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Influence of Cultivation Conditions and Processing Technologies on the Composition and Quality of *Origanum Vulgare* L. Essential Oil: a systematic review

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Abstract

The article presents a systematic analysis of global experience in cultivating *Origanum vulgare* L. to scientifically justify its introduction into the agro-climatic conditions of the Precarpathian region. The influence of the "genotype – environment – technology" interaction on the qualitative and quantitative parameters of essential oil is examined. It has been established that the phenotypic plasticity of the crop allows for the regulation of secondary metabolite biosynthesis through stress factor management. The phenomenon of the "dilution effect" under intensive nitrogen fertilization and the mechanisms of eustress (hormesis), where moderate water or salt stress stimulates carvacrol accumulation, are analyzed in detail. Based on the review of modern agricultural technologies, the expediency of transitioning to integrated nutrient management systems is substantiated. It is shown that combining organic fertilizers with the application of exogenous biostimulants (melatonin, humic acids) allows for minimizing oxidative stress and stabilizing yields. A conclusion is drawn regarding the strategic importance of creating regional adaptive models for *Origanum vulgare* L. cultivation to ensure the resource independence of the domestic pharmaceutical industry.

Keywords: *Origanum vulgare* L., planting density, phenological stage, adaptation, plant introduction, acclimatization.

1. INTRODUCTION

Current trends in the global pharmaceutical, food, and perfumery-cosmetic industries demonstrate a steady reorientation toward the use of natural plant-based raw materials. Among a broad spectrum of medicinal crops, *Origanum vulgare* L. occupies a prominent position due to its unique biological activity profile, attributed to a high content of essential oils, phenolic compounds, and flavonoids (Chishti et al. 2013). The global demand for standardized *Origanum vulgare* L. oil is steadily increasing, necessitating a transition from the extensive harvesting of wild plants to controlled industrial cultivation.

The introduction of *Origanum vulgare* L. into new soil-climatic zones is a strategically important scientific and practical objective. Firstly, the intensive exploitation of natural populations leads to the depletion of genetic diversity and ecosystem degradation (Stefanaki et al. 2016). Secondly, expanding the geography of cultivation ensures regional “resource independence,” reduces import dependency, and guarantees processing enterprises stable volumes of raw materials regardless of natural cataclysms in traditional growing zones, such as the Mediterranean (D'Antuono et al. 2000).

The success of introduction largely depends on understanding the plant's adaptive strategy. *Origanum vulgare* L. is characterized by high phenotypic plasticity; thus, its morphometric parameters (biomass, leaf surface area, bush habit) are a direct reflection of growing conditions (Eleftheriou et al. 2021). Scientific investigation into the influence of agronomic factors, planting schemes, mineral nutrition systems, water regimes, is critical, as they act as regulators of physiological processes. Optimizing these parameters allows for maximizing yield and ensuring the economic viability of crop cultivation (Eleftheriou et al. 2021).

The most complex challenge is the variability of the essential oil chemical composition. It is established that the biosynthesis of secondary metabolites, particularly key quality markers (carvacrol, thymol, gamma-terpinene), serves as a chemical defense mechanism modulated by environmental stress factors (Figueiredo et al. 2008). The relevance of studying the impact of abiotic (temperature, insolation, altitude) and technological factors (drying and extraction methods) is driven by the necessity for standardization. Uncontrolled conditions can result in raw materials with an unpredictable chemotype, rendering them unsuitable for use in the pharmaceutical industry (Lukas et al. 2015). Despite the significant number of publications dedicated to *Origanum vulgare* L., most existing works focus either exclusively on pharmacological activity or on botanical description.

A review of recent literature indicates a consistent surge in scholarly interest regarding *Origanum vulgare* L.(Fig. 1). Between 2000 and 2010, 614 studies focused on its biological and antioxidant properties. This interest intensified from 2010 to 2015, with the number of publications reaching 715. A significant escalation occurred between 2016 and 2020, during which 1,300 studies were conducted - effectively doubling the output of the preceding five-year period.

Research reached its peak in 2024 and 2025, specifically investigating the plant's morphological characteristics and the efficacy of its essential oils, with 476 and 488 publications, respectively. This trend reflects the growing demand for organic compounds in medicine, pharmacy, and agronomy. Overall, the cumulative research volume for the latest five-year period (2021–2025) has reached 2,105 studies.

