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Influence of soil on oregano (*Lippia graveolens* Kunth.) essential oil chemotypes from two regions of Saucillo, Chihuahua

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ABSTRACT

Objective: This study aimed to assess the association between soil properties, chemotypic expression, and essential oil (EO) yield in *Lippia graveolens* Kunth (Mexican oregano) from two distinct regions in Saucillo, Chihuahua.

Design/Methodology/Approach: A comparative analysis was conducted at two sites: a wild population established on Chernozem soil (Carboneros) and a cultivated population grown on Vertisol (Subida Alta). Soil characterization followed NOM-021-SEMARNAT guidelines, including texture, pH, electrical conductivity (EC), organic matter (OM), carbonates, and available nutrients. Essential oil yield was quantified via hydrodistillation using a Clevenger apparatus, while volatile compound profiling was performed through gas chromatography-mass spectrometry (GC-MS) following steam distillation. Statistical differences between sites were evaluated using ANOVA and t-tests ($\alpha=0.05$) with SAS 9.4 software.

Results: The wild population site featured a higher clay content, increased OM, and a slightly alkaline pH (7.88), correlating with a chemotype enriched in aromatic precursors (p-cymene 25.04%; γ-terpinene 9.46%). In contrast, the cultivated site exhibited a sandy-loam texture, reduced OM, a more alkaline pH (8.04), elevated levels of available potassium (K^+), and a predominance of phenolic monoterpenes (carvacrol 29.15%; thymol 10.27%). EO yield was 3.53% at the wild site and 3.00% at the cultivated site. Variations in texture, pH, OM, and K^+ content were consistent with a transition from precursor-rich profiles to phenolic compound-dominated profiles.

Limitations/Implications: The study's scope was constrained by the absence of controlled variables, such as genotype and agronomic history, due to the wild nature of oregano populations. Additionally, extraction techniques may have influenced both EO yield and chemical profiles observed via GC-MS. Experimental manipulation of soil parameters would enhance reproducibility and strengthen causal inferences.

Findings/Conclusions: The findings suggest that soil characteristics significantly influence chemotypic expression in *L. graveolens*. Finer-textured soils with higher OM content favored the accumulation of p-cymene

and γ-terpinene, whereas sandy-loam, alkaline soils with increased K^+ availability were associated with higher concentrations of carvacrol and thymol.

Keywords: yield; hydrodistillation; p-cymene; chemical variability; edaphic factors; chemotype.

INTRODUCTION

Essential oils (EOs) from aromatic plants are complex mixtures of secondary metabolites with high commercial value and wide applications in the food, pharmaceutical, and cosmetic industries. Approximately 5% of the dry plant matter consists of essential oils, which are valuable secondary compounds localized in fruits, flowers, stems, leaves, and buds (Khan *et al.*, 2023). Chemically, these oils are typically composed of intricate blends of flavonoids, monoterpenes, isoflavones, alkaloids, phenolic acids, carotenoids, and aldehydes (Mei *et al.*, 2019). One of the most significant aromatic plants is oregano, which, according to Cheikhyoussef (2020), is classified into four main groups: Turkish oregano (*Origanum onites*), Spanish oregano (*Coridothymus capitatus*), Greek oregano (*Origanum vulgare*), and Mexican oregano (*Lippia graveolens*). The chemical composition of *L. graveolens* essential oil (EO) varies due to genetic, environmental, and agronomic factors. This variability gives rise to different chemotypes, characterized by varying thymol/carvacrol ratios and the accumulation of p-cymene (Krause *et al.*, 2021; Rubio *et al.*, 2023). However, the heterogeneity of plant material and the frequent harvesting from wild populations hinder the standardization of *L. graveolens* EO (Souza, 2006). In this context, the present study evaluated the yield and composition of EO in both wild and cultivated *L. graveolens* populations from two regions in Saucillo, Chihuahua, Mexico, along with the influence of edaphic conditions, with the aim of establishing criteria for the differentiation and standardization of this species.

