UNIVERSITATEA BABEȘ-BOLYAI

Facultatea de Fizică

Şcoala Doctorală de Fizică

- PhD Thesis Summary

Physicochemical investigations and optimization of the biological properties of *Larix decidua*

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ABSTRACT

This study investigates, through an interdisciplinary approach, the interaction between the chemical structure of forest biomaterials and the action of external physical factors on the germination of Larix decidua seeds. The primary objective is to evaluate the physicochemical modifications induced by thermal treatments, electric and electromagnetic fields, and gamma radiation, and to correlate these changes with the biological response of the seeds.

Fourier Transform Infrared (FT-IR) spectroscopy revealed that the chemical structure of the bark is influenced by both its provenance and the applied thermal regime. The obtained spectra confirmed the presence of the main lignocellulosic components (lignin, cellulose, and hemicelluloses), while the observed spectral variations indicated modifications in functional groups, particularly a reduction in hydroxyl absorption bands and transformations involving carbonyl groups. These findings demonstrate that thermal treatments induce molecular reorganization processes rather than merely promoting moisture removal.

The results obtained by Gas Chromatography–Mass Spectrometry (GC-MS) confirmed that both temperature and provenance significantly affect the chemical composition of the bark. Increasing temperature led to the volatilization of low-molecular-weight compounds and to the predominance of more thermally stable constituents, particularly terpenoid and phenolic compounds, while also promoting the formation of secondary products. These transformations reflect changes in the chemical equilibrium of the system, governed by energy transfer processes and molecular stability.

The investigation of the effects of electric and electromagnetic fields on seed germination demonstrated that these treatments act through physical mechanisms involving molecular polarization, charge redistribution, and

modifications in ionic transport at the cellular level. The experimental results identified an optimal range of field parameters in which seed germination was enhanced, suggesting a nonlinear response of the biological system and an efficient utilization of the absorbed energy. Exceeding this optimal range resulted in inhibitory effects associated with structural perturbations.

Exposure to gamma radiation produced a dose-dependent biological response, characterized by stimulatory effects at low doses and inhibitory effects at higher doses. From a physical perspective, these effects are explained by ionization processes and the generation of free radicals, which influence molecular integrity and cellular functionality. The identification of an optimal irradiation dose highlights the potential for the controlled application of ionizing radiation to enhance seed germination.

The integration of FT-IR, GC-MS, and biological experimental results demonstrates that seed response is governed by the interaction between chemical structure and external physical stimuli. The molecular stability of chemical constituents influences the ability of biological systems to adapt to energetic excitation, while the variability observed among provenances reflects differences in dielectric properties and in energy absorption and dissipation mechanisms. Overall, this study confirms the relevance of integrating physicochemical methods into the investigation of biological processes and provides new perspectives for optimizing forest reproductive material production technologies through the controlled application of physical treatments.

1 CHAPTER 1 - Physicochemical Methods for the Investigation of Biological Systems

The application of physical methods enables the investigation of the chemical composition, molecular structure, and reaction mechanisms of biological systems with high precision, thereby contributing to the development of interdisciplinary approaches at the interface of physics, chemistry, and biology (Skoog & David, 2017).

Among the most widely established analytical techniques for the separation and identification of a large number of compounds are mass spectrometry-based methods, including gas chromatography–mass spectrometry (GC–MS), liquid chromatography–mass spectrometry (LC–MS), and high-performance liquid chromatography (HPLC), as well as other complementary analytical techniques. These methods allow the determination of molecular mass, the elucidation of molecular structures, and the analysis of complex mixtures, making them indispensable tools in numerous scientific fields (Figure 1.1).

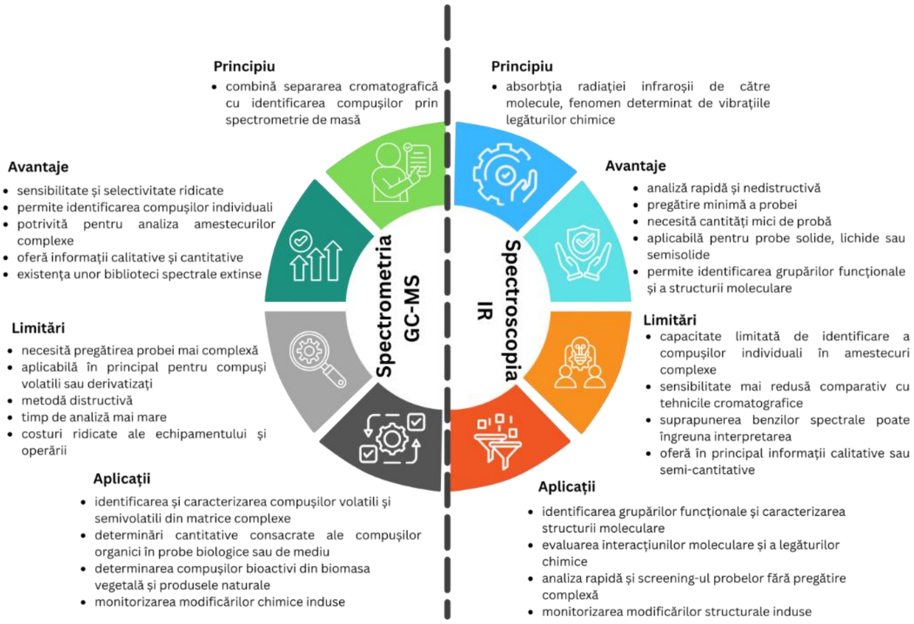

Figure 1.1. *Overview of the principles, advantages, limitations, and applications of gas chromatography–mass spectrometry (GC–MS) and infrared (IR) vibrational spectroscopy in the investigation of biological systems.*

Both mass spectrometry and vibrational spectroscopy constitute the subject of the present doctoral thesis and are employed for the characterization of biological materials.

In addition, gamma radiation, a form of high-energy electromagnetic radiation, interacts with matter through fundamental physical processes. These interactions may generate free radicals and induce molecular modifications which, depending on the absorbed dose, can exert either inhibitory or stimulatory effects on biological processes. In agriculture and forestry, low-dose gamma irradiation has been extensively investigated as a method for enhancing seed germination and improving seed vigor.

Therefore, the integration of physical methods into the study of plant systems provides the opportunity to investigate not only the chemical composition of biological materials but also the mechanisms through which physical factors influence physiological processes. This interdisciplinary approach is essential for understanding the mechanisms underlying the interactions between physical phenomena and biological systems, thereby contributing to the development of applications in fields such as agriculture, forestry, and the sustainable utilization of plant biomass.

1.1 Infrared Spectroscopy in the Characterization of Biological Systems

The absorption of infrared radiation by molecules occurs only when the molecular vibration is accompanied by a change in the dipole moment. This selection rule provides infrared (IR) spectroscopy with high selectivity toward specific chemical bonds and their structural environment. Molecular vibrations are classified into stretching vibrations (symmetric and asymmetric) and bending vibrations (scissoring, rocking, and internal rotational motions).

An IR spectrum is represented as absorbance or transmittance as a function of wavenumber and contains characteristic absorption bands corresponding to functional groups. The position, intensity, and shape of these bands enable the identification of chemical composition and the characterization of intermolecular interactions, such as hydrogen bonding, crystallinity, and molecular orientation.

Modern infrared spectroscopy is based on the Fourier Transform Infrared (FT-IR) technique, which employs a Michelson interferometer and the Fourier transform to generate the infrared spectrum. The measured absorbance is related to the concentration of the absorbing species through the Lambert–Beer law: $A = c \cdot \varepsilon \cdot l$ *(1)*

The technique provides a high signal-to-noise ratio, excellent spectral accuracy, and short analysis time. Different measurement modes, including attenuated total reflectance (ATR), transmission, and diffuse reflectance, enable the analysis of solids, liquids, and powders, as well as rapid in situ measurements using portable systems. Sample preparation depends on the physical state of the material. Solid samples may be analyzed directly using the ATR technique or in the form of KBr pellets, whereas liquid samples are examined using dedicated liquid cells. The resulting infrared spectrum exhibits characteristic absorption bands whose positions provide information about the functional groups present in the analyzed material.

Figure 1.2. *Workflow for IR spectral acquisition. Depending on the physical state of the sample, the analysis may be performed by direct placement on the ATR crystal, preparation of a KBr*

pellet, or the use of a liquid cell. The interaction between the sample and the infrared radiation generates the corresponding IR spectrum.

Due to its non-destructive nature and the minimal sample preparation required, infrared (IR) spectroscopy is widely employed for physicochemical characterization and analytical monitoring (Simionescu, 2012). Its traditional fields of application include materials science and chemical synthesis, where the technique is extensively used for the analysis of functional materials, polymers, and biomaterials (Hsu, 1997; Parker, 2012).

The range of applications has expanded to encompass environmental studies, including the detection and quantification of pollutants and air quality assessment, as well as the food industry, where IR spectroscopy is applied for quality control, product authentication, and the identification of adulterants. Furthermore, it has become an important analytical tool in applied physics for investigating vibrational dynamics and intermolecular interactions (Rodriguez-Saona & Allendorf, 2011; Valand et al., 2020; Candoğan et al., 2021). In agriculture, IR spectroscopy is widely used to evaluate biomass composition, determine moisture content, and identify abiotic and biotic stress in plants (Liu et al., 2015; Tsuchikawa et al., 2022).

In tree research, IR spectroscopy enables the identification of species-specific chemical markers and the monitoring of wood degradation processes. The technique is also employed for bark characterization, providing information on functional groups associated with cellulose, hemicellulose, lignin, and phenolic compounds. Recent studies have demonstrated the usefulness of FT-IR spectroscopy for the analysis of tannins in pine species (Chupin et al., 2013) and for investigating structural changes occurring in wood over time (Bouramdane et al., 2022).

Fourier Transform Near-Infrared (FT-NIR) spectroscopy complements these analyses by enabling the rapid assessment of chemical composition as a function of processing conditions. This technique has been successfully applied to plant-derived products to evaluate the effects of thermal treatments and drying conditions on their biochemical composition (Xu et al., 2023).

1.2 Theoretical Aspects and Applications of Gas Chromatography–Mass Spectrometry (GC–MS)

Gas chromatography–mass spectrometry (GC–MS) is a well-established analytical method for the detection and quantification of a wide range of chemical compounds in virtually any type of sample matrix. This technique enables the determination of molecular masses, the elucidation of molecular structures, and the analysis of complex mixtures, making it one of the most widely applied analytical methods across numerous scientific disciplines. GC–MS combines the physicochemical separation of volatile and semi-volatile compounds by gas chromatography with their identification by mass spectrometry based on their mass-to-charge ratio (m/z) (Beale et al., 2018). GC–MS offers high selectivity, sensitivity, and analytical accuracy, which explains its extensive use in chemical, biological, environmental, pharmaceutical, and food science research. Although sample preparation, processing, and instrumental analysis are considerably more laborious (Figure 1.3) than those required for vibrational spectroscopy, GC–MS remains the reference technique for the precise identification of chemical constituents in complex matrices. A comparative overview of the two analytical methods is presented in Figure 1.4.

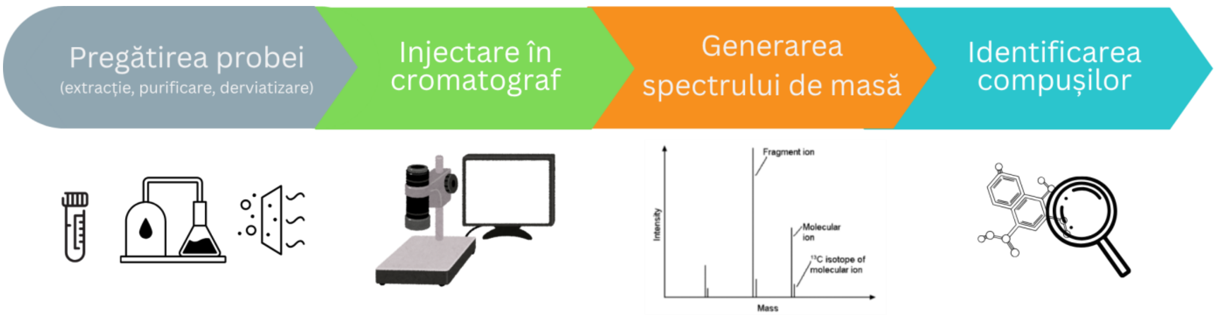

Figure 1.3. *GC–MS analytical workflow. The sample undergoes careful preparation to extract the compounds of interest from the sample matrix and produce a homogeneous extract suitable for GC–MS injection. After injection, the analytes are separated in the chromatographic column, ionized in the mass spectrometer, detected, and finally represented as a mass spectrum.*

From a theoretical perspective, gas chromatography–mass spectrometry (GC–MS) integrates two complementary processes: gas chromatographic separation and mass analysis, which is based on the motion of ions in electric fields. This combination provides high selectivity and sensitivity, making GC–MS one of the most widely used analytical techniques for the characterization of molecular compounds (Beale et al., 2018).

Gas chromatography separates compounds according to their distribution between the gaseous mobile phase (typically helium or nitrogen) and the stationary phase within the capillary column. The separation process depends on the volatility of the analytes and their intermolecular interactions with the stationary phase, with each component being characterized by a specific retention time.

Following chromatographic separation, the compounds are introduced into the mass spectrometer, where they are ionized, accelerated, and separated according to their mass-to-charge ratio (m/z). Ionization is commonly achieved by electron impact (EI) at an energy of approximately 70 eV, producing molecular ions and characteristic fragment ions that enable the structural identification of the analyzed compounds. $M + e^- \rightarrow M^{+\bullet} + 2e^-$ (2)

The molecular ion formed is generally unstable and undergoes fragmentation processes, generating characteristic fragment ions. These fragments reflect the molecular structure and constitute the basis for compound identification. The ionization process involves the transfer of kinetic energy from the incident electrons to the molecules, providing sufficient energy to overcome their ionization potential. The subsequent fragmentation pattern is governed by the distribution of internal energy within the molecular ion and by the stability of the resulting fragment ions.

The ions produced are subsequently accelerated and separated according to their mass-to-charge ratio (m/z) using a mass analyzer, such as a quadrupole analyzer or an ion trap. Ion separation is based on their motion within oscillating or static electric fields, with their trajectories being determined by the equations of motion governing charged particles. The motion of ions is described by the fundamental laws of electromagnetism, particularly the Lorentz force: $\vec{F} = q(\vec{E} + \vec{v} \times \vec{B})$ (3)

where q is the ion charge, $\vec{E}$ is the electric field, and $\vec{B}$ is the magnetic field.

In quadrupole mass spectrometers, which are widely used in GC–MS systems, ion separation is achieved by applying oscillating electric fields that allow only ions with a specific and stable mass-to-charge ratio (m/z) to pass through the analyzer. These ions reach the detector, whereas all other ions are either deflected or removed. This selection process is based on the stability of ion trajectories within a time-varying electric field. The transmitted ions are subsequently detected by a detector, typically an electron multiplier, which converts the impact of the ions into an electrical signal proportional to their abundance. The final output is a mass spectrum, representing ion intensity as a function of the mass-to-charge ratio (m/z).

A GC–MS mass spectrum provides valuable information about the molecular structure of the analyzed compounds. The molecular ion (M^+) indicates the molecular mass, whereas the fragment ions reflect the fragmentation pathways, which are governed by electronic stability and bond dissociation energies. This characteristic fragmentation pattern enables compound identification through comparison with reference spectral libraries (Beale et al., 2018).

Sample preparation is an essential step to ensure compatibility with GC–MS analysis, which is primarily intended for volatile and thermally stable compounds. This process generally includes analyte extraction, removal of interfering substances, and, when required, the derivatization of polar compounds to increase their volatility. Extraction is performed using solvents selected according to analyte polarity, such as hexane, dichloromethane, or methanol, or by employing modern extraction techniques including solid-phase extraction (SPE) and solid-phase microextraction (SPME). Derivatization methods, such as silylation and methylation, convert polar functional groups into more volatile and chemically stable derivatives suitable for GC–MS analysis.

