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ABSTRACT OF DOCTORAL THESIS

Research on the biology of plant taxa belonging to the genus *Taxus* L. - sources of plant extracts with biocontrol potential evaluated under experimental conditions

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LIST OF ABBREVIATIONS

T1 – *Taxus baccata* – spontaneous

T2 – *Taxus baccata* – cultivated

T3 – *Taxus baccata* '*Robusta'* – cultivated

IV – April (strobili production)

VI – June (intense vegetative growth)

IX – September (biomass allocation to roots)

A – photosynthesis rate

E – transpiration rate

R – respiration rate

Qleaf – P.A.R. incident on the leaf surface

tleaf – leaf surface temperature

Ci – sub-stomatal CO_2

gs – stomatal conductance of H_2O

WUE – water use efficiency

E/gs – the ratio of transpiration rate to stomatal conductance

* – statistically significant differences for $p < 0.05$

** – statistically significant differences for $p < 0.01$

*** – statistically significant differences for $p < 0.001$

**** – statistically significant differences for $p < 0.0001$

INTRODUCTION

Interactions between plants and biotic and abiotic factors have led, in the course of their establishment and consolidation, to the synthesis in their organs of a large number of secondary metabolites, products with specific biological properties, of great interest to the pharmaceutical industry or agriculture, which are currently being intensively researched locally, nationally and internationally.

In this context, it is now considered that the plant kingdom represents, through its diversity of species, a rich source of compounds with herbicidal potential still insufficiently explored and used by practitioners, and *allelopathy* has emerged as a pragmatic solution to solve problems in modern agriculture. Thus, multiple approaches such as crop rotation, cover crops, intercropping, mulching, incorporation of crop residues, and application of aqueous extracts are just some of the research directions developed with allelopathic themes to manage agricultural pests, mitigate abiotic and biotic stresses on plants of interest, and improve and increase agricultural production (Farooq et al., 2013).

Viewed from the perspective of the practical effects sought and expected by farmers, allelopathy is a natural ecological phenomenon in which different organisms affect the functioning of other neighboring organisms negatively or positively (Rice, 1984) through the release of secondary metabolites (Farooq et al., 2011a), at the level of plant-plant interactions the links between partner organisms are generated by chemicals produced and released by them into the environment through various pathways (volatilization, exudation, dissolution, etc.) (Wier et al., 2004).

In plant metabolism, synthesized compounds have various roles: informational, structural, energetic, synthetic, and signaling (Soltys et al., 2013), with a particular category being compounded with defensive or competitive functions. Among these compounds, there are numerous categories, such as alkaloids, flavonoids, tannins, organic acids, or volatile compounds, substances that can induce stimulation at high concentrations or inhibition at low concentrations of germination and growth of surrounding plants (Narwal, 1999; Li et al., 2010). Thus, phenolic compounds and terpenoids may act differently about different plant organisms: they may inhibit lipid and protein synthesis, alter photosystem I (Hirata et al., 2003) and photosystem II (Einhellig, 1993; Dayan and Duke, 2006), inhibit nutrient processing or germination, or alter transpiration and respiration rates (Rimando et al., 1998; Abrahim et al., 2003; Dayan and Duke, 2006).

In light of the above, several *Taxus* taxa have been chosen for study as potential sources of biocontrol compounds (Reigosa et al., 1999), as the genus is rich in compounds with phytotoxic activity (phenols, flavonoids, alkaloids) (Das et al., 1998; Parmar et al., Taxus baccata L. - yew - is a dioecious gymnosperm, occurring throughout Europe, with scattered populations extending as far as North Africa and the Middle East (Thomas and Polwart 2003). The species is known to be one of the most shade-tolerant tree species in the European flora (Ellenberg et al., 1992; Thomas and Polwart, 2003; Iszkulo and Boratynski 2006, cf. Devaney et al., 2015); its seedlings survive in the intense shade of mature yews and frequently grow under dense crowns of *Tilia*, *Acer*, *Carpinus*, *Picea* and *Abies*, but also under lighter canopies of *Quercus* or *Pinus* and even in full sun (Iszkulo and Boratynski, 2006, Thomas and Polwart, 2003).

From an ecological preference point of view, yew is generally found in areas with an oceanic-mountain variety of climate zones where it grows well in sheltered and shady conditions (forests) with relatively high atmospheric humidity. The species is also sensitive to drought (Șofletea et al., 2007, cf. Bîrsan et al., 2017). In the Romanian region, it is considered a vulnerable and rare species (Oltean et al., 1994), being a legally protected species and declared a monument of nature sporadically distributed in pure beech forests, but also in mixed conifer and beech forests where it plays significant ecological and environmental roles (Sârbu et al., 2013; Alavi et al., 2020).

In the context of the above, the present research aims, on the one hand, to highlight some aspects related to the biology of some taxa of the genus *Taxus* L. spontaneous and cultivated in the NE area of the country according to specific influencing factors (internal, leaf surface and external factors), through anatomical, micro-morphological, physiological and biochemical determinations, carried out during three phenological phases of its ontogenetic cycle. On the other hand, the results of our analyses evaluate the possible allelopathic (bioherbicidal) potential of aqueous extracts of different concentrations obtained from different organs of the respective taxa, interpreting the effects induced by these extracts, under experimental conditions of cultivation in the laboratory, on the germination process and development of the resulting seedlings in two test plant species: *Amaranthus retroflexus* L. (broadleaf weed species) and *Lycopersicon esculentum* Mill., variety Silvia (crop species).

PART I - THEORETICAL CONSIDERATIONS

CHAPTER 1. THE EFFECT OF SUBSTANCES OF PLANT ORIGIN ON GERMINATION AND GROWTH PROCESSES IN PLANTS

1.1. Ecological considerations

In different regions of the globe, plants do not grow haphazardly, respectively randomly, but form well-defined ecological associations and communities, adapted to specific living conditions, characteristic of a particular geographical area, which has specific constitutive peculiarities (Corbu and Cachiță-Cosma, 2010). Certain substances released into the environment by plants exert on them or other plants (or on many other living organisms in their immediate vicinity) a variety of more or less severe influences. The action of such metabolic by-products may favor or disfavor the life of other neighboring organisms, which receive the intercepted 'chemical messages' differently, depending on their nature, their genus, species or genotype, and their physiological state. This complex phenomenon has been termed ***allelopathy***, a natural phenomenon in which different organisms affect the functioning of other neighboring organisms, negatively or positively, by releasing secondary metabolites (Bhadoria, 2011; Bajwa, 2014; Kostina Bednarz et al., 2023) set of factors in which environmental conditions have a significant role (Bhadoria, 2011).

The term *'allelopathy'* is derived from the Greek words '*allelon*' = reciprocal and '*pathos*' = suffering; it defines the distress caused to a plant by another plant (Rizvi et al., 1992). The term was first used by the Austrian physiologist Hans Molisch in 1937 in his book *Allelopathie* to include biochemical harmful as the beneficial interactions between all types of plants, including microorganisms (Scavo et al., 2018).

Following previous definitions of allelopathy and attempts to explain its mechanism of action, the International Allelopathy Society (IAS) has defined ***allelopathy*** as the science that *'studies any process involving secondary metabolites produced by plants, algae, bacteria, and fungi that influence the growth and development of agricultural and biological systems'* (Duke 2010; Kostina Bednarz et al., 2023).

Notably, one of the earliest and most notable examples of *allelopathy* is that of black walnut (*Juglans nigra* L.), as juglone (5-hydroxy-1,4-naphthoquinone) was the first allelopathic compound described. Juglone is obtained mainly from the leaves, roots, and fruit peels of this species and has shown growth inhibitory effects on the whole plant in species such as tomato, alfalfa, soybean, and cucumber (Durán et al., 2019 Rietveld et al., 1983;

Terzi et al., 2003; Babula et al., 2014; Macías et al., 2020). Pliny the Elder (23-79 AD) was the first to observe that *the 'shade of the walnut tree is poisonous to all plants within its range'* (Durán et al., 2018).

Another example of a species with allelopathic activity is sorghum (*Sorghum bicolor* L.), which produces a variety of allelopathic compounds, the most important of which are hydrophobic p-benzoquinone (sorgoleone), phenols, and acyanogenic glycosides (Abbas et al., 2021). Sorgoleone and its resorcinol (1,4-hydroxylated) form account for 90% of the compounds present in sorghum root exudates (Głąb et al., 2017). It is toxic to numerous other plant species, such as weeds like *Phalaris minor* L., *Chenopodium album* L., *Rumex dentatus* L., and *Convolvulus arvensis* L. (Bhadoria, 2011).

1.2. Phytotoxic substances and modes of action

Allelopathy is considered to be a multidimensional phenomenon, occurring constantly in natural and anthropogenic ecosystems (Gniazdowska et al., 2005), defined as the interaction that takes place between plants and microorganisms through a variety of compounds, usually referred to as *allelopathins*, *allelochemicals* or *allelopathic compounds*. The practical determination of the quality and quantity of the direct or indirect effects of allelopathic compounds on plant communities or microorganisms in the natural environment is very complicated due to the multidimensional nature of these interactions, which is why it has been necessary to develop analytical techniques that allow better specification of the direct effects of *allelopathins*, thus moving research on this phenomenon from the field to the laboratory.

'Allelopathy' refers more to interactions that occur in the natural environment (Soltys et al., 2013). In studies with plant extracts, allelopathins isolated from plant tissue, collected from exudates, or even synthetic compounds identical to natural ones, the term 'phytotoxicity' has been defined to distinguish allelopathy (as a phenomenon occurring in the natural environment) from studies conducted in the laboratory.

However, the phytotoxic activity of certain species and extracts obtained from them is due to the presence of chemicals and the effects they produce, mainly secondary metabolites derived from primary metabolic pathways (Weir et al., 2004; Iqbal et al., 2012). Defensive secondary metabolites involved in allelopathic interactions comprise an enormous range of chemical compounds, the most representative of which are phenolic compounds (simple phenols, flavonoids, quinones, coumarins, etc.), terpenoids (mono-, di- and triterpenes, sesquiterpenes and steroids) and compounds containing a nitrogen atom (e.g. benzoxazinoids) (Scavo and Mauromicale, 2021).

Many potentially allelopathic substances show poor biological activity on soil plants due to the rapid leaching of highly water-soluble compounds and their instability or rapid degradation by microorganisms (Mehdizadeh and Mushtaq, 2019). Recent research has shown that the synergism of allelopathic substances can increase bioavailability due to preferential distribution, leading to increased persistence of allelochemical mixtures in soil composition (Kostina-Bednarz et al., 2023).

Numerous studies with extracts from different plant species have shown that the toxic effects of allelopathic substances are based on delayed seed germination and inhibition of seedling growth. Growth inhibition can also lead to anatomical and morphological deformations of the root tip. Phenolic acids, like coumarins, show similar inhibitory effects on plant growth and induce morphological root deformities (Kumar et al., 2020).

Allelopathic compounds affect the germination and growth of neighboring plants by disrupting various physiological processes, including photosynthesis, respiration, water regime, and hormone balance, mainly by inhibiting enzyme activity. According to the literature, the ability of an allelochemical to both inhibit or retard plant growth and or seed germination is usually defined as its 'allelopathic' (or 'phytotoxic') potential (Soltys et al., 2013).

1.3. The significance of allelopathic interactions in natural and anthropized ecosystems

Chemical communication between plant organisms, but also between plant organisms and other types of organisms (micro-organisms, animals, etc.), is a phenomenon that arose and developed during the evolution of ecosystems. Co-evolutionary effects have shaped biocenoses through this communication and other types of interactions (Sinkkonen, 2006). The involvement of allelopathic relationships in the organization of ecosystems applies not only to terrestrial ecosystems but also to aquatic (Erhard, 2006) and marine ecosystems (Granéli and Pavia, 2006).

Allelopathic interactions have been used in agriculture since ancient times, with one of the main problems of large-scale crop cultivation being the presence of invasive (segetal) species, which can cause significant crop losses; as a result, their control has necessitated the use of a wide range of herbicides over time. Such substances currently used are of synthetic origin, which introduces several drawbacks, including contamination of drinking water with toxic substances, contamination of the food chain with pesticides, and the emergence of herbicide-resistant segetal plants (Liu et al., 2008).

Allelopathic interactions in agroecosystems form the basis of techniques to increase productivity and reduce the number of individuals belonging to segetal species. Such methods, including crop rotation, intercropping, and mulching, rely on chemicals released by plants during their development or present in plant residues. In these farming systems, various plant residues or plant preparations can also be used for controlling pests such as phytopathogenic fungi, insects, or nematodes (Narwal, 2006).

In forest ecosystems, allelopathic interactions play a particular role in processes such as the invasion of exotic species, inhibition of seedling growth, and alteration of soil physico-chemical and biological properties (Reigosa and González, 2006). For this reason, identifying the existence of these interactions and deciphering their mechanisms are fundamental for establishing effective agroforestry practices.

1.4. Allelopathic substances used as bioherbicides

1.4.1. Advantages and disadvantages of bioherbicides

The mode of action of some allelopathic substances is similar to that of synthetic herbicides, and the use of these substances in weed management as bioherbicides is a highly topical area of research. So far, only 3% of the approximately 400,000 compounds known to have allelopathic activity have been recognized to act as bioherbicides, although over 2000 plant species exhibit strong allelopathic effects (Li et al., 2019).

Allelopathic compounds are highly appealing as new classes of herbicides due to a wide variety of advantages, although in the perspective of obtaining allelopathic-based bioherbicides, the effects caused by these compounds on target plants are also classified as being 'phytotoxic' (Soltys et al., 2013).

Characterized by their broad diversity, allelopathic substances can be valuable tools in the fight against weeds in crops; thanks to their properties, they can be used in efforts to discover new specific target sites in acceptor plants. In addition to the ability to inhibit photosynthesis or respiration processes, these allelopathic compounds can also bind to proteins at different loci compared to synthetic herbicides (Nimbal et al., 1996; Dayan et al., 2009), which may give them the ability to eliminate weeds already resistant to synthetic herbicides with the same mode of action currently marketed on the agricultural market.

Despite their many advantages, allelopathic substances have some limitations for direct use as bioherbicides. The different modes of action of each class of allelopathic compounds and how environmental conditions affect their activity are difficult to determine. Also, when designing new bioherbicides, it must be taken into account that allelopathic effects vary between varieties or genotypes, as plants with close taxonomic proximity need not necessarily have similar allelopathic actions (Motmainna et al., 2021).

1.4.2. Biotechnologies for investigating natural herbicides

Studies exploring allelopathic interactions have developed considerably over time, benefiting from the effort of large teams of specialists and increasingly advanced biochemical investigation techniques, which have improved the identification of allelopathic compounds and have complemented the knowledge about their nature, biosynthesis and mode of action by accepting, based on clear evidence, that these secondary metabolites synthesized and released by plants can take part in highly complex inter- and intraspecific ecological interactions in the environment (Soltys et al., 2013).

The development of bioherbicides is much more complicated than synthetic bioherbicides because bioactive substances must first be adequately isolated from plant extracts, and the extractable mass of recovered compounds is usually low compared to the simple process of producing large quantities of herbicides by chemical synthesis (Soltys et al., 2013; Kostina-Bednarz et al., 2023).

Allelopathic compounds could be used as templates for synthesizing new herbicides. The literature shows several studies that have been conducted to develop bioherbicides using coumarins due to their bioactivity, and the results showed that some of the compounds had the same inhibitory effect on weeds as the commercial herbicide acetochlor (Zhao et al., 2021). A series of new phenoxypyridine derivatives containing the natural product coumarin were designed and synthesized in the research by Zhao et al. (2021). These compounds showed excellent allelopathic activity under greenhouse conditions, similar to the commercial herbicide oxyfluorfen; crop selectivity tests showed that maize, cotton, and soybean had excellent tolerance to the new compound, but rice and wheat were affected (Zhao et al., 2021; Kostina-Bednarz et al., 2023).

Allelopathy could be used for two principal purposes: agriculture and phytomedicine. In agriculture, extracts or enriched plant fractions, plant residues or pure bioactive compounds, or newly developed formulations by encapsulation methods can be applied for controlling weeds in an environmentally friendly way, increasing the growth and yield of other plants, combating insect pests, controlling diseases caused by microorganisms; improving the penetration and solubility of pesticides in soil (Farooq et al, 2011; Sunulahpašić et al., 2017; Lim et al., 2019; Walker et al., 2018; Macías et al., 2019) or within the phytomedicine field (Macias et al., 2008). These approaches could also be used to develop new drugs by synthetically modifying the main compounds, improving bioavailability, and applying an enriched extract in search of more efficient and effective medicines for treatments against some diseases (Macias et al., 2020).

1.5. In vitro testing for phytotoxic effects

Evaluation of the phytotoxic action of various chemical compounds, including those of plant origin, can be carried out under in vitro conditions, using test plant species that meet specific requirements: increased speed of germination, sensitivity to the respective test compounds (Dayan and Duke, 2006; Reigosa et al., 2013). It is also recommended to test the activity on several test species with different characteristics (morphology, anatomical structures, biochemical composition, etc.) to capture information on the phytotoxicity of the compounds. These species include radish, cucumber, lettuce, tomato, etc.

In this context, in the practical determinations that are the subject of this research, seeds of two plant species were chosen for germination: the redroot pigweed - *Amaranthus retroflexus* L., a broad-leaved relative species present in crops - and tomato - *Lycopersicon esculentum* Miller, a crop plant, - species with rapid germination, sensitive to the action of external factors (stressors), whose seeds differ morphologically and structurally (smaller in *Amaranthus* and larger in *Lycopersicon*, with a much thicker integument).

CHAPTER 2. GENERAL CONSIDERATIONS ON THE GERMINATION PROCESS

2.1. The seed - origin and composition

Seed is the beginning of a new life. It contains the individual in its incipient, embryonic form, endowed with all that is needed, including the food supply for the early stages of growth and development.

After formation, the seed grows and develops, acquiring the size, weight, physiological, and hereditary characteristics that make it capable of reproducing the organism on which it was formed. Under environmental conditions appropriate to each species, the seed must pass from dormant to active life and produce new plants. Germination is the process by which the plant emerges from dormancy, whether imposed by environmental or endogenous conditions.

2.1.1. Ontogenetic origin

The seed originates from the ovule following the double fecundation process in angiosperms. The embryo will arise from the main zygote, the secondary (accessory) zygote will give rise to a nourishing tissue - the albumen or secondary endosperm, and the integuments of the egg will form the seed coat (seminal). This tissue complex of embryo, endosperm, and tegument is called seed. All the transformations that the egg undergoes after fertilization are intended to ensure the nourishment and defense of the embryo until it reaches favorable conditions to reproduce a new individual (Bădulescu, 2009).

2.1.2. Parts of the seed

With all variations in shape, size, color, and number, etc., a mature seed comprises the following components: seminal tegument, embryo, endosperm or albumen, and perisperm (where present) (Ungurean, 1966, cf. Bădulescu, 2009).

2.2. Seed germination

Seed germination is the process by which it initiates a new cycle of ontogenetic development when favorable environmental conditions are provided (Burzo et al., 2004, cf. Bădulescu 2009).

2.2.1. Germination factors

Seed germination is determined by the action of different factors in the external environment, the structure of the seeds, their stage of development, the presence of various substances, and primarily enzymes in their composition. Thus, seed germination is affected by several factors, which can be grouped into **internal and external factors**.

