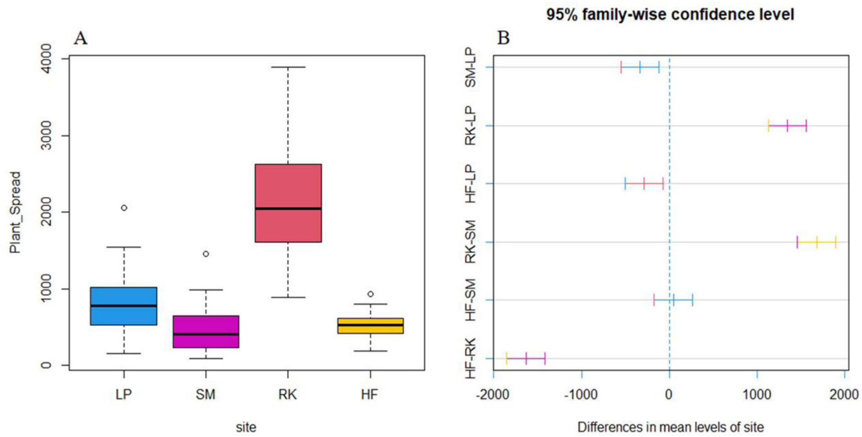


**Fig. 4.** Comparison of plant spread between the study sites- A) Boxplot showing the data distribution of sites B) Comparison of plant spread between all possible combinations of sites.

and Tripathi 2019), it was found to be in increasing manner where the moisture was increased in the sites of LP to HF with 0.22±0.02% and 0.54±0.02%.

**Table 5.** Estimation of essential oil and yield (%) from selected sites.

| Standardization of data | Lengpui | Sumsuih | Reiek | Hmuifang |
|---|---|---|---|---|
| Wt. of the plants (in g) | 250 | 250 | 250 | 250 |
| Quantity of essential oil (in ml) | 5.9±0.31 | 4.3±0.2 | 5.2±0.1 | 3.47±0.65 |
| % yield of essential oil | 2.3±0.12 | 1.72±0.08 | 2.08±0.04 | 1.39±0.26 |

Cation exchange Capacity (CEC) of selected sites were analyzed and found to have a range of K+ between 0.57 to 0.21 Meq/100 g, $Na^+$ was found similar in all the sites ranging with 0.10-0.17 Meq/100 g, whereas, $Ca^{2+}$ was found higher in lower elevations of LP and SM with 8.32 Meq/100 g and least in HF

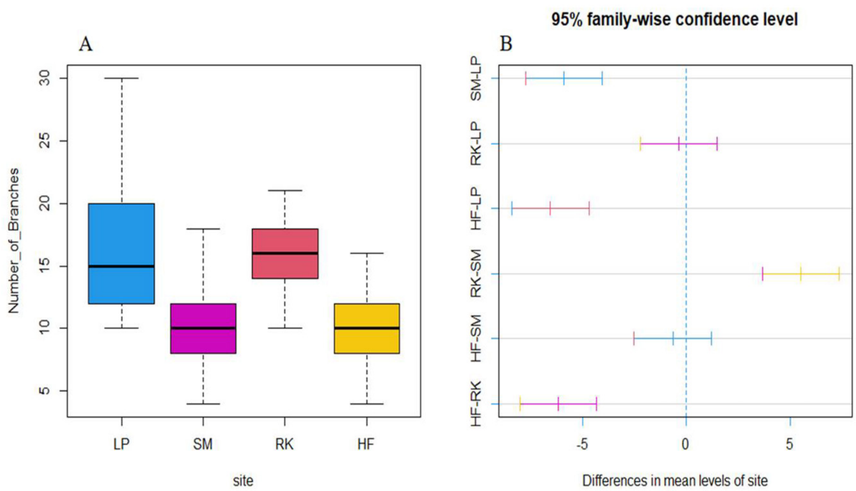


**Fig. 5.** Comparison of no. of branches between the study sites- A) Boxplot showing the data distribution of sites, B) Comparison of no. of branches between all possible combinations of sites.

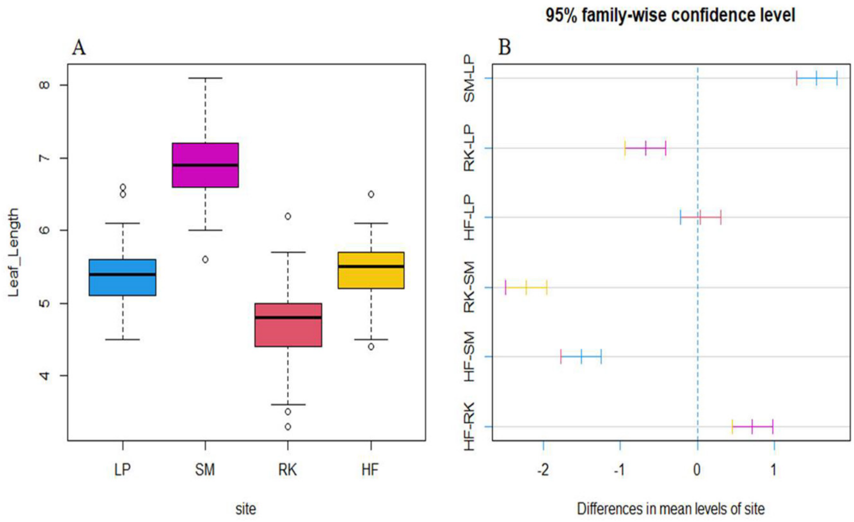


**Fig. 6.** Comparison of leaf length between the study sites- A) Boxplot showing the data distribution of sites, B) Comparison of leaf length between all possible combinations of sites.

