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RESEARCH ON THE GENUS *CLINOPODIUM* L. THROUGH THE PRISM OF MODERN SCIENTIFIC METHODOLOGY

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This study aims to examine the theoretical foundations and methodological framework applied in the study of the biomorphological characteristics and essential oil content in species of the genus *Clinopodium* L., which are recognized for their applications in the pharmaceutical industry, aromatherapy, phytotherapy and gastronomy. The study integrates a comprehensive set of approaches, including systematic classification, the introduction and conservation of biological material under both *ex situ* and *in situ* conditions, developmental biology analysis, the assessment of morphological and anatomical traits, the dynamics of essential oil accumulation and detailed phytochemical analyses. In addition, propagation strategies are evaluated with the objective of developing a standardized cultivation protocol suitable for industrial-scale production. The integration of classical and contemporary methodologies, selected in accordance with the specific research objectives, enables a thorough and coherent assessment of the biological and phytochemical potential of the investigated species.

Keywords: *research, Clinopodium, conservation, biological phenomenon, method, introduction, species, technique.*

CERCETAREA GENULUI *CLINOPODIUM* L. PRIN PRISMA METODOLOGIEI ȘTIINȚIFICE MODERNE

Prezenta lucrare vizează analiza fundamentului teoretic și al cadrului metodologic științific aplicat în studiul particularităților biomorfologice și a conținutului de ulei volatil la speciile genului *Clinopodium* L., recunoscute pentru valoarea lor aplicativă în industria farmaceutică, aromaterapie, fitoterapie și gastronomie. Studiul integrează un complex de metode de investigare, incluzând încadrarea sistematică, introducerea și conservarea materialului biologic din condiții *ex situ* în *in situ*, analiza biologiei dezvoltării, evaluarea caracterelor morfologice și anatomomorfologice, determinarea dinamicii uleiului volatil, precum și investigații fitochimice. De asemenea, sunt analizate metodele de multiplicare a speciilor, în vederea elaborării unei fișe tehnologice de cultivare pe suprafețe industriale. Aplicarea metodelor clasice și moderne, selectate în funcție de obiectivele specifice ale cercetării, permite o evaluare complexă și coerentă a potențialului biologic și fitochimic al speciilor studiate.

Cuvinte-cheie: *cercetare, Clinopodium, conservare, fenomen biologic, introducere, metodă, specie, tehnică.*

Introduction

Biology, as the science of life, is based on the observation, analysis, and interpretation of biological phenomena, with the aim of elucidating the mechanisms that govern the structure and functioning of living organisms [4]. The quality and scientific value of research are largely determined by the degree of methodological rigor applied throughout the investigative process. Scientific research comprises a system of logical, empirical and conceptual approaches that facilitate the generation of valid, reproducible and reliable knowledge [7].

In the context of a doctoral thesis in the biological sciences, the research methodology constitutes a fundamental component, requiring a detailed presentation of the methods and techniques employed to achieve the research aim and objectives. Moreover, it provides the framework for testing the proposed hypotheses and ensuring the validity of the experimental outcomes. A clear and detailed description of the applied methods, interpretation of the obtained data and statistical processing of the results are essential for maintaining scientific transparency and for enabling the reproduction of experiments by other researchers.

At the initial stage of the research, the identification and formulation of the methodological framework require a clear definition of the scientific problem, grounded in a critical and comprehensive review of the relevant literature. In this context, the genus *Clinopodium* L. is of particular interest from both fundamental

and applied perspectives, encompassing aromatic and medicinal species, both native and introduced, characterized by a complex profile of biologically active compounds, particularly essential oils. According to data reported in the specialized literature, these species are utilized across multiple sectors of the national economy, including the pharmaceutical industry, phytotherapy, aromatherapy and gastronomy.

From an ecological standpoint, species of the genus *Clinopodium* L. exhibit a high adaptive capacity to the pedoclimatic conditions of the Republic of Moldova. Besides, an assessment of the scientific novelty indicates that, under local conditions, no comprehensive studies have been conducted to evaluate the aromatic, medicinal, phytochemical, melliferous and technological potential of this genus. In this regard, the present research is directed toward the identification, acclimatization and recommendation of suitable species for sustainable cultivation, particularly in the context of climate change, with an emphasis on taxa demonstrating enhanced drought tolerance.