The final sample must be clear and homogeneous, filtered, and, when necessary, concentrated prior to analysis. Quantitative determination is based on calibration curves correlating chromatographic peak areas with analyte concentrations. Following sample injection, compounds are identified according to their retention time, mass-to-charge ratio (m/z), and comparison with reference spectral databases, while quantification is achieved by comparing chromatographic peak areas with those obtained from calibration standards.

GC–MS provides high sensitivity, selectivity, and analytical accuracy, making it particularly suitable for the analysis of complex mixtures. However, its main limitations include its applicability primarily to volatile and thermally stable compounds and the requirement for derivatization of certain analytes. Furthermore, electron impact ionization (EI) may produce extensive fragmentation, which can complicate the interpretation of mass spectra for some compounds.

GC–MS has numerous applications in agriculture, where it is employed for the analysis of metabolites and pesticide residues; in the food industry, for aroma profiling and authenticity assessment; and in environmental science, for monitoring organic pollutants. In the study of plant materials, GC–MS is widely used for the characterization of volatile compounds present in bark and oleoresins, including those of *Larix decidua*, allowing the identification of chemical and functional differences among different provenances.

1.3 Application of Physical Methods in the Study of Seed Germination and Cellular Viability

Improving seed quality has become a major research topic due to the continuous need to enhance seed resilience to environmental stress and to increase germination efficiency. In this context, a growing body of research has demonstrated that physical treatments, including ionizing and non-ionizing radiation, magnetic and electric fields, microwaves, infrared radiation, and ultraviolet light, can significantly improve seed germination, seedling vigor, and tolerance to both biotic and abiotic stresses (Araújo et al., 2016; Bera et al., 2022) (Figure 1.4).

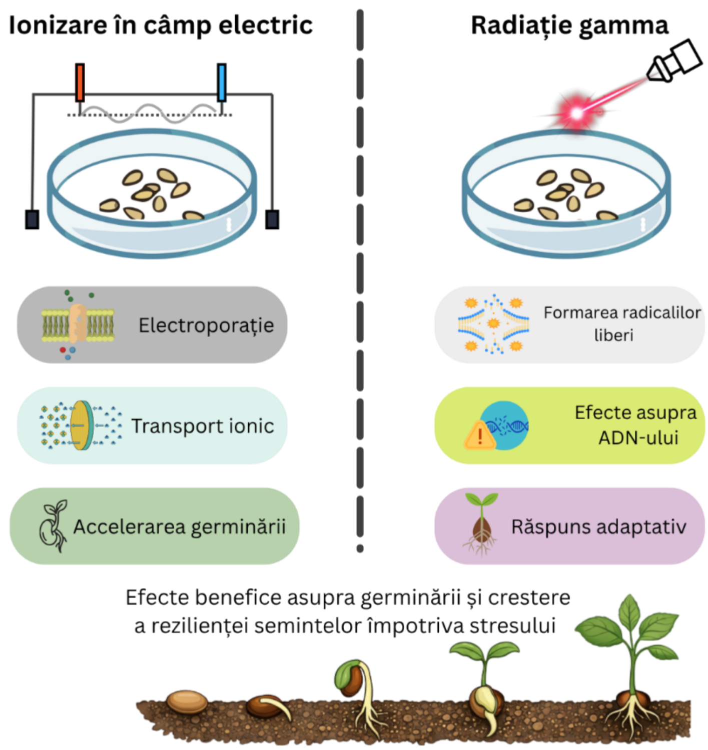

Figure 1.4. *Effects of electric field treatments and gamma irradiation on seed germination aimed at enhancing plant performance and quality.*

The effects of physical treatments on seed quality can be investigated at multiple levels, ranging from morpho-structural changes to molecular modifications. These treatments fall within the concept of seed priming, whereby low-intensity physical stimuli act as controlled stress factors that activate pre-germinative metabolism, antioxidant defense mechanisms, and DNA repair processes (Araújo et al., 2016; Bera et al., 2022).

Conventional seed priming involves controlled hydration, including hydropriming and osmopriming, to improve germination performance and enhance tolerance to abiotic stress. However, limitations associated with cost, storage, and environmental impact have stimulated the development of physical priming methods, such as electric fields, gamma and X radiation, ultraviolet radiation, and microwave treatments.

Electric fields interact with electric charges and molecular dipoles, inducing polarization and changes in the transmembrane potential without causing ionization. In contrast, gamma radiation is an ionizing, high-energy form of electromagnetic radiation capable of inducing structural modifications at the molecular level. Consequently, these two treatment modalities operate within fundamentally different energy regimes.

Electric field treatments provide precise control of the applied parameters and generally induce reversible effects, making them suitable for stimulating seed germination while maintaining cellular viability. In contrast, gamma irradiation produces cumulative effects and is primarily employed for mutagenesis or sterilization. At low doses, however, gamma radiation may exert stimulatory effects through radiation hormesis, although it also carries an increased risk of genomic alterations at higher exposure levels.

1.3.1 Physical Mechanisms Involved in the Stimulation of Seed Germination by Electric Fields

From a physical perspective, fundamental biological processes, such as cellular viability and seed germination, are governed by multiple physical phenomena. The application of electric fields to biological systems, particularly seeds, represents an interdisciplinary approach at the interface between physics and biology, providing a non-chemical tool for modulating these processes. Electric fields can influence the distribution of electric charges, membrane potentials, and ionic transport, all of which are essential for maintaining cellular viability and initiating the germination process.

An electric field is a force field surrounding an electrically charged particle (Griffiths & Nilson, 1999). It is defined as the force exerted on a unit positive charge and is mathematically expressed by the following equation: (4): $\vec{E} = \frac{\vec{F}}{q}$ (4) where $\vec{F}$ s the electric force and q is the electric charge. In biological media, electric fields interact with free charges, molecular dipoles, and polar structures, leading to their redistribution.

From a biophysical perspective, plant cells and seeds can be regarded as heterogeneous dielectric–conductive systems, in which the cell membrane acts as a thin dielectric layer separating conductive media represented by the cytoplasm and the extracellular environment. The application of an external electric field induces the polarization of these structures, resulting in the generation of an additional transmembrane electric potential, described by Equation (5): $\Delta V = f \cdot E \cdot r$ (5) where E is the electric field strength, r is the cell radius, and f is a geometry-dependent factor.

When the transmembrane potential exceeds a threshold of approximately 0.5–1.0 V, membrane permeability increases through the formation of transient pores, a phenomenon known as electroporation. Under controlled conditions, electroporation is reversible and facilitates ionic exchange without compromising cellular integrity, making it particularly relevant for stimulating seed germination.

At the seed level, electric fields influence water uptake, enzymatic activation, and cell division by modifying the distribution of ions such as Ca^{2+}, K^+, and H^+ and by altering transmembrane ionic fluxes. In addition, electric fields may induce electroosmotic and electrophoretic phenomena, thereby enhancing the transport of water and nutrients toward the embryo.

Changes in the resting membrane potential and in the activity of membrane proteins, including ion channels and ion pumps, accelerate metabolic activation and reduce the latency period preceding germination. Consequently, the electric field acts as a controlled physical stimulus capable of enhancing germination without the addition of chemical substances.

The main advantages of electric field treatment include precise control of operational parameters (field strength, exposure time, and frequency), its non-invasive nature, and the absence of chemical residues, making this approach highly compatible with sustainable agricultural practices. Experimental studies have reported improvements in germination rate, emergence uniformity, and seed viability following appropriate electric field treatments.

However, limitations arise when critical threshold values are exceeded, causing irreversible electroporation, which compromises membrane integrity and cellular viability. Furthermore, the biological response depends on the plant species and the experimental conditions employed, while the interpretation of results may be influenced by secondary effects, such as Joule heating.

1.3.2 Application of gamma radiation in the study of seed germination

Gamma (γ) radiation is a form of high-energy electromagnetic radiation characterized by extremely short wavelengths ($<10^{-11}$ m) and photon energies in the keV–MeV range, originating from nuclear processes. Gamma photons possess neither mass nor electric charge and exhibit a high penetrating capability.

The interaction of gamma radiation with matter occurs through three principal mechanisms: the photoelectric effect, Compton scattering, and electron–positron pair production. These interactions result in ionization and the generation of secondary electrons. In biological systems, gamma irradiation induces the radiolysis of water, leading to the formation of reactive oxygen species (ROS), which are responsible for the oxidation of biomolecules and the alteration of cellular structures.

The biological effects of gamma radiation depend primarily on the absorbed dose, expressed in gray (Gy). At low doses, an adaptive response known as radiation hormesis may occur, whereas higher doses generally produce cytotoxic effects. In seeds, low-dose gamma irradiation has been reported to stimulate germination and enhance tolerance to environmental stress, provided that the irradiation parameters are carefully controlled.

An important parameter describing the interaction of gamma radiation with matter is the linear attenuation coefficient, which represents the probability of photon absorption or scattering per unit path length. The attenuation of gamma radiation in a material follows an exponential law, expressed by Equation (6): (6): $I = I_0 \cdot e^{-\mu x}$ (6) where I_0 is the incident radiation intensity, I is the transmitted intensity, μ is the linear attenuation coefficient, and x is the thickness of the material. This relationship illustrates the high penetrating power of gamma radiation and the necessity of using high-density shielding materials, such as lead, for effective radiation protection.

Within the context of biological systems, gamma irradiation represents a physical stimulation technique based on the application of high-energy ionizing electromagnetic radiation, capable of interacting extensively with biological structures. Gamma radiation is widely employed for sterilization, mutation induction, and the investigation of cellular responses to oxidative stress. In seeds, exposure to low doses may stimulate germination by activating DNA repair mechanisms and enhancing metabolic activity, whereas high doses cause DNA damage and inhibit seedling development. Therefore, a thorough understanding of the physical mechanisms governing the interaction between gamma radiation and matter is essential for its controlled application in biological and agricultural research.

2 Biological material and experimental system

The selection of an experimental system for studies on plant biomaterials is determined by the interaction between molecular structure and external physical factors. Plant materials are heterogeneous systems composed of natural polymers, including cellulose, hemicelluloses, and lignin, together with a wide range of secondary metabolites. These materials exhibit dielectric and structural properties that influence the absorption, distribution, and transformation of energy at the molecular level.

In the context of climate change and the increasing pressure on forest ecosystems, optimizing seed germination has become an important research objective. Physical treatment methods offer several advantages over conventional approaches owing to their precise control of treatment parameters and their reduced environmental impact (Danek et al., 2022). *Larix decidua* represents an appropriate experimental model because of its ecological importance and the variability of its germination response among different provenances. The seeds of this species can be regarded as complex physical systems, in which germination processes are governed by energy transfer mechanisms and the dynamics of molecular interactions.

Electric and electromagnetic fields interact with biological materials through polarization and charge redistribution, resulting in modifications of membrane properties and ionic transport. These effects depend on the intensity, frequency, and duration of exposure. Owing to their ionizing nature, gamma rays induce excitation and ionization processes at the molecular level, producing structural and functional changes that are dependent on the absorbed radiation dose. The physicochemical characterization of the plant material is performed using complementary analytical techniques. Fourier Transform Infrared (FT-IR) spectroscopy enables the identification of structural changes in functional groups, whereas gas chromatography–mass spectrometry (GC–MS) provides detailed information on the chemical composition and stability of individual compounds. The integration of these techniques allows the correlation of molecular structure, energy transfer processes, and biological responses.

The aim of this study is to investigate the influence of physical fields on the germination of *Larix decidua* seeds by evaluating the effects of treatment parameters and identifying the optimal conditions for germination stimulation. The proposed approach seeks to establish correlations between measurable physical parameters and biological responses, thereby contributing to the development of efficient and sustainable strategies for forest regeneration.

2.1 Taxonomy and morphological characteristics of *Larix decidua*

Larix decidua Mill. belongs to the Gymnosperms, a group of woody plants characterized by the production of naked seeds and the development of cones as their reproductive structures. From a taxonomic perspective, this species is classified within the Pinaceae family, one of the most important families of woody plants in the Northern Hemisphere, comprising numerous species of considerable ecological and economic importance.

The taxonomic classification of *Larix decidua* is as follows:

Subdivision: Gymnospermae

Class: Coniferopsida

Order: Coniferales

Family: Pinaceae

Genus: Larix

Species: *Larix decidua* Mill.

The Pinaceae family comprises resinous trees characterized by a monopodial stem and needle-like leaves, while the wood consists predominantly of tracheids. A distinctive feature of the genus Larix is its deciduous needles, an adaptation that reduces water loss during the winter season.

The genus Larix includes approximately 10–15 species distributed throughout the Northern Hemisphere, such as *Larix sibirica, Larix occidentalis*, and *Larix kaempferi. Larix decidua* is native to the mountainous regions of Central Europe, particularly the Alps and the Carpathians, and is widely used in forest plantations because of its high-quality timber (Holonec & Truța, 2015).

From an ecological perspective, L. decidua is well adapted to mountain climates characterized by low temperatures and pronounced seasonal fluctuations. Mature trees may reach 40–50 m in height and typically exhibit a straight trunk and a relatively open pyramidal crown, allowing efficient light penetration.

The root system is initially characterized by a taproot, which later develops into a highly branched system adapted to local soil conditions. The bark changes from a smooth texture in young trees to a thick, deeply fissured rhytidome with age and contains tannins and phenolic compounds (approximately 7–16%), which are responsible for its antioxidant properties.

The wood is dense, moisture-resistant, and rich in resin canals, containing numerous terpenoid compounds that can be characterized by FT-IR spectroscopy and GC–MS analysis. The shoots are differentiated into two distinct types: long, slender shoots and short, thicker shoots bearing well-developed buds.

***Figure 2.1. Larix decidua**: (a) stem with bark; (b) transverse section of the wood.*

The buds are nearly spherical in shape and are covered by protective scales coated with a thin layer of resin, which contributes to their protection against environmental factors (Holonec & Truța, 2015).

The leaves are soft, deciduous needles measuring 1–3 cm in length. They are arranged spirally on the long shoots and in clusters of 30–40 needles on the short shoots (Figure 2.2). In autumn, the needles turn golden yellow before being shed, thereby contributing to the accumulation of organic matter in the soil (Danek et al., 2022).

Larix decidua is a monoecious species bearing unisexual flowers. The male flowers are yellow catkins, whereas the female flowers are ovoid to spherical and reddish or violet in color (Figure 2.2). Flowering occurs in spring (April–May), simultaneously with needle emergence.

The cones are relatively small (3–4 cm long), with imbricate scales, and may remain attached to the tree for 2–4 years after seed maturation. The seeds are small and equipped with a membranous wing that facilitates wind dispersal (anemochory) (Figure 2.2). Approximately 160,000 seeds are contained in one kilogram (Teodosiu et al., 2023).

Seed maturation occurs during the autumn of the first growing season, while the germination rate is relatively low (20–45%), which justifies the application of seed stimulation treatments to improve germination performance.

Figure 2.2. ***Larix deciuda****, shoot with needles, cones and seeds, and reproductive structures.*

The longevity of European larch is remarkable, with mature individuals capable of reaching 600–700 years of age under favorable growing conditions. The high structural stability and biological resistance of this species, together with the morphological and chemical characteristics of its plant tissues, make *Larix decidua* an appropriate biological model for investigating the interactions between electromagnetic radiation, physical fields, and plant biomaterials, particularly within the context of physicochemical research.

2.2 Physicochemical properties of *Larix decidua* bark

The bark of *Larix decidua* can be regarded as a natural composite material with a hierarchical structure whose behavior depends on both its chemical composition and external factors, such as temperature, electromagnetic radiation, and electric fields (Aleksandrovna & Redzhinaldovich, 2021). From a physical perspective, bark is a heterogeneous and anisotropic system, whose properties—

including density, porosity, and polarizability—vary according to its internal organization (Bianchini et al., 2025).

The bark matrix consists primarily of the structural biopolymers cellulose, hemicellulose, and lignin. Cellulose exhibits semi-crystalline domains stabilized by hydrogen bonds, which are responsible for its mechanical rigidity and well-defined vibrational modes (Frejaville et al., 2013; Agarwal et al., 2021). Hemicellulose has an amorphous structure and contributes to material flexibility and to its response to variations in external energy (Cáceres et al., 2018). Lignin, a three-dimensional aromatic polymer, possesses high polarizability and plays an important role in the interaction with electromagnetic radiation and in energy dissipation processes (Wagner et al., 2015; Vangeel et al., 2023).