2.2.2. Germination metabolism

During germination, complex biochemical transformations occur in the seed, and the substances stored in the reserve tissues are gradually used in the processes carried out during this time. Germination, which initiates a new cycle of ontogenetic development, comprises four main stages with specific procedures.

CHAPTER 3. THEORETICAL CONSIDERATIONS ON PHYSIOLOGICAL PROCESSES IN PLANTS (CONCERNING WOODY PLANTS)

The national and international literature that we have had the opportunity to consult generally presents the physiological processes carried out by herbaceous plants, describing some functional trials recorded in woody plants, especially angiosperms, with a large leaf area, quite easily accessible to field research, "*in vivo*" and contains much fewer data on the functions of conifers, including trees of the *Taxaceae* family, considered one of the oldest enigmas in gymnosperm systematics, with a much controversial place among conifers, including those of the genus *Taxus*, a genus with a particular taxonomic position, still debated by specialists at present.

As a result, this chapter attempts a synthetic presentation of information on the nutritional functions of higher plants, putting together available data from national literature, with some additions taken from international articles and treatises referring to woody plants and conifers, with examples from taxa of the genus *Pinus*, by far the most studied genus from this point of view, but also from the genus *Taxus*, a genus extremely difficult to delimit into species, subspecies and varieties based on morphological traits alone.

3.1. Water Regime

3.1.1. Water regime in tree life

The availability of sufficient water is a primary factor for plant survival, growth, and development. Water supply, light, and temperature values are major ecological factors in the distribution of forest vegetation, determining its altitudinal and latitudinal zonation (Grudnicki, 2006).

The water regime of plants has three moments: *absorption*, *circulation*, and *elimination into the external environment*. This is the ratio between the amount of water the plants take in and the amount of water they remove in a unit of time, which is the *water balance*. Water loss must be compensated by water uptake to maintain a healthy balance. If, under certain conditions, the amount of water lost exceeds that acquired by absorption, a water deficit occurs in the tissues (Murariu, 2002).

3.1.2. Water uptake by woody plants

Woody plants have a root system that differs according to the species and soil conditions under which they grow. Extension depends on aeration conditions and the soil's composition. To achieve the maximum absorption area, the root has a rich branching and, at least in the young stages, a high number of absorbent hairs. These are present in the seedling rhizomes and at the tips of plant root branches and have a cell wall rich in cellulose and a thin peptide layer or gelatinous coating on the outside, which constitutes a wet phase involved in water exchange between soil and root (Parascan et al., 2001).

3.1.2.1. Environmental factors affecting water uptake

a. Soil water and its accessibility to plants. Plants can absorb water from the soil when it comes into contact with the active absorption zone and if the water-holding force of the soil particles does not exceed the suction force of the roots.

b. Soil solution concentration. In regions with a regular rainfall regime and those covered by forests, the osmotic force of the soil solution is not limiting in the water absorption process.

c. Soil aeration. On soils with excess moisture or those with a low number of capillaries, the root system suffers from a lack of oxygen and the accumulation of excess carbon dioxide. Only plants with specific adaptations can grow in these conditions, as the lack of oxygen over a sustained period leads to root poisoning.

d. Soil temperature. Between 15°C and 40°C temperatures, water uptake is typical for most plants. Lowering the temperature increases the viscosity of the protoplasm and decreases its permeability to water. Temperatures exceeding 40°C also cause a stiffening of the protoplasm and hence decreased permeability (Parascan et al., 2001).

3.1.3. Water circulation in plant bodies

In woody plants, absorbed water travels long distances from the root tissues through the stem to the mesophyll of the leaves, where, after use in physiological processes involving its presence, it is eliminated to the outside. The ascent of water through the xylem vessels from the root to the last nerves in the leaves is mainly by suction of water due to transpiration and by its pushing due to root pressure.

3.1.4. Water balance. Plant wilting

There is a ratio between the amount of water absorbed and the amount sweated, called the *water balance*. Water balance is balanced on days with regular humidity and not too hot because sweating corresponds to water absorption. On summer days with high insolation, transpiration increases, and water deficit occurs in plants.

Among the causes of water deficit are disharmony between the functions of the foliar and root systems or lack of water in the soil. When the ground is well supplied with water, water deficit in plants sometimes occurs only during the hottest days, with values as low as 5-10%. Wilting can be *temporary and permanent*.

3.2. The transpiration processes

The transpiration process - the elimination of water from the plant body into the atmosphere in the form of vapor - is an evaporation process controlled by leaf structure and stomatal activity, which depend on the plant's adaptation to environmental conditions.

Literature data indicate that plant transpiration accounts for about 61% of global evapotranspiration (Schlesinger and Jasechko, 2014); most water loss occurs through leaves, and stomata largely control foliar transpiration. The regulation of the stomatal aperture is very complex because stomata respond to a variety of environmental (for example, light, humidity, temperature, CO_2 concentration) and endogenous (for example, hormone production and release in roots and leaves, age) influences (Pallardy, 2008).

3.2.1. Factors that influence transpiration

A. External factors

The intensity of sweating depends on environmental factors, of which light, temperature, atmospheric pressure, air currents, soil, and air humidity influence significantly.

B. Factors relating to the nature of the plant

While the transpiration process is controlled mainly by physical factors, it does not follow a similar pathway in all woody plants growing in the same environmental conditions since it is influenced by internal factors such as leaf area, root-to-leaf area ratio, degree of stomata opening, number, size and position of stomata, etc.

3.2.2. Particularities of tree transpiration

The transpiration of woody plants is influenced by both the characteristics of each species and environmental factors. When there is sufficient water in the soil, transpiration is correlated with climatic factors, especially temperature, in the steppe and forest, where soil moisture is present at suitably low levels, and transpiration is primarily coupled to the soil water reserve.

About age, it was found that the transpiration rate in seedlings is higher than in adult trees and that, about time and unit leaf area, the transpiration rate is lower in resinous trees, whose leaves are better adapted to stop this process than in deciduous trees. At the same time, the leaf area of a coniferous tree is much bigger than that of a deciduous tree, so that at crowns of similar size, coniferous trees lose more water than deciduous trees (Burzo et al., 2005).

Gout, as a process of water removal by plants in the form of droplets, occurs when, after a warm period, during which water supply has been carried out as usual, the environment is filled with cold air masses, conditions in which the temperature of the air decreases so that the relative humidity of the air increases to values close to saturation, which inhibits the transpiration process. Simultaneously, root water uptake from the soil is proceeding normally, and root pressure causes the raw sap to rise to leaf level, where it is eliminated as droplets. Gout is of very little importance to woody plants and is only encountered accidentally when the intensity of transpiration is too low. In conifers, the guttation process does not occur (Burzo et al., 2005).

3.3. Photosynthesis process

Plant photosynthesis is a complex biological, physical, and chemical process in which plants convert light energy into chemical energy and carry out the synthesis of organic matter, which is the premise and basis of all plant physiological activities, and the level of photosynthetic efficiency reflects not only the efficiency of light energy use in plants but also their growth potential and ability to withstand stress (Iszkulo, 2010; Adams et al., 2018; Yin et al., 2022).

Woody plants are photosynthetically classified as type C3, specific to temperate plants. Photosynthesis varies between species as much as within a single plant due to the tree crown, where light capture by leaves differs in time and space. For example, young leaves of Pinus taedea achieve positive photosynthesis about 40 days after bud break, so that three months after bud break, the photosynthetic intensity equals or exceeds that of leaves one year older. When leaves have reached more than 90% of their maximum size, the photosynthetic process is at its highest intensity (Radoglou and Teskey cf. Burzo et al., 2005).

The functional parameter called **assimilation rate**, which represents the amount of organic matter accumulated per unit of leaf area, varies for young coniferous plants between 3-10 mg dry matter/dm2/day during the growth period and between 10-50 mg/dm^2/day during the maximum accumulation period.

3.3.1. Importance of photosynthesis in nature

Photosynthesis is the process by which solar energy is absorbed by assimilating pigments in the leaf, followed by its conversion into chemical energy needed to synthesize organic substances by reducing carbon dioxide captured from the atmosphere.

The carbon is found in 90-95% of the structural substances of plant organisms, and the green plants' principal carbon source is carbon dioxide taken from the atmosphere. As a large-scale process, green land plants absorb about 20 billion tonnes of carbon dioxide every year, with large quantities of coal, oil, and natural gas being stored as a result of the photosynthetic process over long periods of evolution of life on Earth.

3.3.2. Factors Affecting Photosynthesis

Photosynthesis Variations are determined by internal factors (which are related to the nature of the plant) and a series of external factors (environmental factors), with which they are closely linked.

3.4. Respiration process

In the process of respiration, part of the photosynthesized products are consumed by plants to gradually obtain, through reactions carried out in the presence of oxygen in the air, the biochemical energy necessary to carry out vital processes, together with simpler intermediate compounds used as support material for the growth process at the cellular level (Burzo et al., 2005).

In all cells, respiration, a process that happens both day and night, occurs within the cells, and some energy is lost as heat energy. This production of heat, which can be as much as 90% of the energy released during respiration of the budding seeds, is intended to provide the plant as a single unit with a temperature that favors the spread of volatile odorous substances and stimulates pollinating insects.

3.4.1. Respiration of the tree and its parts

Under the same growing conditions, respiration in woody plants differs according to species, organ under study, or age. Light plants have a higher respiration rate than shade plants, and deciduous plants breathe more intensively than resinous plants, but respiration in organs of the same tree has different values (Burzo et al., 2005). Although they represent the smallest part of the tree mass, leaves perform the most intense respiration because they contain the highest proportion of living, intensely active tissues. Respiration is generally low in the main stem and thick branches (Parascan et al., 2001).

3.4.2. Factors influencing respiration

Factors influencing respiration are the physiological state of the plant (amount and nature of the organic substrate of respiration, enzyme activity, internal water regime, age) and environmental factors (temperature, light, concentration of oxygen and carbon dioxide in the air).

3.5. Resistance to thermal and hydric stress

A drought is a meteorological phenomenon that manifests as little or no rainfall, high temperatures, high evapotranspiration, and dry and strong winds. As a result, the humidity of the physical environment becomes insufficient to meet plant needs.

Water deficit can affect the soil (edaphic drought), the air environment (atmospheric dryness), or it can include the physical environment as a whole (mixed drought). In situations where the water deficit in plants is not caused by a lack of water in the soil but rather by an inability to absorb it due to too cold, poorly aerated soils, high water retention capacity, or damaged roots, we can speak of a 'physiological dryness'. The manifestations are similar to those produced by climatic dryness (Parascan et al., 2001).

CAPITOLUL 4. SITE OF COMMUNITY IMPORTANCE DEALU MARE - HÂRLĂU ROSCI 0076 - OVERVIEW

4.1. Site characterization

The Dealu Mare - Hârlău site - an area that includes the **Tudora Yew Station**, the vegetation area of the Taxus baccata L. taxon under study - is part of the continental bioregion, the Central Plateau ecoregion of Moldavia, with a geographical location of 47° 30' 59" lat. N and 26°44'52" long. E. It covers an area of 25,112 hectares, located on the administrative territory of 3 counties: Botoșani county in the localities of Copălău, Corni, Cristești, Curtești, Flămânzi, Frumușica, Tudora, Vlădeni, Vorona; Suceava county in the locality of Dolhasca and Iasi county, in the localities of Deleni, Hârlău, Lespezi and Sirețel (Catalogue of habitats, species and Natura 2000 sites in Romania, 2013).

4.2. Overview

The site is notable for its high degree of forest cover (97%), with old-growth forests and patches of beech, oak, oak, hornbeam, and ash trees. The old trees favor a rich fauna. The high degree of shading results in the creation of ecological niches with a lot of moisture, in which various species of invertebrates or their larvae live, which are the preferred food of many species of birds and other vertebrates. The general state of conservation of the woodlands is very high as the human impact in the old-growth areas is extremely low, with the Humosu beeches being an example.

4.3. Geology/Geomorphology

The site is located in the Suceava Plateau - Dealu Mare - Hârlău. It is situated on slopes with medium relief energy, with slopes of 10 - 12^0. The whole area is located in the Moldovan Platform and is composed on the surface of almost horizontal sedimentary deposits. In the Dealu Mare region, there are two layers of oolitic limestones.

4.4. Pedologie

The soils within the site belong to the podzols category and are pseudo-glaciated agriloiluvial brown. The clayey soils include brown alluvial soils and brown clayey silty soils. The substratum on which these soils are formed consists of clays and clays, sometimes alternating in thin layers of clays, marls, sands, or gravels. The soils are deep to very deep, rarely medium deep.

4.5. Hidrologie

The water network within the site is very dense. During spring, numerous torrents form in the valleys of the area due to snowmelt. Most of these dry up in summer or have a low flow. Gârla Morii and Vorona with its affluents Teișu, Moscalu, Iezeru, Chișcovata, Tudora, Fundoaia, Pleșa, Tisa, Râpa Dracului, Turbățica are affluents of the Siret, and the main affluent of the Prut is Jijia, with its affluents Miletinul and Bahluiul. Many of the streams in the southern part of the site flow into the Bahlui. The water flow direction is generally from NW to SE (Catalogue of habitats, species, and Natura 2000 sites in Romania, 2013).

4.6. Climate aspects

The territory of the site is climatically part of the forested plateau of Moldavia. The climate is temperate continental with excessive or continental, temperate maritime influences. The average annual temperature is 8-9°C and the average annual precipitation ranges between 600-700 mm (Posea et al., 1982, cf. Bîrsan et al., 2017).

4.7. Vegetation

A large number of mosses were identified in the site, 65 in total, most of them characteristic of mixed beech forests in the hilly regions of Moldova. Among the rare species, it is important to mention *Dicranum viride*, a species of Community interest not included in the standard form. The flora is mostly composed of secular beech of the hill type. There are also frequent occurrences of hornbeam silver linden and yew. Other tree species include the juniper, lowland ash, birch, cherry, ash, forest apple, forest pear, trembling aspen, oak, and field elm. The shrub layer is made up of sandberry, hazel, hawthorn, sallow, wild buckthorn, dogwood, deer thorn, mace, blackberry, and raspberry. Among the species of community interest present in the site, it is worth mentioning a very rare species of orchid, namely *Cypripedium calceolus* L. - lady's slipper (Catalogue of habitats, species, and Natura 2000 sites in Romania, 2013).

4.8. Fauna

The fauna of the site is characteristic of the lowland and hill regions. Among the gastropod mollusks are the orchard snail and the limax. Insects are well represented, the old trees being ideal habitats for many valuable species such as the small nosegay beetle, the stink bug, the large peacock's-eye butterfly, the striped butterfly, the large oak croaker, the duckweed, the alpine croaker. The amphibians are represented by the wood frog, the red mountain frog, the great lake frog, the little frog, the yellow-bellied pond turtle, and among the reptiles are the water turtle, the hazel snake, the house snake, the gnatcatcher, the grey lizard.

The forests covering the site are ideal nesting and feeding grounds for many bird species, such as the Lesser Spotted Woodpecker, Great Spotted Woodpecker, Deer Gnatcatcher, Green Woodpecker, Garden Woodpecker, Oak Woodpecker, Black Woodpecker, various species of woodpecker, the blackthorn, the linnet, the woodlark, the wood thrush, the linnet, the raven, the nightingale, the black-headed nightingale, the yellow warbler, the sand martin, the skylark, the field lark, the field skylark, the red-rumped warbler, the field honeyeater, the little honeyeater, the woodlark, the black throat. The field chrysanthemum is rare in the meadows to the north of the site. Among the day and night birds of prey, the common buzzard, hen harrier, little eagle, wasp, cuckoo, wood thrush, capercaillie, common loon, and little owl are common. Mammals are represented by hedgehogs, moles, kestrels, deer, roe deer, wild boar, wild boar, hazel grouse, squirrel, wood mouse, collared mouse, wild cat, jer, badger, and fox. The gopher, a species of Community interest, is also present here (Catalogue of habitats, species and Natura 2000 sites in Romania, 2013).

4.9. Tudora yew reserve, Botoșani county

Nature Reserve 2.231 Tudora Forest (RONPA0248) is a protected natural area of national interest, corresponding to IUCN category IV (forest type nature reserve), declared by Law no. 5 of 6 March 2000, which is part of the Site of Community Importance Dealu Mare - Hârlău ROSCI 0076. The Tudora Forest is one of the most visited protected natural areas in Botoșani County, due to the existence, in its perimeter, of the yew tree - *Taxus baccata* L., a species declared a monument of nature.

CHAPTER 5. SPECIES STUDIED – OVERVIEW

5.1. Taxa of the genus Taxus L.

5.1.1. Taxonomic classification

Taxus baccata belongs to the Class *Pinate* (*Pinopsida)*, Order *Taxales*, Family *Taxaceae,* and genus *Taxus* (Sârbu et al., 2013). The Order *Taxales* is a small group of plants, comprising five genera and 20 species: *Amentotaxus* (4 spp.), *Austrotaxus*, *Pseudotaxus* (both monotypic), *Taxus* (8 spp.) and *Torreya* (6 spp.) (Biswas and. Johri, 1997) included in a single-family - *Taxaceae* (Dallimore and Jackson, 1966, cf. Dempsey and Hook, 2000). The phylogenetic position for the family *Taxaceae* is one of the oldest enigmas in gymnosperm systematics, its place among conifers being controversial (Chamberlain, 1935; Florin, 1948a, 1951; Sporne, 1965, cf. Ghimire et al., 2014).

Recent data from the international literature states that the genus *Taxus* is considered extremely difficult to delimit between species using morphological traits alone. So, taxonomies differ widely, from the recognition of a single species with subspecies and varieties (Pilger, 1903) to the recent studies recognizing 24 species with 55 varieties (Spjut, 2007). This genus may be a better example of species groups for which it would be revealing to apply a more explicit methodology than intuitive taxonomy based on careful observation of specimens, as has been practiced so far.

The 24 species and 55 varieties of *Taxus* recognized by Spjut (2007) are classified into three groups (Spjut, 1998, 2000; Spjut in Hageneder 2007), two of which are native to North America:

► ***Baccata* Group**, represented by T. *canadensis* and many varieties related to T. *baccata*, T. *biternata*, T. *caespitosa*, T. *cuspidata*, T. *recurvata* and T. *umbraculifera*.

►***Wallichiana* Group**, represented by native Florida yew (T. *globosa* var. *floridana*), Mesoamerican yew (T. *globosa* var. *globosa*), and Pacific yew (T. *brevifolia*), including two varieties (var. *polychaeta* and var. *reptaneta*).

► **The *Sumatrana* group**, appears more evolved in the leaves, which have a wider abaxial marginal zone of differentiated epidermal cells, often glossy red in herbarium specimens. This marginal bare zone varies in width from 8 to 36 cells, of which about half of the cells closest to the stomatal band may develop papillae but usually not more than 12 cells.

Two of the species groups are further divided into two subgroups or alliances: the ***Wallichiana* Group** includes the ***Chinensis* and *Wallichiana* subgroups/alliances**, and the other group - **the *Baccata* Group** - is divided into two geographic subgroups/alliances, which are distinguished by the fact that the abaxial leaf surface between the stomatal band and the margin is largely papillose (***Baccata* Alliance**) or smooth (***Cuspidata* Alliance**) (Spjut 1992, 1993, 1998, 2000a, c) (http://www.worldbotanical.com/Introduction.htm, date accessed:13.04.2021).