MATERIALS AND METHODS

Plant Material

Oregano plants were collected from two regions in Saucillo, Chihuahua, Mexico. The wild collection site was located at 28° 03' 16" N, 105° 13' 52" W, at an elevation of 1,249 meters above sea level, covering an area of 1.62 hectares with a perimeter of 1.28 km, in a region known as Carboneras. The second collection site was located in the Subida Alta region, where the National Forestry Commission (CONAFOR, 2021) donated 250,000 L. graveolens plants for transplantation over a 5-hectare area. This initiative supported 487 families across 7 communal land units (ejidos) within the municipality, organized under the Asociación Oreganeros del Conchos SPR de RL de CV, as part of a program promoting EO extraction for commercial sale and export. This cultivated site is located at 28° 05' 28" N, 105° 16' 16" W, at an elevation of 1,194 meters above sea level, with an area of 4.93 hectares and a perimeter of 0.94 km.

Soil analysis

Soil types at both sites were classified according to the Compendio de Información Geográfica Municipal de los Estados Unidos Mexicanos by INEGI (2010), which identifies

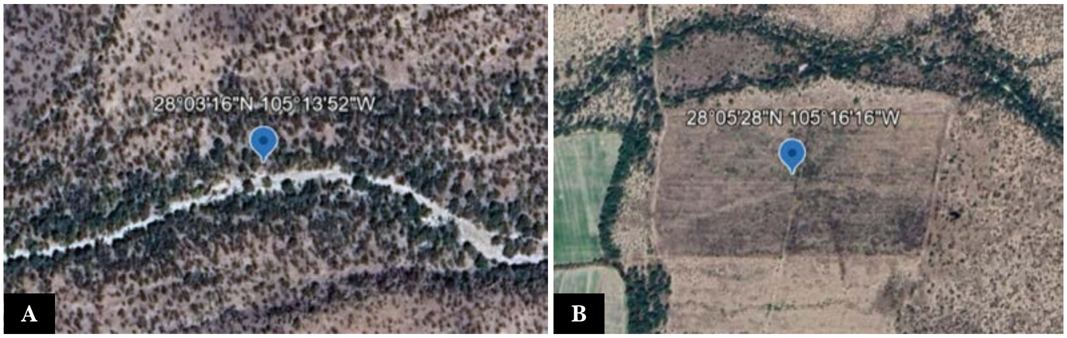

Figure 1. Sampling sites in Saucillo, Chihuahua. a) Wild population (Carboneras) (A), Cultivated population (Subida Alta) (B).

the wild site as Chernozem and the cultivated site as Vertisol based on their geographical location. According to the technical soil classification manual by the Soil Survey Staff (2022), Chernozem soils are typically found under temperate grasslands and are characterized by a dark mollic epipedon rich in humus and base cations, with carbonate accumulation in the subsoil. These soils are fertile and generally neutral to slightly alkaline. In contrast, the cultivated site contains Vertisol, a soil type commonly found in plains or depressions experiencing pronounced wet-dry cycles. Vertisols contain expansive clays that swell when wet and contract upon drying, forming deep cracks and polished, striated surfaces. They are typically fertile, with neutral to slightly alkaline pH and high base saturation.

Soils were analyzed following standard procedures outlined in the Mexican Official Standard NOM-021-SEMARNAT (SEMARNAT, 2002). At each site, soil samples were collected from a 30 cm depth using a zigzag pattern, with ten subsamples per site. The methodologies for each parameter included: texture via the hydrometer method (Bouyoucos); pH in saturation paste using a potentiometer (HI5521-01, Hanna Instruments, USA); electrical conductivity measured with a multiparameter meter (Orion Star A215, Thermo Scientific, USA); organic matter via Walkley-Black; total carbonates ($CaCO_3$) by calcimetry; bulk density via the core method; soluble salts (Ca^{2+}, Mg^{2+}, Na^+, K^+, CO_3^{2-}, HCO_3^-, Cl^-, SO_4^{2-}) extracted in a saturated paste and quantified by titration (Cl^- via argentometry) and turbidimetry for SO_4^{2-} using a 2100Q turbidimeter (Hach, USA). Available nutrients were measured as follows: phosphorus (Olsen method) via UV/Vis spectrophotometry (DR2800, Hach, USA); nitrate (NO_3^-) by colorimetric method (UV/Vis); and exchangeable cations/micronutrients (Fe, Zn, Cu, Mn, and K^+) by atomic absorption spectrophotometry (AAnalyst 200, PerkinElmer, USA) following extraction. All analyses were reported in accordance with the units and interpretive criteria specified by NOM-021.