The bark also contains a variety of extractive compounds, including tannins, phenolic compounds, and terpenes, which influence its physicochemical properties because of their high molecular mobility and the presence of polar functional groups (–OH and C=O). These constituents significantly affect both the dielectric behavior and the spectroscopic response of the material (Baldan et al., 2017).

The thermal behavior of the bark depends on the stability of its individual components. At moderate temperatures, reversible processes, such as structural reorganization and the redistribution of chemical constituents, predominate. In contrast, exposure to elevated temperatures leads to irreversible processes, including thermal degradation and oxidation (Sillero et al., 2018; Bianchi et al., 2015). The extractive fraction is the most thermally sensitive component, and its transformations can be readily detected by FT-IR spectroscopy and GC–MS analysis.

When exposed to electric fields, the material exhibits dielectric polarization and charge redistribution, both of which depend on the frequency of the applied field and the temperature (Kain et al., 2018). Under the action of electromagnetic radiation, energy absorption occurs through molecular vibrational excitations, particularly within the infrared region of the electromagnetic spectrum, thereby enabling the characterization of the material's molecular structure (Tumenjargal et al., 2020).

Therefore, *Larix decidua* bark represents a complex physicochemical system, in which its macroscopic properties arise from the interaction between its molecular structure and external energetic factors.

2.3 Chemical composition of *Larix decidua* bark and its relevance for spectroscopic analysis

The spectroscopic analysis of biomaterials is based on the interaction between electromagnetic radiation and molecular structure, resulting in characteristic energy transitions. In the case of *Larix decidua* bark, its complex chemical composition produces a distinctive spectral response, with each infrared (IR) absorption band corresponding to specific molecular vibrations (Baldan et al., 2017).

In the infrared region, radiation absorption is associated with the vibrational modes of chemical bonds, which depend on both the atomic masses of the bonded atoms and the bond force constants. The FT-IR spectrum of lignocellulosic materials reflects the combined contributions of cellulose, hemicellulose, lignin, and extractive compounds (Kain et al., 2018). The broad absorption band centered around 3400 cm^{-1} is attributed to the stretching vibrations of hydroxyl (–OH) groups and the hydrogen-bonding network, making it particularly sensitive to structural changes and the degree of hydration. The absorption band near 2900 cm^{-1} corresponds to the C–H stretching vibrations of aliphatic chains, whereas the 1700–1750 cm^{-1} region is characteristic of carbonyl (C=O) groups, whose intensity may be influenced by oxidation processes or other chemical transformations. Furthermore, the 1500–1600 cm^{-1} region reflects the vibrational modes of the aromatic rings in lignin, while the 1000–1200 cm^{-1} region is associated with the polysaccharide framework, providing valuable information on the structural organization of cellulose and hemicellulose.

The interpretation of FT-IR spectra is complemented by GC–MS analysis, which enables the identification of individual chemical constituents. The combined application of these analytical techniques facilitates the interpretation of structural and energetic changes, including the reorganization of hydrogen-bonding networks and induced chemical transformations.

Therefore, infrared spectroscopy provides not only detailed information regarding the chemical composition of *Larix decidua* bark but also valuable insight into the energetic state and molecular-level physical behavior of the biomaterial.

3 Experimental methodology

The experimental methodology was designed to investigate the relationship between the physicochemical properties of plant biomaterials and their biological response to external physical factors under controlled laboratory conditions. The approach is based on the interaction between radiation and matter, examining how different forms of energy influence molecular structure and seed germination processes. Conceptually, the study focuses on energy transfer to the biological system and its effects at the molecular, subcellular, and physiological levels, enabling the correlation of physical parameters, including field intensity, exposure duration, radiation dose, and frequency, with the biological response of the plant material.

The experimental design was organized into two complementary research directions (Figure 3.1). The first focused on the physicochemical characterization of Larix decidua bark using FT-IR spectroscopy to analyze functional groups and GC–MS to identify volatile compounds. The second investigated the effects of physical fields on seed germination by applying treatments involving electric fields, magnetic fields, and gamma radiation.

Figure 3.1. Experimental design of the doctoral thesis.

By integrating these two experimental approaches, the methodology enables the establishment of correlations between the structural and compositional changes in the plant biomaterial, revealed through physicochemical analytical techniques, and the biological response of the seeds, as reflected by their germination parameters. This multidisciplinary approach provides a coherent framework for investigating the relationship between the applied physical factors and the dynamics of biological processes, thereby contributing to a deeper understanding of the mechanisms through which physical energy modulates the functioning of plant systems.

3.1 Origin of the biological material

The biological material consisted of seven clones of *Larix decidua* Mill. originating from different regions of Romania: Gura Humorului, Brașov (Valea Cetății), Săcele, Brașov (Valea Popii), Sinaia, Anina, and Latorița (Figure 3.2). The clones were derived from plus trees selected for their superior phenotypic characteristics. They were propagated by grafting and established in the Baciu seed orchard (Cluj-Napoca) in 1975.

For the physicochemical analyses, larch bark samples were collected, whereas the germination experiments were performed using seeds subjected to treatments with electric fields and gamma radiation. The selected provenances encompass an ecological gradient ranging from low-altitude, warmer environments to cold mountainous regions, thereby allowing the assessment of biological variability under different environmental conditions.

For each provenance, ten healthy trees were selected. Bark samples were collected at 1.3 m above ground level (diameter at breast height, DBH) (Figure 3.3a,b), after which they were stored under controlled conditions and transported to the laboratory. Sampling was carried out during the active growing season to ensure the stability of the chemical composition of the collected material.

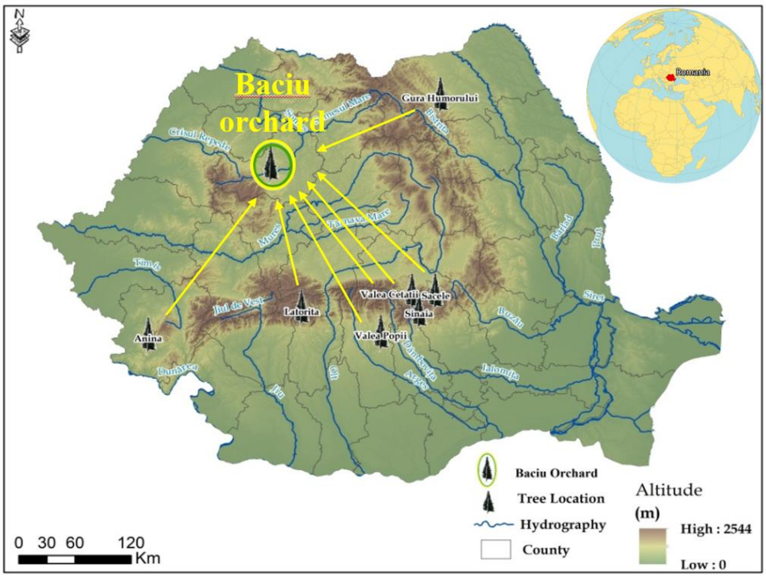

Figure 3.2. *Geographic location of the Larix decidua provenances and the Baciu seed orchard.*

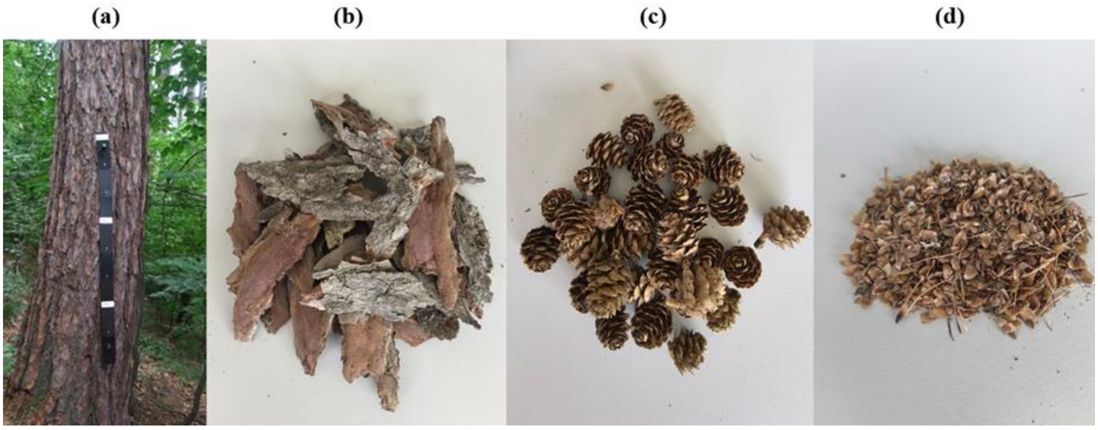

Figure 3.3. *Collection and preparation of Larix decidua biological material: (a) bark sampling from the trunk at breast height (1.3 m); (b) bark fragments; (c) mature cones; (d) seeds extracted from the cones.*

To reduce within-provenance variability, bark samples collected from trees belonging to the same clonal group were homogenized, resulting in one composite sample for each provenance. This approach ensured both a sufficient quantity of material for analysis and the biochemical representativeness of each provenance.

The seed material used for the germination experiments was obtained from cones collected from mature trees (approximately 50 years old) belonging to the same provenances. The cones were dried at 20–25°C, after which the seeds were manually extracted and used in the experimental investigations (Figure 3.3c,d).

3.2 Preparation of *Larix decidua* bark samples for FT-IR and GC–MS analysis

The bark samples were prepared to ensure the homogeneity and reproducibility of the analytical measurements. Drying was carried out under four temperature regimes: control (untreated), 60°C, 80°C, and 100°C, each for 2 h. These temperatures facilitate water removal, the reorganization of hydrogen-bonding networks, and the mobilization of volatile compounds without causing significant degradation of the structural components (Ruiz-Aquino et al., 2023; Morar et al., 2024).

Subsequently, the samples were ground using a Retsch Grindomix GM 200 mill and sieved to obtain particles smaller than 300 μm. Reducing the particle size increases the specific surface area, improves extraction efficiency for GC–MS analysis, and ensures a more uniform interaction with infrared radiation during FT-IR measurements, thereby minimizing light-scattering effects.

3.2.1 FT-IR Technique used for the investigation of *Larix decidua* bark

Fourier Transform Infrared (FT-IR) spectroscopy is a widely used technique for investigating molecular structure, based on the interaction between infrared radiation and matter, combined with the application of the Fourier transform to the recorded interferometric signal. Infrared absorption occurs when the energy of the incident radiation matches the vibrational energy transitions of chemical bonds, producing characteristic absorption bands in the spectrum (Gierlinger et al., 2004). Owing to its ability to identify functional groups, FT-IR spectroscopy is extensively employed in the analysis of lignocellulosic biomaterials.

In the present study, FT-IR spectroscopy was used to characterize the chemical composition of Larix decidua bark. Samples were prepared by mixing approximately 3 mg of finely ground bark powder with 200 mg of calcined potassium bromide (KBr), followed by homogenization and compression into transparent pellets using a hydraulic press (Specac Ltd., United Kingdom).

From a physical perspective, chemical bonds can be modeled as harmonic oscillators, in which atoms vibrate around their equilibrium positions. The vibrational frequency depends on the atomic masses and the stiffness of the chemical bond, allowing FT-IR absorption bands to be correlated with specific bond types. In biomaterials such as Larix decidua bark, the vibrations of O–H, C=O, and C–H functional groups generate a complex spectrum that is particularly sensitive to intermolecular interactions, especially hydrogen bonding (Piqueras et al., 2020).

FT-IR spectra were recorded using a Jasco FT/IR-4100 spectrometer (JASCO, Japan) over the spectral range of 4000–350 cm^{-1}, with a spectral resolution of 4 cm^{-1} and 256 accumulated scans. The different spectral regions provide information on polysaccharides, lignin, and extractive compounds, while the fingerprint region contains characteristic features of the overall molecular structure.

The FT-IR technique employs a Michelson interferometer, which simultaneously records all infrared frequencies in the form of an interferogram. This signal is subsequently converted into an absorption spectrum by means of the Fourier transform, providing high spectral accuracy and an excellent signal-to-noise ratio.

Solid samples were analyzed as KBr pellets, prepared by dispersing finely ground bark powder within an infrared-transparent potassium bromide matrix. Careful control of particle size and sample homogeneity was essential to obtain highly reproducible spectra.

The spectral data were processed using Spectra Manager (JASCO) and further analyzed with OriginLab software through baseline correction and peak fitting. Spectral interpretation was performed by comparison with published literature data (Vârban et al., 2021; Morar et al., 2024).

Although FT-IR spectroscopy provides comprehensive information on the overall chemical structure of the biomaterial, the overlap of absorption bands limits the identification of individual compounds. Therefore, FT-IR was used in combination with GC–MS analysis. In the present study, this complementary approach enabled the evaluation of structural modifications in *Larix decidua* bark as a function of provenance and the applied treatments.

3.2.2 GC–MS Analysis for the characterization of *Larix decidua* bark

The volatile and semi-volatile constituents of Larix decidua bark were analyzed using gas chromatography–mass spectrometry (GC–MS), a technique that combines chromatographic separation with structural identification and is particularly suitable for investigating lipophilic fractions that cannot be adequately characterized by FT-IR spectroscopy.

The bark samples were dried, ground, and sieved to obtain particles smaller than 400 μm, thereby increasing the specific surface area and improving extraction efficiency. Approximately 3 g of bark powder were subjected to ultrasound-assisted extraction (UAE) with 5 mL of dichloromethane using an ultrasonic bath operating at 40 kHz and 135 W. The extraction process relies on acoustic cavitation, which promotes the disruption of cellular structures and facilitates the release of intracellular compounds.

Dichloromethane was selected as the extraction solvent because of its high efficiency in recovering lipophilic compounds, including terpenoids and fatty acids. To minimize the loss of volatile constituents, extraction was carried out at 0–5°C. Following extraction, the samples were centrifuged at 10,000 rpm for 10 min, and the resulting supernatant was collected for GC–MS analysis.

GC–MS analyses were performed using a Shimadzu QP-2010 PLUS system equipped with an autosampler. Chromatographic separation was achieved on a ZB-5MS Plus capillary column (30 m × 250 μm × 0.25 μm), which is suitable for the analysis of volatile and semi-volatile organic compounds.

The oven temperature program was optimized to separate compounds with different volatilities. The initial temperature was maintained at 40°C for 2 min, followed by a temperature ramp of 20°C min^{-1} up to 300°C, with a final holding time of 5 min. This temperature profile enabled the efficient elution of compounds ranging from highly volatile molecules to high-molecular-weight constituents, resulting in well-resolved chromatograms.

Samples were injected in splitless mode at an injector temperature of 250°C, using helium as the carrier gas at a constant flow rate of 0.8 mL min^{-1}. Splitless injection allows the entire sample to enter the chromatographic column, making it particularly suitable for analytes present at low concentrations.

Following chromatographic separation, the analytes were identified by mass spectrometry using electron impact ionization (EI) at 70 eV, which produces characteristic fragmentation patterns for each compound. Mass spectra were acquired over the m/z 35–800 range, with the ion source maintained at 220°C. Compound identification was performed by comparison with the NIST and Wiley spectral libraries, while retention times were used as an additional criterion for confirmation.

The GC–MS technique enables the identification and quantification of volatile and semi-volatile compounds present in *Larix decidua* bark, thereby complementing the structural information obtained through FT-IR spectroscopy and providing a comprehensive characterization of the bark's chemical composition.

3.3 Influence of electric fields, electromagnetic fields, and gamma radiation on the germination processes of *Larix decidua* seeds

The germination of Larix decidua seeds exposed to electric fields, electromagnetic fields, and gamma radiation was investigated in relation to the morphometric characteristics of the cones and seeds. The mass and dimensions of the cones, the number of seeds per cone, as well as the mass and dimensions of the seeds were determined, since these parameters are closely associated with seed viability and germination potential.