The study of the genus *Clinopodium* L. requires an integrated methodological approach combining botanical, phytochemical, ecological and technological analyses [6]. A rigorous methodological framework is essential for generating reliable and comparable data, supporting the scientific basis for the establishment of these species as valuable plant resources. This interdisciplinary approach aims not only to advance knowledge of *Clinopodium* L. but also to guide strategic directions for its sustainable utilization.

These considerations underscore the pivotal role of scientific research in supporting environmentally sustainable, competitive agricultural systems, aligned with national and European strategic priorities. The anticipated results are expected to make a significant contribution to expanding current knowledge of the aromatic and therapeutic potential of the genus *Clinopodium* L., diversifying the range of medicinal plants cultivated organically in the Republic of Moldova, and promoting contemporary directions in sustainable rural development and bioeconomy.

Applied Methods and Materials

The research is supported by a robust theoretical and methodological framework, enabling a comprehensive analysis of species within the genus *Clinopodium* L, integrating fundamental aspects – ecological, systematic, morphological, anatomical and phytochemical [2] and applied ones, such as ecological cultivation, economic valorization and promotion of sustainable development [11,12].

The research will be conducted within the "Plant Resources" Laboratory of the Botanical Garden, with experimental trials established within the aromatic plant collection-exhibition. Three species of the genus *Clinopodium* L., present in both *ex situ* collections and the spontaneous flora of the Republic of Moldova, served as research subjects. Phenological observations and biomorphological studies will be conducted on 25 plants at three-day intervals throughout the entire growing season, following established methodologies [10]. During these observations, key climatic parameters, including air temperature, precipitation and humidity, will be taken into account. Morphological analyses will be performed on 10 normally developed plants [8], and the ontogenetic stages and life periods of the plants will be clearly delineated.

Herbage yield will be determined during the full flowering stage of the plants. For this purpose, the herbage will be harvested from an area of 1-3 m² in 2 repetitions.

Essential oil extraction will be carried out using the steam distillation method, employing a Clevenger-type hydrodistillation apparatus [3], which is a standardized hydrodistillation technique used for obtaining and quantitatively determining essential oils from plant material. The Clevenger EIDG-002-001 apparatus separates the oil through boiling, condensation and continuous decantation, being considered the official standard for quality control, with direct heating and rigorous monitoring of operating parameters.

The chemical composition of the essential oil will be determined by gas chromatography coupled with mass spectrometry (GC-MS), using an Agilent Technologies 6890N gas chromatograph connected to a 5975 inert XL Mass Selective Detector [1]. This is a modern method for extracting volatile compounds without distillation, in which volatile compounds are adsorbed onto a fiber and subsequently analyzed by GC-MS. No solvents are used; the method is rapid and sensitive, making it ideal for highly volatile compounds. Such modern techniques constitute the core methodology for the analysis of *Clinopodium* essential oil, enabling both quantitative determination and identification of individual constituents. Complementary

approaches, including HPLC, FT-IR, NMR and SPME, provide detailed chemical characterization, yielding insights into the structure and complex composition of the volatile oils.

The essential oil and plant extracts of *Clinopodium* will also be tested for insecticidal and aphid-repellent activities, as documented in the literature [5].

The type and distribution of essential oil secretory structures across plant organs will be determined using both temporary and permanent preparations [9].

A part of the investigations will be carried out in collaboration with national and international research centers and institutions, ensuring high accuracy and comparability of results. Depending on the research objectives, a quantitative experimental design will be adopted, incorporating elements of comparative and explanatory analysis. This experimental approach allows for the investigation of underlying biological processes and the testing of hypotheses within a controlled and reproducible framework.

Obtained Results and Discussions

Scientific research in the natural sciences, with a particular focus on aromatic and medicinal plants, necessitates the application of a broad spectrum of interdisciplinary methodologies that enable an integrated and multidimensional understanding of biological processes. In this context, the present study combines documentary analysis, which provides theoretical grounding based on both classical and contemporary concepts in plant systematics, with an examination of the taxonomic position of the genus *Clinopodium* L. within the family Lamiaceae, alongside theoretical perspectives on the ontogeny and development of the investigated species.

A central focus of this research is the assessment of plant – environment interactions and the adaptive capacity of newly introduced *Clinopodium* L. species under the pedoclimatic conditions of the Republic of Moldova, approached as a complex, experimental and comparative process. This evaluation integrates a comprehensive set of ecological, morphological, physiological and biochemical indicators, providing a holistic view of plant responses to environmental factors and their acclimatization potential.