The *Taxus baccata* group is divided into two species alliances based on leaf anatomical data: (1) a Euro-Mediterranean *T. baccata* alliance, possibly derived from an ancestral T. *contorta/wallichiana* complex, and (2) an East Asian *T. cuspidata* alliance, which has affinities with a *T. chinensis/umbraculifera* complex. These alliances differ in stomatal band type, defined by stomatal width and density. Differences in stomatal density are seen in the frequency of stomata within and between strands, generally measured by the number of stomatal strands in a band relative to the absence of papillae on the marginal cells. As for **Alliance *Baccata***, it has species with leaves with a lower number of stomata in slightly wider bands - at least 4 cells wide - in which stomatal bands usually extend up to 4 cells from the margins, while **Alliance *Cuspidata*** has species with leaves with a higher density of stomata in narrower bands, most often delimited by a marginal zone 8-18 cells wide. (http://www.worldbotanical.com/Introduction.htm, date accessed:13.04.2021).

Taxus baccata includes numerous varieties or forms (Beissner, 1891; Carrière, 1855, 1867; Elwes and Henry, 1906; Gordon, 1858, 1875; Knight, 1850; Krüssmann, 1985; Lawson et al., 1851; Loudon, 1844; den Ouden and Boom, 1965; Pilger, 1903, 1916), mainly recognized in horticulture. More distinct taxa have been treated in the works of authors Bailey (1923, 1933), Carrière (1855, 1876), Elwes and Henry (1906), Gordon (1858, 1875), and Pilger (1903, 1916). Before Beissner (1891) and Pilger (1903), these were often considered varieties; Rehder (in Bailey, 1902, 1923, 1933), for example, treated them initially as varieties but later as "garden forms" (Rehder 1940, 1949). (http://www.worldbotanical.com/Introduction.htm, date accessed:13.04.2021).

The great diversity of growth forms that is evident in European yew, together with a wide range of differences in anatomical leaf characters, points to a long evolutionary history of the *Taxus* species, for which it has been suggested that species traits were more distinct in the past than in the present (Spjut, 2007a). This observation is further evidenced by comparing the taxa from geographical areas with higher diversity to those with lower diversities.

Another approach (the current one) is genetic (DNA analysis), needless to say, but in this specific case, the comprehensive sample of all the dispersed areas where this genus naturally grows becomes a main obstacle in obtaining credible results. Because of the extensive cultivation of the thyme and the plant's vulnerability to genetic introgression, genetically uncontaminated material is only guaranteed in natural populations isolated from any other taxon mentioned, as well as in cultivated plants (Farjon, 2017).

5.1.2. Morphological characters

Genus *Taxus* L.

Includes trees or shrubs with reddish or reddish-brown, scaly bark, and buds with imbricate scales. The leaves are acicular, persistent, spiral more or less pectinate, linear, more or less falcate, without resinous canals. Flowers are axillary, dioecious, and exceptionally monoecious; the male flowers are solitary, globose, pedunculate, formed of 6-14 stamens with broadened, shield-shaped filaments at the tip, each with 5-9 concentric pollen sacs; the female flowers are ovoid, each formed of an erect ovule, slightly thickened at the base, covered with numerous scales; the seed is ovate, 5-7 mm long and 5 mm wide, surrounded by a reddish sheath (aril). Maturation is annual.

The genus comprises eight species, native to the northern hemisphere, often considered as geographic races of the species *T. baccata*, of which four (one spontaneous) occur in our culture.

Taxus baccata L. - yew

History: The tree is a Tertiary relict, which appears mentioned in the works of Theophrastus and Julius Caesar. In the area of our country, archaeological excavations have proved that since ancient times (BC) in the Getic household, in the full age of wood, yew wood was used to make plowing tools, household vessels, support posts for houses, shingles, bows, arrows, and other objects.

The European or English yew (*Taxus baccata* L.) is native to most European habitats. It is an extremely long-lived tree, with specimens known to be 5000 years old (Milner, 1992, after Benham, 2016). However, as it is difficult to determine the age of the tree accurately - ring-based estimation becomes impractical for the oldest specimens that have scorbutus trunks (Eckenwalder, 2009, after Benham, 2016) - opinions on the exact age of the oldest species are divided (Thomas and Polwart, 2003).

Yew is mostly dioecious; although there are also monoecious trees, they are rare and usually have branches of different sexes. The male flowers are like small green globules grown on the underside of the previous year's shoots, while the female flowers are small green flowers that appear in the axil of the leaf grown the year before. Aeolian pollination gives rise to false, scarlet fruits, about 7 mm in diameter, with a fleshy, red aril (the only non-toxic part of the tree (Wilson et al., 2011) surrounding the central, dark seed. The seeds are dispersed by birds feeding on the red core/pulp and carrying the red seeds away. Yew is also capable of vegetative reproduction.

The toxicity of this tree's organs has been known since ancient times. The fact is that all parts of the plant, except the aril, contain an alkaloid called taxine and the glucoside taxicatine. The bark, wood, and seeds are relatively non-toxic, but the leaves are the most toxic parts of the plant. Taxine ($C_{37}H_{51}NO_{10}$) was isolated from leaves in 1836 by Lucas; this alkaloid has a bulbar action comparable to morphine (analgesic and narcotic). Taxicanthin ($C_{37}H_{22}O_7$) is bitter-tasting and dissolves in water, alcohol, and acetic ether. This glucoside, produced by hydrolysis glucose and taxigenin, has a cardiac action (Zanoschi et al., 1981).

In the past, the pharmacological activity of compounds isolated from yew has led to its widespread exploitation. For this reason, but also due to some ecological peculiarities, the species currently has endangered status in several countries, including Romania (Oltean et al., 1994; Oprea, 2005), and natural habitats with yew are listed in Annex II of the Habitats Directive (Casals et al., 2015).

5.1.3. Anatomical characters

The anatomical characteristics of the species studied are determined by the structural features of the wood (stem) and leaves. The histo-anatomical features of the leaves of most conifers are mostly original, but also the consequence of the adaptation of these species to the particular conditions in which they live. Their acicular leaf shape reduces the surface area of transpiration, which is advantageous for plants in the cold season when water absorption is almost impossible and prevents excessive water loss. The thickened walls of the epidermal and hypodermal cells, the stomata sunken into the hypodermis, or even into the mesophyll, covered with resin, also contribute to moderating or stopping transpiration. The structure of the mesophyll, often made up of septate cells, is a helpful feature for photosynthesis: the surface area of the walls along which the chloroplasts are arranged is thus increased considerably by the formation of septa and thus increases the number of chloroplasts in each assimilating cell.

In *Taxus* taxa, a leaf cross-section shows the mesophyll differentiated into palisading and spongy tissue. Resin channels are absent (distinguishing them from conifers). The bundle sheath consists of a ring of prominent, regularly arranged cells. The leading bundle is large and flattened dorsoventrally, and the parenchyma develops between the rows of xylem and phloem; the transforaminal tissue occurs around the leader bundle, and the tracheids and some of the included parenchyma are limited to wing-like extensions lateral to the xylem. Most parenchyma, however, also extend adaxially and abaxially to the bundle. Albuminous cells are particularly prominent in this region, with those within the sheath of the fasciculus being very large and also vesicular. The stomata are hypostatic, and the subsidiary cells develop papillary extensions that form a deep epistomal chamber so that the guard cells appear deep within it (Biswas and Johri, 1997).

The leaves of Taxus baccata are needle-shaped. No fibers are present in the tissues, and no resin channels could be identified. The abaxial surface of the leaf has a thicker cuticle than the adaxial surface. Palisading parenchyma (one to two layers) is present in the abaxial epidermis only. Numerous transfixing tracheids are present around the leading fascicles. The leaf possesses only one conducting bundle (Hamidipour et al., 2011).

5.2. Test species

5.2.1. *Amaranthus retroflexus* L.

Species description: Pivoting root; stem erect, tall to 100 cm, thick and finely hairy, especially at the top, green, sometimes reddish. Flowers spiciformly clustered; female perigonal laciniae linear-cuneate or spatulate, white-membranous. Flowering, VII-IX. Fruit, dehiscent compressed ellipsoidal capsule. The seed is seed small (1 mm) and lenticular.

Agriculture: Cultivator pest where it settles. Biological consumption considerably depletes the soil of water and mineral elements while shading outcrop plants. Decreases agricultural production. It is used only in some areas for pig feed (Pârvu, 2005).

5.2.2. *Lycopersicon esculentum* Miller

Species description: Pivoting, branched root, penetrates deep into the ground. Stem tall, 30 - 300 cm, depending on variety and variety, erect, with high shoot capacity, forming shoots at the underside of the leaves.

The stem forms adventitious roots in contact with the soil. Leaves interrupted-impernate-compound, covered with glandular hairs with a characteristic smell. The leaves are oval, lanceolate, of various sizes, with an embossed or smooth surface arranged alternately. Yellow flowers are arranged in racemes with a bunch-like appearance.

Autogamous pollination, in rare cases allogamous entomophilous pollination (5%). Flowering in months VII-VIII. Fruit fleshy berries of various shapes and colors (yellow, red, pink), weighing 30-500 g. Seeds are oval-roundish, flattened, and covered with grey or silvery peristyles (Pârvu, 2004).

PART II - PERSONAL RESEARCH

AIM AND OBJECTIVES OF THE RESEARCH

PURPOSE

The research that is the subject of this thesis aims, on the one hand, to highlight some aspects of the biology of some spontaneous and cultivated taxa of the genus *Taxus* L., about specific influencing factors (external factors, leaf surface factors, internal factors) and to evaluate the possible allelopathic (bioherbicidal) potential of aqueous extracts obtained from various organs of these taxa, interpreting their effects on the germination process, and seedling development of two test plant species under experimental laboratory conditions: *Amaranthus retroflexus* L. (broad-leaved weed species) and *Lycopersicon esculentum* Mill, variety Silvia (crop species).

OBJECTIVE:

→ **O1 -** morpho-anatomical investigations on the *yew* taxa studied: biometric determinations and micro-morphological analyses (on leaves), histo-anatomical analyses (on leaves and stems), to assess their degree of variability, as an effect of the adaptation reactions of the supporting plants to the specific conditions of the living environment;

→ **O2 -** physiological determinations of the *yew* taxa studied about specific influencing factors;

→ **O3** – interpretation of the level of metabolic rhythms of *yew* plants belonging to the taxa considered during three different moments of their phenological cycle and analysis of specific biochemical components of the fruits (arils), as the only non-toxic, edible organs of the plants;

→ **O4** - determination of the chemical composition of the volatile oils synthesized in the leaves of the studied spontaneous and cultivated taxa of the genus *Taxus* at the time of intense vegetative growth and identification of the presence at this level of taxol - a compound specific to the genus *Taxus*, with high therapeutic value;

→ **O5** – production of plant extracts (aqueous and alcoholic) of different concentrations (1 and 5%) from vegetative (bark, leaves) and reproductive (arils, and seeds) organs belonging to the three *yew* taxa considered at different times of their phenological cycle, and their biochemical characterization;

→ **O6** – evaluation, under experimental laboratory growing conditions, of changes in some indices of germination and seedling development in the test plant species *Amaranthus retroflexus* L. (broad-leaved ruderal crop species) and *Lycopersicon esculentum* Mill., variety Silvia (crop species) as a result of treatments with aqueous extracts prepared from vegetative and reproductive organs of the *yew* taxa under study;

→ **O7** – evaluation of possible anti-denaturing effects on animal proteins produced by alcoholic extracts prepared from vegetative and reproductive organs of *yew* taxa studied at different times of their phenological cycle.

CHAPTER 6. MATERIAL AND METHODS OF USE

6.1. Biological material

The biological material analyzed is represented by different organs (bark, leaves, arils, and seeds) collected from female yew individuals belonging to the spontaneous species *Taxus baccata* L. (**T1**), from the Yew Reserve - Tudora Forest, Botoșani County (lat. 47.524909° N, long. 26.691887° E, alt. 444 m) (access to the Reserve was ensured based on ANANP agreement no. 8147/26.07.2019 issued by the National Agency for Protected Natural Areas) and from cultivated taxa: *Taxus baccata* (**T2**) and *Taxus baccata 'Robusta'* (**T3**), supplied by Pepiniera S.C. Doropad S.R.L. Suceava based in Dorohoi, commercialized according to the certificate of quality and conformity issued on 31.07.2019 and cultivated in a private space in Vorniceni commune, Botoșani county (lat. 47.986328° N, long. 26.663299° E, alt. 185 m).

After the determination and authentication of the plant material by biologist Dr. Irina Irimia, vouchers for the selected taxa were submitted to the Herbarium of the Faculty of Biology of "Alexandru Ioan Cuza" University of Iasi, with corresponding identification numbers: 186539 - taxon **T1** (*Taxus baccata* L. - spontaneous species), 186537 - taxon **T2** (*Taxus baccata* - cultivated taxon), 186538 - **taxon T3** (*Taxus baccata 'Robusta'* - cultivated taxon); for cultivated specimens the taxon names were kept according to the certificate of quality and conformity issued on 31.07.2019 (date of their acquisition).

6.2. Design experimental

The research was carried out in two directions: field and laboratory analysis. The main physiological processes (photosynthesis, transpiration, respiration) were determined in the study specimens in the "*in vivo*" area at certain defining moments of the ontogenetic cycle during the 2021 calendar year, respectively in April (IV), June (VI), and September (IX).

The research was carried out in two directions: field and laboratory analysis. The main physiological processes (photosynthesis, transpiration, respiration) were determined in the specimens in the "*in vivo*" field at certain defining moments of the ontogenetic cycle during the 2021 calendar year, in April (IV), June (VI), and September (IX). This corresponds, according to the literature (Robakowski et al., 2018), to the specific seasons of the phenological cycle of the analyzed species: strobili production in spring, intensive vegetative growth in summer, and biomass allocation to roots in autumn. We specify that no investigations were carried out during the winter resting period due to the specific weather conditions of winter 2021 in the Yew Reserve in Tudora Forest (heavy snowfall, strong wind, consistent snow cover), which did not allow access to the field.

For further laboratory analysis, fresh material was collected from each taxon and processed in the research facilities of the Faculty of Biology of the "Alexandru Ioan Cuza" University of Iasi, using equipment provided by the Plant Biology Laboratory and the Integrated Centre for Environmental Science Studies for the North-East Development Region (CERNESIM), organized with funds obtained through grant No. 257/28.09.2010, SMIS/CNR 13984/901.

Biological material (bark, leaves, arils, and seeds) collected for analysis was used fresh (leaves), harvested at the stage of intense vegetative growth for extraction of volatile oils by hydrodistillation; for the other determinations it was subjected to the following conditioning operations: conservation in 70% ethyl alcohol (leaves) for histo-anatomy; refrigeration or freezing (arils) for part of the biochemical analyses; thermal fixation (bark, leaves, seeds) in a ventilated oven 60°C for 60 minutes for enzymatic inactivation, followed by drying at room temperature in adequately ventilated areas, protected from direct sunlight, and grinding/transformation into powder to obtain alcoholic, aqueous and acetone extracts, further used as follows:

- alcoholic extracts of 1% and 5% concentration, respectively, for a qualitative summary spectrophotometric evaluation;
- aqueous extracts of the same concentration as the alcoholic extracts, to investigate, under experimental laboratory conditions, their possible allelopathic effects on the germination and development processes of newly formed seedlings, using seeds belonging to the test species *Amaranthus retroflexus* L. -

(redroot pigweed, ruderal species) and *Lycopersicon esculentum* Mill. variety Silvia - tomato, crop plant), species with rapid germination stimulated by ambient light (Boldor et al.,1983; Asaad et al., 2017);

- acetone extracts, for the separation, identification, and quantification of taxol by high-performance liquid chromatography (HPLC).

6.2.1. Preparation of extracts

Alcoholic extracts

They were prepared using 70 % ethyl alcohol as an extractant. According to the literature, extracts prepared using solvents of various polarities (water, ethyl alcohol, methyl alcohol, acetone, chloroform) allow the extraction of both water-soluble and hydrophobic or hydrosoluble compounds from plant material (Naczak, 2004). Extraction was carried out by maceration for 48 h in closed plastic Falcon tubes, stored in a dark place to avoid photodegradation of the constituent compounds.

Aqueous extracts

Aqueous extracts of bark, leaves, arils, and seeds in concentrations of 1% and 5% were prepared using 0.5 and 2.5 grams of powdered plant material suspended in 49.5 and 47.5 ml of distilled water, respectively. The Erlenmeyer flasks with the obtained mixture (plant material and solvent) were kept for 150 minutes in the water bath at 50°C to facilitate the extraction of the bioactive compounds and to avoid their degradation due to the high temperature. Subsequently, the extracts were filtered through filter paper and stored at 4°C away from any light source to prevent the decomposition of biologically active components until use (Lobiuc et al., 2016).

Acetone extracts

For the separation, identification, and quantification of taxol by high-performance liquid chromatography (HPLC), acetone extracts of 99.5% concentration were made according to the method described by Sadeghi-aliabadi et al. (2009).

6.2.2. Extraction of volatile oils

Fresh plant material was extracted from leaves (approximately 300 g per sample) harvested during intense vegetative growth. Extraction was performed by hydrodistillation using a NeoClevenger extractor according to the method described by N. Radulović et al. (2010), Burzo and Toma (2012), Stefanović et al. (2016).

6.3. Research methods and techniques

6.3.1. Field investigation methods – "*In vivo*" determination of the intensity of photosynthesis, transpiration, and respiration processes

For "*in vivo*" determination of specific functional parameters at leaf level - photosynthetic rate (A) (μmoles CO_2 $m^{-2}s^{-1}$), transpiration rate (E) (mmol H_2O $m^{-2}s^{-1}$) and, by particular adaptation for dark conditions, respiration rate (R) (μmoles CO_2 $m^{-2}s^{-1}$), together with the degree of illumination available to the plant photosynthetic apparatus (Qleaf) (μmol $m^{-2}s^{-1}$), leaf surface temperature (tleaf) (°C), sub stomatic CO_2 concentration (Ci) (vpm) and stomatal water conductance (gs) (mol $m^{-2}s^{-1}$), the portable LC*i* Photosynthesis System (ADC BioScientific, 2007) was used. The system is equipped with an infrared gas analyzer, and the determinations were carried out under environmental conditions specific to the year 2021. At the same time, water use efficiency (WUE) (A/E) and the ratio of transpiration rate to stomatal conductance (E/gs) were calculated for all investigated taxa at each time point of their ontogenetic cycle specified above.

Registration of functional parameters was performed on clear and bright days, between 10:00 AM and 12:00 AM, on a sample of 15 one-year-old leaves randomly chosen for each individual analyzed. Leaves of the investigated taxa were enclosed in the leaf chamber under ambient conditions, held for 3 min for acclimatization, and then readings were recorded at 15-second intervals for 3 min. At the time of investigations, air temperature (T) (°C) and atmospheric humidity (RH) (%) were measured using a thermos-hygrometer (Testo 625).

6.3.2. Laboratory investigation methods

6.3.2.1. Morphological determinations - leaf area measurement

Morphological measurements were performed on fresh leaves. Twenty leaves (needles) from one-year-old shoots of each investigated taxon were separated from the leaves and scanned with the handheld compact AM 300 ADC Bioscientific determination machine, used to accurately determine leaf area (LA) (mm^2) and associated parameters: length (LL) (mm) and width (LW) (mm), calculating the ratio between these two parameters (LWR).