All measurements were performed using an analytical balance and a digital caliper on representative samples from each provenance. The morphometric data were correlated with the germination percentage to evaluate the relationships between seed morphology and the response to the applied physical treatments, allowing the identification of possible associations between seed size and sensitivity to physical stimuli.

The analyses were conducted in accordance with the International Seed Testing Association (ISTA) standards and the Food and Agriculture Organization (FAO) guidelines, ensuring methodological rigor and comparability. The results are presented as mean values accompanied by appropriate statistical indicators to evaluate variability and to assess the influence of provenance and treatment on seed germination.

3.3.1 Effect of electric field treatments on seed germination

The influence of electric fields on the germination of Larix decidua seeds was investigated in the Biophysics Laboratory of the University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, using an experimental system consisting of two electrodes with an adjustable separation connected to a variable-voltage power supply.

The application of electric fields to Larix decidua seeds is based on the interaction between the external electric field and electrically charged biological structures, particularly cell membranes and the enzymatic systems involved in the initiation of germination. From a physical standpoint, the electric field is characterized by its field strength, defined as the ratio between the applied voltage and the distance separating the electrodes, which enables the precise quantification of the electrical stimulus applied to the seeds. Under the experimental conditions employed, with an electrode separation of 7.3 cm, the applied voltages (10 V, 30 V, and 50 V) generated low-intensity electric fields that were nevertheless sufficient to induce biophysical modifications at the cellular level without causing destructive effects.

The experiment was conducted under controlled laboratory conditions using parallel circular electrodes (diameter 26 cm). The seeds were positioned between the electrodes within a uniform electric field, without direct contact with the electrode surfaces, thereby preventing electrical discharges. Careful control of both the applied voltage and electrode spacing ensured the reproducibility and comparability of the experimental results.

A factorial experimental design was adopted, considering two independent variables: electric field intensity and exposure time. Three voltage levels (10, 30, and 50 V) and three exposure periods (15, 35, and 60 min) were tested, resulting in nine treatment combinations in addition to an untreated control. This design enabled the evaluation of both the individual effects of each factor and their interaction.

At the biophysical level, the applied electric field modifies the distribution of electrical charges across the cell membrane, increasing membrane permeability and facilitating ion transport. These effects accelerate water imbibition and activate embryonic metabolism. Simultaneously, the activity of hydrolytic enzymes, including amylases, proteases, and lipases, is stimulated, thereby promoting the mobilization of seed storage reserves.

Changes in the cellular electrical potential also influence cell division and cell elongation, contributing to more rapid radicle development. The effects of the treatments were evaluated using standard germination parameters, including germination percentage, germination energy, mean germination time, and germination uniformity, following the recommendations of the International Seed Testing Association (ISTA).

The results, expressed as mean values, demonstrated clear relationships between the applied physical parameters and the biological response of the seeds. Electric field treatment proved to be an effective, reproducible, and environmentally friendly method for stimulating seed germination without the use of chemical agents.

3.3.2 Effect of electromagnetic field (EMF) treatment on seed germination

The influence of the electromagnetic field (EMF) on the germination of Larix decidua seeds was investigated under controlled laboratory conditions at the Ioan Ursu Institute, Faculty of Physics, Babeș-Bolyai University. A Helmholtz coil configuration, consisting of two identical circular coils positioned coaxially at a distance equal to their radius, was employed to generate a uniform magnetic field. This configuration provides an almost constant magnetic induction within the central region, ensuring homogeneous seed exposure while eliminating spatial variations in field intensity that could otherwise influence the germination response.

The electromagnetic field was generated by supplying the coils with a direct current of 0.680 A, producing a magnetic induction of 0.4 mT within the central region. This magnetic flux density falls within the range of weak magnetic fields, which are known to exert stimulatory biological effects without causing structural damage to cells.

Uniformly selected *Larix decidua* seeds were placed in the region of homogeneous magnetic field in a single layer without overlapping, ensuring identical exposure conditions for all samples. The experimental protocol included an untreated control and three exposure durations (10, 20, and 30 min) while maintaining a constant magnetic field intensity, allowing the effect of exposure time to be evaluated independently.

Following treatment, the seeds were germinated under standard laboratory conditions according to ISTA protocols, and germination parameters were monitored daily. Replicate experiments were performed for each treatment variant to ensure statistical reliability.

From a physical perspective, the electromagnetic field influences membrane permeability and ionic transport, thereby facilitating water uptake and enzymatic activation during germination. Consequently, the differences observed among the experimental variants can be directly attributed to the effects of the applied electromagnetic field.

3.3.3 Effect of gamma radiation treatments on seed germination

Seed irradiation was performed under controlled conditions at the Ioan Ursu Institute, Faculty of Physics, Babeș-Bolyai University, using a Gamma Chamber 900 equipped with a ^60Co gamma source. The circular arrangement of the radioactive source (approximately 900 Ci) ensured a uniform radiation field and highly reproducible experimental conditions.

The gamma rays emitted by cobalt-60 (^60Co) are high-energy electromagnetic radiation with considerable penetrating power. These photons induce ionization and electronic excitation within biological tissues, affecting nucleic acids, proteins, and cellular membranes, and consequently influencing the physiological processes involved in seed germination.

Four irradiation doses (1, 1.5, 2, and 4 Gy) were applied together with an untreated control. This dose range was selected to evaluate both the stimulatory and the inhibitory effects of gamma radiation on germination.

Uniformly selected *Larix decidua* seeds were placed inside the irradiation chamber under conditions that ensured homogeneous exposure to the radiation field. The irradiation time was adjusted according to the dose rate in order to achieve the prescribed absorbed doses.

After irradiation, the seeds were germinated under standard laboratory conditions following ISTA protocols. Germination was monitored daily, and radicle emergence was used as the criterion for successful germination. Replicate samples were included for each treatment to ensure the statistical validity of the results.

The treatment effects were evaluated using standard germination parameters, including germination percentage, germination index, germination energy, and coefficient of germination velocity, while the final germination percentage was determined after 30 days. The results enabled a direct comparison of the different irradiation doses, revealing stimulatory responses at low doses and inhibitory effects at higher doses. The strict control of all experimental parameters ensured that the observed differences could be attributed specifically to the effects of gamma radiation.

3.4 Data processing and statistical analysis

The data obtained from the FT-IR, GC–MS, and seed germination experiments were statistically analyzed to evaluate the influence of the experimental factors. The results are presented as arithmetic means, while variability was estimated using the standard error of the mean (SEM).

For the analysis of the bark chemical composition (FT-IR and GC–MS), a multifactor analysis of variance (ANOVA) was performed to assess the effects of provenance, drying temperature, and their interaction. The data were tested for normality and homogeneity of variances, and statistically significant differences among treatments were identified using Duncan's multiple range test ($\alpha < 0.05$). For the morphometric characteristics and germination parameters, a one-way analysis of variance (ANOVA) was applied, and comparisons among treatment means were performed using Tukey's honestly significant difference (HSD) test ($\alpha = 0.05$).

To investigate the relationships among the measured variables, several multivariate statistical methods were employed. Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were used to evaluate the chemical composition of the bark, whereas Canonical Correspondence Analysis (CCA) was applied to examine the relationships between seed characteristics and germination performance. The results were visualized using PCA score plots, hierarchical dendrograms, and heat maps. All statistical analyses were performed using PAST software, version 4.17 (Hammer et al., 2001).

The evaluation of seed germination was performed using several standard germination indices, including:

GP (Germination percentage - %)(Fetouh & Hassan, 2014):

$$GP = \frac{\text{Numărul de semințe germinate pe zi}}{\text{Numărul total de semințe puse la germinat}} \times 100 \quad (7)$$

Germination Index (GI) (AOSA, 1983), calculated using the following equation,

$$GI = \frac{\text{Numărul de semințe germinate}}{\text{Numărul de zile de la prima evaluare}} + \cdots + \frac{\text{Numărul de semințe germinate}}{\text{Numărul de zile de la ultima evaluare}} \quad (8)$$

SE (Germination Speed (Germination Energy) (Islam et al., 2009), where,

$$SE = \frac{\text{Numărul de semințe germinate în prima zi de germinare}}{\text{Numărul de semințe germinate în ultima zi de germinare}} \times 100.$$

CRG (Coefficient of Velocity of Germination (CVG), where,

$$CRG = \frac{n_1 + n_2 + \cdots + n_n}{n_1 \times T_1 + n_2 \times T_2 + n_3 \times T_3 + \cdots + n_n \times T_n} \times 100 \quad (9)$$

n_1 = number of seeds germinated on day 1 (T1), n_2 = number of seeds germinated on day 2 (T2), n_n= number of seeds germinated on day n (Tn) (Bewley & Black, 1985; Chiapusio et al., 1997).

4 Experimental results from the study of *Larix decidua* bark

4.1 FT-IR spectra of *Larix decidua* bark

The analyses demonstrate that the variations in the chemical composition of Larix decidua bark can be interpreted not only as biochemical differences but also as changes in the physical properties of the lignocellulosic system resulting from the interaction between its molecular structure and the thermal energy applied during the drying process. The differences observed among the provenances indicate the existence of distinct initial states of the macromolecular network, characterized by variations in bond density, the distribution of functional groups, and their energetic stability.

The FT-IR spectra reflect the vibrational response of the lignocellulosic system to infrared radiation, with each absorption band corresponding to specific modes of molecular vibration. The broad absorption region between 3390 and 3412 cm^{-1}, assigned to the O–H stretching vibrations, reflects the behavior of the hydrogen-bonding network, which plays a fundamental role in maintaining the structural stability of cellulose and hemicelluloses. The aliphatic C–H stretching bands (2915–2851 cm^{-1}) indicate the contribution of hydrocarbon chains from both structural constituents and extractive compounds, whereas the carbonyl band (1732–1741 cm^{-1}) is associated with the C=O stretching vibrations of esters, carboxylic acids, and oxidized compounds. The 1600–1000 cm^{-1} region comprises a complex overlap of vibrational modes attributed to the aromatic structures of lignin and the polysaccharide backbone. A complete assignment of these absorption bands is presented in Table 4.1.

Table 4.1. Characteristic FT-IR absorption bands (4000–350 cm⁻¹) of Larix decidua bark under different drying treatments.

Wave number (cm^{-1})	Band origin	References
617	Stretching vibration of C-S	Tsaousis *et al*., 2025
770-784	C–C Alkanes skeletal vibrations; Vibration of mannan in hemicellulose and CH out-of-plane bending in phenyl rings	Santos *et al*., 2021; Evans *et al*., 1992; Yue *et al*., 2023
1046-1059	C-O valence vibration mainly from C3-O3H (Cellulose) Aromatic C-H in-plane deformation, a guaiacyltype lignin and C-O deformation, primary alcohol	Schwanninger *et al*., 2004 Faix, 1991; Rana *et al*., 2008; Cuello *et al*., 2020
1104-1123	COH in plane deformation (celluloses and hemicelluloses); aromatic C-H in plane deformation (typical syringyl units); aromatic skeletal and C–O stretch; C–O–C stretching in cellulose and hemicellulose	Pandey and Pitman, 2003; Popescu *et al*., 2010; Popescu *et al*., 2007; Piqueras *et al*., 2020; McCann *et al*., 1997; Zhang *et al*., 2010;
1154-1167	C–O–C asymmetric stretching in cellulose and hemicellulose	Faix and Bottcher, 1992; Popescu *et al*. 2007
1262-1276	C–O vibration in guaiacyl rings	Popescu *et al*., 2007; Chen *et al*., 2010
1336-1366	C–H deformation in cellulose and hemicelluloses	Colom and Carrillo 2005; Popescu *et al*., 2007; Evans *et al*., 1992; Mohebby, 2008
1439-1448	C–H asymmetric deformation in –OCH3 groups, for lignins, asymmetric in CH3 and CH2 in pyran for hemicellulose C–H deformation; asymmetric in –CH3 and –CH2–	Popescu *et al*., 2007; Chen *et al*., 2010; Traore *et al*., 2018; He *et al*., 2022; Faix, 1991
1510-1520	C=C stretching of the aromatic ring, C=O bond vibrations in extractive compound such as aldehydes, ketones, fatty acids, esters and oxidized terpenoids.	Popescu *et al*., 2007; Zhou *et al*., 2015
1580	Aromatic skeletal vibration plus C-O stretch	Faix, 1991; Faix *et al*., 1991; Rana *et al*., 2008
1614	C=O stretching conjugated to the aromatic ring, and in carboxylic groups in lignin, carboxylic acid and ester compounds Aromatic skeletal vibrations plus C=O stretching	Zhao *et al*. 2014; Traore *et al*., 2018 Schwanninger *et al*., 2004

	in lignin	
1732-1741	C=O stretch in unconjugated ketones, carbonyls and in ester groups (frequently of carbohydrate origin)	Bodirlau and Teaca, 2009; Zhou *et al*., 2015; Faix, 1991; Pandey and Pitman, 2003; Popescu *et al*., 2007; Schwanninger *et al*., 2004
2845-2851	Symmetric C–H stretching of –CH2 and –CH3 groups, associated with cellulose, hemicelluloses, and lignin	Faix, 1991; Schwanninger *et al*., 2004; Popescu *et al*., 2007; Fackler *et al*., 2010
2915-2929	C–H (asymmetric stretching) of –CH2 and –CH3 groups from aliphatic chains present in lignin and hemicelluloses	Faix, 1991; Schwanninger *et al*., 2004; Pandey and Pitman, 2003; Fackler *et al*., 2010; Longo *et al*., 2020; Santos *et al*., 2021
3392-3412	O–H and N–H stretching, predominantly from absorbed water, and to a lesser extent from lignin hydroxyl groups	Zhao *et al*, 2014; Longo *et al*., 2022; Faix, 1991; Pandey and Pitman, 2003; Schwanninger *et al*., 2004; Popescu *et al*., 2007

Although the overall spectral structure is preserved across all provenances, the drying temperature induces systematic changes in both the position and intensity of the absorption bands, indicating variations in the physical parameters of the lignocellulosic system, such as the force constants of chemical bonds and the effective reduced masses of the vibrating molecular systems. The observed shifts of the O–H stretching bands toward higher wavenumbers suggest a weakening of the hydrogen-bonding network, resulting from the reduction of intermolecular interactions and thermally induced structural reorganization. Similarly, the changes observed in the C–H region (770–784 cm^{-1}) indicate adjustments in the configuration of the aliphatic chains, whereas variations in the carbonyl region reflect oxidation processes or the rearrangement of functional groups. These transformations do not imply major structural alterations of the biomaterial but rather indicate a reorganization of its internal vibrational environment.

4.1.1 Conclusions on the characterization of *Larix decidua* bark by FT-IR spectroscopy

FT-IR spectroscopy revealed temperature-dependent shifts in the spectral regions associated with the functional groups of the principal lignocellulosic components of the bark. The progressive shift of the O–H stretching band, together with the changes observed in the regions corresponding to C–H deformation and C=O stretching, indicates modifications of the hydrogen-bonding network and partial structural reorganization within the cellulose–hemicellulose–lignin matrix.

Several bark extractives, particularly phenolic compounds, lignans, and terpenoid alcohols containing hydroxyl groups, contribute to the characteristics of the O–H absorption band observed in the FT-IR spectra. These compounds are well known for their role in antioxidant activity, temperature response, and the chemical interactions that occur during bark processing.

Similar spectral behavior has been reported in studies of conifer bark and wood subjected to moderate heating, where the removal of bound water and increased polymer mobility result in subtle shifts of the absorption bands without causing complete degradation of the constituent biopolymers (Pandey & Pitman, 2003; Schwanninger et al., 2004; Traoré et al., 2018).

The observed spectral changes are consistent with polymer relaxation phenomena and the mobilization of extractive compounds, which can modify vibrational intensities through the reorientation of aromatic and aliphatic functional groups. The provenance-specific variability in band shifts further suggests the existence of intrinsic clonal differences in lignin composition, the degree of polysaccharide crystallinity, and the abundance of extractive compounds. Similar differences have been associated, in other larch populations, with adaptive responses to local environmental conditions (Rana et al., 2008).