The investigative approach aims to determine the degree of adaptability of introduced *Clinopodium* species to climatic conditions (temperature regime, precipitation distribution and the frequency and intensity of drought events) and edaphic conditions (soil type, soil pH and fertility level), and to compare them with native species, used as biological controls. In this context, assessment is performed through experimental cultivation of the species under controlled and comparable conditions over three consecutive growing seasons, allowing the evaluation of the stability of adaptive responses under variable climatic conditions.

The research methodology employs a comprehensive set of indicators**.** Morphological indicators reflect the structural adaptation of plants and include parameters such as plant height, number of shoots, leaf area and accumulated vegetative biomass. Phenological indicators enable evaluation of the synchronization of the biological cycle with environmental conditions, through analysis of the onset of vegetation, flowering period and duration of the development cycle. Physiological indicators reveal the functioning of the metabolic apparatus and the capacity to respond to stress, measured through parameters such as photosynthetic activity, tissue water content and resistance to water stress. Furthermore, biochemical indicators, particularly relevant for aromatic plants, focus on quantifying essential oil content and analyzing its chemical composition, reflecting the level of secondary metabolism expression.

The integration of these indicator categories enables the establishment of functional relationships between environmental factors and the biological responses of plants, facilitating the identification of adaptation mechanisms and the ecological limits of the studied species. A *Clinopodium* L. species is considered well-adapted when it exhibits normal and complete development, progresses through all phenological stages in accordance with local conditions, demonstrates high tolerance to abiotic stressors (particularly drought and extreme temperatures) maintains stable and high productivity, and preserves a significant content of essential oil with a chemical composition suitable for practical applications.

This methodological framework provides a scientific foundation for the acclimatization and cultivation of *Clinopodium* L. species, contributing to the expansion of aromatic plants adapted to local pedoclimatic conditions and supporting the development of sustainable and efficient agricultural systems.

Furthermore, the research integrates phytochemical concepts and methods that are essential for the quantitative and qualitative assessment of bioactive compounds, particularly for determining essential oil content. The essential oil content of *Clinopodium* L. species is quantified using hydrodistillation, a classical pharmacopeial technique recognized for its accuracy and reproducibility, with results expressed as a percentage of the dry plant material. This approach ensures the extraction of a representative volatile fraction, forming the basis for comparative evaluation of the phytochemical potential of the studied species. Comparative analyses of the essential oil content of native and introduced species allow assessment of the influence of ecological and genetic factors on the biosynthesis of secondary metabolites. Observed differences in essential oil yield and composition may reflect both species adaptation to local pedoclimatic conditions and intraspecific genetic variation, expressed as distinct chemotypes. Notably, essential oil accumulation can vary widely – from less than 0.1% to several percent, depending on species, environmental conditions and the developmental stage of the plant.

In parallel, the research involves the development of preliminary cultivation protocols for *Clinopodium* L. species, adapted to the pedoclimatic conditions of the Republic of Moldova. These protocols include identifying the optimal sowing or planting time, determining the optimal planting density, selecting propagation methods (either generative – through seeds or vegetative), optimizing irrigation and fertilization regimes, and establishing the necessary agronomic practices for crop maintenance, including weed control.

A novel aspect of this study is the first-time application of naturally derived biostimulators, including cyanobacteria, in the cultivation technology of *Clinopodium* L. species. The use of these biostimulators aims to evaluate their influence on seed germination, plant growth and development, and the metabolic quality of the resulting biomass. According to the literature, naturally derived biostimulators enhance physiological and metabolic processes, stimulate vegetative growth, increase plant resistance to abiotic stressors (particularly drought and extreme temperatures) and promote the accumulation of secondary metabolites, including essential oils. Therefore, integrating these biostimulators into cultivation practices has the potential to increase both productivity and the quality of plant raw material while strengthening the adaptive capacity of the studied species to environmental conditions.