6.3.2.2. Micromorphological and histo-anatomical determinations

Micromorphological interpretations were made by taking photographs of the leaves under the scanning electron microscope in the Electron Microscopy Laboratory of the Faculty of Biology. Thus, leaf samples prepared by the air-drying method were examined under the scanning electron microscope (Tescan Vega II SBH) using the VegaTC Software program, taking photographs of both leaf surfaces (adaxial and abaxial) (Hynninen et al., 2018). According to the working protocol of the Laboratory of Plant Morphology and Anatomy Academician Constantin Toma of the Faculty of Biology, the plant material, preserved in 70% ethyl alcohol, was processed for histo-anatomical analysis. The obtained preparations were analyzed under an optical microscope (Euromex bScope BS.1153Pli) and photographs were taken with the built-in camera, Euromex HD Ultra DC 1357.

6.3.2.3. Biochemical determinations

6.3.2.3.1. Determination of water and dry matter content

It consists of measuring the loss of water from the mass of the sample to be analyzed by heating it to 105º C (Boldor et al., 1983).

6.3.2.3.2. Determination of ash (mineral elements) and organic matter content

For the quantitative estimation of the mineral content, the material from the dry matter determination is calcined for 3 hours at 550°C (Boldor et al., 1983) to obtain ash.

6.3.2.3.3. Determination of foliar photoassimilate pigment content

The amount of photoassimilatory pigments in leaves are extracted with acetone of 80% concentration and the absorbance of the solution is read in a spectrophotometer at wavelengths 663 nm, 646 nm, and 470 nm versus acetone (Boldor et al., 1983; Sumanta et al., 2014).

6.3.2.3.4. Determination of anthocyanin pigment content in

Anthocyanins are extracted with an acidic alcohol solution and the absorbance of the solution is measured in a spectrophotometer at the specific wavelength 515 nm. The reagents used are 70% ethyl alcohol and concentrated HCl (density 1.19) (Artenie and Tănase, 1981).

6.3.2.3.5. Determination of carotenoid pigment content in arils

The plant material is triturated with a mixture of anhydrous sodium sulfate and calcium oxide, which act to retain colored substances other than carotenoid pigments. Anhydrous sodium carbonate is added to the mixture to prevent the decomposition of the carotene pigments in an acid medium. The carotenoid pigments are extracted by crushing with acetone and removal in petroleum ether. The content of carotenoid pigments in the extract obtained is determined spectrophotometrically (Artenie and Tănase, 1981).

6.3.2.3.6. Determination of vitamin C content of arils

In an acidic environment, vitamin C reduces potassium ferricyanide to potassium ferrocyanide, which in the presence of trivalent iron ions forms ferric ferrocyanide or Berlin blue. If fluorine ions are present in the reaction medium, then Berlin blue does not precipitate and a blue solution is obtained, the intensity of which can be measured with a spectrophotometer at a wavelength of 680 nm (Artenie and Tănase, 1981).

6.3.2.3.7. Determination of total polyphenol content in alcoholic extracts

A 1:9 dilution was made from the initial 5% alcoholic extracts and the Folin-Ciocalteu method was used for determinations (Herald et al., 2012).

6.3.2.3.8. Determination of flavonoid content in alcoholic extracts

Determination of flavonoid compound content was carried out on alcoholic extracts diluted 1:9 by the method taken from Jia et al. (1999) and Herald et al. (2012).

6.3.2.3.9. Determination of the antioxidant activity of alcoholic extracts

The method used was taken from Thaipong et al. (2006) and Herald et al. (2012). For the determinations, a 60μM DPPH alcohol solution is prepared and the sample to be analyzed is obtained by mixing 0.1 ml diluted plant extract (1:9) with 2.9 ml DPPH solution. The resulting mixture is incubated in the dark at room temperature for 3 hours. The extinction readings are taken at λ= 515 nm against ethanol.

6.3.2.3.10. Evaluation of the anti-denaturing activity of alcoholic extracts on proteins

Evaluation of the anti-denaturing activity of alcoholic plant extracts on proteins included the use of egg white albumin as reaction substrate. Albumin, upon exposure to high temperatures, denatures an effect that can be assessed by following the change in turbidity of an albumin solution.

*
*   *

All biochemical determinations were carried out in three replicates/triplicates; the results obtained represent their arithmetic mean and are presented in the results chapter of the paper in the form of tables or graphs, and the raw values of all analyses are given in the appendices in tables numbered and named according to the name of the methods applied.

6.3.2.4. Qualitative evaluation of absorption spectra of aqueous and alcoholic hydrosols and extracts

For the qualitative evaluation of the chemical composition of the hydrosols and the aqueous and alcoholic extracts prepared, their UV-vis absorption spectra were determined (Baciu et al., 2013), using a Beckman DU - 730 spectrophotometer in the range 190 - 700 nm. Before evaluating the absorption spectra, it was necessary to dilute the aqueous and alcoholic extracts of 1% and 5% concentration respectively (25µl extract + 675 µl solvent).

6.3.2.5. Determination of the chemical composition of volatile yew oils by GC-MS

The GC-MS (gas chromatography-mass spectrometry) method was used for the qualitative analysis of the volatile oils. The oil samples obtained were diluted in hexane with a dilution of 100 and analyzed using the GC/MS gas chromatograph coupled mass spectrometer/Agilent Series GC/MS system consisting of GC 7890B Gas Chromatograph, MS 5977A System, GS Sampler 80 Injector. The analyses were carried out in the laboratories of the Research Centre for the Study of the Quality of Agrifood Products-HORTINVEST of the Faculty of Horticulture of the University of Agronomic Sciences and Veterinary Medicine of Bucharest.

6.3.2.6. Identification of taxol by high-performance liquid chromatography (HPLC)

The separation, identification, and quantification of taxol from yew acetone extracts were performed by high-performance liquid chromatography (HPLC) using a Shimadzu Prominence HPIM system (2x LC20AD pumps, SIL20AC autosampler, CT20AC oven, SPD M20A DAD detector) coupled to a Chromolith HR RP - C18 end-point column (150 mm long, 4. For HPLC detection, taxol (purity > 95%, HPLC), purchased from PhytoLab (phytolab.com), was used as standard.) For the detection of taxol, acetonitrile was used as mobile phase A and water for mobile phase B, in a ratio of 40:60, according to the method described by Sadeghi-aliabadi et al. (2009). 20µl of the sample was injected for detection, elution was performed at a flow rate of 1 ml/min, and taxol was detected at 227 nm and eluted at 17.57 min. Chromatographic data were obtained using Shimadzu LC solution Software and interpreted manually. Analyses were performed in the Biochemistry and Molecular Biology Laboratory of the Faculty of Biology, "Alexandru Ioan Cuza" University of Iasi.

6.3.2.7. Experimental conditions for testing aqueous extracts

To test the effects of aqueous extracts on the germination process, seeds of *Lycopersicon esculentum* Mill., variety Silvia - tomato, crop species - purchased from Agrosel, and redroot pigweed - *Amaranthus retroflexus* L., broadleaf weed, were used.

Seed sterilization was carried out in two steps: a first step with 2.5% sodium hypochlorite for 3 minutes, followed by three successive rinses with sterile distilled water, and a second step with 3% hydrogen peroxide for 3 minutes, followed by three consecutive rinses with distilled water.

Two sets of experimental variants were organized for each species; each variant consisted of 6 tubes with equal volumes of 5 ml of aqueous extracts of 1% and 5%

concentrations of bark, leaves, arils, and yew seeds, respectively, and one tube of distilled water (control variant). The aqueous extracts prepared from the plant material corresponded to the specific harvesting times (months IV - April, VI - June, IX - September for leaves and bark, respectively, and IX - September for arils and seeds.

Two sets of experimental variants were organized for each species; each variant consisted of 6 tubes with equal volumes of 5 ml of aqueous extracts of 1% and 5% concentrations of bark, leaves, arils, and yew seeds, respectively, and one tube of distilled water (control variant). The aqueous extracts prepared from the plant material corresponded to the specific times of harvesting of the plant material (months IV - April, VI - June, IX - September for leaves and bark, respectively, IX - September for arils and seeds).

After sterilization, the seeds of the test plants (15 *Amaranthus* and 10 *Lycopersicon* seeds for each experimental variant, respectively) were soaked for 24 hours in each working variant. At the end of the imbibition period, the seeds were placed on filter paper in 9 cm diameter Petri dishes previously sterilized in an oven at 180°C for 2 hours to avoid microbial contamination, the handling of the biological material being carried out in a vertical 700 laminar flow hood.

This resulted in 98 experimental variants (48 treatments x 2 test species + 1 control x 2 test species), each of the 98 variants being set up in replicates of three plates. The filter paper in the Petri dishes was moistened with 2 ml of sterile distilled water at the start of the experiment. For the duration of the test, the paper was moistened with 1 ml of distilled water as needed, several times a day, so that the germinating plant material did not dry out and drowning was avoided. Petri dishes with seeds were initially maintained for 24 hours in the dark in a thermostat at 24°C and then for 13 days in the laboratory at room temperature (approximately 26°C). The experiment was 14 days (336 hours), including the 24 hours of imbibition.

6.3.2.7.1. Germination index analysis

The effects of applying aqueous extracts on the germination process in the test species were evaluated by recording the total number of germinated seeds in each working variant at 24-hour intervals throughout the experiment. The values thus obtained were used to calculate the following germination indices according to the formulae described by Boldor et al. (1983), Chiapusio et al. (1997), and Mominul Islam and Kato-Noguchi (2014), the results were calculated as the average of the values obtained in triplicate for each experimental variant.

6.3.2.7.2. Biometric determinations of newly formed seedlings

To evaluate the influence of aqueous extracts on the initial growth of newly formed seedlings through the seed germination process of the test plants, the following seedling parameters were measured: radicle length (mm), hypocotyl length (mm), and seedling mass (g) (Dayan and Duke, 2006). Measurements were made 336 hours after the experiment was set up on 15 seedlings of the shoot and up to 10 seedlings of the tomato, or the maximum number of seedlings available for each variant, using Image.

6.3.3. Statistical analysis

The data reported for all parameters represent the mean value ± standard error (SEM). Statistically significant differences between variables were assessed using one-way ANOVA and Tukey's multiple comparisons tests using GraphPad Prism 9.2.0. Significant differences between variants were considered at $p \leq 0.05$ and are marked on graphs and tables as follows: **** = $p<0.0001$, *** = $p<0.001$, ** = $p<0.01$, * = $p<0.05$.

CHAPTER 7. RESULTS AND DISCUSSION

7.1. Research on the "*in situ*" and "*ex situ*" biology of some taxa of the genus *Taxus* L.

7.1.1. Leaf morphological changes in response to environmental factors

Morphological changes occurring at the foliar level under the action of environmental factors were followed by macroscopic determinations on mature yew leaves, including leaf area (SA), length (LL), width (LW), and length-to-width ratio (LWR), and mean values ± standard error of the mean (SEM) for each taxon investigated are shown in Table 7.1.

Table 7.1. Variation of leaf area and associated parameters in the leaves of the *Taxus* taxa analyzed during June (intense vegetative growth phase)

Analysed parameters	TEST SPECIMENS		
	T1 *Taxus baccata* – spontaneous	**T2** *Taxus baccata* - cultivated	**T3** *Taxus baccata* '*Robusta*' cultivated
Leaf area (SA) mm^2	**73.35** ± 3.17 ****	**38.05** ± 1.51 ****	**50.65** ± 2.8 ***
Leaf length (LL)(mm)	**27.28** ± 0.16 ****	**16.75** ± 0.51 ****	**19.84** ± 0.74 **
Leaf width (LW)(mm)	**3.36** ± 0.09 **	**2.94** ± 0.08	**3.26** ± 0.11
Length-width ratio of leaf (LWR)	**8.19** ± 0.24 ****	**5.77** ± 0.21 ****	**6.16** ± 0.25

Each value represents the calculated mean of twenty independent measurements ± SEM; only statistical differences have been marked with an asterisk: * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$; **** = $p<0.001$

According to data presented in the literature, needle-shaped yew leaves vary in length; they usually reach a 16-25 mm length, but in some trees, they can be as short as 10 mm or as long as 45 mm, and their usual width is 2-3 mm (Hageneder, 2013). In this context, the practical determinations carried out confirmed the data presented in the literature (Dempsey and Hook, 2000; Perrin and Mitchell, 2013), showing that **T1** specimens exhibit high plasticity in terms of leaf morphology, with shade-grown plants having almost double the leaf area of unshaded **T2**, **T3** cultivated specimens.

Preliminary considerations:

- Increasing photoassimilate surface area during the ontogenetic cycle is an important and necessary process for efficient metabolic processes in plants; the rate of leaf area formation in representatives of the genus *Taxus* varies in correlation with the degree of leaf illumination, with higher levels of shading resulting in higher leaf area formation;
- Practical determinations made are consistent with the data presented in the literature on the high plasticity of the surface morphology of the leaf surface of yew specimens, highlighting the degree of adaptation of spontaneous taxon **T1** specimens to the vegetation conditions together with different deciduous species in their area, species that modify quantitatively and qualitatively, through their rich canopy, the light spectrum available to yew trees. The results of the study of the morphology of the leaf surface of yew trees highlight the degree of adaptation of specimens of the spontaneous taxon **T1** to the conditions of vegetation together with different deciduous species in their area, these species altering quantitatively and qualitatively, through their rich canopy, the light spectrum available to yew trees.

7.1.2. Micromorphological and histo-anatomical characteristics of leaves and stems as a function of the action of environmental factors

7.1.2.1 Micromorphological characteristics

- <u>Micromorphology of the upper epidermis surface</u>

The usual epidermal cells are long, rectangular, dimensionally unequal, with relatively straight lateral walls, covered by a smooth cuticle, on which rare local filiform deposits of epicuticular wax/local deposits of amorphous epicuticular wax (very rarely) are observed for the leaf epidermis of the spontaneous specimen (**T1**) (Figure 7.1).

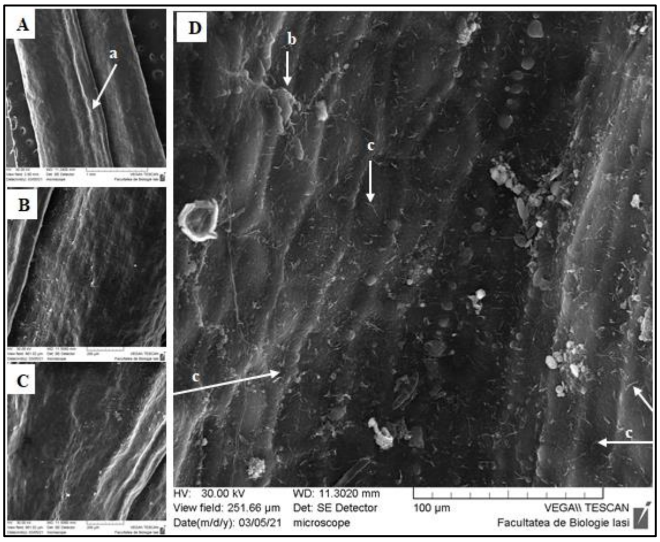


Figure 7.1. Micromorphology of the upper epidermal surface (S.E.M.) – **T1**
A, B, C – upper epidermis; D – upper epidermis detail;
a – midrib; b – amorphous epicuticular wax; c – filiform epicuticular wax

- Micromorphology of the lower epidermis surface

The territorial organization of this epidermis, taking the midrib as a reference point, consists of the presence on either side of the midrib of two bands of stomata, each band containing about 7-8 rows of stomata. Each of the two leaf margins and the stomatal bands consists of a band of ordinary epidermal cells, generally comprising five rows of cells of rectangular outline, radially elongated, covered by a smooth cuticle, devoid of epicuticular wax.

The stomatal apparatus consists of two stomatal cells and the ostiole between them, the attached cells flanking each stomatal cell, the auxiliary epidermal cells immediately adjacent to the attached cells, plus the subsidiary cells surrounding the stomatal pore and forming what is known in the literature as *Florin's ring*. The two stomatal cells and the two attached cells lie below the outer layer of the epidermis and are protected by *Florin's ring*. The genus *Taxus* L. is defined by papilliform hypostatic bands (auxiliary epidermal cells, subsidiary cells) in contrast to the midrib epidermal cells and marginal epidermal band cells (spontaneous specimen **T1**) (Figure 7.2.).

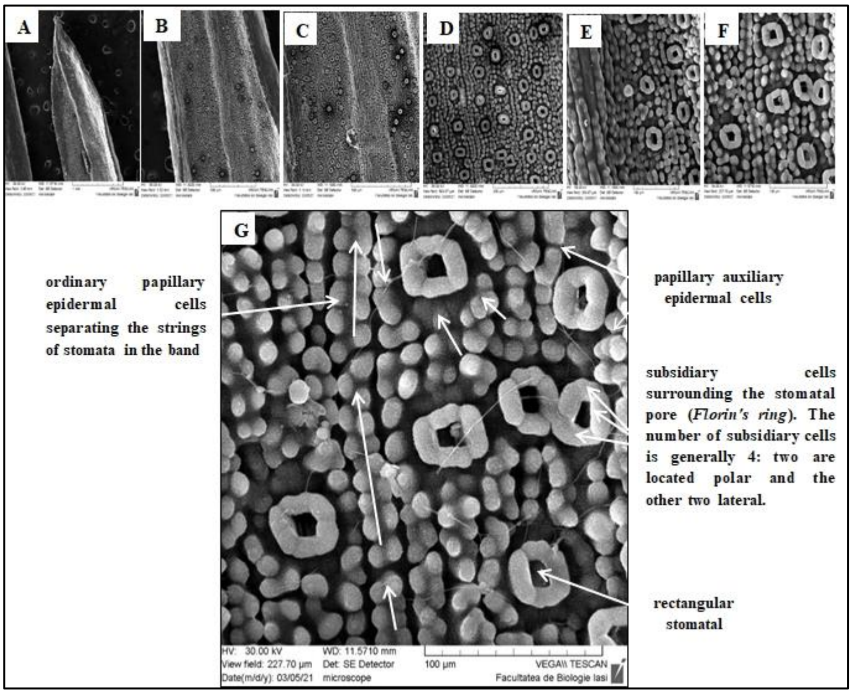


Figure 7.2. Micromorphology of the surface of the lower epidermis (S.E.M.) – **T1**
A, B, C – median nerve; D, E, F – stoma; G – stoma detail

Preliminary considerations:

Following the S.E.M. investigations on the epidermis (upper and lower) of the leaves belonging to the yew specimens under consideration, we can state the following:

- The micromorphology of the upper epidermis is relatively similar in all three variants analyzed (**T1**, **T2**, and **T3**), consisting of long, rectangular, dimensionally unequal epidermal cells with straight lateral walls and outer walls covered by a cuticle of smooth, thin cuticle, on which sparse local filiform deposits of epicuticular wax are observed;
- organization of the lower leaf epidermis is architecturally influenced in all three taxa analyzed by the bands of stomata located on either side of the midrib, an arrangement characteristic of the genus Taxus and with definite micromorphological diagnostic value.
- Sometimes (**T2** and **T3** cultivated variants), phenomena of fluctuating asymmetry in the disposition of their epidermal structures about the midrib correlated with possible stress factors.

7.1.2.2. Histo-anatomical characteristics

♦ *Leaf*

In the analyzed plant material, the histo-anatomical characteristics of the epidermis (upper and lower), mesophyll, and midrib were observed in cross-section through the leaf (Figure 7.3.).