with 2.28 Meq/100g. $Mg^{2+}$ was found higher similar in lower range of LP and to RK with 1.76 to 2.14 Meq/100 g and low in RK with 0.10 Meq/100 g. Zn was found to be decreasing pattern with 1.48 ppm in LP to 0.51 in HF, and similar report has been found in other study by Satisha *et al.* (2000). The content of $Fe^{2+}$ was found fairly high in all the sites with least in HF 4.60 ppm and 12.07 ppm in SM (Table 3).

## Morphological variations between the sites

According to the data collection from the experimental sites (Lengpui, Sumsuih, Reiek, Hmuifang), the morphological data include, plant height, plant spread, number of branches, leaf length, leaf breadth, petiole length and flower length (Table 4). The ANNOVA comparison suggested significant differences in term of p-value $< 0.05$. Pairwise ANNOVA comparison of plant height show significant difference between all combination of site (SM-LP, HF-LP, HF-RK, HF-SM, RK-LP and RK-SM) (Fig. 3) that

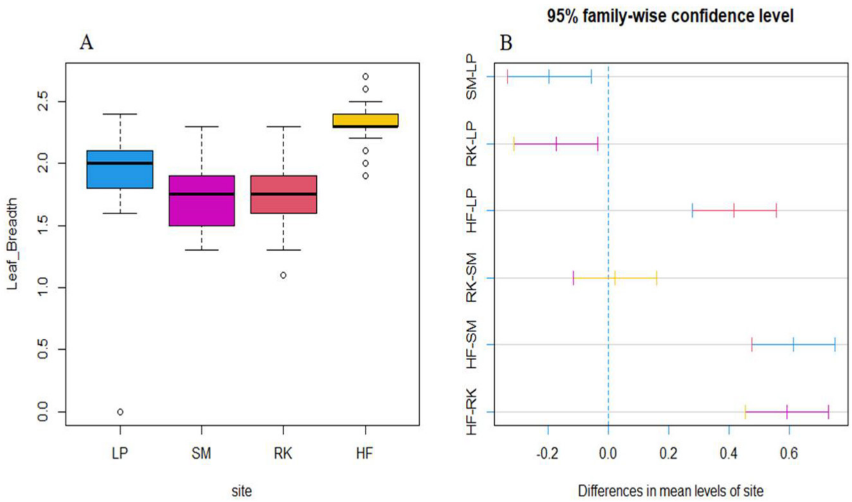


**Fig. 7.** Comparison of plant leaf breadth between the study sites- A) Boxplot showing the data distribution of leaf breadth collected from the sites, B) Comparison of leaf breadth differences between all possible combinations of sites.

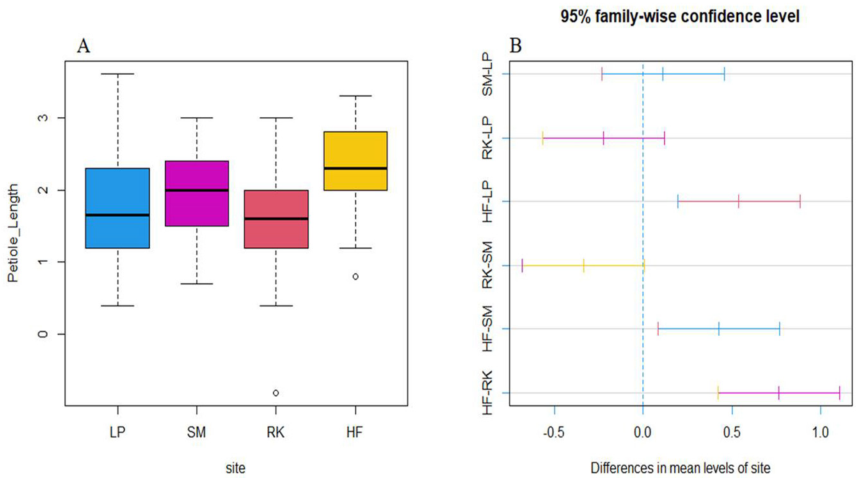


**Fig. 8.** Comparison of petiole length between the study sites- A) Boxplot showing the data distribution of petiole length of sites, B) Comparison of petiole length differences between all possible combinations of sites.