4.2 GC–MS analysis for the identification of compounds present in *Larix decidua* bark

GC–MS analysis complements the FT-IR results by identifying the volatile and semi-volatile compounds present in *Larix decidua* bark, thereby providing valuable information on the energetic distribution and thermal stability of secondary metabolites. The identified compounds include α-pinene, larixol, tributyl acetylcitrate, 9-octadecenamide, cycloartanol acetate, kauren-18-ol acetate, caryophyllene oxide, and retinol acetate. These molecules exhibit different molecular masses and chemical structures, and their volatility and thermal stability depend on their bond dissociation energies and intermolecular interactions.

The consistent presence of these compounds across all provenances indicates the existence of a stable metabolic core, whereas quantitative variations among provenances reflect differences in the energetic equilibrium and in the kinetics of thermal degradation and transformation processes. The complete analytical dataset is presented in Tables 4.2 and 4.3.

Table 4.2. Effect of the interaction between provenance and drying regime on the physicochemical properties of Larix decidua bark

No*	Provenance	Tratament	α-Pinene (%)	Larixol (%)	Tributyl acetylcitrate (%)	9-Octadecenamide (%)	Cycloartanol acetate - (%)
1	Gura Humorului	Control	0.74±0.06 bcd	10.77±0.64 o	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a
2		60 °C	1.22±0.07 cd	1.67±0.07 a	0.00±0.00 a	0.00±0.00 a	2.34±0.33 u
3		80 °C	0.37±0.02 ab	12.65±0.49 t	2.15±0.16 j	4.47±0.63 o	1.48±0.24 g
4		100 °C	0.00±0.01 a	11.09±0.32 q	4.06±0.20 r	8.02±0.83 q	1.54±0.20 j
5	Brașov V.C	Control	0.31±0.01 ab	11.87±0.90 s	0.00±0.00 a	0.00±0.00 a	2.65±1.12 x
6		60 °C	0.34±0.01 d	8.84±0.61 k	3.08±0.44 p	2.14±0.24 g	1.68±0.59 o
7		80 °C	0.47±0.01 ab	8.31±0.42 h	3.02±0.44 o	0.00±0.00 a	0.00±0.00 a
8		100 °C	0.49±0.01 ab	7.14±0.30 e	2.86±0.40 n	2.52±0.25 i	0.99±0.25 c
9	Săcele	Control	0.73±0.00 bcd	13.45±0.60 w	0.00± 0.00 a	0.00±0.00 a	2.34±0.62 u

10		60 °C	0.18±0.00 ab	9.66±0,56 l	0.00±0.00 a	1.23±0.18 c	2.63±0.74 w
11		80 °C	0.36±0.02 ab	14.62±0,67 y	2.53±0.31 m	3.96±0.47 m	2.25±1.28 t
12		100 °C	0.32±0.01 ab	8.62±0.24 j	1.43±0.20 f	4.08±0.50 n	1.67±0.92 n
13	Brașov V.P	Control	0.61±0.02 abc	10.62±0.55 n	0.00±0.00 a	0.00±0.00 a	1.49±0.29 h
14		60 °C	0.46±0.02 ab	11.18±0.88 r	0.86±0.08 d	0.00±0.00 a	2.50±0.95 i
15		80 °C	0.33±0.01 ab	11.06±0.91 pq	1.24±0.13 e	2.11±0.33 f	1.83±0.67 q
16		100 °C	0.41±0.01 ab	9.76±0.67 m	1.69±0.05 h	3.81±0.43 l	1.58±0.51 m
17	Sinaia	Control	0.51±0.03 ab	12.84±0.82 u	0.00±0.00 a	0.00±0.00 a	1.07±0.50 d
18		60 °C	1.20±0.04 cd	5.86±0.63 b	1.55±0.17 g	3.19±0.25 j	1.55±0.39 k
19		80 °C	0.66±0.03 bc	7.35±0.71 f	1.74±0.19 i	3.46±0.30 k	1.33±0.38 f
20		100 °C	0.19±0.02 ab	14.1±0.94 x	1.23±0.10 e	0.00±0.00 a	2.39±0.80 v
21	Anina	Control	0.49±0.03 ab	11.02±0.94 p	0.00±0.00 a	0.00±0.00 a	1.09±0.35 e
22		60 °C	0.36±0.03 ab	6.69±0.64 c	3.95±0.48 q	5.58±0.00 p	2.21±0.51 s
23		80 °C	0.00±0.00 a	8.55±0.77 i	2.19±0.21 k	2.22±0.18 h	0.95±0.22 b
24		100 °C	0.00±0.00 a	7.94±0.72 g	2.03±0.20 l	1.97±0.10 e	1.56±1.41 l
25	Latorița	Control	0.67±0.02 bc	13.13±0.98 v	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a
26		60 °C	0.32±0.02 ab	9.65±0.68 l	0.00±0.00 a	0.00±0.00 a	2.94±0.61 y
27		80 °C	0.13±0.01 ab	6.84±0.73 d	0.79±0.21 b	1.07±0.07 p	1.71±0.42 p
28		100 °C	0.11±0.01 ab	8.85±0.71 k	0.83±0.22 c	1.84±0.09 d	1.98±1.47 r

Means within a column followed by different lowercase letters differ significantly according to Duncan's Multiple Range Test (MRT) (p < 0,05).

Table 4.3. Effect of the interaction between provenance and drying temperature regime on the physicochemical properties of Larix decidua bark.

No*	Origine	Trat.	Bicyclo[4.4.0]dec-2-ene-4-ol, 2-methyl-9-(prop-1-en-3-ol-2-yl) - (%)	Kauren-18-ol, acetate, (4beta) (%)	1-Phenanthrene carboxylic acid (%)	Caryophyllene oxide (%)	Retinol Acetate (%)
1	Gura Humorului	Control	0.00±0.00 a	63.75±4.55 o	0.00±0.00 a	0.14±0.01 b	0.00±0.00 a
2		60 °C	0.00±0.00 a	6.33±0.12 d	0.00±0.00 a	1.32±0.13 e	0.00±0.00 a
3		80 °C	0.37±0.12 d	68.5±4.61 t	0.00±0.00 a	7.70±0.70 h	0.00±0.00 a
4		100 °C	0.00±0.00 a	50.85±3.12 g	0.00±0.00 a	6.85±0.56 g	0.00±0.00 a
5	Brașov V.C	Control	0.71±0.17 h	63.03±4.01 m	0.91±0.15 c	6.63±0.43 f	0.00±0.00 a
6		60 °C	0.00±0.00 a	0.00±0.00 a	11.34±2.04 n	0.00±0.00 a	0.00±0.00 a
7		80 °C	0.00±0.00 a	55.67±3.11 i	2.99±0.33 k	21.13±3.92 n	0.00±0.00 a
8		100 °C	0.00±0.0 a	0.00±0.00 a	2.80±0.26 j	19.89±2.84 m	0.00±0.00 a
9	Săcele	Control	0.00±0.00 a	63.03±3.02 m	1.11±0.16 e	0.00±0.00 a	0.43±0.02 b
10		60 °C	0.01±0.00 a	68.07±3.03 s	1.35±0.43 g	13.15±2.21 k	0.00±0.00 a
11		80 °C	0.00±0.00 a	65.44±2.97 q	0.00±0.00 a	9.29±0.1.95 i	0.00±0.00 a
12		100 °C	0.03±0.01 b	63.07±2.72 n	1.12±0.11 f	14.85±2.42 l	0.00±0.00 a
13	Brasov V.P	Control	0.49±0.14 f	68.72±4.12 u	0.00±0.00 a	0.00±0.00 a	1.47±0.14 c
14		60 °C	0.42±0.14 e	72.61±4.15 y	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a
15		80 °C	0.00±0.00 a	69.01±4.01 w	0.00±0.00 a	0.00±0.00 a	0.40±0.02 b
16		100 °C	0.00±0.00 a	69.56±4.01 x	0.00±0.00 a	11.31±1.26 j	0.00±0.00 a
17	Sinaia	Control	0.79±0.18 i	60.68±2.15 l	1.06±0.12d	0.00±0.00 a	0.00±0.00 a
18		60 °C	0.00±0.00 a	72.74±3.02 z	0.00±0.00 a	0.00±0.00 a	1.94±0.07 d

19		80 °C	0.33±0.08 c	68.96±2.00 v	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a
20		100 °C	1.17±0.26 j	64.45±1.95 p	2.11±0.18 i	0.00±0.00 a	0.44±0.02 b
21	Anina	Control	0.00±0.00 a	9.93±1.11 f	0.68±0.05 b	0.00±0.00 a	0.43±0.02 b
22		60 °C	0.00±0.00 a	67.97±3.45 r	00.00±0.00 a	0.00±0.00 a	2.81±0.24 f
23		80 °C	0.00±0.00 a	1.04±0.09b	5.16±1.70 l	0.00±0.00 a	0.00±0.00 a
24		100 °C	0.00±0.00 a	4.88±0.72 c	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a
25	Latorița	Control	0.00±0.00 a	6.75±1.12 e	8.13±1.93m	0.00±0.00 a	0.00±0.00 a
26		60 °C	0.00±0.00 a	53.38±3.20 h	3.00±0.42 k	0.00±0.00 a	2.25±0.26 e
27		80 °C	0.42±0.07 e	57.97±3.26 k	1.76±1.11 h	0.47±0.01 c	6.88±0.96 h
28		100 °C	0.00±0.00 a	56.15±3.25 j	8.12±1.93 m	0.95±0.02 d	4.87±0.78 g

Means within a column followed by different lowercase letters differ significantly according to Duncan's Multiple Range Test (MRT) ($p < 0,05$).

The interaction between provenance and drying temperature demonstrates that each biological system exhibits a distinct thermochemical behavior, governed by its internal structure and the energetic distribution of its constituent compounds. For example, the variations observed in α-pinene and larixol indicate differences in the activation energy of volatilization and degradation processes, whereas the increased abundance of esters and amides at higher drying temperatures suggests molecular reorganization or the secondary formation of these compounds through thermally induced reactions. These differences may be interpreted as variations in the energetic stability of the identified compounds among provenances, thereby confirming the existence of distinct initial molecular structures.

The independent analysis of provenance (Table 4.4) shows that the distribution of the identified compounds is governed by intrinsic characteristics of the biological system, including lignin composition, the degree of polysaccharide polymerization, and the content of extractive compounds. Likewise, the results presented in Table 4.5 reveal significant variations in the abundance of bicyclic alcohols, diterpenoid acetates, and oxidized compounds, suggesting differences in their biosynthetic pathways and chemical stability.

Table 4.4. Provenance-dependent variations in the structure and physicochemical properties of Larix decidua bark

No*	Origine	α-Pinene (%)	Larixol (%)	Tributyl acetylcitrate (%)	9-Octadecenamide (%)	9,19-Cyclolanostan-3-ol, acetate, (3beta) - (%)
1	Gura Humorului	0.58±0.37 B	9.05±0.59 B	1.55±0.21 E	3.12±0.22 G	1.34±0.85 B
2	Brașov V.C	0.65±0.95 B	9.04±0.30 B	2.24±0.33 G	1.17±0.18 B	1.33±0.40 A
3	Săcele	0.40±0.02 AB	11.59±0.55 F	0.99±0.14 C	2.32±0.30 E	2.22±0.83 G
4	Brasov V.P	0.45±0.31 AB	10.65±0.54 E	0.95±0.09 B	1.48±0.19 C	1.60±0.32 E
5	Sinaia	0.65±0.30 AB	10.04±0.65 D	1.13±0.13 D	1.66±0.15 D	1.59±0.25 D
6	Anina	0.21±0.01 A	8.55±0.63 A	2.11±0.14 F	2.44±0.25 F	1.43±0.21 C
7	Latorița	0.31±0.01 AB	9.62±0.65 C	0.41±0.07 A	0.73±0.04 A	1.66±0.20 F

Mean values reflect the effect of provenance. Means followed by different uppercase letters differ significantly according to Duncan's Multiple Range Test (MRT) ($p < 0,05$).

Drying temperature acts as an energetic perturbation factor, influencing the distribution of compounds through volatilization, degradation, and chemical transformation processes. The behavior of α-pinene, which reaches its maximum abundance at 60 °C before subsequently declining, can be explained by the balance between its release from the lignocellulosic matrix and its loss through volatilization. In contrast, the progressive increase in compounds such as tributyl acetylcitrate and 9-octadecenamide suggests their enhanced stability or secondary formation at elevated temperatures. The variations observed for cycloartanol acetate, kauren-18-ol acetate, caryophyllene oxide, and retinol acetate (Tables 4.6 and 4.7) further confirm that each class of compounds exhibits a distinct thermal response, governed by its bond dissociation energy and structural stability.

Table 4.5. Provenance-dependent variations in the structure and physicochemical properties of Larix decidua bark.

No*	Origine	Bicyclo[4.4.0]dec-2-ene-4-ol, 2-methyl-9-(prop-1-en-3-ol-2-yl)- (%)	Kauren-18-ol, acetate, (4beta)- (%)	1-Phenanthrenecarboxylic acid (%)	Caryophyllene oxide (%)	Retinol Acetate (%)
1	Gura Humorului	0.09±0.02 C	47.36±2.97 D	0.00±0.00 A	4.00±0.35 D	0.00±0.00 A
2	Brașov V.C	0.31±0.08 F	29.68±1.98 B	4.51±0.26 E	11.91±2.70 F	0.00±0.00 A
3	Săcele	0.01±0.00 B	64.90±3.71 E	0.89±0.06 C	9.32±0.38 E	0.11±0.04 B

4	Brasov V.P	0.23±0.07 E	69.97±3.90 G	0.00±0.00 A	2.83±0.30 C	0.47±0.16C
5	Sinaia	0.57±0.16 G	66.71±3.70 F	0.79±0.03 B	0.00±0.00 A	0.60±0.24 D
6	Anina	0.00±0.00 A	20.95±2.50 A	1.46±0.26 D	0.00±0.00 A	0.81±0.33 E
7	Latorița	0.11±0.01 D	43.56±3.00 C	5.25±0.41 F	0.36±0.02 B	3.50±0.84 F

Mean values reflect the effect of provenance. Means followed by different uppercase letters differ significantly according to Duncan's Multiple Range Test (MRT) ($p < 0,05$).

Table 4.6. Influence of the drying temperature regime on the stability and distribution of volatile and Semi-volatile compounds in Larix decidua bark

No*	Treatment	α-Pinene (%)	Larixol (%)	Tributyl acetylcitrate (%)	9-Octadecenamide (%)	9,19-Cyclolanostan-3-ol, acetate, (3beta)- (%)
1	Control	0.58±0.02 B	11.96±0.75 D	0.00±0.00 A	0.00±0.00 A	1.23±0.25 A
2	60 °C	0.73±0.03 B	7.65±0.44 A	1.35±0.17 B	1.73±0.11 B	2.11±0.66 D
3	80 °C	0.33±0.01 A	9.91±0.62 C	1.95±0.28 C	2.47±0.19 C	1.36±0.27 B
4	100 °C	0.22±0.01 A	9.64±0.62 B	2.06±0.30 D	3.18±0.21D	1.67±0.31 C

Mean values reflect the effect of the treatments. Means followed by different uppercase letters differ significantly according to Duncan's Multiple Range Test (MRT) ($p < 0,05$).

Table 4.7. Influence of the drying temperature regime on the stability and distribution of volatile and Semi-volatile compounds in Larix decidua bark

No*	Treatment	Bicyclo[4.4.0]dec-2-ene-4-ol, 2-methyl-9-(prop-1-en-3-ol-2-yl)- (%)	Kauren-18-ol, acetate, (4beta)- (%)	1-Phenanthrenecarboxylic acid (%)	Caryophyllene oxide (%)	Retinol Acetate (%)
1	Control	0.28±0.06 D	47.98±3.02 B	1.70±0.09 B	0.97±0.07 A	0.33±0.04 A
2	60 °C	0.06±0.01 A	48.73±3.03 C	2.24±0.05 D	2.07±0.28 B	1.00±0.09 C
3	80 °C	0.16±0.03 B	55.23±3.25 D	1.42±0.09 A	5.51±0.62 C	1.04±0.09 D
4	100 °C	0.25±0.03 C	44.14±2.90 A	2.02±0.07 C	7.69±0.74 D	0.76±0.05 B

Mean values reflect the effect of the treatments. Means followed by different uppercase letters differ significantly according to Duncan's Multiple Range Test (MRT) ($p < 0,05$).