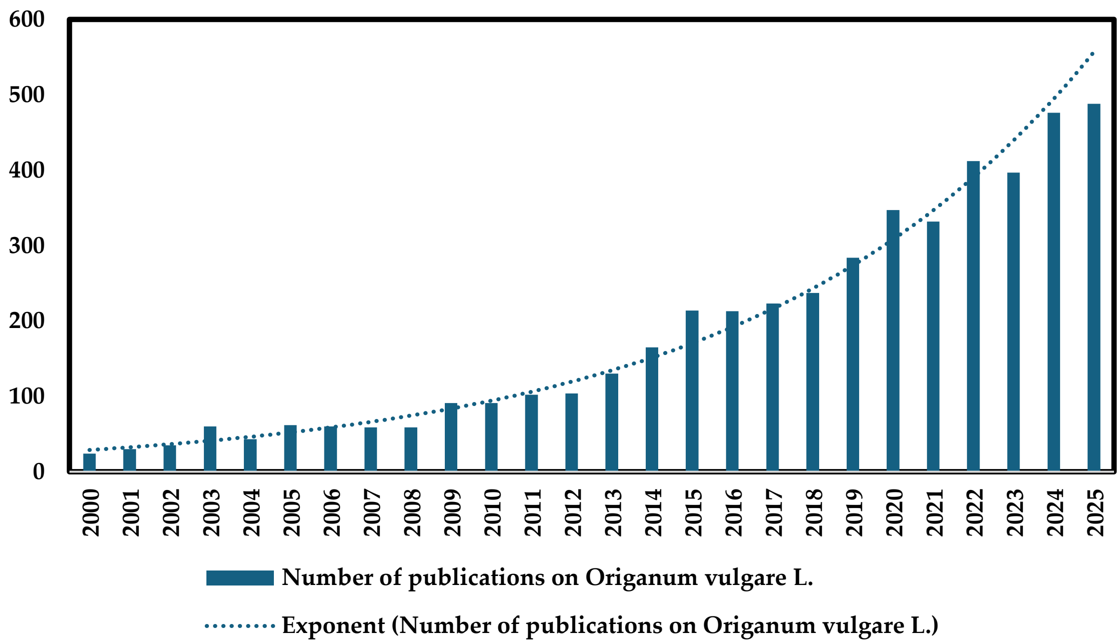

Fig. 1. Annual distribution of publications on *Origanum vulgare* L.

The scientific novelty of this review lies in the systemic combination of the analysis of agronomic cultivation conditions and technological processing as a single, continuous chain. For the first time within a single study, scattered data on how the interaction of the "genotype – environment – technology" system shapes the quality of the final product are synthesized. The aim of the article is the systematization and critical analysis of scientific data regarding the influence of cultivation conditions, ecological factors, and post-harvest processing methods on the qualitative and quantitative indicators of *Origanum vulgare* L. essential oil to substantiate technologies for obtaining products with predicted properties.

2. LITERATURE REVIEW: GENERALIZATION OF MAIN STATEMENTS

Analysis of scientific publications indicates that research on the ecological-biological plasticity of *Origanum vulgare* L. is typically realized through two methodological approaches: monitoring natural populations along altitudinal gradients (in situ) and experimental cultivation of cloned material under controlled conditions in climatic chambers or on experimental plots with varying exposure (ex situ). This differentiated approach has allowed researchers to isolate the influence of abiotic factors from the genetic component (Lukas et al. 2015). Results from transplantation experiments, where plants of high-altitude ecotypes were transferred to lowland conditions, demonstrated a substantial change in chemotype as early as the first growing season, confirming the dominant role of the environment over the genotype in regulating terpene biosynthesis (Tuttolomondo et al. 2013).

A detailed analysis of the influence of the temperature regime has shown that the sum of active temperatures is a critical factor for the initiation of flowering and biomass accumulation. Studies using phytotrons established that maintaining daytime temperatures at +25…+30°C stimulates the proliferation of peltate glandular trichomes on the leaf epidermis, which serve as the primary reservoirs of essential oil (Bosabalidis 2002). Conversely, under conditions of reduced temperatures (+10…+15°C), characteristic of highlands or northern latitudes, the activation of defense mechanisms is observed: plants form a more compact habit and reduce the evaporation area yet simultaneously intensify the synthesis of phenolic compounds (carvacrol) as a response to cold stress (Morshedloo et al. 2018).