Essential oil extraction and yield calculation (% v/w, dry weight basis)

Hydrodistillation (HD) is a conventional technique for extracting essential oils (EOs) from plant material using water or steam. In the Clevenger apparatus, the mixture is brought to a boil, causing the vapors to rise to a condenser, where they liquefy and collect in a graduated trap. Due to immiscibility and density differences, the oil separates from the aqueous phase. The condensed water recirculates to the boiling flask, maintaining volume and enabling direct measurement of the recovered EO (Fagbemi *et al.*, 2021). This method is endorsed by the European Pharmacopoeia and ISO 6571 standards for spices, providing reliable and traceable results (European Pharmacopoeia, 2023; ISO, 2008).

The experiment used dried *L. graveolens* leaves from both wild and cultivated populations to determine EO yield. Three replicates per treatment were performed using 50 g of dry material. Samples were macerated and placed in 1 L flasks with 500 mL of distilled water, then boiled at 100 °C for 3 hours. After distillation, the EO volume was measured directly from the Clevenger column. Yield was expressed as % v/w (mL/100 g dry weight). For statistical analysis, results were reported as mean ± standard deviation, and differences between sites were evaluated using one-way ANOVA and Student's t-test ($\alpha=0.05$) in SAS 9.4 (SAS Institute Inc., 2013).

Essential oil extraction for volatile compound analysis

Essential oil was obtained by steam distillation using a VEVOR apparatus. Steam passed through the plant material, releasing volatile compounds, which were collected as hydrosol after condensation. The hydrosol was extracted with hexane in a separatory funnel to isolate the organic phase rich in EO. This fraction was concentrated using a rotary evaporator (Science Med, SM100-PRO, Finland) to remove the solvent. The resulting oil was stored in amber vials at 4 °C until analysis (Nahar, 2019).

Biosynthesis of thymol, carvacrol, and p-Cymene

The biosynthetic pathway for thymol and carvacrol (Figure 4) begins in plastids via the MEP/DOXP pathway (2-C-methyl-D-erythritol-4-phosphate/1-deoxy-D-xylulose-5-phosphate), producing IPP (isopentenyl pyrophosphate) and DMAPP (dimethylallyl pyrophosphate). These intermediates are condensed by GPPS (geranyl pyrophosphate synthase) to form GPP (geranyl pyrophosphate), a C_{10} prenyl donor and monoterpene precursor. GPP is cyclized by TPS (terpene synthase) to produce γ-terpinene, the direct precursor of thymol and carvacrol (Degenhardt *et al.*, 2009). γ-terpinene is oxidized by cytochrome P450 enzymes (CYP71D subfamily), introducing a hydroxyl group and forming unstable cyclohexadienol intermediates. These are further dehydrogenated by SDR (short-chain dehydrogenase/reductase) to allylic ketones, which through keto-enol tautomerism and rearomatization, yield thymol (if hydroxylation occurs at C-3) or carvacrol (at C-6) (Krause *et al.*, 2021).

Volatile compounds

Volatile compound concentrations were analyzed by gas chromatography-mass spectrometry (GC-MS) using solid-phase microextraction (SPME). A 1.5 mL aliquot of oregano EO was placed in 4 mL vials. During agitation, an SPME fiber (65 μm, PDMS-DVB, Agilent, USA) was exposed to the headspace for 60 minutes at room temperature. The fiber was then thermally desorbed in the GC-MS injector for 5 minutes at 200 °C. The GC-MS system (Agilent) was equipped with a DB-WAX column (60 m×0.25 mm, 0.25 μm, Agilent, USA). Chromatographic conditions included: injector temperature 200 °C; oven temperature held at 33 °C for 5 minutes, increased to 50 °C at 2 °C min^{-1}, then to 250 °C at 5 °C min^{-1}, held for 6.5 minutes. Helium was used as the carrier gas at a linear velocity of 30 cm s^{-1}. Mass spectra were acquired via electron ionization at 70 eV. Transfer line and ion trap temperatures were set at 250 °C and 180 °C, respectively. Compound identification was achieved by comparing sample spectra with the NIST MS 2014 library. Results were expressed as relative concentrations, calculated as the ratio of each compound›s base peak area to the total peak area.

Statistical analysis

A one-way analysis of variance (ANOVA) and Tukey's multiple comparison test ($p \leq 0.05$) were performed using SAS 9.4 statistical software (SAS Institute Inc., 2013).