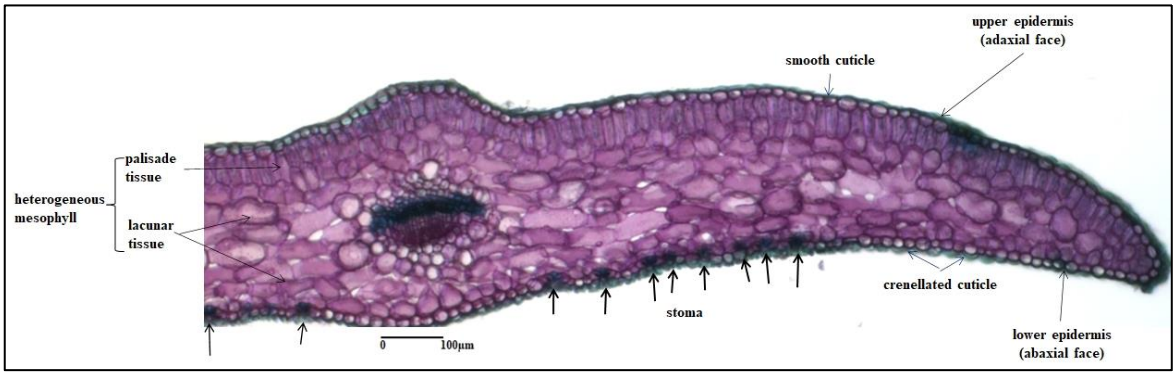


Figure 7.3. Cross section through leaf blade at **T1**

The upper epidermis is unistratified, with slightly radially elongated cells having slightly thicker outer walls than the others and covered by a smooth cuticle.

The heterogeneous mesophyll is differentiated into unistratified, sometimes bistratified palisading tissue with radially elongated but not very tall cells (this tissue occupies about 1/5 of the thickness of the mesophyll) and pluristratified lacunar tissue (4-5 layers of tangentially flattened cells with small aerial spaces - meats - that decrease in size from the upper to the lower epidermis); hence, the leaf blade has a heterofacial (dorsiventral) bifacial structure.

The lower epidermis, also unistratified, has regular epidermal cells, the outer walls of which are covered by a crenelated cuticle, and on either side of the midrib there are two groups of 8 stomata located below the external level of the epidermis; the leaf blade is therefore hypostatic.

The median midrib of the leaf is represented by a single free-lemnoid conducting fascicle, with the wood facing the upper epidermis and the free facing the lower epidermis. The woody is formed by tracheids (imperfect woody vessels, characteristic of gymnosperms in general), and the free is represented by ridged cells arranged in radial rows. Small expansions of tracheid woody parenchyma are observed on either side of the conducting bundle.

Preliminary considerations:

Correlating literature data with our results, from a histo-anatomical point of view, the yew leaf has the following **distinct seven features of diagnostic value**:

- is hypostatic, the stomata being arranged in bands in varying numbers per band on either side of the midrib;
- has a dorsiventral (heterofacial) bifacial structure;
- Leaf elasticity and flexibility are due to the absence of lignified sclerenchyma hypodermis;
- Resiniferous channels are missing;
- The midrib is represented by a single free-woody conducting bundle bounded by a parenchymal endoderm, easily observed;
- Transfusion tracheal parenchyma is poorly represented and is seen mainly in the lateral part of the leading bundle in the form of small expansions that exceed the limits imposed by the perfectly overlapping liberian tissue over the woody tissue;
- The crenelated appearance of the cuticle is because it molds to the external wall protrusions of the lower epidermal cells (papillary cells).

♦ *Stem (branch)*

At the level analyzed, the outline of the cross-section is irregular, comprising ribs of different sizes (two of them widest and two, three others of smaller amplitude) separated by valleys of medium depth.

The epidermis is unistratified, with small, slightly tangentially flattened cells, the outer walls thicker than the others, and covered by a thin, relatively thin cuticle. In places, stomata are observed slightly below the external level of the epidermal cells, with a discrete suprastomatal chamber (Figure 7.4.).

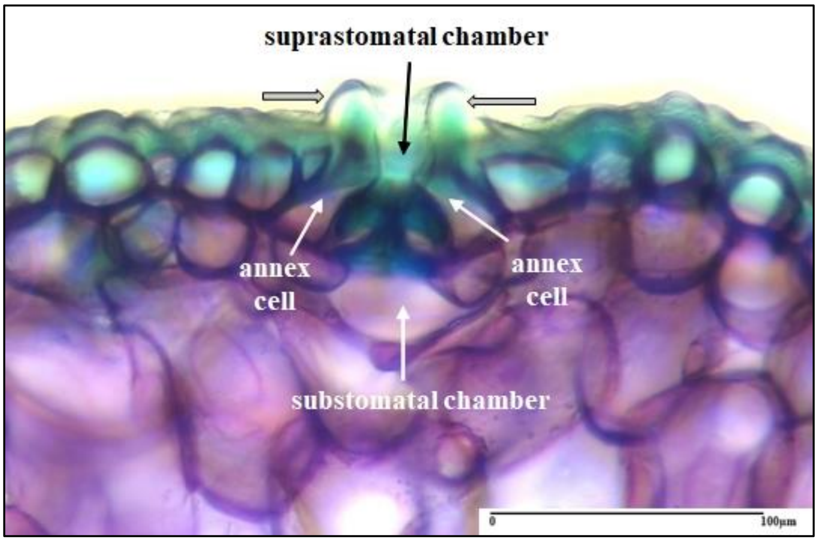


Figure 7.4. Stomata from the epidermis of the branch of **T1** (detail)

The bark is differentiated into three distinct sub-areas:

- external, unistratified, with small, polygonal cells with all walls slightly thickened and lignified;
- middle, multilayered, with large, radially elongated, rounded cells leaving meaty aerial spaces between them (in places, these cells appear disorganized, tangentially flattened, forming small aerial cavities);
- internal, unistratified, constituting an amyloid endodermoid, continuous around the secondary free ring.

A central cylinder has already passed to the secondary structure on account of the activity of a cambial that, operating bifacially, has generated outwards a ring of secondary free, which pushes the islands of primary free with flattened, hardly identifiable elements. The (primary and secondary) vacuole consists of flattened ridged cells arranged in radial rows. The inwardly formed secondary wood consists only of tracheids (homoxyl wood) arranged in radial rows, separated in places by uniseriate medullary rays with radially elongated cells with lignified walls. The cambium in the material analyzed has been functioning for two successive years, as two annual rings with spring tracheid and autumn-winter tracheid are distinguished. Small islands of primary wood (9-10) are observed towards the pith, projecting into the medullary parenchyma, which means that in the primary structure, a central cylinder contained 9-10 conducting collateral-open fascicles (Figure 7.5).

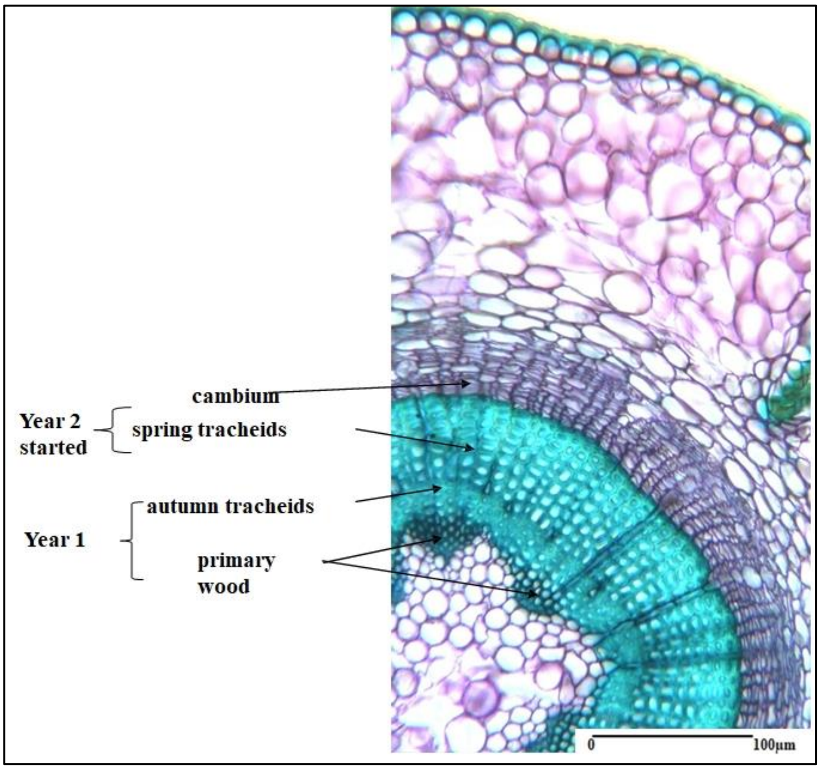


Figure 7.5. Cross section through stem at **T1** (sector; detail)

The marrow has a trapezoidal-uneven outline and is parenchymatous, with rounded cells that increase in size from the periphery inwards (Figure 7.6.).

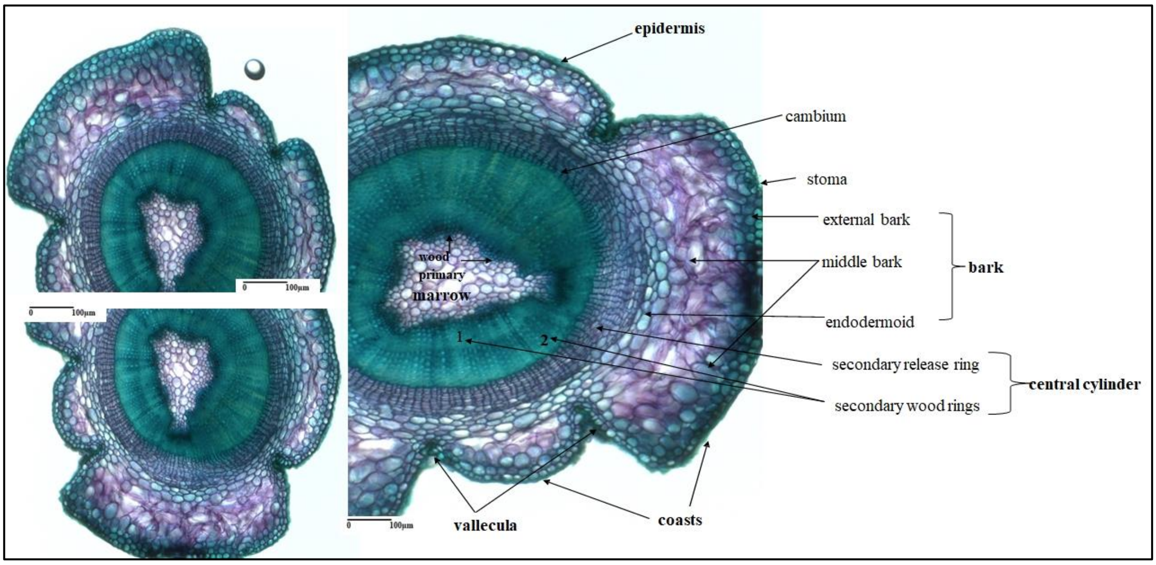


Figure 7.6. Cross section through the stem at **T1**

Preliminary considerations:

Correlating the literature data with our results, from a histo-anatomical point of view, the branches between 1 and 3 years of age of the yew show the following three distinct characteristics:

- irregular contour (in cross-section) with two coasts of larger amplitude, alternating with 2-3 ribs of smaller amplitude in all variants investigated (**T1**, **T2**, and **T3**);
- local disorganization of the middle bark, with the formation of small aerial cavities; exceptionally in the variant of the **T2**, the disorganization of the assimilatory parenchyma already in the first year of life of the branch may be due to an exaggerated supply of water, either by artificial sprinkling or by natural rainfall increased in quantity in a short period or to a chemical treatment applied against fungi/pests;
- the evolution of the woody body goes from the initial presence of few uniseriate medullary rays to numerous medullary rays, ending with its fragmentation into two islands with concavities facing each other by two multiseriate secondary medullary rays (at the age of 3 years this aspect is already generalized).

7.1.3. Physiological responses at leaf level in correlation with internal leaf factors, leaf surface factors, external (environmental) factors

Photosynthesis, as a physiological process, is regulated simultaneously by several external factors related to the quality of the plant's living environment, such as light, temperature, humidity, availability of CO_2 and other gases, quantity and quality of nutrients (Teskey et al., 1995, cf. Wieser, 2007), but also several internal factors that determine the rate of photosynthesis that can be achieved *in situ* during a growing season at a given ambient CO_2 concentration and under optimal conditions of temperature, light irradiance, water vapor pressure deficit, and soil water availability (Larcher, 2001, cf. Wieser, 2007).

In the investigations carried out on the taxa under study, we followed the simultaneous evolution of the three fundamental physiological processes (photosynthesis, transpiration, and respiration) during three defining moments of the phenological cycle of the yew, representing their evolution graphically (Figure 7.7.).

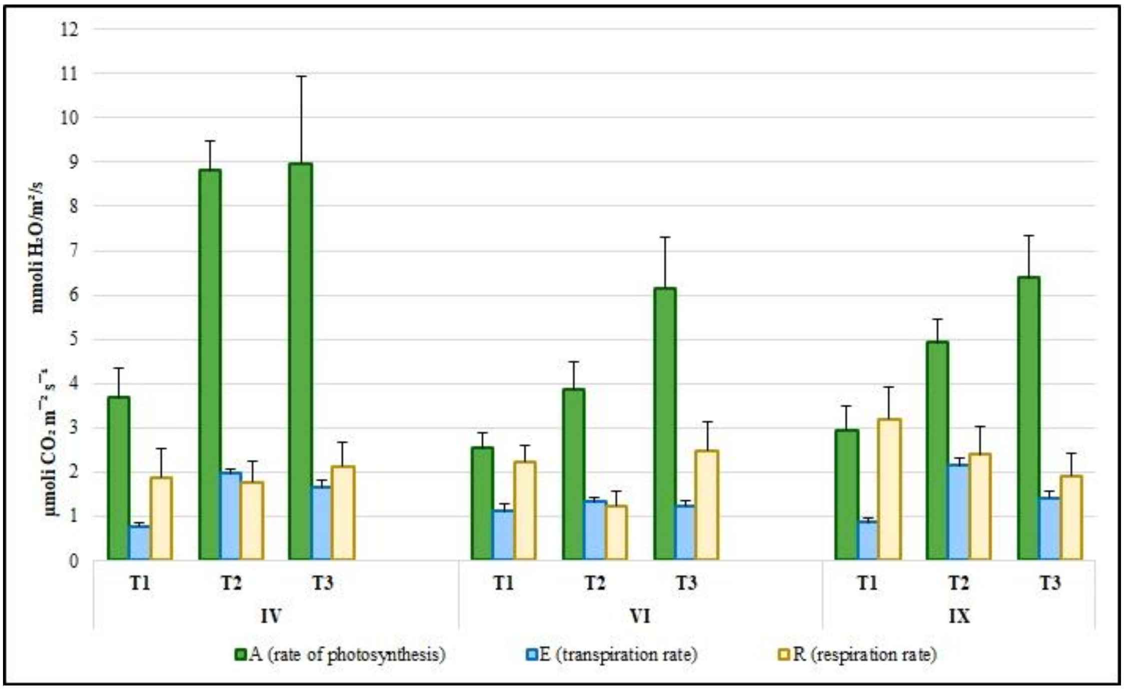


Figure 7.7. Variation in fundamental physiological processes in taxa of the genus *Taxus* L. during the proper ontogenetic cycle of vegetation corresponding to the calendar year 2021
(IV- April; VI- June; IX- September)

Preliminary considerations:

Following the research carried out on the plant material available and under the given growing/cultivation conditions, we can state that the physiological and biochemical determinations carried out in <u>the field</u> - "*in vivo*" and in <u>the laboratory</u> aimed at completing the functional picture of the taxa of the genus *Taxus* investigated, in correlation with the environmental conditions available in the habitat where they grow spontaneously or are cultivated, during three defining moments of their ontogenetic cycle in the 2021 growing year; the interpretation of the data thus obtained allows us to highlight the following aspects:

- the content of foliar photoassimilate pigments shows quantitative variations depending on the biological particularities of the investigated taxa, these variations being determined by the information baggage of the respective plant organisms, by the metabolic level specific to the ontogenetic moments captured in the course of their life cycle, as well as by the variation of microclimatic factors (light, temperature, atmospheric humidity);
- the degree of hydration and leaf dry matter content determined reveals that the plant individuals analyzed carry out fundamental physiological processes (photosynthesis, respiration, transpiration) with increasing intensity towards the time of fruiting (aril ripening) and that these processes will continue to provide them with the biological basis for existence and survival during the final phases of the ontogenetic cycle (senescence), as well as resistance, through underground organs, from one year of vegetation to the next;

7.1.4. Variation in the content of biochemical compounds in arils in response to environmental factors

For species of the genus *Taxus*, oval-shaped arils with a diameter of about 10 mm and a gelatinous texture surrounding the single seed are the only non-vegetable part of the plant (Sârbu et al., 2013) and are frequently described as edible, fleshy, and energy-rich (Cope, 1998; Herrera, 1987, cf. Schex et al., 2021; Dumitraș et al., 2022).

7.1.4.1. Anthocyanin pigments (anthocyanins) content

Analyses carried out on the arils harvested from the taxa studied (Figure 7.8.) show that the arils harvested from taxon **T3** record a significantly higher quantity of anthocyanins, 37.45 mg/g fresh material, unlike the other two taxa investigated, which record 24. 85 mg/g and 22.70 mg/g fresh material (**T1** and **T2**, respectively), which are in practical agreement with the literature, which indicates anthocyanin pigments in the range of 33.3 - 59.3 mg/g fresh material (Tabaszewska et al., 2021).

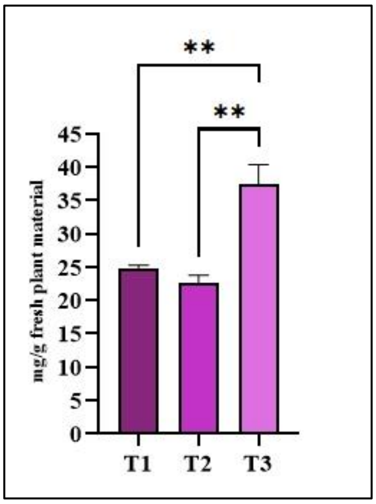


Figure 7.8. Variation in anthocyanin/anthocyanin pigment content of arils of taxa of the genus *Taxus* L. analyzed

7.1.4.2. Carotenoid pigments (carotenes) content

The data obtained practically from the biological material analyzed (Figure 7.9.) show that the arils harvested from the cultivated taxon **T3** have the highest carotene content (4.29 mg/100g fresh material), followed by the other cultivated taxon analyzed (**T2**), with a value of 3.30 mg/100g and, finally, by the spontaneous taxon **T1**, with the lowest content of 1.87 mg/100g fresh material.

The practical results thus obtained agree with the data presented in the literature consulted, respectively a content ranging between 3.30 - 5.42 mg /100g fresh material in the case of the study conducted by Tabaszewska et al. (2021), which concerned Taxus taxa from 4 localities in Poland, and with the data obtained by Dumitraș et al. (2022) for yew arils collected from 3 different localities in Cluj - Napoca, the taxa presented a carotene content that varied between 3.37 - 3.38 mg /100g fresh material.