A separate body of research is dedicated to studying the photobiological reactions of the crop. Experiments with artificial modeling of light spectral composition proved that ultraviolet radiation (UV-B), the intensity of which increases with altitude, acts as a potent elicitor of secondary metabolism (De Falco et al. 2013). Plants subjected to dosed UV irradiation demonstrated an increase in the concentration of essential oil and flavonoids, which function as a chemical "screen" to protect the photosynthetic apparatus from radiation damage.

At the same time, comparative agronomic experiments on cultivating *Origanum vulgare* L. under shading conditions (40–50% reduction in insolation) recorded not only a decrease in the total oil yield but also a shift in its qualitative profile towards a reduced proportion of valuable monoterpenes. This makes the selection of southern slope exposure a critically important condition for establishing industrial plantations (De Falco et al. 2013).

The productivity of essential oil crops depends not only on genetic potential and climate but also on cultivation technology. Studies confirm that manageable factors (irrigation regime, type of fertilization, plant density, harvest timing, and drying method) are capable of significantly altering both the quantitative yield of raw material and the chemotype of the essential oil.

Agronomic measures, ranging from irrigation strategies to fertilization specifics, serve as critical regulators of both total biomass yield and the qualitative composition of *Origanum vulgare L.* secondary metabolites; notably, the plant response to these factors often exhibits a non-linear character.

Experimental data regarding irrigation regimes (1, 2, and 3-week intervals) combined with varying rates of organic fertilizers (0–30 t/ha) demonstrate that moderate water deficit, induced by extended irrigation intervals, functions as a potent elicitor of essential oil synthesis (Gerami et al. 2016).

In response to water stress, the plant activates defense mechanisms accompanied by an increase in the concentration of endogenous proline and phenolic compounds in tissues; however, without adequate nutrient compensation, such a regime leads to significant inhibition of growth processes and a reduction in leaf surface area (Morshedloo et al. 2017).

Concurrently, investigations into the impact of nitrogen (N) nutrition have revealed a complex inverse correlation between vegetative biomass accumulation and the percentage content of essential oil (Ninou et al., 2021).

The application of high nitrogen rates (up to 80 kg/ha) stimulates intensive shoot growth and a 40–59% increase in dry matter; however, this often results in the so-called "dilution effect" (Fig. 2). This phenomenon occurs because the total population of glandular trichomes fails to increase proportionally to the rapid expansion of leaf area. Consequently, this reduces the oil concentration on a dry matter basis, although the total oil yield per hectare may still rise due to the high gross biomass harvest.

This premise is corroborated by research (Azizi 2009) establishing that while nitrogen fertilizers (in the form of ammonium nitrate) linearly increase plant height and branch number, the maximum percentage content of essential oil was observed in control groups without fertilization or at minimal nitrogen doses.