RESULTS AND DISCUSSION

The use of essential oils (EOs) and their versatility enable their application across various sectors, including food, industrial, and pharmaceutical fields. This is largely due to their minimal side effects when administered at appropriate doses (Posadzki *et al.*, 2012). While mild allergic reactions and slight phototoxic effects may occur, only a limited number of oils exhibit necrotic, narcotic, nephrotoxic, hepatotoxic, or carcinogenic effects. Nevertheless, most adverse reactions are attributable to improper use, typically resulting from incorrect dosing or self-medication (Bunse *et al.*, 2022). A key limitation in the use of EOs is the chemical instability of their constituents, influenced by edaphic, climatic, and other environmental factors (Răileanu *et al.*, 2013). Additionally, achieving a consistent EO composition even from identical plant material remains a significant challenge (Machado *et al.*, 2022).

Hydrodistillation and yield using the clevenger apparatus

Table 1 presents the EO yield obtained by treatment. Hydrodistillation (HD) produced a significantly higher yield in wild oregano (3.53% v/w) compared to cultivated oregano (3.00% v/w). These values align with previously reported yields for *L. graveolens* obtained via HD, which typically range between 2-3%, consistent with those observed in the cultivated samples (Bautista *et al.*, 2021). The increased yield in wild oregano may be attributed to environmental conditions, phenological stage, or stress factors such as water availability and light exposure, as well as soil characteristics all of which are known to modulate EO content (Morshedloo *et al.*, 2018). Moreover, higher yields (3-4% or even up to 4.4%) have been recorded under specific conditions or through steam distillation at the laboratory

Table 1. Essential Oil Yield (EOY).

Treatment	Yield (mm)	% v/w	n
Wild	1.77 ± 0.12^{a}	3.53	3
Cultivated	1.50 ± 0.10^{a}	3.00	3

n=number of samples, results are presented in % v/w, dry basis, millimeter (mm).

scale. These findings suggest that yield may be further enhanced by employing alternative extraction methods or extending the distillation time to 240 minutes, as demonstrated by Zheljazkov (2012). However, excessive distillation time may result in decreased yields due to potential degradation or chemical alteration of EO constituents under prolonged heat exposure (Zhao *et al.*, 2013).

Chemical profile of wild essential oil (EO)

Volatile compounds in the wild EO were grouped by class as follows: aromatic monoterpenes (p-cymene, p-cymenene) accounted for 63.90%; oxygenated monoterpenes for 17.95%; non-aromatic monoterpenes (α/β-pinene, camphene, myrcene, α-terpinene, limonene, terpinolene, terpinen-4-ol) for 11.96%; and sesquiterpenes for 5.69%. The total compound sum was 99.50%, with the discrepancy attributed to rounding. Within the sesquiterpene group, β-caryophyllene (3.29%) and α-humulene (1.94%) were predominant, with aromadendrene also present (0.46%). At the individual compound level, p-cymene was the most abundant (63.52%), followed by thymol (6.22%), carvacrol (4.85%), and terpinen-4-ol (4.60%).

Chemical profile of cultivated essential oil (EO)

In the cultivated EO, the volatile profile consisted of oxygenated monoterpenes (51.57%), sesquiterpenes (25.07%), aromatic monoterpenes (15.28%), and non-aromatic monoterpenes (5.03%), totaling 96.95% (discrepancy attributed to rounding and trace compounds). Phenolic ethers were detected in higher proportions methyl thymol ether (3.74%) and methyl carvacrol (3.54%) along with elevated levels of terpinen-4-ol and p-cymen-8-ol (0.55% *vs.* 0.15% in the wild EO). The cultivated oil exhibited a phenolic chemotype, with carvacrol (29.15%) and thymol (10.27%) as major constituents, while p-cymene decreased to 14.59%. Among the sesquiterpenes, β-caryophyllene (14.22%) and α-humulene (10.85%) were prominent. The volatile profile was dominated by p-cymene (63.52%), accompanied by γ-terpinene (4.60%), thymol (6.22%), and carvacrol (4.85%) among the oxygenated monoterpenes. In the sesquiterpene fraction, notable compounds included cis-β-caryophyllene (3.29%) and α-humulene (1.94%). Non-aromatic monoterpenes (α/β-pinene, camphene, β-myrcene, α-terpinene, D-limonene, terpinolene) summed to 12.0%, while aromatic monoterpenes (p-cymene + p-cymenene) reached approximately 63.9%; oxygenated monoterpenes totaled ~18.0%, and sesquiterpenes ~5.7%, for an overall profile completeness of ~99.5%.