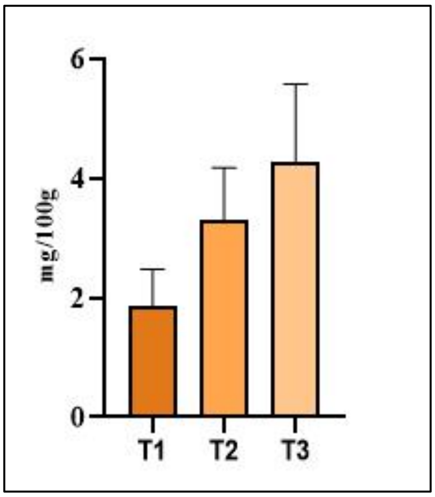


Figure 7.9. Variation in carotenoid/carotene pigment content of arils of *Taxus* L. taxa analyzed

7.1.4.3. Vitamin C content

The ascorbic acid content identified in the arils of the yew taxa analyzed (Figure 7.10.) records approximately the same value in all three taxa investigated: ascorbic acid 3.16 mg/100 g fresh material at **T3**, or 3.16 mg/100 and 3.33 mg/100 g at **T1** and **T2**, respectively.

The literature consulted reports an ascorbic acid content in yew arils ranging from 60.7 - 145 mg/ 100 g fresh plant material for arils collected from four different locations in Poland, with differences in the content of this compound explained as probably determined by variation in various environmental conditions such as temperature, water availability, intensity of natural lighting and exposure to the action of air currents (wind) (Cocco et al., 2015, cf. Tabaszewska et al., 2021).

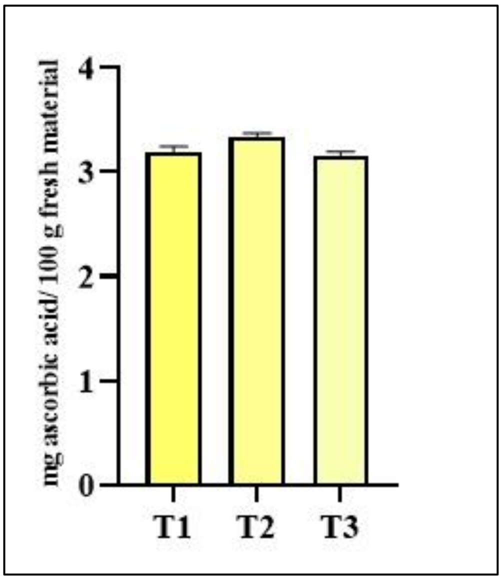


Figure 7.10. Variation in vitamin C content of arils of taxa of the genus *Taxus* L. analyzed

Preliminary considerations:

Biochemical investigations carried out on plant material (arils) collected from the three taxa of the genus *Taxus* taken in the study lead us to state the following:

- the anthocyanin content varies significantly between taxa, with the arils collected from the cultivated taxon **T3** recording the highest amount (37.45 mg/ g fresh material), data that correspond to the values reported in the literature consulted;
- the arils collected from the cultivated taxon **T3** also showed the highest quantity of carotenes, followed by the arils collected from taxa **T2** and **T1**;
- the ascorbic acid content in the arils of the three taxa analyzed showed roughly equal values in all taxa analyzed;

7.2. Investigations on the "in situ" and "ex situ" biochemical composition of some taxa of the genus Taxus **L.**

7.2.1. Biochemical composition of volatile oils extracted from leaves

Volatile oils are naturally volatile compounds with complex structures. They are transparent, rarely colored, soluble in organic solvents, with a density less than the density of water, and fat-soluble. They protect plants against attacks by weeds, bacteria, viruses, and fungi and attract pollinators (Bakkali et al., 2008).

Analyzing data obtained by GC/MS analysis of volatile oils extracted by hydrodistillation from available plant material, it is considered that, due to their chemical composition, volatile oils are of importance in different economic branches, such as pharmaceuticals, cosmetics, or agriculture.

Thus, for the spontaneous yew species, **T1**, several 32 chemical compounds were detected, of which 31 were identified and are presented in Table 7.3 in the case of the oil obtained from the leaves of the cultivated *Taxus baccata* L. taxon (**T2**), 42 chemical compounds were detected and identified, and the volatile oil obtained from the leaves of the cultivated taxon **T3** (*Taxus baccata ' Robusta'*) showed several 36 chemical compounds.

Table 7.3. Percentage chemical composition of volatile oils obtained from leaves of studied wild and cultivated yew taxa harvested in the intense vegetative growth phenophase (June 2023)

No	Compound	Chemical formula	Retention time (RT)	Percentage (%)		
				T1	*T2*	*T3*
1.	2-Butene-1,4-diol	$C_4H_8O_2$	11.882	-	1.7	-
2.	1-Octen-3-ol	$C_8H_{16}O$	12.186	1.03	25.61	-
3.	3-Octanol	$C_8H_{18}O$	13.048	-	0.21	-
4.	Octanol	$C_8H_{18}O$	16.660	1.8	2.73	0.92
5.	Linalool	$C_{10}H_{18}O$	17.912	-	0.19	1.38
6.	Nonanal	$C_9H_{18}O$	18.123	-	0.42	-
7.	α-Terpineol	$C_{10}H_{18}0$	21.593	-	-	0.59
8.	Methyl salicylate	$C_8H_8O_3$	21.724	-	0.68	-
9.	Myrtenol	$C_{10}H_{16}O$	21.816	-	1.83	0.31
10.	Nonane	C_9H_{20}	22.038	-	-	0.21
11.	p-Menth-2-en-7-ol, cis	$C_{10}H_{18}O$	22.170	-	0.45	-
12.	Nerol	$C_{10}H_{18}O$	23.084	-	0.91	0.24
13.	p-Mentha-1(7),8(10)-dien-9-ol	$C_{10}H_{16}O$	23.810	-	4.05	-

No	Compound	Chemical formula	Retention time (RT)	Percentage (%)		
				T1	*T2*	*T3*
14.	Geraniol	$C_{10}H_{18}O$	24.094	-	1.16	0.6
15.	trans-2-Decenal	$C_{10}H_{18}O$	24.325	0.64	1.27	0.65
16.	1-Decanol	$C_{10}H_{22}O$	24.784	2.68	0.76	0.53
17.	Perilla alcohol	$C_{10}H_{16}O$	25.645	-	055	-
18.	Tridecane	$C_{13}H_{28}$	25.713	2.02	-	2.02
19.	Eugenol	$C_{10}H_{12}O_2$	27.708	-	1.1	-
20.	Farnesane	$C_{15}H_{32}$	28.331	1.25	-	1.46
21.	β-Elemene	$C_{15}H_{24}$	28.862	-	-	2.91
22.	Tetradecane	$C_{14}H_{30}$	29.108	3.7	-	4.16
23.	Benzene. 1,2,4-trimethyl-	C_9H_{12}	29.240	-	0.71	-
24.	10-Undecin-1-ol	$C_{11}H_{20}O$	30.646	-	1.4	-
25.	1.9-Decadiyne	$C_{10}H_{14}$	30.793	-	7.92	-
26.	*Neidentificat*		31.022	4.02	-	-
27.	Germacrene D	$C_{15}H_{24}$	31.726	-	5.13	-
28.	a-Bergamotene	$C_{15}H_{24}$	32.160	-	3.36	3.19
29.	α-Bergamotene	$C_{15}H_{24}$	32.275	1.42	-	-
30.	Humulene	$C_{15}H_{24}$	32.474	-	-	5.87
31.	a-Farnesene	$C_{15}H_{24}$	32.562	-	-	6.47
32.	α-Farnesene	$C_{15}H_{24}$	32.566	0.9	5.4	
33.	cis-α-Bisabolene	$C_{15}H_{24}$	33.606	0.59	1.8	-
34.	Nerolidol	$C_{15}H_{26}O$	34.218	3.02	0.38	0.55
35.	cis-3-Hexenyl benzoate	$C_{13}H_{16}O_2$	34.429	-	0.46	-
36.	Hexadecane	$C_{16}H_{34}$	35.252	0.5	-	-
37.	Pentadecanal	$C_{15}H_{30}O$	35.664	0.79	-	-
38.	α-Bisabolol	$C_{15}H_{26}O$	37.732	-	0.33	-
39.	Heptadecane	$C_{17}H_{36}$	38.068	0.57	-	-
40.	Hexadecanal	$C_{16}H_{32}O$	38.473	1.16	-	-
41.	Hexa-hydro-farnesol	$C_{15}H_{32}O$	38.913	2.26	-	-
42.	Hexa-hydro-farnesol	$C_{15}H_{32}O$	38.927	-	0.4	1.1
43.	Benzyl Benzoate	$C_{14}H_{12}O_2$	39.847	-	0.84	-

No	Compound	Chemical formula	Retention time (RT)	Percentage (%)		
				T1	***T2***	***T3***
44.	Octyl caprylate	$C_{16}H_{32}O_2$	40.196	-	-	0.35
45.	1,14-Tetradecanediol	$C_{14}H_{30}O_2$	41.173	0.9	-	-
46.	Hexahydrofarnesyl acetone	$C_{18}H_{36}O$	41.966	33.03	5.15	17.81
47.	Salicylic acid, benzyl ester	$C_{14}H_{12}O_3$	42.568	0.87	0.93	-
48.	Nonadecane	$C_{19}H_{40}$	43.297	0.96	-	-
49.	Isophytol	$C_{20}H_{40}O$	44.484	0.72	-	-
50.	Phthalic acid, hex-3-yl isobutyl ester	$C_{18}H_{26}O_4$	44.930	1.4	-	-
51.	geranyl-α-terpinene	$C_{20}H_{32}$	45.025	-	1.71	-
52.	Manoyl oxide	$C_{20}H_{34}O$	46.123	-	10.86	1.46
53.	aR-abietatriene	$C_{20}H_{30}$	47.152	3.03	0.53	1.16
54.	Heneicosane	$C_{21}H_{44}$	48.095	2.52	0.55	2.96
55.	Phytol	$C_{20}H_{40}O$	48.397	0,97	0.87	0.49
56.	Docosane	$C_{22}H_{46}$	50.281	1,92	0.69	3.63
57.	Tricosane	$C_{23}H_{48}$	52.414	6.36	1.72	8.45
58.	Tetracosane	$C_{24}H_{50}$	54.469	3.1	0.93	5.34
59.	Pentacosane	$C_{25}H_{52}$	56.443	5.04	1.31	7.37
60.	Hexacosane	$C_{26}H_{54}$	58.342	3.45	0.93	6.57
61.	Heptacosane	$C_{27}H_{56}$	60.180	7.37	2.11	11.27
TOTAL				**100**	**100**	**100**

7.2.1.1. Qualitative evaluation of hydrosols/hydrosols

Hydrosols/hydrosols are the secondary compounds of hydrodistillation of different parts of aromatic plants, which separate from the volatile oil phase at the end of the distillation process (Politi et al., 2022). In the literature, hydrosols are found under various names, including hydrolate, hydrochlorate, hydroflorate, aromatic water, floral water, and essential aromatic water (Rajeswara Rao, 2013; D'Amato et al., 2018).

To qualitatively estimate the composition of the hydrosols resulting from the hydrodistillation of leaves collected from the three yew taxa studied, which were in the period of intense vegetative growth (VI), a spectrophotometric analysis of their hydrosols was performed (Figure 7.11.); this revealed higher values of the concentration of constituent compounds corresponding to the 220 - 240 nm region of the spectral region, an area indicating the presence of aromatic compounds, and in the 260 - 280 nm region, an area corresponding to the presence of phenolic compounds (Kumar, 2006; Pretsh et al, 2009; Butnariu, 2014). Of the three yew taxa analyzed, the cultivated species **T2** shows the highest absorbance, especially in the 220 - 240 nm region, so its hydrosol can be considered the most concentrated in aromatic compounds.

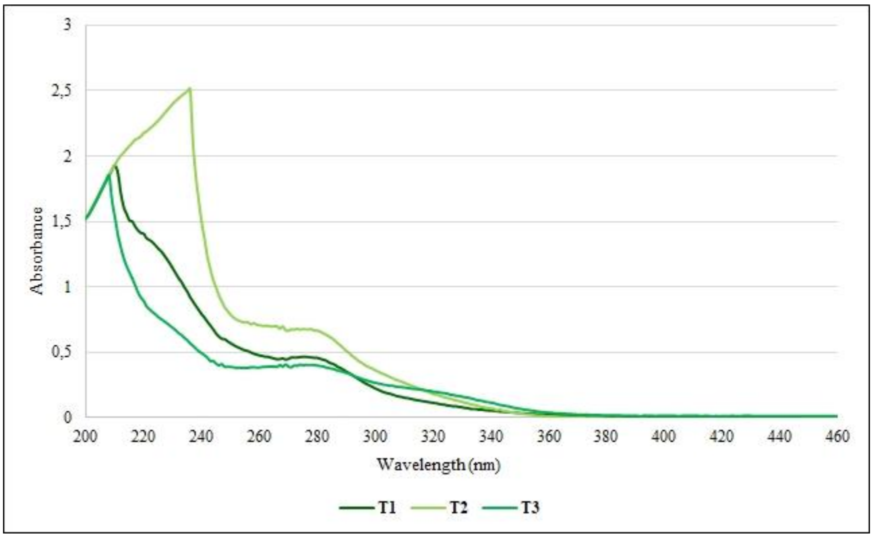


Figure 7.11. Absorption spectra of hydrosols obtained by hydrodistillation of leaves of the three *Taxus* taxa analyzed

Preliminary considerations:

Following the quantitative and qualitative analysis of the volatile oils and hydrosols obtained from the leaves of the *Taxus* taxa under consideration, we can deduce the following:

- the chemical composition of the volatile oil extracted from leaves harvested during the period of intense vegetative growth (VI) from the three taxa studied showed qualitative and quantitative variations:
    - oil extracted from spontaneous taxon **T1** showed 31 compounds, of which the highest concentration was hexahydrofarnesyl acetone (33.03%);
    - the oil extracted from the cultivated taxon **T2** contained 42 chemical compounds in its composition, the compound 1-Octen-3-of having the highest concentration (25.61%);
    - oil extracted from cultivated taxon **T3** recorded 36 chemical compounds in its constitution, the highest concentration being reached, as in the case of spontaneous taxon **T1** by hexahydrofarnesyl acetone (17.81%);

- the main groups of chemical compounds identified in the hydrodistillation volatile oils of the yew taxa studied were alkanes (48.81%), alcohols (45.34%), and hydrocarbons (31.04%);
- the qualitative evaluation of the hydrosols obtained by hydrodistillation, carried out by analyzing their absorption spectra, indicates the presence in their composition of aromatic compounds, identified in the 220-240 nm region, and phenolic compounds, detected in the 260-280 nm region of their absorption spectra.

7.2.2. Identification and isolation of taxol from acetone extracts

In the practical determinations performed within the present research theme, acetone extracts were prepared from the leaves, bark, seeds, and arils of the three *Taxus* taxa under consideration, with the biological material being harvested during the phenological periods specific to their ontogenetic cycle.

The extracts were subjected to taxol identification analysis by high-performance liquid chromatography (HPLC) using a taxol Standard with a retention time (RT) of 17.570 and wavelength of 229 nm for comparison of results (Figure 7.12.).

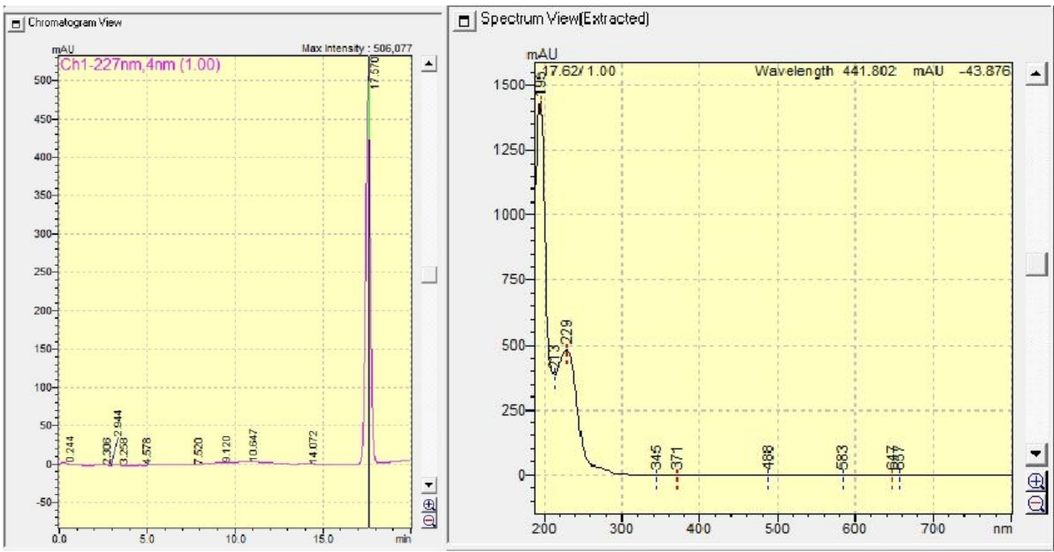


Figure 7.12. HPLC chromatogram and absorption spectrum of taxol used as working standard (concentration 5mg/ml; May 2023)

The only valid result obtained in the determinations carried out was for the extract prepared from the leaves of spontaneous taxon **T1**, corresponding to the period of formation of the strobili (April); the chromatogram thus obtained revealed, in addition to the taxol identified, corresponding to retention time 17.560 and wavelength = 225 nm, the presence of other compounds presents in the extract under analysis (Figure 7.13.).

This research approach validates the method used for the extraction, identification, isolation, and quantification of taxol (Sadeghi-aliabadi et al., 2009), a methodology taken from the literature, the impediments encountered during the processing of the samples taken in work being probably caused by the low concentration of the acetone extracts analyzed, which requires improvement at the time of resuming the determinations, to allow a precise detection of this compound on the working column of the HPLC existing in the Biochemistry Laboratory of our faculty and a correct calculation of its concentration in the newly prepared extracts.

The chromatogram obtained for the analyzed extract showed, in addition to the taxol identified at retention time 17.560 and wavelength = 225 nm, the presence of other compounds in the extracted extract (Figure 7.13.).