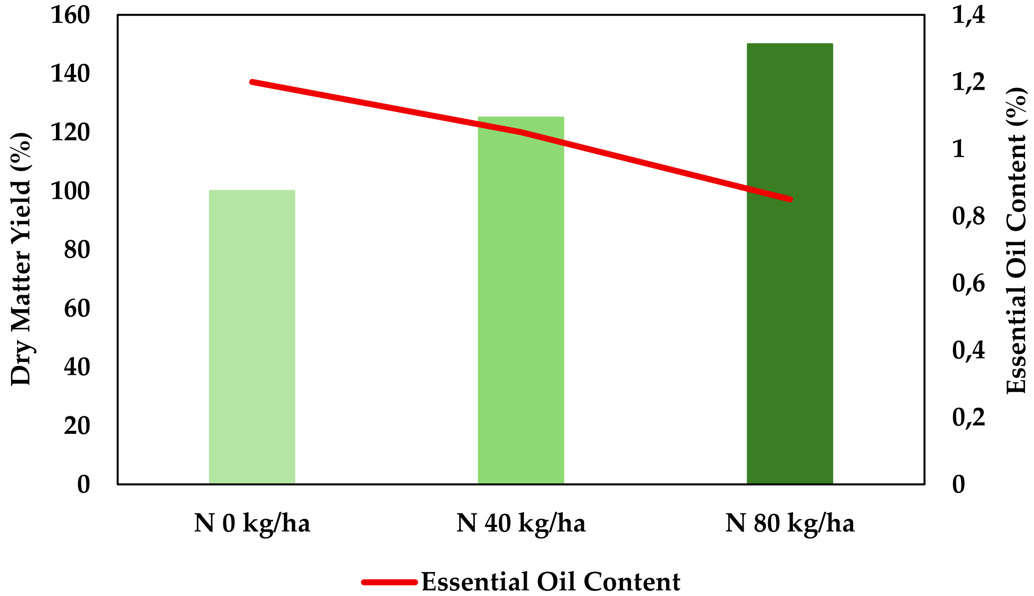

Fig. 2. Impact of nitrogen fertilization on biomass accumulation and essential oil concentration in *Origanum vulgare* L.: The "Dilution Effect"

Moreover, excess nitrogen can shift the plant's metabolic balance according to the Carbon-Nutrient Balance Hypothesis (CNBH): under high nitrogen availability, resources are directed toward protein synthesis for primary biomass growth, while the synthesis of non-nitrogenous secondary metabolites (terpenes, phenols) is inhibited (Giannoulis et al. 2020).

However, it is important to note that optimized nitrogen doses (approximately 40–60 kg/ha) can positively influence the oil's qualitative composition, particularly by increasing the carvacrol fraction through the stimulation of synthase enzyme activity, making precise N dosage a critical technological factor.The response of *Origanum vulgare* L. to soil salinity is complex and multifactorial, as salt stress affects the plant through two primary mechanisms: osmotic water deficit and specific ion toxicity.

Current research (Azimzadeh et al. 2021) confirms that the crop's response is non-linear and varies significantly depending on the intensity of the stress factor.Analogous physiological patterns are observed under salt stress (NaCl) conditions, which, similar to drought, induce osmotic stress in the plant organism. In the initial stages (at low salt concentrations of approximately 25 mM), this stress can stimulate an increase in carvacrol and gamma-terpinene content as an adaptive response of the antioxidant system (Azimzadeh et al. 2021). However, with intensified stress load (exceeding 50 mM), chlorophyll degradation, disruption of photosynthetic activity, and a sharp decline in productivity occur, indicating a narrow range for the positive influence of abiotic stress on secondary metabolism.

The mechanism of this dual response consists of two phases. The stimulation phase ("Eustress") manifests during moderate salinity (25 mM NaCl), where a hormesis effect is observed. In response to the osmotic signal, the expression of monoterpene biosynthesis genes, such as *Ovtps2* (gamma-terpinene synthase), is activated (Azimzadeh et al. 2022). Studies indicate that under these conditions, essential oil yield can increase to 0.8% (compared to control), and the proportion of carvacrol within its composition can rise (reaching 30–72% depending on the subspecies), as this compound acts as an effective antioxidant for membrane protection (Azimzadeh et al. 2022).

The inhibition phase ("Distress") manifests as salt concentrations increase to 50–100 mM, where the toxic impact of Na+ and Cl- ions becomes dominant. This results in a reduction of photosynthetic pigment content; for instance, the chlorophyll index (SPAD) may decline, and total plant biomass may decrease by 30–50%. Furthermore, the expression of key biosynthesis genes (*DXR*, *CYP71D180*) is inhibited, negating any potential rise in secondary metabolite concentration (Azimzadeh et al. 2022).

Therefore, the application of moderate salt stress can serve as a tool for enhancing raw material quality, but only within a narrow range (up to 25–30 mM), beyond which irreversible inhibition of productivity occurs (Tab. 1).

Table 1. Comparative characterization of *Origanum vulgare L.* physiological responses to different levels of salt stress

Parameter	**Moderate Stress (25 mM NaCl)**	**Severe Stress (>50 mM NaCl)**
Response Type	Eustress (stimulation/hormesis)	Distress (inhibition)
Primary Mechanism	Osmotic signaling, antioxidant system activation	Ion toxicity (Na^+, Cl^-), membrane disruption
Gene Expression (*Ovtps2*)	Upregulated (Increased expression)	Downregulated (Suppressed expression)
Essential Oil Content	Increase (or stability)	Decrease
Chlorophyll Content	Minor / Negligible changes	Significant degradation
Plant Biomass	Slight decrease	Sharp decline (by 30–50%)

Modern agronomic strategies are increasingly based on the use of integrated nutrition systems that combine organic fertilizers and exogenous biostimulants to enhance plant resilience. Recent studies (Shabankareh et al. 2025) have shown that foliar application of melatonin (at concentrations of 100–200\,mM) under water deficit conditions significantly reduces oxidative stress. Melatonin acts as an antioxidant and growth regulator, increasing chlorophyll content and improving relative water content (RWC), which allows for biomass preservation even under limited irrigation.