Volatile compounds in wild and cultivated EO

Table 2 shows the relative composition of volatile compounds in both wild and cultivated EO samples. The cultivated EO displayed a phenolic chemotype, with carvacrol (29.15%) and thymol (10.27%) as the dominant compounds, followed by p-cymene (14.59%), cis-β-caryophyllene (14.22%), and α-humulene (10.85%). The distribution by chemical class was as follows: non-aromatic monoterpenes 5.0%, aromatic monoterpenes 15.3%, oxygenated monoterpenes 51.6%, and sesquiterpenes 25.1%, summing to 96.9%.

Table 2. Relative Composition of Wild and Cultivated *Lippia graveolens* Essential Oil (EO).

Monoterpenes

RT	Compound	Wild (%)	Cultivated (%)
9.71	1R-α-Pinene	0.33±6.28	N.D.
10.87	Camphene	0.72±14.86	N.D.
11.99	L-β-Pinene	0.23±4.26	N.D.
13.51	β-Myrcene	2.14±42.66	1.05±0.92
14.06	α-Terpinene	1.82±33.10	0.31±0.03
14.65	D-Limonene	1.83±39.39	2.58±1.95
16.23	γ-Terpinene	4.60±193.51	1.09±0.61
17.18	Terpinolene	0.29±3.10	N.D.

Aromatic monoterpenes

RT	Compound	Wild (%)	Cultivated (%)
16.84	p-Cymene	63.52±88.07	14.59±7.93
21.14	p-Cymenene	0.38±6.51	0.69±0.43

Oxygenated monoterpenes

RT	Compound	Wild (%)	Cultivated (%)
15.10	Eucalyptol (1,8-cineole)	1.58±16.52	0.53±0.13
23.83	Linalool	0.88±1.61	1.17±0.33
26.37	γ-Terpineol	N.D.	0.32±0.01
31.05	p-Cymene-8-ol	0.15±0.32	0.55±0.07
37.16	Thymol	6.22±12.23	10.27±0.87
38.47	Carvacrol	4.85±10.29	29.15±2.56
25.10	Thymol methyl ether	2.04±5.28	3.74±0.81
25.41	Methyl carvacrol	0.67±1.14	3.54±0.98
27.14	Ipsdienol	0.14±0.05	N.D.
27.95	Camphor	1.06±41.46	1.41±0.21
31.82	carvacryl acetate	0.21±6.34	0.35±0.05
32.91	o-Isopropylphenetol	0.15±4.25	0.54±0.04

Sesquiterpenes

RT	Compound	Wild (%)	Cultivated (%)
25.84	β Caryophyllene	3.29±6.02	14.22±1.45
27.54	α-Humulene	1.94±14.11	10.85±1.00
25.94	Aromandendrene	0.46±24.13	N.D.

Notes: RT=retention time (min); N.D.=not detected. Values are expressed as mean ± standard deviation of the relative abundance (%).

Figure 2 illustrates the predominant compound classes in wild and cultivated *Lippia graveolens* essential oil.

The main predominant volatiles in (A) Wild EO and (B) Cultivated EO are shown in Figure 3.

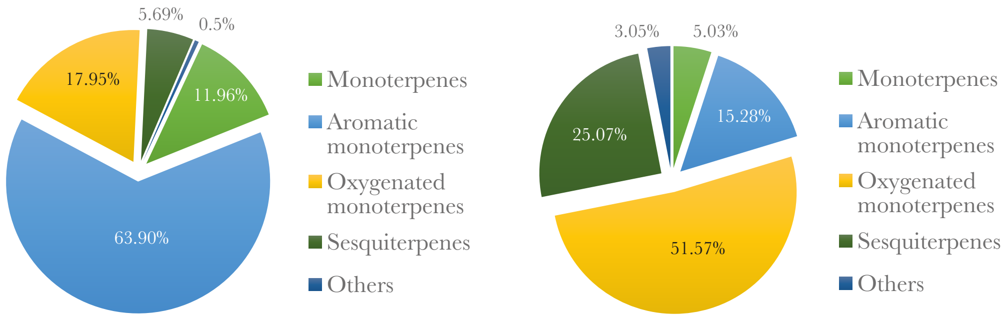

Figure 2. Predominant compound classes in (A) Wild EO, (B) Cultivated EO.