Multivariate hierarchical cluster analysis (Figure 4.2) demonstrates that these differences are not random but follow well-defined patterns, suggesting the existence of structural and energetic relationships among the identified compounds. The clustering of provenances according to the similarity of their chemical profiles reflects their proximity within the physicochemical parameter space, whereas the co-occurrence of specific compounds indicates possible correlations in their biosynthetic pathways or in their response to thermal treatment.

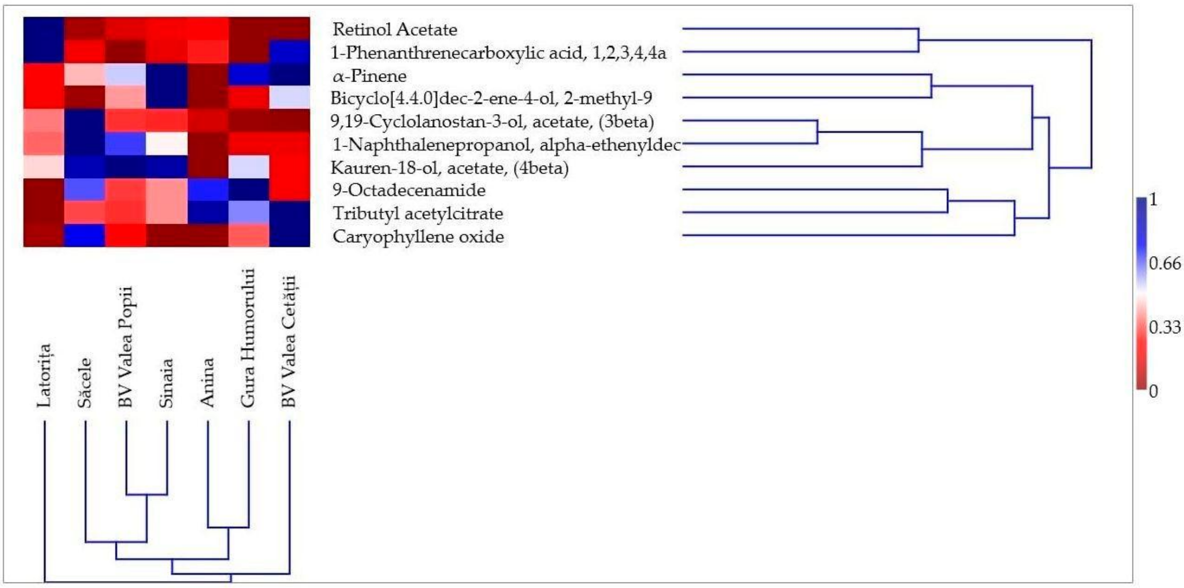

Figure 4.1. *Heat map and UPGMA hierarchical cluster analysis of the distribution of volatile and semi-volatile compounds, highlighting structural differences among Larix decidua provenances.*

Overall, the results demonstrate that both provenance and drying temperature govern the physical and chemical properties of *Larix decidua* bark by modifying its vibrational environment, energetic stability, and the distribution of chemical compounds, without altering the fundamental structure of the lignocellulosic system. This approach highlights that plant biomass can be interpreted as a complex physical system in which chemical processes are governed by the transfer and redistribution of energy at the molecular level.

4.2.1 Conclusions on temperature-induced changes in volatile and semi-volatile compounds

Thermal treatment exerted a pronounced effect on the chemical profile of Larix decidua bark, confirming previous findings that temperature influences volatilization, oxidation, esterification, and degradation processes in conifer tissues (Czajka et al., 2020; Kain et al., 2020).

The results indicate that moderate heating (60–80 °C) promotes the release and stabilization of compounds such as monoterpenes, esters, terpenoid alcohols, and certain triterpenoid acetates. Several compounds, including α-pinene and kauren-18-ol acetate, reached their highest concentrations at intermediate temperatures, a phenomenon associated with the partial mobilization of resin constituents and the increased availability of extractive compounds.

Exposure to higher temperatures (100 °C) favored the formation of oxidized sesquiterpenoids, fatty acid-derived amides, and aromatic acids, reflecting thermally induced oxidative transformations. These patterns are consistent with the known volatilization thresholds of monoterpenes and the oxidative susceptibility of resin acids, as temperatures above 90–100 °C promote a transition from the simple release of volatile constituents to their degradation or structural reorganization (Jiang et al., 2023; Shao et al., 2019). The significant increases in 9-octadecenamide and caryophyllene oxide at elevated temperatures are likewise consistent with lipid oxidation processes commonly triggered under thermal stress conditions.

4.2.2 Influence of ecological conditions and geographic origin on the biochemical composition of bark

Provenance significantly influenced the chemical composition of Larix decidua bark, reflecting both genetic variability and adaptation to local ecological conditions. Provenances originating from cooler, mid-elevation regions (Săcele and Sinaia) exhibited higher concentrations of terpenoids and resin-related compounds, which are associated with protective physiological mechanisms. In contrast, provenances from warmer regions (Anina and Latorița) showed a greater abundance of oxidized derivatives and lipid esters, consistent with higher ambient temperatures and enhanced metabolic activity.

These biochemical differences are ecologically relevant, as secondary metabolites contribute to plant defense against both abiotic and biotic stress (Ferreres et al., 2018; Jiang et al., 2023). At the same time, they indicate the existence of valuable genetic variability that may be exploited in future tree breeding and selection programs.

The environmental conditions at the Baciu seed orchard, which are warmer and drier than those prevailing in the original habitats of the provenances, influenced acclimation processes and the synthesis of secondary metabolites. Moderate environmental stress promoted the accumulation of terpenoids and phenolic compounds, whereas the mountain provenances retained adaptive biochemical characteristics typical of cool and humid environments.

The provenance × temperature interactions further reveal differences in thermochemical plasticity, with certain provenances exhibiting greater metabolic stability than others. These findings underscore the importance of selecting genotypes adapted to variable climatic conditions.

Finally, the multivariate analyses confirm that provenance-specific chemical signatures remain detectable even after thermal treatment, supporting the fundamental role of genetic factors in determining the chemical composition of *Larix decidua* bark.

4.3 Conclusions on the characterization of *Larix decidua* bark using physicochemical methods

The results obtained through FT-IR and GC–MS analyses demonstrate that *Larix decidua* bark represents a valuable source of extractive compounds with potential applications in bio-based materials, adhesives, functional coatings, and natural antioxidants. The identification of provenances with high concentrations of terpenoids and lipid-derived compounds supports the selection of superior genotypes for breeding programs and the optimization of drying and extraction processes.

The Săcele and Sinaia provenances exhibited high thermochemical stability, indicating considerable potential for industrial applications, particularly in resin production. Phenolic compounds and lignin-derived constituents contribute to adhesive properties and thermal stability, whereas terpenoids provide significant antioxidant potential.

The integration of FT-IR and GC–MS analyses enabled the correlation between the chemical structure of the bark and its molecular composition. Although the use of composite samples reduces experimental variability, it may limit the assessment of individual variation; nevertheless, the statistical analyses confirm the robustness and reliability of the obtained results.

Overall, the chemical composition of *Larix decidua* bark is governed by the interaction between genetic factors and environmental conditions, providing a scientific basis for genotype selection and the efficient utilization of forest biomass in high-value applications.

5 Experimental results on the study of *Larix decidua* seeds

5.1 Characterization of larch cones

The results reveal variations in cone characteristics (length, width, weight, and number of seeds per cone) among the seven Larix decidua provenances analyzed.

Cone length was greatest in the Gura Humorului and Latorița provenances (approximately 3.4–3.5 cm) and lowest in Brașov V.C. (approximately 2.5 cm). The greater variability observed in the Gura Humorului and Latorița provenances indicates a higher degree of phenotypic heterogeneity (Figure 5.1a).

Cone width reached its maximum value in Brașov V.C. (approximately 2.5 cm) and its minimum in Anina (approximately 1.08 cm), with the latter provenance also exhibiting the highest variability (Figure 5.1b).

Cone weight was highest in the Latorița and Brașov V.P. provenances (approximately 4.6 g) and lowest in Anina (approximately 2.4 g). The greater dispersion of values observed for the Latorița and Brașov V.P. provenances suggests a higher level of phenotypic diversity (Figure 5.1c).

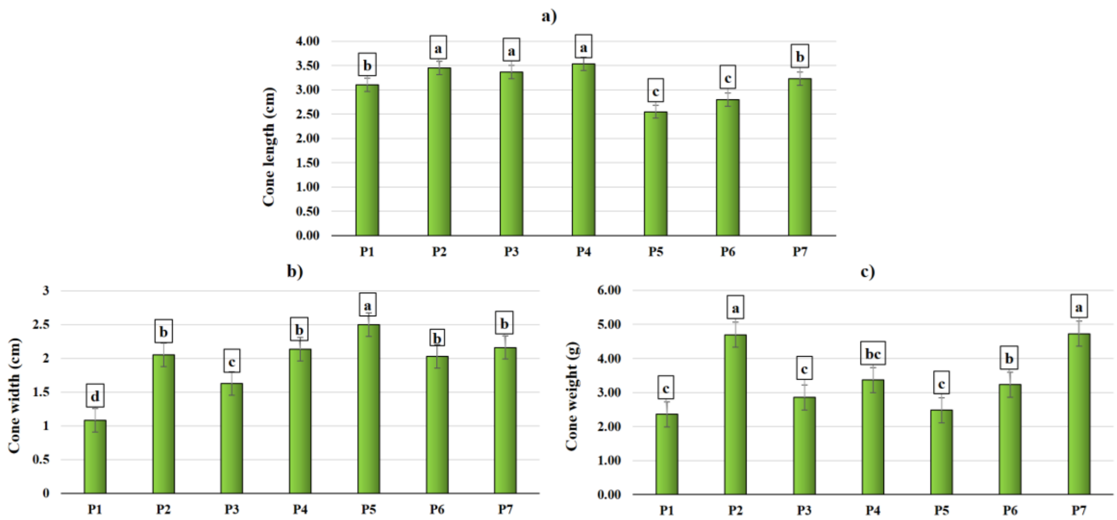

Figure 5.1. *Main cone characteristics of Larix decidua according to provenance. Bars represent the mean ± standard error (SE) (n = 30). For each trait analyzed, different letters above the bars indicate significant differences among provenances according to Tukey's test (α = 0.05). P1*

(Anina), P2 (Latorița), P3 (Sinaia), P4 (Gura Humorului), P5 (Brașov V.C.), P6 (Săcele), and P7 (Brașov V.P.).

The results reveal morphological differences among the analyzed provenances, suggesting the combined influence of genetic and environmental factors on the development of Larix decidua cones and seeds. The Latorița and Brașov V.P. provenances were characterized by larger trait values and greater variability, whereas the Anina provenance exhibited smaller dimensions, possibly reflecting local adaptation.

The seed characteristics also varied significantly among provenances. The greatest seed length was recorded in the Gura Humorului provenance (approximately 4.2 mm), whereas the smallest was observed in Săcele (approximately 3.2 mm), with the former exhibiting greater variability (Figure 5.2a). Seed width was highest in Sinaia (approximately 2.4 mm) and lowest in Latorița (approximately 2.0 mm), with moderate variation among provenances (Figure 5.2b). Seed weight reached its maximum in Sinaia (approximately 74.3 mg) and its minimum in Brașov V.C. (approximately 54.2 mg), while the remaining provenances displayed intermediate values (Figure 5.2c).

The number of seeds per cone was highest in the Latorița provenance (approximately 70 seeds per cone) and lowest in Brașov V.C., indicating differences in reproductive potential among provenances (Figure 5.2d).

In general, larger and heavier seeds are associated with higher germination capacity and greater seedling vigor owing to their larger nutrient reserves. In this context, the Sinaia, Latorița, and Anina provenances exhibited the most favorable seed characteristics, whereas Brașov V.C. showed comparatively lower values. These differences reflect both genetic variability and adaptation to local environmental conditions, with important implications for the selection of biological material in breeding and reforestation programs.

The morphological characteristics observed in the seeds of different forest tree species further emphasize the importance of seed origin (provenance) (Roman et al., 2022a). Similar studies have previously highlighted the relevance of understanding reproductive traits and genetic diversity among different populations (Vilcan (Truța) et al., 2017; Gramazio et al., 2018; Todea et al., 2019; Roman et al., 2022b).

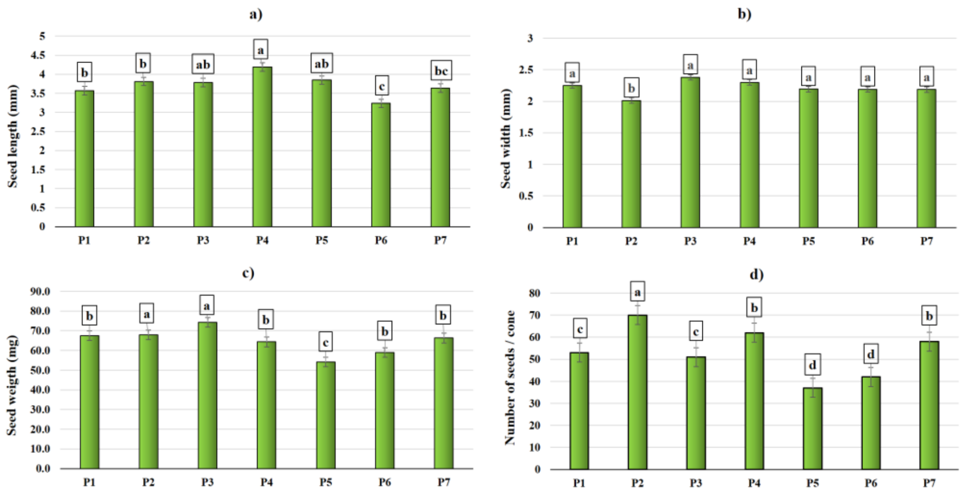

Figure 5.2. *Main seed characteristics of Larix decidua according to provenance. Bars represent the mean ± standard error (SE) (n = 30). For each trait analyzed, different letters above the bars indicate significant differences among provenances according to Tukey's test (α = 0.05). P1 (Anina), P2 (Latorița), P3 (Sinaia), P4 (Gura Humorului), P5 (Brașov V.C.), P6 (Săcele), and P7 (Brașov V.P.).*

These studies are important because they provide valuable insights into how populations adapt to their specific environments and interact with other species within ecosystems. Understanding reproductive characteristics can guide conservation efforts, particularly in the context of environmental change and habitat degradation. Furthermore, genetic diversity plays a crucial role in enhancing the ability of populations to withstand abiotic stress and to adapt to changing climatic conditions.

5.2 Analysis of the effects of electric field treatment on the germination of *Larix decidua* seeds

The germination capacity of Larix decidua seeds varied according to provenance, electric field intensity, and exposure duration (Figure 5.3), reflecting the interaction between the applied electric field and the biophysical properties of the seeds. The P4 and P3 provenances exhibited the highest germination values, whereas P7 and P6 showed the lowest, suggesting differences in their dielectric properties and in the manner in which they couple with the applied electric field.

The electric field intensity significantly influenced seed germination. Treatments at 10 V and 50 V resulted in higher germination percentages, whereas the 30 V treatment produced a comparatively weaker response, indicating a nonlinear behavior and the existence of optimal stimulation thresholds.

The exposure duration had a cumulative effect on germination. An exposure time of 60 min produced the highest germination percentages, whereas 15 min and 35 min were less effective, suggesting that polarization processes and ionic transport require sufficient time to reach a stable state.

The interaction among these factors indicates that the highest germination rates were achieved under combinations of higher electric field intensity and longer exposure duration, whereas certain provenances remained less responsive, most likely because of intrinsic structural limitations within the seed tissues (Figure 5.3).