Work by (Aytaç et al. 2022) demonstrated that the soil application of humic acids not only improves soil structure but also stimulates essential oil synthesis (increasing carvacrol and γ-terpinene content), as well as enhancing the antimicrobial activity of the raw material.

These data align with results from field experiments regarding organic fertilization. Integrated cultivation systems demonstrate the highest agronomic efficiency, where the negative impact of water or salt stress is mitigated by the application of organic substrates or biostimulants, such as humic acids or melatonin. In experiments, this approach allowed for achieving maximum yield indicators (66.62 kg/ha of oil) specifically through a combination of infrequent irrigation (once every 3 weeks) and high doses of manure (30 t/ha) (Matłok, Stępień 2020). In this context, organic fertilizers improve the soil's water-holding capacity and provide prolonged nutrition, stabilizing plant metabolism and enabling the utilization of stress-induced oil accumulation without the loss of vegetative biomass.

Thus, combining traditional organics (manure) with modern biostimulants (melatonin, humates) represents the most promising pathway for obtaining stable *Origanum vulgare L.* yields under climate change conditions.

3. CONCLUSIONS

The conducted systemic analysis of global research confirms the scientific feasibility and high potential of introducing *Origanum vulgare* L. into the agro-climatic conditions of the Precarpathian region. Data generalization allows for the formulation of a cultivation strategy for this region. Adaptation to regional conditions. The crop's high phenotypic plasticity indicates that specific climatic factors of the Precarpathian region (temperature regime, humidity, insolation) will be dominant in shaping the plant chemotype. This necessitates a departure from replicating Mediterranean technologies in favor of developing adaptive regional models. Quality management through agronomy. Maintaining a balance between biomass accumulation and essential oil synthesis is critically important for Precarpathian conditions. It has been established that intensive nitrogen nutrition causes a "dilution effect" (reduced oil content), whereas moderate controlled stress (eustress) stimulates carvacrol accumulation. Nutrition strategy. The most effective approach for the region is the utilization of integrated nutrition systems. The combination of organic fertilizers and modern biostimulants (melatonin, humates) will enable plants to effectively withstand potential abiotic stresses characteristic of the introduction zone, ensuring a stable yield of high-quality raw material. Thus, the introduction of *Origanum vulgare L.* in the Precarpathian region is a strategically justified step for ensuring the resource independence of the domestic pharmaceutical industry, provided that technologies accounting for the complex interaction within the "genotype – environment – technology" system are implemented.

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Data availability. Not Applicable

Declarations

Conflict of interest. The authors have no competing interests to declare relevant to this article's content.

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Анотація
У статті здійснено системний аналіз світового досвіду культивування *Origanum vulgare* L. з метою наукового обґрунтування її інтродукції в агрокліматичні умови Передкарпаття. Розглянуто вплив взаємодії в системі «генотип – середовище – технологія» на якісні та кількісні показники ефірної олії. Встановлено, що фенотипова пластичність культури дозволяє регулювати біосинтез вторинних метаболітів через управління стресовими факторами. Детально проаналізовано явище «ефекту розбавлення» при інтенсивному азотному живленні та механізми еустресу (гормезису), коли помірний водний або сольовий стрес стимулює накопичення карвакролу. На основі огляду сучасних агротехнологій обґрунтовано доцільність переходу до інтегрованих систем живлення. Показано, що комбінування органічних добрив із застосуванням екзогенних біостимуляторів (мелатоніну, гумінових кислот) дозволяє мінімізувати оксидативний стрес та стабілізувати врожайність. Зроблено висновок про стратегічну важливість створення регіональних адаптивних моделей вирощування *Origanum vulgare* L. для забезпечення ресурсної незалежності вітчизняної фармацевтичної галузі.

Ключові слова: *Origanum vulgare* L., фенологія, адаптація, інтродукція рослин, акліматизація.