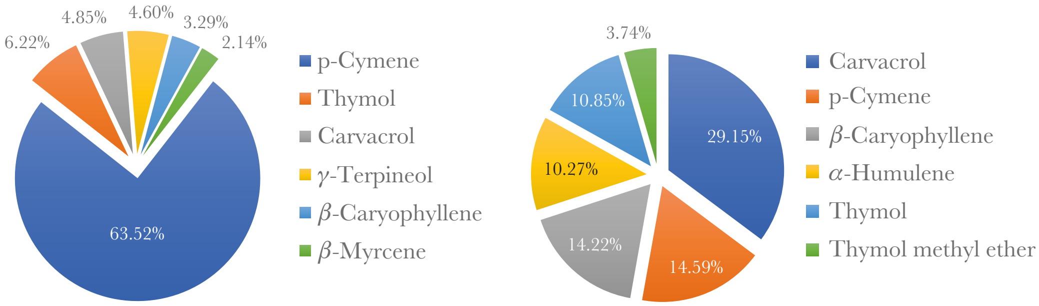

Figure 3. Main predominant volatiles in (A) Wild EO, (B) Cultivated EO.

Soil characterization and influence on EO

The wild site exhibited a clay-loam texture (25.6% sand, 32.3% silt, 41.0% clay), slightly alkaline pH (7.88), and high organic matter (OM) content (3.78%). The cultivated site had a sandy-loam texture (64.4% sand, 18.6% silt, 17.1% clay), moderately alkaline pH (8.04), and low OM content (0.83%). Electrical conductivity was very low at both sites. Among soluble salts, the most pronounced difference was in K^+ (2.45 mEq L^{-1} in cultivated *vs.* 0.52 mEq L^{-1} in wild); HCO_3^- and SO_4^{2-} also varied, whereas Ca^{2+}, Mg^{2+}, Na^+, and Cl^{-a}showed no significant differences. In terms of available nutrients, the cultivated site had higher K (1046 ppm) and NO_3^-, but lower levels of Fe, Zn, Cu, and Mn compared to the wild site. Superscript letters a/b in Tables 1-3 indicate statistically significant differences between sites (ANOVA and Tukey's *post hoc* test, $\alpha=0.05$): significant differences were found for sand, silt, clay, pH, OM, K^+ (salts), HCO_3^-, SO_4^{2-}, NO_3^-, K, Fe, Zn, Cu, and Mn. No significant differences were observed for Ca^{2+}, Mg^{2+}, Na^+, Cl^-, or bulk density. Class and range interpretations followed NOM-021-SEMARNAT-2000 guidelines. These edaphic gradients were reflected in the EO profile: the cultivated site, characterized by higher K^+ and NO_3^- levels, alkaline pH, and low OM, exhibited a phenolic chemotype (carvacrol 29.15%, thymol 10.27%) and a higher sesquiterpene fraction (β-caryophyllene 14.22%, α-humulene 10.85%). Conversely, the wild site, with lower K^+ and NO_3^-, a finer texture (silt + clay), and higher OM, showed an aromatic-monoterpene profile (p-cymene 63.52%, terpinen-4-ol 4.60%). This chemotypic distribution aligns with previous findings for *Lippia graveolens* populations across edaphic and climatic gradients in Mexico, where both phenolic (thymol/carvacrol) and non-phenolic/aromatic (p-cymene/terpinen-4-ol) chemotypes coexist (Bautista *et al.*, 2021).

Parameters: Texture, pH, and Organic Matter

The wild site presented a clay-loam texture (25.6% sand, 32.3% silt, 41.0% clay), slightly alkaline pH (7.88), and high OM content (3.78%). The cultivated site exhibited a sandy-loam texture (64.4% sand, 18.6% silt, 17.1% clay), moderately alkaline pH (8.04), and low OM content (0.83%). Electrical conductivity (EC) was very low at both sites. Interpretation followed NOM-021-SEMARNAT-2000 (SEMARNAT, 2002). Among parameters with significant differences texture, pH, and OM a potential impact on volatile profiles is anticipated, as reflected in Table 3, which shows the differing parameters likely to influence volatile composition.