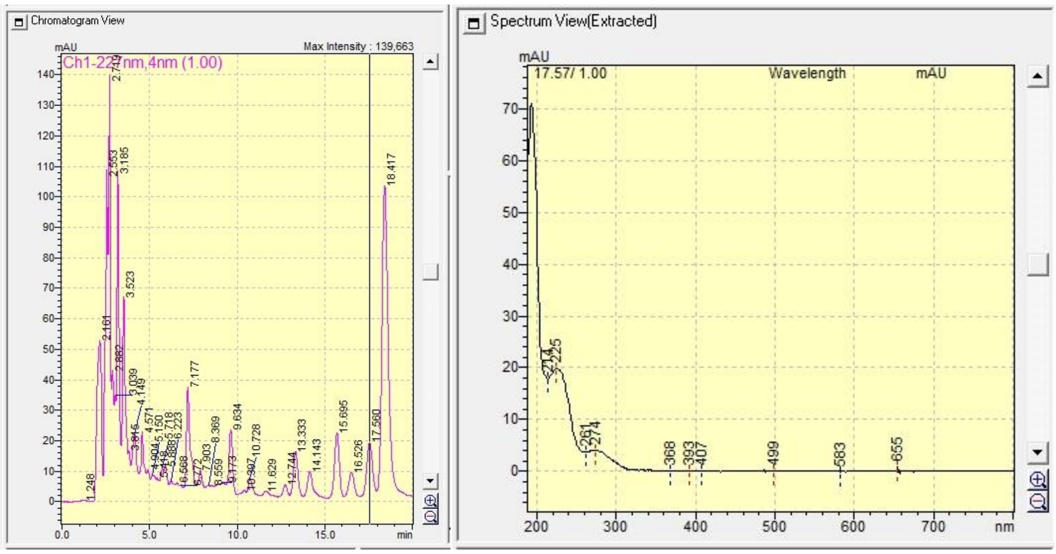


Figure 7.13. HPLC chromatogram and absorption spectrum of taxol identified in leaves of spontaneous taxon (T1) - period of strobili formation (VI-April)

Preliminary considerations:

- The information presented in the literature indicates the presence of low concentrations of taxol, a compound with recognized medicinal properties, in yew trees growing in different parts of the world, including Romania.
- The costs of extracting and purifying the natural product and the large amount of plant material required as raw material to obtain it from the organs of yew trees support the usefulness of developing and perfecting modern methods of chemical synthesis of this compound.
- Through the partially positive result obtained, the working method applied for the identification, isolation, and quantification of taxol present in the plant material

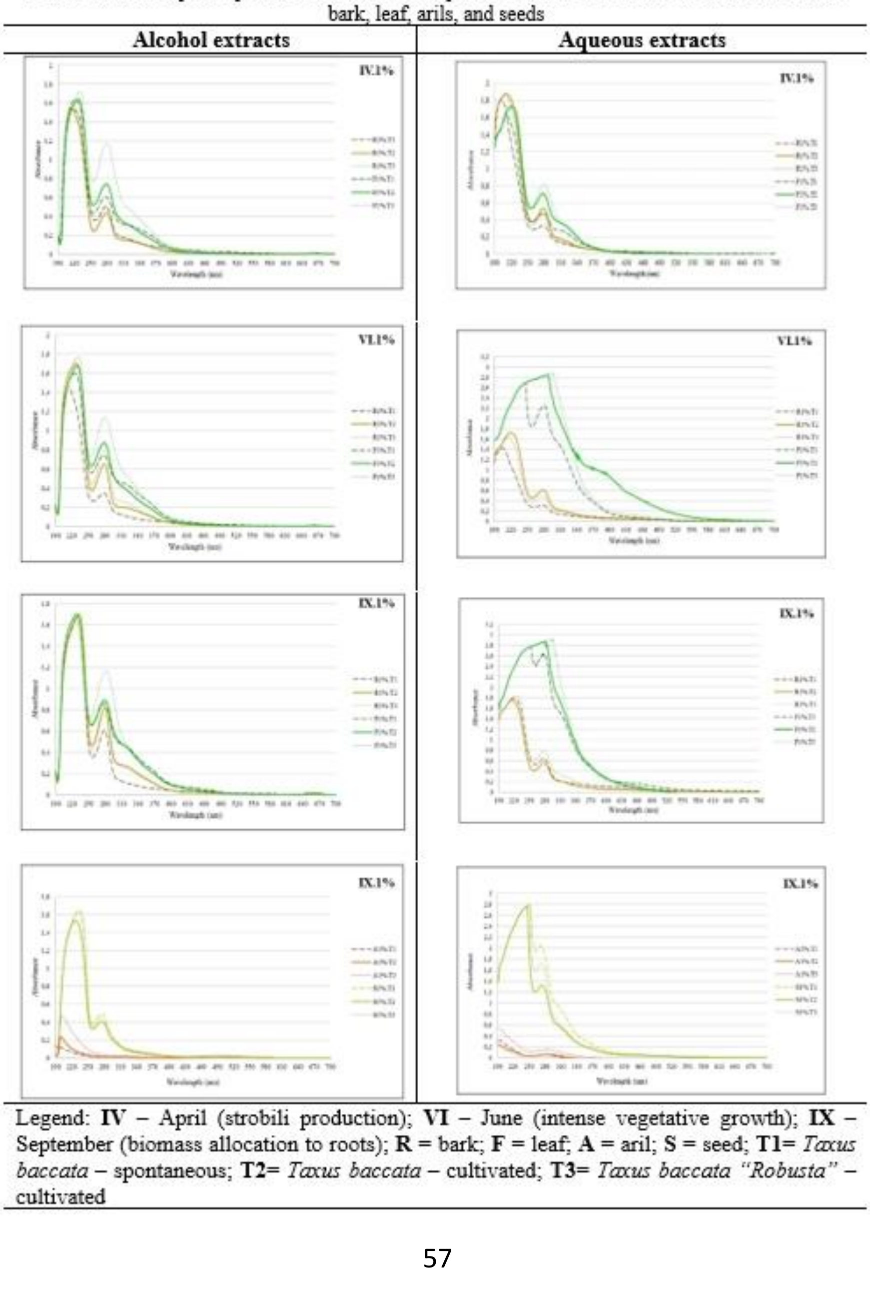
(vegetative and reproductive organs belonging to the three yew taxa analyzed) is valid and replicable under the conditions of improving the concentration of the plant extract for analysis, such a research approach not yet reported in the Romanian literature.

7.3. The evaluation of qualitative, quantitative, and possible effects of some plant extracts obtained from taxa of the genus Taxus L.

7.3.1. Qualitative evaluation of extracts from vegetative and reproductive organs

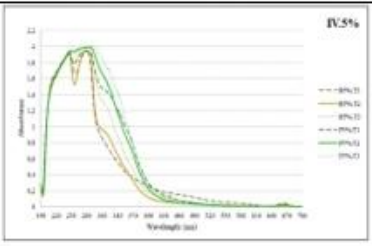
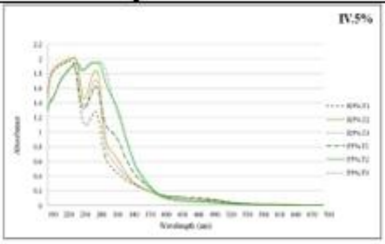
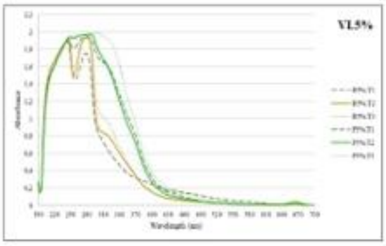
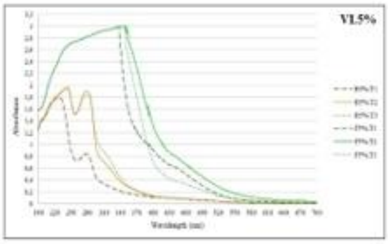
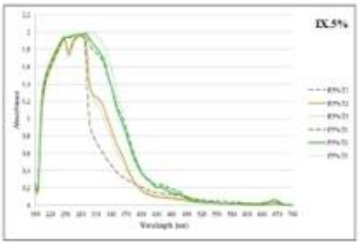
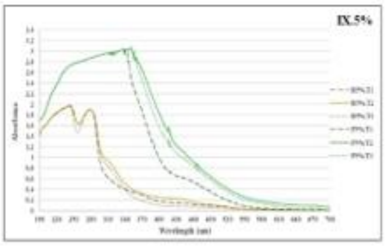
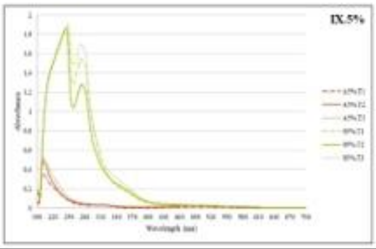
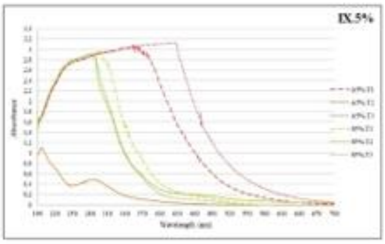
Spectrophotometric analysis of aqueous and alcoholic yew extracts was performed in the UV-visible range (190 - 700 nm). The values obtained indicate the presence of several types of compounds, with variations according to taxon, season/time of investigation, organ, extract type, and extract concentration (Tables 7.5. and 7.6.).

Table 7.5. Absorption spectra of alcoholic and aqueous extracts of 1% concentration from *Taxus* bark, leaf, arils, and seeds



Legend: **IV** – April (strobili production); **VI** – June (intense vegetative growth); **IX** – September (biomass allocation to roots); **R** = bark; **F** = leaf; **A** = aril; **S** = seed; **T1**= *Taxus baccata* – spontaneous; **T2**= *Taxus baccata* – cultivated; **T3**= *Taxus baccata "Robusta"* – cultivated

Table 7.6. Absorption spectra of alcoholic and aqueous extracts of 5 % concentration from *Taxus* bark, leaf, arils, and seeds

Alcohol extracts	Aqueous extracts
	
	
	
	

Legend: **IV** – April (strobili production); **VI** – June (intense vegetative growth); **IX** – September (biomass allocation to roots); **R** = bark; **F** = leaf; **A** = aril; **S** = seed; **T1**= *Taxus baccata* – spontaneous; **T2**= *Taxus baccata* – cultivated; **T3**= *Taxus baccata "Robusta"* – cultivated

7.3.2. Quantitative evaluation of alcoholic extracts

7.3.2.1. Total polyphenol content of alcoholic extracts

The practical results obtained during the present experiment on the total polyphenol content of the alcoholic extracts analyzed showed variations between taxa, organs, and analysis times (Figure 7.14.).

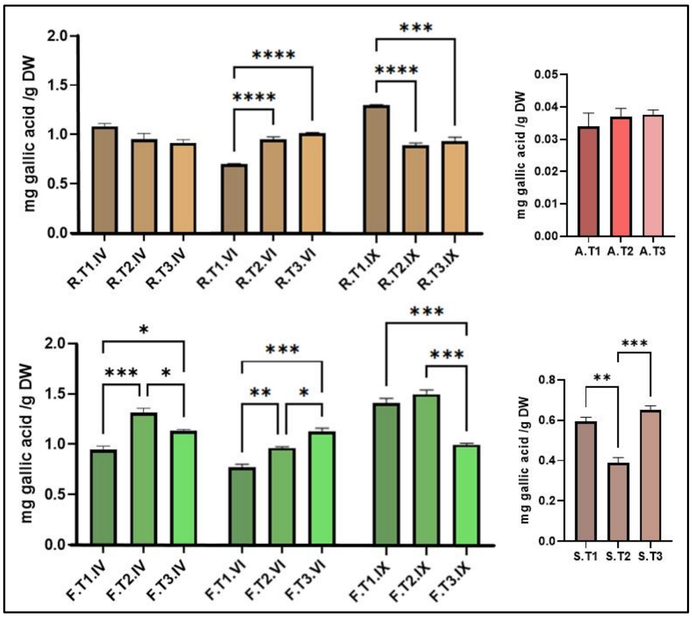


Figure 7.14. The total polyphenol content of *Taxus* taxa analyzed
R = bark; **F** = leaf; **A** = aril; **S** = seed; **T1** = *Taxus baccata* - spontaneous; **T2** = *Taxus baccata* - cultivated; **T3** = *Taxus baccata 'Robusta'* - cultivated; **IV** - April (strobili formation); **VI** - June (intense vegetative growth); **IX** - September (biomass allocation to roots)

7.3.2.2. Total flavonoid content of alcoholic extracts

The practical results obtained during the experiments carried out under the present research theme (Figure 7.15.) show that the bark and leaves of *Taxus* are the most flavonoid-rich organs.

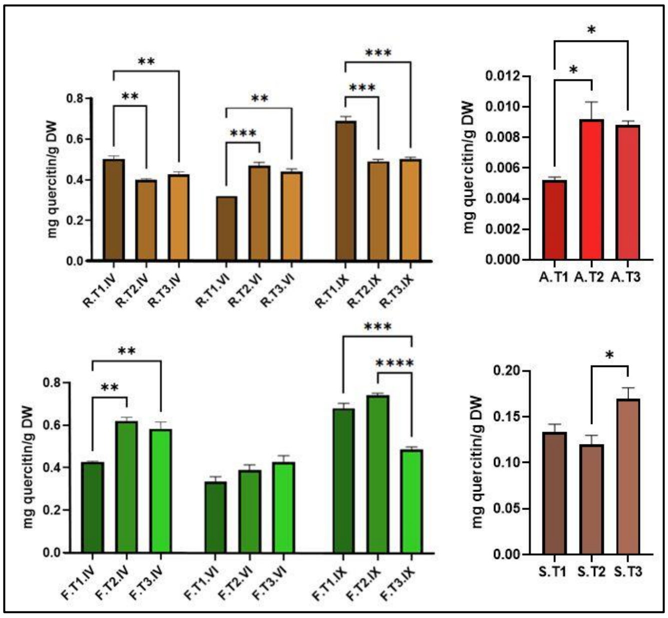


Figure 7.15. The total flavonoid content of *Taxus* taxa analyzed
R = bark; **F** = leaf; **A** = aril; **S** = seed; **T1** = *Taxus baccata* - spontaneous; **T2** = *Taxus baccata* - cultivated; **T3** = *Taxus baccata 'Robusta'* - cultivated; **IV** - April (strobili formation); **VI** - June (intense vegetative growth); **IX** - September (biomass allocation to roots)

Significant quantities of such compounds are found in the plant material analyzed during the autumn (9th - September) for the spontaneous taxon **T1** and the cultivated taxon **T2**. At the same time, the seeds of the cultivated taxon **T3** show significantly higher flavonoid contents compared to the other two taxa studied, and the arils of the cultivated taxa **T2** and **T3** show significantly higher contents compared to the spontaneous taxon **T1**.

7.3.3. Evaluation of antioxidant and anti-denaturing effects of alcoholic extracts

7.3.3.1. Antioxidant activity of alcoholic extracts

The antioxidant activity and its intensity in plants depend on the existence of different compounds in the respective plant species. The antioxidant and free radical scavenging activities of flavonoid compounds in plant organisms are well studied and reported in the literature (Das et al., 1993).

Data obtained practically in our research using alcoholic extracts from various organs of the Taxus taxa studied (Figure 7.16.) show the highest values of antioxidant activity for extracts obtained from leaves, followed by those from bark, seeds, and finally arils.

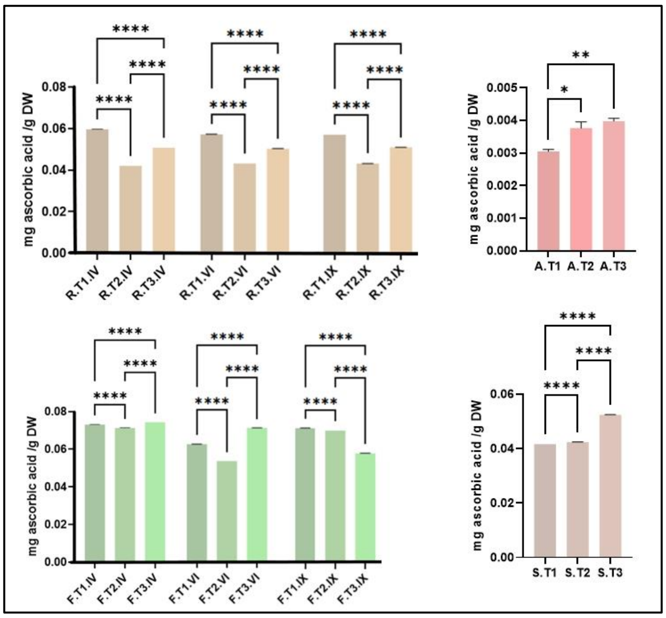


Figure 7.16. Antioxidant activity of alcoholic extracts in *Taxus* taxa tested
R = bark; **F** = leaf; **A** = aril; **S** = seed; **T1** = *Taxus baccata* - spontaneous; **T2** = *Taxus baccata* - cultivated; **T3** = *Taxus baccata 'Robusta'* - cultivated; **IV** - April (strobili formation); **VI** - June (intense vegetative growth); **IX** - September (biomass allocation to roots)

7.3.3.2. Anti-denaturing activity of alcoholic extracts

The experiments carried out to evaluate the possible anti-inflammatory effect of yew extracts were expressed as equivalent mg/ g salicylic acid (Table 7.7.), the aim of this test being to follow the anti-denaturing effects of proteins determined by extracts of different concentrations (1%, 2%, 3%, 4%, 5%) obtained from ryegrass, leaves, arils and seeds harvested from the three taxa taken for analysis at three defining moments of the ontogenetic cycle of the yew and to determine which organ, which taxon and at which moment of the study the most effective anti-denaturing effects of the proteins are attributed.

Table 7.7. The anti-denaturing activity of alcoholic extracts prepared from vegetative and reproductive organs of the *Taxus* taxa tested expressed as equivalent mg/ g salicylic acid

Season / TEST	IV			VI			IX		
	T1	*T2*	*T3*	*T1*	*T2*	*T3*	*T1*	*T2*	*T3*
R. 1%	**0.54** ± 0.05	**0.19** ± 0.04	**0.08** ± 0.03	**0.05** ± 0.02	**1.10** ± 0.07	**0.50** ± 0.14	**4.44** ± 1.83	**1.00** ± 0.42	**1.57** ± 0.64
	***			*			*ns*		
R. 2%	**0.99** ± 0.03	**0.26** ± 0.11	**0.12** ± 0.08	**0.29** ± 0.03	**0.51** ± 0.10	**1.08** ± 0.16	**0.34** ± 0.13	**3.18** ± 0.41	**1.33** ± 0.05
	***			**			***		
R. 3%	**1.52** ± 0.03	**0.75** ± 0.28	**0.85** ± 0.12	**0.58** ± 0.04	**0.52** ± 0.13	**1.16** ± 0.14	**1.52** ± 0.62	**5.96** ± 0.53	**1.42** ± 0.23
	*			*			***		
R. 4%	**1.83** ± 0.26	**2.06** ± 0.19	**1.86** ± 0.50	**0.64** ± 0.05	**1.51** ± 0.30	**1.29** ± 0.19	**1.57** ± 0.61	**5.58** ± 0.51	**4.64** ± 1.83
	ns			*ns*			*ns*		
R. 5%	**2.02** ± 0.37	**2.16** ± 0.02	**2.05** ± 0.29	**0.96** ± 0.10	**4.73** ± 1.49	**2.51** ± 0.20	**3.49** ± 1.25	**7.36** ± 0.55	**3.75** ± 0.21
	ns			*ns*			*		
F. 1%	**0.26** ± 0.29	**0.08** ± 0.11	**0.24** ± 0.09	**0.34** ± 0.16	**0.36** ± 0.15	**0.78** ± 0.17	**2.08** ± 0.26	**0.10** ± 0.10	**0.09** ± 0.15
	ns			*ns*			***		
F. 2%	**1.42** ± 0.76	**1.12** ± 0.27	**1.42** ± 0.11	**0.91** ± 0.19	**1.36** ± 0.27	**1.88** ± 0.14	**4.10** ± 0.12	**0.15** ± 0.06	**1.17** ± 0.26
	ns			*			****		
F. 3%	**2.96** ± 0.26	**2.67** ± 0.15	**2.43** ± 0.25	**4.10** ± 0.52	**6.31** ± 4.53	**4.78** ± 0.49	**4.66** ± 0.41	**3.09** ± 1.42	**4.38** ± 0.41
	ns			*ns*			*ns*		
F. 4%	**5.09** ± 0.68	**4.93** ± 0.05	**2.90** ± 0.32	**13.03** ± 2.14	**9.22** ± 1.15	**8.42** ± 1.50	**4.83** ± 0.23	**2.88** ± 0.30	**5.17** ± 0.63
	*			*ns*			*		
F. 5%	**9.91** ± 0.54	**5.49** ± 0.86	**8.89** ± 1.14	**22.63** ± 0.56	**10.27** ± 5.05	**19.87** ± 0.73	**12.12** ± 1.21	**4.55** ± 0.46	**9.68** ± 0.94
	*			*ns*			**		
A. 1%	-	-	-	-	-	-	**0.13** ±	**0.12** ±	**0.10** ±

Table 7.7. The anti-denaturing activity of alcoholic extracts prepared from vegetative and reproductive organs of the *Taxus* taxa tested expressed as equivalent mg/ g salicylic acid

							0.05	0.02	0.05
							ns		
A. 2%	-	-	-	-	-	-	**0.10** ± 0.06	**1.05** ± 0.06	**0.15** ± 0.01
							****		
A. 3%	-	-	-	-	-	-	**1.35** ± 0.01	**1.35** ± 0.07	**1.13** ± 0.02
							*		
A. 4%	-	-	-	-	-	-	**1.37** ± 0.07	**1.49** ± 1.10	**1.31** ± 0.03
							ns		
A. 5%	-	-	-	-	-	-	**1.57** ± 0.03	**1.69** ± 0.08	**1.34** ± 0.07
							*		
S. 1%	-	-	-	-	-	-	**0.01** ± 0.00	**0.09** ± 0.06	**0.03** ± 0.01
							ns		
S. 2%	-	-	-	-	-	-	**0.11** ± 0.09	**0.41** ± 0.04	**0.26** ± 0.09
							ns		
S. 3%	-	-	-	-	-	-	**0.28** ± 0.10	**0.49** ± 0.03	**1.62** ± 0.05
							****		
S. 4%	-	-	-	-	-	-	**1.53** ± 0.59	**1.36** ± 0.07	**2.31** ± 0.09
							ns		
S. 5%	-	-	-	-	-	-	**2.58** ± 0.27	**1.49** ± 0.09	**4.33** ± 0.11
							****		

Legend: IV - April (strobili formation); **VI** - June (intense vegetative growth); **IX** - September (biomass allocation to roots); **T1** = *Taxus baccata* - spontaneous; **T2** = *Taxus baccata* - cultivated; **T3** = *Taxus baccata 'Robusta'* - cultivated; **R** = bark; **F** = leaf; **A** = aril; **S** = seed; 1%, 2%, 3%, 4%, 5% - concentration of extracts; statistical differences have been marked with an asterisk: * = p<0.05; ** = p<0.01; *** =p<0.001; **** = p<0.0001.