decided based on adjusted p-value $< 0.001$. Similarly, the comparison based on the plant spread shows significant differences between HF-RK, RK-SM and RK-LP but the ANNOVA comparison shows no significant difference at the label of mean between HF-SM, p-value $\geq 0.944$ and minute mean differences between HF-LP and SM-LP (Fig. 4). Further we tried to examine the number of branches between the sites and statistical analysis indicated significant differences between HF-RK, RK-SM, HF-LP and SM-LP and pairwise comparison between RK-LP and HF-SM shows no significant differences in term of p-values $> 0.05$ (p-value $> 0.950$) (Fig. 5). Comparison in the growth of leaf length suggested that significant differences in all combination of sites (SM-LP, HF-RK, HF-SM, RK-LP and RK-SM) except in HF-LP found no difference in term of p-value $\leq 0.976$ (Fig. 6). The growth in leaf breadth is also measured and ANNOVA pairwise comparison between the sites also indicated significant differences in all combination of sites (SM-LP, HF-RK, HF-SM, RK-LP and HF-LP) except RK-SM found no difference in p-value $\leq 0.976$ (Fig.

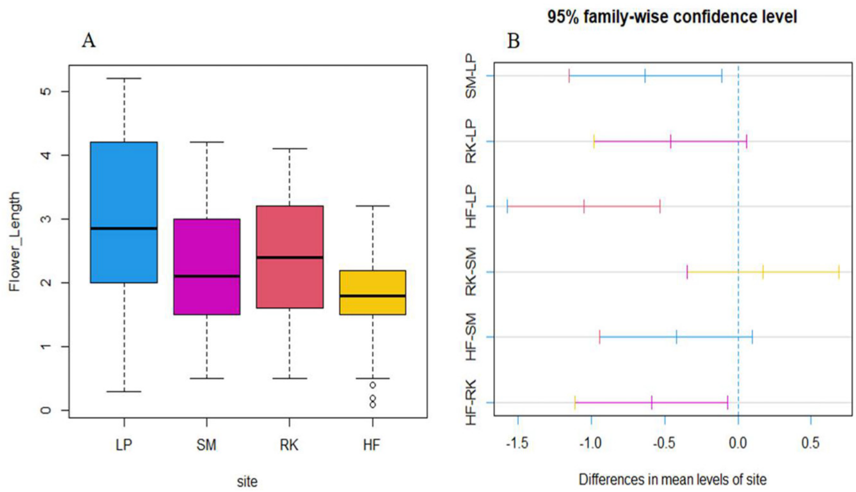


**Fig. 9.** Comparison of flower length between the study sites- A) Boxplot showing the data distribution of flower length of sites, B) Comparison of petiole length differences between all possible combinations of sites.

**Table 6.** Correlation coefficients between essential oil yield and environmental parameters.

| Yield | Effect of different parameters Altitude | Temperature | Rainfall |
|---|---|---|---|
| | 0.743 | 0.741 | 0.739 |

7). Where the measurement of petiole length shows the difference between HF-LP, HF-SM, and HF-RK found the p-value < 0.05 and SM-LP, RK-LP and RK-SM, the p-value we found ≥ 0.832, ≥ 0.330 and ≥ 0.057, respectively. (Fig. 8) and lastly, we compared the flower length between the sites and found major difference in HF-LP among all the sites considered with p-value less than 0.05 (Fig. 9).

*Elsholtzia* essential oil concentrations varied significantly between different sites (p<0.01) in terms of average content. Plants from the sites LP and HF were observed to have the highest 2.3% and lowest 1.39% levels, respectively. It has been observed in this study that the essential oil yield was more in the lower altitudes and found decreasing in manner with the increase in altitude (Table 5).

According to these results, there was a strong positive and negative association between the yield of essential oils and the parameters for temperature and altitude respectively (Table 6).

According to former research, environmental factors including temperature and precipitation rate had an impact on the yield of essential oils present in aromatic plants (Barra 2009, Sangwan *et al.* 2001, Kumar *et al.* 2011). To access the high essential oil yield, *Elsholtzia communis* plants should be grown in areas with low elevation and a sufficient watering schedule.

Inverse relationships existed among altitude, temperature and rainfall as environmental variables, mean with their annual report compared with the annual yield. The pearson correlation study suggested that with the decrease in altitude the temperature, and rainfall was found high, resulting in the increase in yield production. For instances the total essential oil with 511 m altitude was higher in Lengpui than in Hmuifang site with 1433 m in plants of *E. communis.*

## CONCLUSION

The present study has conducted at four different altitudinal variations comprising of 50 populations each of *Elsholtzia communis* in the *Jhum* Lands of Mizoram. The results from this study explore the altitudinal aspects, soil physico-chemical variations, the plant morphology and yield of essential oil. It is evident and the study revealed from the outcome that altitude has significant effect on plant morphology which resulted variations in the yield of essential oil in *Elsholtzia communis.* It was also recorded that the yield of essential oil increased gradually as temperature rose, hence, it was most abundant at areas with low elevation and high precipitation rates. Moreover, the systematic soil physico-chemical parameters study revealed that the varying degree of results along the altitudinal gradients and with the understanding of the nutrients required a great ideal parameter could be set up for future cultivation aspects directing the species towards higher yield potential in a local as well as commercial level.


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