Regarding soluble salt parameters, the cultivated site exhibited a significantly higher K^+ concentration (2.45 mEq L^{-1}) compared to the wild site (0.52 mEq L^{-1}). Literature consistently reports that edaphic factors modulate EO composition. In Lamiaceae, mineral nutrition studies indicate that K^+ can shift the chemical profile toward chemotypes rich in oxygenated monoterpenes and increase carvacrol levels in certain Origanum species under N-P-K regimes. In *Lippia graveolens*, chemotypic variation has been linked to edaphoclimatic activity (Kumar *et al.*, 2022). Thus, the elevated K^+ content in the cultivated soil aligns with the higher carvacrol concentration observed (29.15%), although the mechanism does not appear to be direct: other parameters (pH, texture, OM) likely exert a greater influence than K^+ alone. The remaining ions, being similar in range and interpretation, were not associated with noticeable differences in EO composition (Valkovszki *et al.*, 2023).

Soluble salts and nutrients

The moderately alkaline pH (8.0) and sandy-loam texture with low organic matter (OM) content at the cultivated site explain the reduced availability of Fe, Zn, and Mn, as shown in Table 5. At higher pH levels, these micronutrients tend to precipitate or become poorly soluble. Furthermore, the low OM and clay content limit retention capacity,

Table 3. Physical soil parameters of wild and cultivated oregano sites.

Parameter	**Wild**		**Cultivated**	
	Concentration	**Interpretation**	**Value**	**Interpretation**
Sand %	25.60±18.47 b	Clay loam	64.40±2.54 a	Sandy loam
Silt %	32.30±13.59 a		18.60±1.64 b	
Clay %	41.00±7.39 a		17.10±1.10 b	
Apparent Density g/cm^3	1.43±0.09 a	Medium	1.44±0.0 a	Medium
Total CaCOa%	0.36±0.04 a	Very low	0.39±0.08 b	Very low
Organic Matter %	3.78±0.45 a	High	0.83±0.33 b	Low
pH Paste	7.88±0.14 b	Slightly alkaline	8.04±0.11a	Moderately alkaline
EC mmhos/cm^3	0.71±0.04 a	Very low	0.68±0.08 b	Very low

Note: Methods and interpretations follow NOM-021-SEMARNAT-2000.

Table 4. Soluble Salt Parameters.

Parameter (mEq/L)	Wild		Cultivado	
	Concentration	**Interpretation**	**Concentration**	**Interpretation**
Ca^{++}	3.17±0.66 a	Low	3.10±0.47 a	Low
Mg^{++}	0.77±0.22 a	Medium	0.59±0.24 a	Medium
Na^{+}	6.06±0.83 a	Low	6.06±0.83 a	Low
K^{+}	0.52±0.12 b	Medium	2.45±1.84 a	Very High
CO_3	0.04±0.09 a	Low	0.03±0.11 b	Low
HCO_3	3.33±0.67 a	High	2.51±0.48 b	High
Cl^{-}	4.13±0.34 a	Moderate	4.08±0.46 a	Moderate
SO_4	3.15±0.67 a	Low	0.32±0.17 b	Low

Note: Methods and interpretations follow NOM-021-SEMARNAT-2000.

Table 5. Available Soil Nutrient Parameters.

Parameter (ppm)	Wild		Cultivado	
	Concentration	**Interpretation**	**Concentration**	**Interpretation**
NO_3	11.04±8.11 b	Moderate	18.97±5.18 a	Moderate
P	6.16±0.76 a	Very low	3.31±0.66 b	Very low
K	867.50±49.46 b	High	1045.50±40.02 a	High
Fe	2.009±0.37 a	Low	1.10±0.03 b	Low
Zn	0.878±0.76 a	Moderate	0.27±0.04 b	Low
Cu	0.29±0.03 a	Low	0.23±0.03 b	Low
Mn	2.31±0.16 a	High	1.28±0.12 b	Moderate

Note: Methods and interpretations follow NOM-021-SEMARNAT-2000.

affecting the plant's nutritional and physiological status, which may influence thymol and carvacrol synthesis (Miller, 2016; Saleem *et al.*, 2023). Among soluble salts, K^+ was the most contrasting ion: 2.45 mEq L^{-1} in the cultivated site versus 0.52 mEq L^{-1} in the wild site. Regarding available nutrients, the cultivated site had higher levels of K (1046 ppm) and NO_3^-, but lower concentrations of Fe, Zn, and Mn compared to the wild site. This pattern (pH≈8, sandy texture, and low OM) is typical of soils with reduced micronutrient availability (Fe, Zn, Mn) due to precipitation/adsorption and low retention in the fine fraction and OM.