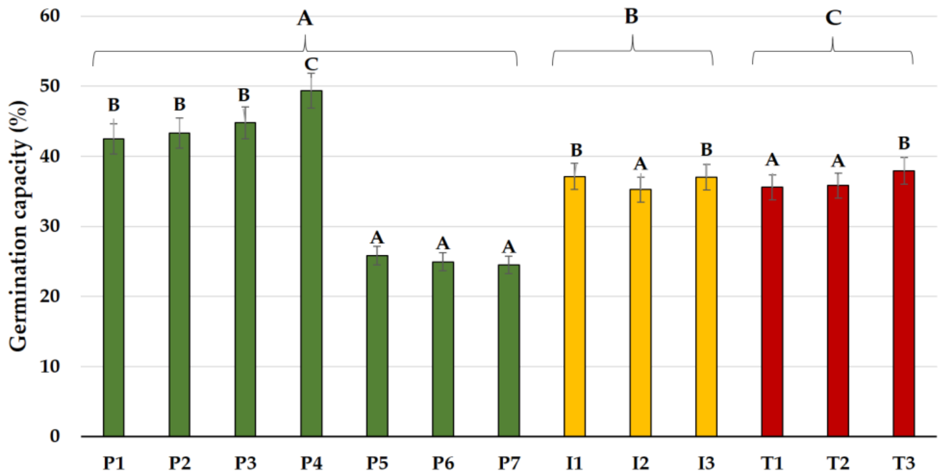

Figure 5.3. *Effects of electric field intensity on the germination capacity (%) of Larix decidua seeds according to provenance (A), electric field intensity (B), and exposure duration (C). Bars represent the mean ± standard error (SE) (n = 30). For each factor analyzed, different letters above the bars indicate significant differences among treatments according to Tukey's test (α = 0.05). P1 (Anina), P2 (Latorița), P3 (Sinaia), P4 (Gura Humorului), P5 (Brașov V.C.), P6 (Săcele), P7 (Brașov V.P.); I1 = 10 V, I2 = 30 V, I3 = 50 V; T1 = 15 min, T2 = 35 min, T3 = 60 min.*

The interaction between provenance (P) and electric field intensity (I) reveals variations in the germination response that depend on the physical characteristics of the seeds. The highest germination percentage observed for the P4I3 combination, together with the high values recorded for P2I1, indicates enhanced efficiency of polarization and ionic transport processes under these treatment conditions. In contrast, the lowest germination values observed for P6I2 suggest inefficient energy dissipation or a non-uniform distribution of the electric field within the seed structure (Figure 5.4). These differences may be associated with variations in cell membrane structure and seed water content, both of which influence the electrical properties of the biological system.

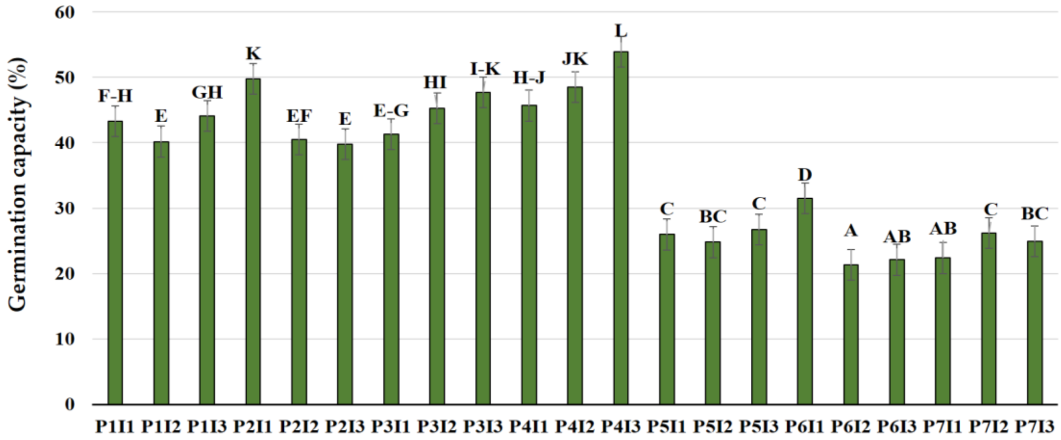

Figure 5.4. *Germination capacity (%) of Larix decidua seeds as a function of the interaction between provenance and electric field intensity. Bars represent the mean ± standard error (SE) (n = 30). For the analyzed interaction, different letters above the bars indicate significant differences among treatments according to Tukey's test (α = 0.05). P1 (Anina), P2 (Latorița), P3 (Sinaia), P4 (Gura Humorului), P5 (Brașov V.C.), P6 (Săcele), P7 (Brașov V.P.); I1 = 10 V, I2 = 30 V, I3 = 50 V.*

The interaction between provenance (P) and exposure duration (T) indicates that the temporal dynamics of seed germination are governed by the ability of the biological system to integrate electrical energy into its physiological processes. The P4 provenance exhibited a consistently high germination response across all exposure durations, reaching its maximum under the T3 treatment, which suggests an efficient adaptation to the electrical stimulus. In contrast, the low germination values observed for the P5T1 combination indicate a limited capacity to respond under short-term exposure conditions (Figure 5.5).

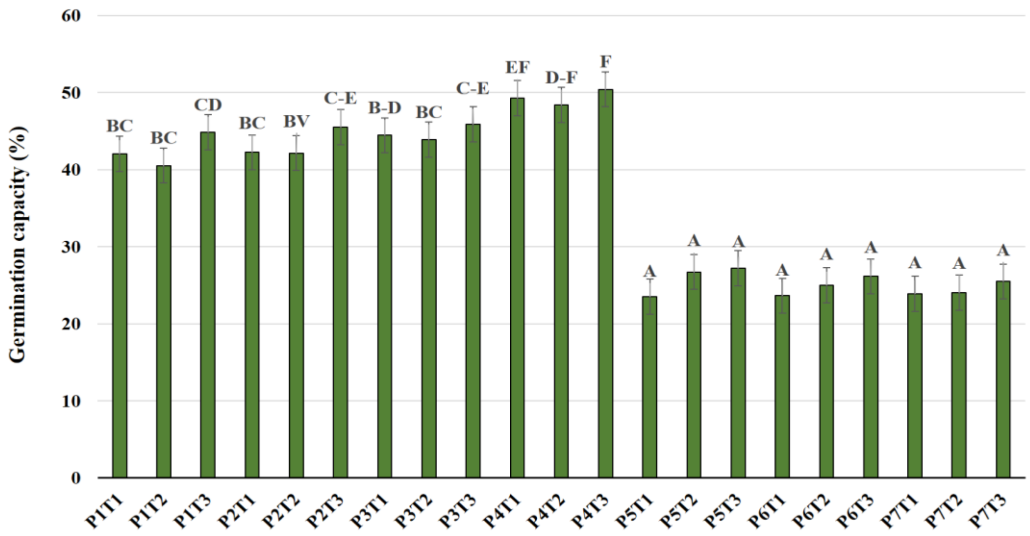

Figure 5.5. *Germination capacity (%) of Larix decidua seeds as a function of the interaction between provenance and exposure duration to the electric field (EF). Bars represent the mean ± standard error (SE) (n = 30). For the analyzed interaction, different letters above the bars indicate significant differences among treatments according to Tukey's test (α = 0.05). P1 (Anina), P2 (Latorița), P3 (Sinaia), P4 (Gura Humorului), P5 (Brașov V.C.), P6 (Săcele), P7 (Brașov V.P.); T1 = 15 min, T2 = 35 min, T3 = 60 min.*

With respect to the interaction between exposure duration (T) and electric field intensity (I), the results demonstrate the existence of optimal combinations of electrical parameters. The highest germination percentages observed for the T3I1 and T3I3 treatments suggest that both moderate and high electric field intensities can effectively enhance germination when the exposure time is sufficient to allow the stabilization of polarization processes and ionic transport. In contrast, the T1I2 and T2I2 combinations resulted in the lowest germination values, indicating suboptimal conditions for energy transfer (Figure 5.6).

Overall, the results indicate that the effect of the electric field on seed germination can be described as a process of energy transfer and redistribution within the biological system, governed by both the electrical field parameters and the physical properties of the seeds. The observed effects are attributed to mechanisms of dielectric polarization, ionic migration, and changes in membrane permeability, which, under optimal treatment conditions, promote both the acceleration and the enhancement of germination efficiency.

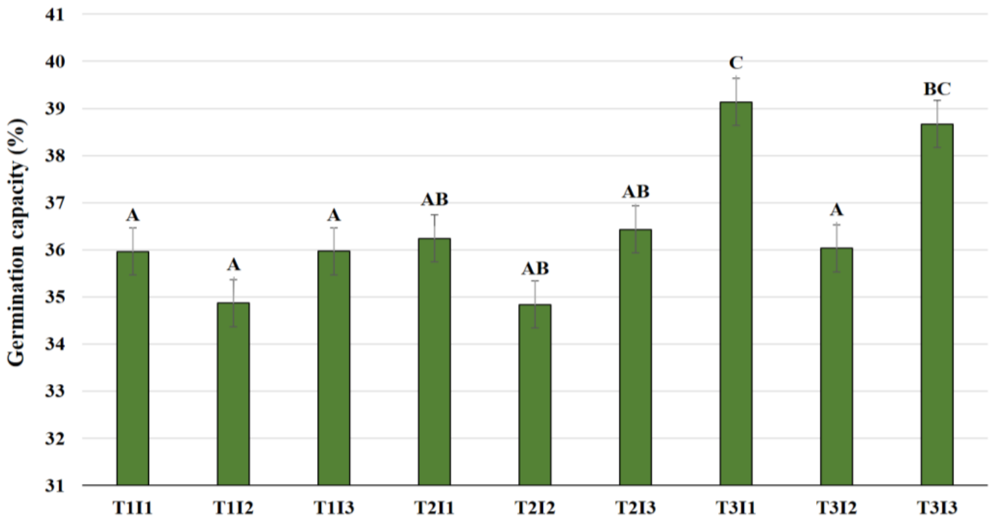

Figure 5.6. *Germination capacity (%) of Larix decidua seeds as a function of the interaction between exposure duration and electric field intensity (EF). Bars represent the mean ± standard error (SE) (n = 30). For the analyzed interaction, different letters above the bars indicate significant differences among treatments according to Tukey's test (α = 0.05). T1 = 15 min, T2 = 35 min, T3 = 60 min; I1 = 10 V, I2 = 30 V, I3 = 50 V.*

5.3 Analysis of the effects of electromagnetic fields

The analysis of the experimental data demonstrates that exposing *Larix decidua* seeds to a low-intensity electromagnetic field (0.4 mT) produces a germination response that depends on both the treatment duration and the provenance of the biological material. This response is reflected in systematic variations of the germination percentage (GP), germination index (GI), germination energy (GE), and coefficient of velocity of germination (CVG). Compared with the control treatment, short-term exposure (10 min) resulted in a clear increase in the germination percentage across all analyzed provenances, with the highest values recorded for the Sinaia and Anina provenances. These findings indicate an effective stimulatory effect of the electromagnetic field on the initiation of the germination process (Table 5.1).

Table 5.1. Germination response of Larix decidua seeds to electromagnetic field treatment as a function of provenance and electromagnetic field parameters, expressed as germination percentage (GP).

No	Treatment/Provenance	Germination indices (%)			
		GP	GI	SE	CRG
Control (no treatment)					
1	Anina	64.28	1.83	7.84	7.12
2	Latorița	58.47	1.00	7.23	7.52
3	Sinaia	61.12	1.31	6.82	7.25
4	Gura Humorului	51.79	0.93	8.30	7.80
5	Brașov V.C.	47.58	1.14	9.24	7.15
6	Săcele	50.47	1.18	7.26	7.34
7	Brașov V.P.	48.17	1.12	8.13	7.13
10 min					
1	Anina	72.26	1.73	5.7	7.63
2	Latorița	62.32	1.49	5.8	7.48
3	Sinaia	76.48	1.82	8.7	7.31
4	Gura Humorului	55.46	1.56	7.3	7.24
5	Brașov V.C.	52.14	1.21	6.2	7.12
6	Săcele	58.36	1.13	6.2	7.33
7	Brașov V.P.	51.72	1.23	6.7	7.21
20 min					
1	Anina	62.45	1.15	5.3	7.15
2	Latorița	57.61	1.21	7.3	7.00
3	Sinaia	61.8	1.11	5.9	7.23
4	Gura Humorului	52.1	1.00	6.8	7.18
5	Brașov V.C.	41.17	1.18	7.2	7.14
6	Săcele	47.26	1.24	7.06	7.23
7	Brașov V.P.	41.23	1.18	7.23	6.97
30 min					
1	Anina	65.21	1.70	5.46	7.23
2	Latorița	53.57	1.21	5.23	7.31
3	Sinaia	58.25	1.61	7.12	7.27
4	Gura Humorului	50.48	1.26	5.43	7.23
5	Brașov V.C.	55.62	1.37	5.82	7.49
6	Săcele	61.3	1.67	5.54	7.72
7	Brașov V.P.	54.12	1.38	7.23	7.24

* GP – germination percentage; GI – germination index; GE – germination energy; CVG – coefficient of velocity of germination.

The increase in germination percentage (GP) was accompanied by higher values of the germination index (GI), indicating not only an improvement in germination capacity but also an acceleration of the germination process through the more rapid activation of seed metabolism.

After 20 min of exposure, a decline in both GP and GI was observed for most provenances, in some cases approaching the values of the control treatment, indicating a nonlinear response and the existence of an optimal exposure duration. At 30 min, these parameters either stabilized or increased slightly but did not reach the maximum values recorded after 10 min of exposure. Germination energy (GE) confirmed an acceleration of germination under optimal exposure conditions, whereas the coefficient of velocity of germination (CVG) remained relatively constant, suggesting that the electromagnetic field induced a functional rather than structural modulation of the germination process.

The differences observed among provenances indicate a substantial genetic component in the response to electromagnetic field treatment. Some provenances, particularly Sinaia and Anina, exhibited a stronger stimulatory response, whereas others responded less markedly. These effects may be explained by the influence of the electromagnetic field on electric charge distribution, ionic mobility, and membrane permeability, thereby facilitating water imbibition during the initial stages of germination.

Compared with other pre-sowing treatments, the electromagnetic field offers the advantages of being non-invasive, easily controlled, and capable of inducing moderate yet stable stimulatory effects. Consequently, it represents an effective physical method for enhancing seed germination without the use of chemical substances and without causing degradation of the biological material. degradare a materialului biologic.

5.4 Effects of gamma radiation on the germination capacity of *Larix decidua* seeds

The variation in the germination indices highlights the combined influence of seed provenance and gamma radiation treatments on the germination behaviour of Larix decidua seeds (Table 5.2). From a physical perspective, these results can be interpreted as the consequence of ionizing energy transfer to the biological system, resulting in molecular and subcellular modifications that influence the initial stages of the germination process.

Tabel 5.2. Germination response (%) of Larix decidua seeds to absorbed γ-radiation dose (Gy) according to provenance.