Methodologically, the research is based on the combined application of classical and modern approaches, selected according to the specific objectives of the study. Botanical methods, including phenological observations, biomorphological descriptions and ontogenetic stage analyses, enable the assessment of adaptive potential, the characterization of biomorphological traits and the determination of vegetative periods of the studied species. Applied ecological methods contribute to evaluating the tolerance of the species to abiotic and biotic factors, assessing the process of introduction and adaptation under local pedoclimatic conditions, and determining the feasibility of large-scale cultivation in the context of climate change. Anatomical methods involve the use of optical microscopy for the identification and localization of secretory structures producing essential oils, as well as their quantitative evaluation.

Phytochemical approaches include the determination of the essential oil content through hydrodistillation. Determining the patterns of essential oil accumulation holds considerable theoretical and practical significance. From a theoretical perspective, such studies elucidate the mechanisms underlying esential oil biosynthesis in plants. The theoretical importance of such studies lies in their ability to explain the processes of essential oil biosynthesis in plants. The practical significance, on the other hand, lies in identifying the phenological stage, specific organ and plant age at which the maximum amount of essential oil is synthesized, thereby enabling the development of harvesting recommendations for plants with the highest essential oil content.

The significance and identity of aromatic plants are reflected in the chemical composition of their essential oils, which have applications across various industries. The biosynthesis of these compounds determines the characteristic features of each plant species and is influenced by both genetic factors and pedoclimatic conditions. These factors can affect not only the quality but also the proportion of chemical constituents present in the oil. Accordingly, in this study, the chemical composition will be analyzed using gas chromatography (GC) or gas chromatography coupled with mass spectrometry (GC-MS).

Testing hydroalcoholic extracts and the essential oil obtained from the studied species allows the evaluation of their biological potential in controlling diseases and pests, indicating possible insecticidal activity.

The obtained experimental data will be systematically collected and recorded, and subsequently subjected to comparative analysis among the investigated species, with the aim of identifying those with superior potential for utilization across various sectors of the national economy. Statistical processing of the data, performed using quantitative methods with specialized software (e.g., Excel, SPSS, R), will enable validation of the results and the identification of significant differences between experimental variants. The results will be presented in the form of tables, graphs and diagrams, as well as chromatograms accompanied by narrative interpretations, facilitating their correlation with data reported in the specialized literature.

Conclusions

The rigorous application of scientific methodology in the development of a doctoral thesis in biology, speciality – botanics, focused on the study of aromatic species of the genus *Clinopodium* L., underscores the necessity of an integrated and comprehensive approach that combines modern techniques in ecological, morphological, anatomical and phytochemical analysis. This approach enables the demonstration of both the fundamental and applied significance of the research findings, by highlighting the economical and ecological potential of the investigated species.

A comprehensive evaluation of the effects of ecological and biological factors on the studied species is an essential element in determining their capacity to adapt to new environmental conditions, providing a solid foundation for their acclimatization and cultivation in the Republic of Moldova. In the current context of climate change, characterized by an increasing frequency of abiotic stress events, these species may represent viable alternatives for diversifying agricultural systems.

Data reported in the international literature confirm that *Clinopodium* L. species are distinguished by a significant content of essential oils, enhanced tolerance to drought conditions and natural resistance to pathogens and pests. These biological and phytochemical traits support their agronomic value and relevance for the development of sustainable cultivation technologies.

The integration of cyanobacteria into agricultural practice represents an innovative, organic approach that contributes to soil fertility improvement and increased plant productivity. Through their ability to fix atmospheric nitrogen, synthesize bioactive compounds and stimulate stress-resistance mechanisms, cyanobacteria have proven particularly effective in optimizing growth and development processes, including in *Clinopodium* L. species.

The results obtained in this study demonstrate the high potential of the investigated species for integration into sustainable and resilient agricultural systems, contributing to the diversification and strengthening of plant resource bases. They also highlight the capacity of these plants to maintain both productive and qualitative performance under variable pedoclimatic conditions.

Implementation of these research conclusions may generate multiple benefits, both economically and ecologically, through the diversification of aromatic and medicinal plant assortments with valuable species.

Overall, this study demonstrates that aromatic species of the genus *Clinopodium* L. are not only valuable components of native and introduced biodiversity but also serve as an effective experimental model for investigating plant – environment adaptation mechanisms, the biosynthesis of secondary metabolites (essential oils) and interactions with natural biostimulators such as cyanobacteria. By integrating ecological, physiological and phytochemical insights, this research provides a robust scientific foundation for developing sustainable, resilient and innovative agricultural strategies capable of making the best use of the economic, ecological and biochemical potential of aromatic plants.

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