Soil K levels were clearly higher in the cultivated site both in soluble form (K^+) and as available K (ppm). Various studies in oregano have shown that adjustments in N-P-K, particularly K, can influence EO composition, increasing carvacrol within specific K ranges and under dose-dependent interactions. For instance, intermediate K levels may boost carvacrol content, while excessive levels may hinder biomass accumulation.

This evidence aligns with the carvacrol concentration observed in the cultivated EO (29.15%) under high soil K availability (Chrysargyris *et al.*, 2025). The cultivated site showed relatively higher K and NO_3^- levels, which corresponded with an increase in oxygenated monoterpenes (carvacrol 29.15%, thymol 10.27%) and a higher sesquiterpene content

(β-caryophyllene 14.22%, α-humulene 10.85%). The wild site, with lower K and NO_3^- levels and higher OM/clay content, retained a more aromatic profile (p-cymene 63.52%; terpinen-4-ol 4.60%). This contrast is consistent with descriptions of *Lippia graveolens* populations across edaphoclimatic gradients. With increased nitrogen availability, plants tend to enhance their photosynthetic capacity and glandular trichome formation/activity, potentially increasing EO content and modulating the terpenoid biosynthetic pathway thereby altering the proportions of thymol, carvacrol, and p-cymene. This effect is most evident at intermediate N levels; excessive nitrogen often prioritizes vegetative growth, not necessarily improving EO quality (Ninou *et al.*, 2021). The soil in the cultivated oregano site had higher K content (1046 ppm) and lower concentrations of Fe, Zn, and Mn. Accordingly, its EO showed a higher proportion of oxygenated monoterpenes (carvacrol 29.15%). The wild site (868 ppm K) retained more precursors (p-cymene 25.04%; terpinen-4-ol 9.46%). This supports findings that N-P-K regimes, particularly K, can modulate EO composition and promote phenolic profiles in Origanum (Chrysargyris *et al.*, 2025). Additionally, nitrogen (N) often increases biomass, while micronutrients (Fe, Zn, Mn) play key roles in terpene biosynthesis (Kumar *et al.*, 2022). In *L. graveolens* (Verbenaceae), chemotypes have been linked to edaphoclimatic conditions, supporting the idea that higher K and lower micronutrient levels contribute to a greater proportion of oxygenated compounds in cultivated EO (Bautista *et al.*, 2021).

Physicochemical Factors That May Enhance the Aromatic Profile

In addition to genetic or enzymatic factors, the conversion of terpinen-4-ol to its aromatic derivative p-cymene may be triggered by exposure to light, oxygen, and heat during drying, storage, or even distillation. These conditions elevate p-cymene levels without necessarily inducing the conversion to thymol or carvacrol. Studies conducted under UV-A exposure have shown that p-cymene is the primary oxidation product of terpinen-4-ol (Bi *et al.*, 2012).

Oxidation markers of EO due to p-Cymene aging

During the natural aging of monoterpene-rich essential oils, p-cymen-8-ol may form due to abiotic factors (O_2, light, time). In *Protium heptaphyllum*, aged batches contain 18.7-43.0% p-cymene and 8.2-31.8% p-cymen-8-ol, compared to fresh samples, indicating that the alcohol arises as a secondary oxidation product of p-cymene (Sawamura *et al.*, 2004). In controlled chemical oxidation of p-cymene, p-cymen-8-ol is the major product, alongside more oxidized derivatives such as p-cuminaldehyde and p-cuminic acid (Albino *et al.*, 2017). In the present study, no degradation attributable to prolonged aging was observed; instead, chemotypic differences were mainly explained by edaphoclimatic factors, particularly soil conditions.

CONCLUSIONS

The results of this study confirm that soil properties play a decisive role in the chemical variability of *Lippia graveolens* essential oil. Specifically, soils with higher clay and organic matter content favored the accumulation of aromatic precursors such as p-cymene and terpinen-4-ol, while sandy-loam soils with higher potassium concentrations and more alkaline pH promoted a phenolic profile characterized by elevated levels of carvacrol and thymol. This chemotypic differentiation, linked to soil type, highlights the importance of considering edaphic factors as a key criterion for selecting cultivation areas and standardizing EO chemical quality. Accordingly, it is recommended to implement technical cultivation strategies in homogeneous soils, systematically characterize local chemotypes, and adopt sustainable and replicable extraction methods. These measures will not only help reduce final product variability but also enhance the added value of Mexican oregano, thereby strengthening its competitiveness in both domestic and international markets.

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