No	Treatment/Proveniență	Indicii germinativi (%)			
		GP	GI	SE	CRG
Control (no treatment)					
1	Anina	46.67	1.08	7.10	7.65
2	Latorița	40	1.04	8.30	7.84
3	Sinaia	53.33	1.21	6.30	7.39
4	Gura Humorului	40	0.93	8.30	7.80
5	Brașov V.C.	33.33	0.73	10	7.586
6	Săcele	36.67	0.88	9.1	7.33
7	Brașov V.P.	36.67	0.9	9.1	8.03
1 Gy					
1	Anina	43.33	0.94	7.7	7.93
2	Latorița	30	0.59	11.1	6.72
3	Sinaia	50	1.1	6.7	7.41
4	Gura Humorului	36.37	0.76	9.1	7.04
5	Brașov V.C.	33.3	0.71	10	7.44
6	Săcele	33.3	0.8	20	7.58
7	Brașov V.P.	36.67	0.83	9.1	7.1
1.5 Gy					
1	Anina	50	1.13	6.7	7.85
2	Latorița	36.67	0.85	9.1	7.01
3	Sinaia	60	1.35	5.6	7.33
4	Gura Humorului	43.3	1.06	7.7	7.39
5	Brașov V.C.	43.33	0.98	7.7	7.41
6	Săcele	43.33	1	7.7	7.1
7	Brașov V.P.	40	0.88	8.3	6.86
2 Gy					
1	Anina	66.67	1.64	5	7.41
2	Latorița	60	1.41	5.6	7.69
3	Sinaia	73.33	1.78	9.1	7.35

4	Gura Humorului	56.67	1.41	5.09	7.66
5	Brașov V.C.	56.67	1.38	5.9	7.39
6	Săcele	60	1.37	5.7	7.06
7	Brașov V.P.	53.33	1.24	6.3	7.17
4 Gy					
1	Anina	56.67	1.46	17.6	7.87
2	Latorița	43.33	0.99	7.7	7.1
3	Sinaia	63.33	1.59	5.3	7.66
4	Gura Humorului	46.67	1.16	7.1	7.73
5	Brașov V.C.	46.67	1.22	7.1	8
6	Săcele	46.67	1.15	7.11	7.53
7	Brașov V.P.	43.33	1.07	7.7	7.65

GP – germination percentage; GI – germination index; GE – germination energy; CVG – coefficient of velocity of germination.

The analyzed parameters (GP, GI, GE, and CVG) reflect both the final germination efficiency and the dynamics of the germination process, serving as indicators of how the absorbed radiation energy is converted into physiological processes.

In the control treatments, GP values ranging from 33.33% to 53.33% revealed inherent differences among provenances, which can be attributed to variations in seed internal structure and biochemical composition.

Exposure to gamma radiation introduced an interaction mechanism governed by ionization processes. At a dose of 1 Gy, a slight reduction in GP and GI was observed, suggesting the occurrence of physiological disturbances without a significant stimulatory effect. At 1.5 Gy, the response became more heterogeneous, indicating a balance between radiation-induced damage and adaptive physiological responses.

The 2 Gy treatment produced the most pronounced stimulatory effects, resulting in significant increases in both GP and GI, with the highest germination percentage recorded for the Sinaia provenance (73.33%). These findings indicate the existence of an optimal level of ionizing energy transfer, capable of enhancing the metabolic processes involved in seed germination. At 4 Gy, the response became variable once again, suggesting that the optimal stimulation threshold had been partially exceeded and that radiation-induced degradation processes had begun to prevail.

Overall, the germination response was dose-dependent and nonlinear, demonstrating the existence of an optimal stimulation range. Canonical Correspondence Analysis (CCA) further revealed clear differences among provenances: Anina and Sinaia were positively associated with GP and GI, whereas Brașov V.P., Brașov V.C., Săcele, and Latorița were more closely associated with GE and CVG, indicating provenance-specific differences in the absorption, transfer, and utilization of ionizing energy during the germination process.

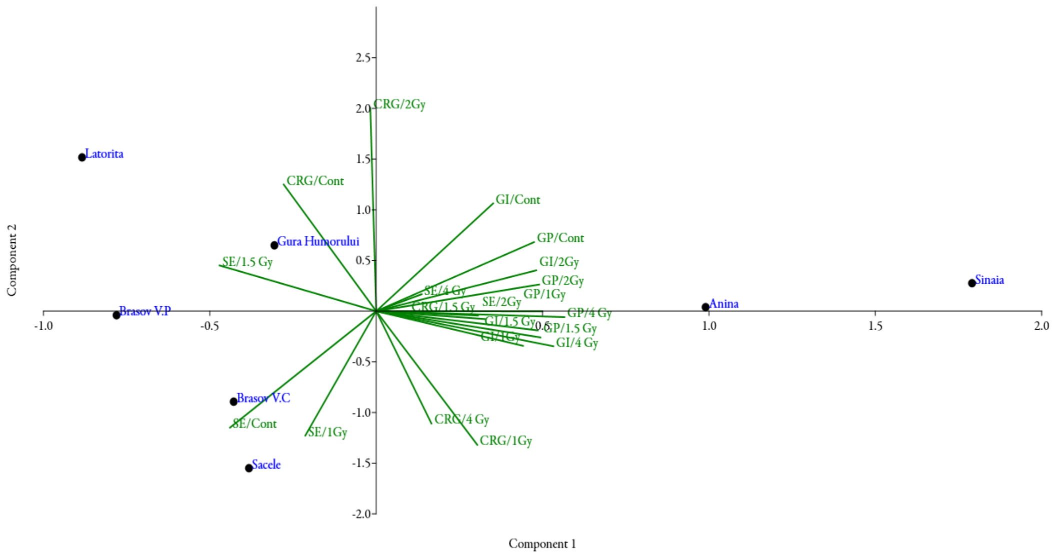

Figure 5.7. *Canonical correspondence analysis (CCA) illustrating the relationships among the seven Larix decidua provenances, γ-radiation treatments, and germination capacity.*

The UPGMA dendrogram (Figure 5.8), constructed using the Gower similarity index, reveals the systematic organization of germination responses according to treatment and provenance. The clustering of the control treatments together with the moderate radiation treatments into a distinct group, clearly separated from the higher-dose treatments, suggests the existence of different energetic interaction regimes. This separation reflects the transition from a regime dominated by natural physiological processes to one significantly influenced by the effects of ionizing radiation.

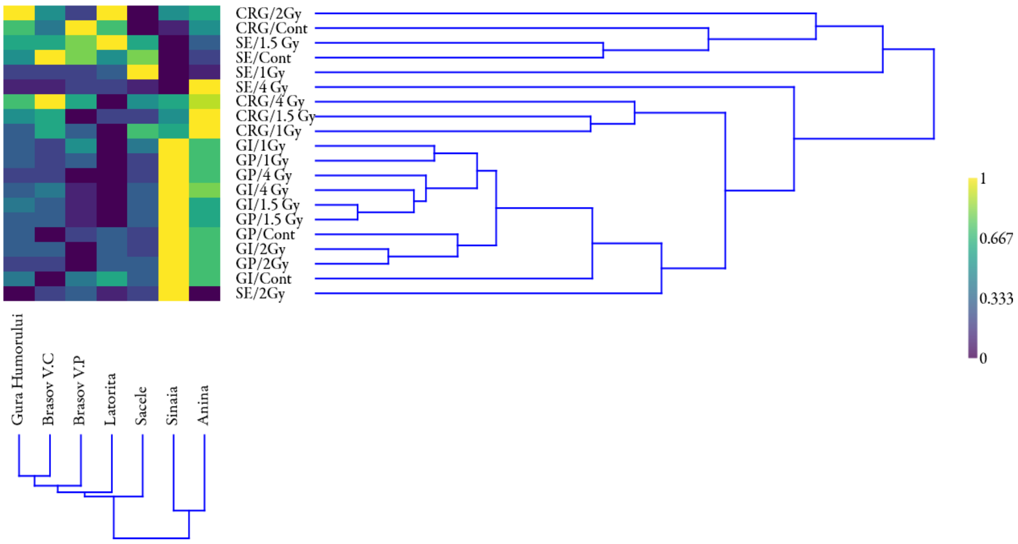

Figure 5.8. *Hierarchical clustering using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) based on the gower similarity index, illustrating the relationships among the seven Larix decidua provenances and the influence of γ-radiation treatments on germination capacity.*

At the same time, the clustering of the Gura Humorului, Brașov V.C., and Brașov V.P. provenances indicates similar responses to gamma irradiation, whereas the Anina and Sinaia provenances exhibited distinct behaviour, suggesting differences in the structural and physicochemical properties of their seeds. These differences may be associated with variations in structural density, water content, or chemical composition, all of which influence the efficiency of ionizing energy absorption.

Overall, the multivariate analyses performed using UPGMA and Canonical Correspondence Analysis (CCA) confirm that the germination response to gamma radiation results from a complex interaction between the physical parameters of the radiation and the intrinsic properties of the biological system. Ionizing radiation acts as an energetic factor capable of modifying both the structure and functionality of the biological system, while the observed effects are determined by the balance among excitation, ionization, repair, and degradation processes, which are dependent on both the absorbed dose and the seed provenance.

5.5 Conclusions on seed germination under electric fields, flectromagnetic fields, and gamma radiation

The analysis of seed characteristics revealed clear differences among the Larix decidua provenances, resulting from the interaction between genetic background and environmental conditions. Provenances such as Latorița and Brașov V.P. exhibited larger morphometric values, whereas Anina was characterized by smaller seed dimensions. These differences influence the manner in which seeds absorb, transfer, and utilize energy during the germination process. Larger seeds, particularly those from the Sinaia, Latorița, and Anina provenances, possess greater energy reserves and consequently exhibit a higher germination potential.

The electric field promotes direct energy transfer through dielectric polarization and ionic transport, and the germination response depends on the relationship between field intensity, exposure duration, and the dielectric properties of the seeds. High germination percentages were obtained under conditions of efficient energy transfer, whereas non-optimal parameter combinations resulted in reduced germination responses.

The electromagnetic field produced more moderate effects, characterized by a nonlinear response and the existence of an optimum at short exposure durations. Its action primarily involves the orientation of molecular dipoles and the modulation of ionic mobility, without inducing major structural alterations in the biological system.

Gamma radiation produced a high level of energy transfer through ionization, generating dose-dependent effects, namely stimulation at moderate doses and inhibition at high doses. The differences observed among provenances reflect variations in their capacity to tolerate, absorb, and dissipate ionizing energy.

Comparatively, the electric field proved to be the most effective method for the direct stimulation of germination, the electromagnetic field provided greater control and stability of the biological response, whereas gamma radiation produced a more variable, dose-dependent response. The optimization of seed germination therefore requires careful adjustment of the physical treatment parameters to the intrinsic characteristics of the biological material, ensuring efficient energy transfer without exceeding the biological tolerance thresholds.

6 Final conclusion

FT-IR analysis demonstrated the influence of both seed provenance and thermal treatments on the chemical structure of Larix decidua bark, as reflected by variations in the characteristic absorption bands. The spectra confirmed the presence of lignin, cellulose, hemicelluloses, and extractive compounds, while increasing drying temperature resulted in a reduction of –OH functional groups, changes in carbonyl absorption bands, and modifications to the lignin structure. These findings demonstrate that the drying process involves not only dehydration but also significant chemical transformations.

GC–MS analysis confirmed that both drying temperature and provenance influence the chemical composition of the bark by promoting the volatilization of low-molecular-weight compounds, increasing the proportion of thermally stable fractions, and facilitating the formation of secondary metabolites. The chemical profile was dominated by terpenoid and phenolic compounds, while the observed differences among provenances indicate variations in molecular stability and in the response to thermal treatments.

Electric and electromagnetic fields act as physical stimuli, influencing dielectric polarization, electric charge distribution, and ionic transport. The results demonstrate the existence of an optimal range of physical parameters within which seed germination is stimulated, followed by a decline in germination efficiency at higher intensities or longer exposure durations, confirming the nonlinear behaviour of the biological system. Gamma radiation produced a dose-dependent response, characterized by stimulation at low and moderate doses and inhibition at high doses through ionization processes and the generation of free radicals. The differences among provenances reflect variations in their capacity for energy absorption, damage tolerance, and cellular repair.

The integration of all experimental results demonstrates that the biological response is governed by the interaction between the chemical structure of the biological material and the applied physical energy. Molecular stability plays a key role in determining tolerance to physical stimuli, whereas more reactive structures facilitate the activation of biological processes. The combined application of FT-IR spectroscopy, GC–MS analysis, and germination experiments provides a coherent physicochemical interpretation of the relationship between molecular structure, energy transfer, and biological response, thereby contributing to a deeper understanding of the adaptive mechanisms of Larix decidua under different physical treatments. Integrarea metodelor FT-IR, GC-MS și a experimentelor de germinare permite o interpretare coerentă a relației dintre structură, energie și răspuns biologic.

List of Published Papers

ISI-Indexed Journal Articles Published within the Scope of the Doctoral Thesis

1. **Truta, P.,** Morar, I. M., Stefan, R., Gal, E., Dan, C., Sestras, P., Adriana F. Sestras, Alina M. Truta, David, L. (2025). Integrated FT-IR and GC–MS Profiling Reveals Provenance-and Temperature-Driven Chemical Variation in *Larix decidua* Mill. Bark. Forests, 17(1), 20. (IF 2025=2.5) WOS: 001671202800001. https://www.mdpi.com/1999-4907/17/1/20

2. **Truta, P.**, Morar, I. M., Truta, A. M., Dan, C., Viman, O., Arion, I. D., Ioras F., Sestras A.F., David, L. (2025). Reproductive trait variability and seed germination responses to electric field and low-dose gamma irradiation in *Larix decidua* Mill. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 53(4), 14811. (IF 2024=1.3) WOS: 001654109200049. https://bnu.repository.guildhe.ac.uk/id/eprint/20776/

International Conference Presentation Related to the Doctoral Thesis

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1. **Petru Truta**, Irina M. Morar, Catalina Dan, Iulia Arion, Roxana L. Stoiandod, Carla Aparaschive, Adriana F. Sestras, Alina M. Truta and David Leontin; Non-Chemical Methods for Improving Larch (*Larix decidua*) Seed Germination; The 23rd International Conference ”Life Sciences for Sustainable Development” 26th– 28th September, 2024 Cluj-Napoca, Romania.

Other ISI-Indexed Journal Articles

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1. Morar, I. M., Stefan, R., Dan, C., Sestras, R.E., **Truta, P.**, Medeleanu, M., Ranga F., Sestras P., Truta A.M., Sestras, A.F. (2024). FT-IR and HPLC analysis of silver fir (*Abies alba* Mill.) bark compounds from different geographical

provenances. Heliyon, 10(5). (IF 2023=3.4) (Q1) WOS: 001206351700001 https://www.cell.com/heliyon/fulltext/S2405-8440(24)02851-2

2. Holonec, R., Viman, O., Morar, I. M., Sîngeorzan, S., Scheau, C., Vlasin, H. D., **Truta, P.,** Criveanu, H., Holonec, L., Truta, A.M. (2021). Non-chemical treatments to improve the seeds germination and plantlets growth of sessile oak. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 49(3). (IF 2021=1.249) WOS: 000704166300006

https://www.notulaebotanicae.ro/index.php/nbha/article/view/12401

Articles BDI

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1. Morar, I., Truta, A., Arion, I., Rebrean, F., Varga, M., **Truta, P.,** Dan, C. (2025). Phenotypic variability of *Picea abies* characteristics in regard with microstationary ecological conditions. Journal of Horticulture, Forestry and Biotechnology, 29(2), 159-166.
2. Truța, A. M., Viman, O., Dohotar, V. D., Sîngeorzan, S., **Truța, P.**, Holonec, L. (2020). The Influence of Certain Types of Substrate and Biochemical Substances in Seed Germination and Plant Development of Spruce (*Picea abies*). Bulletin of the University of Agricultural Sciences Veterinary Medicine Cluj-Napoca. Horticulture, 77(1):128-135, CABI, file:///C:/Users/USAMV-Cluj/Downloads/13762-Article%20Text-54132-1-10-20200603.pdf
3. Holonec, L., Viman, O., Sîngeorzan, S. M., **Truța, P.,** Truța, A. M. (2021). Chemical Methods to Improve Seed Germination and Seedling Growth of Honey Locust (*Gleditsia triacanthos* L.). seeds. Bulletin of the University of Agricultural Sciences Veterinary Medicine Cluj-Napoca. Horticulture, 78(1):63-70, CABI, file:///C:/Users/USAMV-Cluj/Downloads/14103-Article%20Text-55075-3-10-20210604%20(1).pdf

4. Truța, A. M., Viman, O., Dohotar, V. D., Sîngeorzan, S., **<u>Truța, P.</u>,** Holonec, L. (2020). The Influence of Certain Types of Substrate and Biochemical Substances in Seed Germination and Plant Development of Spruce (*Picea abies*). Bulletin of the University of Agricultural Sciences Veterinary Medicine Cluj-Napoca. Horticulture, 77(1):128-135, CABI, file:///C:/Users/USAMV-Cluj/Downloads/13762-Article%20Text-54132-1-10-20200603.pdf