Preliminary considerations:

Following the qualitative and quantitative evaluation of plant extracts obtained from vegetative and reproductive organs of the *Taxus* taxa under consideration at three defining moments of their phenological cycle, the testing of their possible antioxidant effects and anti-denaturing effects on proteins, we can conclude the following:

- phenolic, alkaloid, and carotenoid compounds were found in the composition of alcoholic and aqueous extracts of the investigated taxa;
- according to the literature consulted so far, the analysis and discussion of the absorption spectra of these extracts is a scientific novelty, at least at a national level;
- the amount of polyphenolic and flavonoid compounds and the antioxidant activity of alcoholic extracts obtained from ryegrass, leaves, arils, and seeds of the yew taxa analyzed showed slight quantitative variations between taxa and times of analysis; in this respect, the cultivated taxon **T2**, which presents higher amounts of polyphenolic and flavonoid compounds compared to the spontaneous taxon **T1**, stands out;
- the highest values of antioxidant activity of alcoholic extracts prepared from various organs of *Taxus* taxa rank, for extracts obtained from the bark, the wild species **T1** in the first place, and for extracts obtained from leaves, arils, and seeds, the cultivated taxon **T3**, whose extracts prepared from these organs show the highest antioxidant activity at the times of strobile formation (IV-April) and intense vegetative growth (VI-June);
- evaluation of the possible anti-inflammatory effect of alcoholic yew extracts by determining the anti-denaturing action of animal proteins (albumin) was significant for extracts obtained from:
  - bark of **T1** and **T2** taxa (1%, 2% and 3% concentration extracts);
  - leaves of taxon **T1**, (2%, 1% and 5% concentration extracts);
  - arils of taxon **T2** (2%, 3% and 5% concentration extracts);
  - seeds of taxon **T3** (3% and 5% extracts).

7.3.4. Evaluation under experimental laboratory conditions of the biocontrol potential of plant extracts obtained from *Taxus* L. taxa in test plant species

7.3.4.1. Effects on germination index

The aqueous extracts obtained from the organs of the *Taxus* taxa studied had different influences on the germination indices analyzed (germination percentage, germination rate, cumulative germination rate, germination rate coefficient, emergence rate, germination energy, vigor index) in the two test species, with stronger influences being found in the case of tomato seeds - *Lycopersicon esculentum* Mill, variety Silvia (crop plant), compared to those of redroot pigweed - *Amaranthus retroflexus* L. (broad-leaved rustic species).

For both test species considered, seed germination was monitored over 336 hours (14 days), following the protocol for this type of determination presented in the literature (Yarnia et al., 2009; Singh et al., 2012).

7.3.4.2 Biometric index effects

♦ In the experiments with the test species of the broad-leaved weed, *Amaranthus retroflexus* L., some of the aqueous *Taxus* extracts tested generally inhibited the biometric parameters analyzed. Stronger influences were recorded for this species when applying treatments with bark and leaf extracts from all three yew taxa analyzed.

♦ In the case of the crop species *Lycopersicon esculentum* Mill, variety Silvia, the comparative analysis of the biometric parameters characterizing the newly formed seedlings in the germination process following treatments with aqueous extracts of bark, leaves, arils, and seeds obtained from the three yew taxa studied allows us to consider that they were, in general, statistically significantly reduced compared to the control sample.

Preliminary considerations:

As a result of laboratory investigations carried out to identify the possible effects induced by the application of aqueous extracts obtained from vegetative and reproductive organs belonging to specimens of three *Taxus* taxa (spontaneous and cultivated) at three defining moments of the phenological cycle, the following can be deduced:

- aqueous extracts obtained from the bark, leaves, arils, and seeds of the yew taxa studied, especially those obtained from the bark, exert inhibitory effects on germination parameters (<u>germination percentage, germination rate, cumulative germination rate, germination rate coefficient, emergence rate, germination energy, vigor index</u>) in the test plant species studied;
- application of 5% aqueous extracts obtained from the bark of spontaneous taxon **T1** and cultivated taxon **T3** significantly inhibits <u>root elongation</u> of newly formed seedlings of *Amaranthus retroflexus* L.;
- in the case of the test species *Lycopersicon esculentum* Mill., variety Silvia, the application of aqueous extracts of 1 and 5% concentration obtained from the bark of all yew taxa studied and extracts of 1% concentration prepared from the

leaves of the cultivated taxon **T3** inhibits root elongation of newly formed seedlings;

- hypocotyl elongation of newly formed seedlings by germination in both test plant species investigated was stimulated by the application of 5% aqueous extracts obtained from arils harvested from all three yew taxa investigated;
- seeds of *Lycopersicon esculentum* Mill., variety Silvia showed a higher sensitivity to the applied aqueous extracts compared to seeds of *Amaranthus retroflexus* L.

FINAL CONSIDERATIONS

The research carried out in this doctoral thesis, with a high degree of interdisciplinarity, aimed to highlight, on the one hand, some aspects related to the biology of some taxa of the genus *Taxus* L. spontaneous and cultivated in the north-eastern part of the country, according to specific influencing factors (internal factors, leaf area factors, and external factors), and to evaluate, under laboratory experimental conditions, the possible allelopathic (bioherbicidal) potential of aqueous extracts of different concentrations obtained from vegetative and reproductive organs of the respective taxa, by interpreting their effects on the germination process and the development of the resulting seedlings in test plant species of different groups (weeds and crop plants).

Following the research carried out on the plant material available and under the given growing/cultivation conditions, the research was organized according to the objectives pursued, we highlight the following:

- **Determinations of morphological changes at the leaf level in response to environmental factors** revealed that:
  - leaf area varies in size in representatives of the genus Taxus in correlation with the environmental conditions available in the habitat where they grow spontaneously or are cultivated, mainly represented by the degree of illumination / increased shading of their leaves;
  - practical results obtained are in agreement with the data presented in the literature, highlighting the fact that the specimens of taxon **T1**, growing spontaneously in the shady conditions of the forest in the Tudora Reserve,

clearly show high plasticity in terms of the morphology of the leaf area and its associated indices, a plasticity materialized in the formation of a leaf area almost double compared to that of the cultivated specimens **T2** and **T3**, not shaded.

- **Determinations of micromorphological and histo-anatomical characteristics of leaves and stems as a function of the action of environmental factors show:**
  - **Micromorphological characteristics of leaves:**
    - The surface of the upper epidermis of the leaves belonging to the specimens of the three spontaneous and cultivated yew taxa analyzed is approximately similar, and the organization of the lower epidermis, through the particular arrangement of the stomatal bands about the midrib, provides taxonomic specialists with a valuable micromorphological character with a high diagnostic value in the identification of taxa of the genus *Taxus*;
    - The fluctuating asymmetry phenomena observed in the leaves of **T2** and **T3** cultivated taxon specimens regarding the disposition of their epidermal structures about the midrib agree with the data presented in the current literature and can be correlated with potential stress factors of the cultivation environment of these taxa.
  - **Histo-anatomical characteristics of leaf apparatus and stems (branches):**
    - ***The similarities in leaf structure*** between the taxa analyzed, which also confirm data already existing in the literature, refer to:
      - the presence of stomata only in the lower epidermis (hypostomatic limb);
      - arrangement of stomata in rows forming bands of variable width (5-7-8-9 stomata) on either side of the midrib;
      - complex organization of the stomatal apparatus and presence of *Florin's ring*;
      - absence of lignified sclerenchyma hypodermis;
      - absence of secretory ducts;
      - organization of the leaf vein;
      - the discrete presence of the transfusion parenchyma in the lateral parts of the nerve leading fascicle;
      - lower epidermal cells are papilliform.

- ***Differences in leaf structure*** between the taxa analyzed, which fall, instead, into the category of *structural variations* correlated with ecological conditions, differences considered as adaptive response reactions of these taxa to the vegetation conditions offered to the yew specimens analyzed refer to:
    - the unequally equvifacial bifacial structure, at least on the two outer surfaces of the leaf of specimen **T2**, as opposed to the established heterofacial bifacial leaf structure of specimens **T1** (spontaneous) and **T3** (cultivated), a constant mention in the literature;
    - mesophyll with unistratified, high-cell palisading tissue in the leaf of the spontaneous specimen **T1** and bistratified, sometimes tristratified palisading tissue in the leaf of the cultured specimen **T3**, with low cells;
    - quantitative disproportion between the wood and the free wood of the leader bundles in favor of the former in the leaf of the cultivated specimen **T3**.
- ***Stems (branches):***
    - spontaneous taxon **T1** shows an irregular cross-sectional outline, with ribs of different sizes (two wider and two three smaller) separated by medium-deep valleys, and unistratified epidermis showing stomata with supra stomatal chambers of various sizes, through the intervention of outer walls of adnexal cells +/- elongated, sometimes ridge-like, projecting above the external level of epidermal cells;
    - branches between 1 and 3 years of age in all taxa investigated (**T1**, **T2**, and **T3**) show an irregular cross-sectional outline, with two higher amplitude ribs alternating with 2-3 lower amplitude ribs;
    - taxon **T2** shows particular changes in structure (disorganization of the assimilatory parenchyma from the first year of life), probably caused by +/- favorable growing conditions (excessive successive watering, heavy rainfall in short intervals or specific chemical treatments to combat diseases and pests).

- **Determinations of physiological responses at the leaf level in correlation with internal leaf factors, leaf surface factors, and external (environmental) factors** allowed us to state that:

- fundamental physiological processes (photosynthesis, transpiration, respiration) take place with different intensities, depending on the specific phenological phases of the life cycle of the taxa under consideration, the factors at the leaf level, and the climatic conditions available to them in their natural habitats/location of cultivation.
- The content of foliar photoassimilate pigments shows quantitative variations depending on the particularities of the biological cycle of the investigated taxa, on the variations of the internal factors, on those of their foliar apparatus (Qleaf, tleaf, Ci, gs), as well as on the external factors (temperature, atmospheric humidity) available to the taxa in the environment.
- The content of water, dry matter, mineral elements, and organic substances accumulated in the leaves of the specimens belonging to the taxa analyzed varies according to their genotype and the time of investigation, demonstrating that the plant individuals studied have recorded metabolic levels strictly correlated with their vital needs during the successive phases of their life cycle.

- **Determinations of the variation in the content of some biochemical compounds in arils in response to environmental factors** revealed the following:
  - The anthocyanin and carotenoid pigments accumulated in the arils of the taxa under consideration showed visible quantitative variations, with the cultivated *Taxus baccata 'Robusta'* (**T3**) showing the highest values, while the vitamin C content is maintained at approximately the same level in all three taxa, the results obtained in this regard being consistent with the data presented in the literature consulted;
  - The quantitative differences recorded between the biochemical compounds dosed in the arils collected from the wild and cultivated yew taxa under consideration can be explained by the specificity of their genotypes, which allowed their specific adaptive responses to the various environmental factors available to them in the areas where they grow (Yew Reserve in Tudora Forest, compared to the location of the cultivated taxa), including air temperature, degree of illumination in the environment, soil quality and moisture (water availability), altitude, exposure to the action of air currents (wind).

- **Research on the *in situ* and *ex situ* biochemical composition of some taxa of the genus *Taxus* L**. revealed the following:
    - The chemical composition of the volatile oil and hydrosols obtained by hydrodistillation from the leaves of the three taxa under study harvested during the period of intense vegetative growth (VI-June) showed qualitative and quantitative variations discussed in the literature as being dependent on the species (including factors intrinsic to each taxon), the age of the producing plant, the organ subjected to extraction, the climatic conditions existing in the geographical region where the trees grow;
    - The number of volatile oil components extracted from the leaves of yew specimens belonging to the taxa studied is higher in cultivated taxa (42 compounds - taxon **T2**, respectively 36 compounds - taxon **T3**), compared to wild species **T1** (31 compounds);
    - The qualitative evaluation by spectrophotometry of hydrosols obtained as a by-product of oil extraction by hydrodistillation of plant material (leaves) indicates the existence of aromatic compounds, detected in the 220-240 nm region, and phenolic compounds detected in the 260-280 nm region of their absorption spectra, which may give them possible allelopathic properties;
    - The partially positive results obtained in the process of separation, identification, and quantification of *taxol* in acetone extracts of yew by high-performance liquid chromatography (HPLC) require further research and concentration of working extracts so that the concentration of taxol identified in the plant material can be calculated; such a scientific approach is a novelty in national research, as far as we know, no results in this regard have been reported so far in the Romanian literature;

- **The evaluation of qualitative, quantitative, and possible effects of some plant extracts obtained from taxa of the genus *Taxus* L.** allowed us to consider the following:
    - The chemical characterization of aqueous and alcoholic extracts of 1% and 5% concentration obtained from different organs (bark, leaves, arils, and seeds) of the studied yew taxa revealed the presence of phenolic, alkaloid, and carotenoid compounds, the analysis and discussion of the absorption

spectra of these extracts being, so far, a scientific novelty at least at national level;

- The quantity of polyphenolic and flavonoid compounds and the antioxidant activity of the alcoholic extracts showed slight variations in value between taxa and analysis periods; in this respect, the cultivated taxon **T2**, which showed higher quantities of polyphenolic and flavonoid compounds compared to the spontaneous taxon **T1**, stands out;
- The highest values of antioxidant activity rank the alcoholic extracts prepared from various organs of *Taxus* taxa according to the intensity of their antioxidant activity thus: extracts obtained from leaves, followed by those prepared from bark, from seeds, and, finally, those obtained from arils, with statistically significant differences between all taxa at all times of analysis (months IV, VI, IX).
- evaluation of the possible anti-inflammatory effect of alcoholic yew extracts by determining their anti-denaturing action on proteins was performed on the following (different concentrations) extracts obtained from: bark collected from taxa **T1** (spontaneous) and **T2** (cultivated), leaves of taxon **T1**, arils of taxon **T2** and seeds of taxon **T3**;

Evaluation, under laboratory experimental conditions, of the biocontrol potential of plant extracts obtained from taxa of the genus *Taxus* L. in test plant species demonstrated the following:

- aqueous extracts of 1% and 5% concentration, respectively, prepared from the bark, leaves, seeds, and arils of the three yew taxa studied produced strong inhibitory effects on the germination of the test plants germination, ordering the organs presented as a source of aqueous extract in descending order of the respective extract effects;
- *Lycopersicon esculentum* Mill. seeds, variety Silvia, showed a higher sensitivity to the applied aqueous extracts compared to *Amaranthus retroflexus* L. seeds, a phenomenon evidenced by the level of change in the value of the specific germination process indices;
- elongation of newly formed *Amaranthus retroflexus* L. seedling roots by germination is significantly inhibited by 5% aqueous extracts from bark (taxa **T1** and **T3**) and in *Lycopersicon esculentum* Mill., variety Silvia by both

aqueous extracts from the bark of all taxa tested and 1% extracts from leaves (taxon **T3**);

- hypocotyl elongation of newly formed seedlings by germination in both test plant species analyzed was stimulated by the application of 5% aqueous extracts obtained from arils harvested from all three yew taxa studied.

- The allelopathic effects produced by aqueous extracts obtained from vegetative and reproductive organs of the three yew taxa under consideration can and should be continued and completed by increasing the number and diversity of morpho-anatomical tests, physiological, and biochemical on a broader range of target plants of agricultural interest (weeds and crop plants), as an essential research step in the work to identify, isolate and test biologically active compounds with implications in organic agriculture (possible bioherbicidal potential).

- The research aimed to complete the national and international literature with practical data obtained through laboratory and field experiments on the biology of the *Taxus* taxa studied, whose allelopathic potential is less known, to support the conservation efforts of the spontaneous species *Taxus baccata* L., with reduced ranges at the national level and to encourage the cultivation, for ornamental purposes, of some yew taxa increasingly marketed through authorized horticultural companies, supporting with credible arguments their real value for the natural protection of agriculturally important spontaneous or cultivated species.

- **Research directions opened up by this thesis:**
    - The standardization of the preparation method (by testing different types of extractants and working techniques) of plant extracts from vegetative and reproductive organs of spontaneous/cultivated *Taxus* taxa in various areas of our countries for the identification, isolation, and quantification of taxol by high-performance liquid chromatography (HPLC).
    - further research on the chemical composition and possible applications of volatile oils and hydrosols obtained by hydrodistillation on several samples of plant material (vegetative and reproductive organs) belonging to several yew specimens growing spontaneously / cultivated in Romania, as resources

of biologically active compounds valuable for medicine, agriculture, and ecology.

- further tests to validate the results obtained so far on the possible anti-inflammatory / anti-denaturing effect of proteins manifested by extracts prepared from the bark, leaves, arils, and seeds of yew taxa taken in the study, optimization, and standardization of the extraction method and analysis of these extracts, as a source of biologically active compounds with medicinal potential, applicable to similar determinations in other samples of plant material collected from specimens of yew wild or cultivated in our country.

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ANNEXES