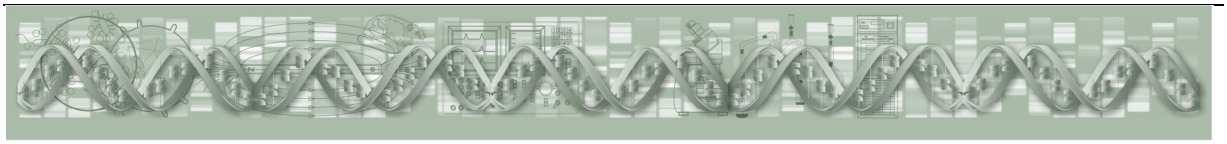

YEW EXTRACTS - A POSSIBLE SOURCE OF BIOACTIVE COMPOUNDS WITH POTENTIAL ALLELOPATHIC PROPERTIES

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Abstract

Representative species of the *Taxus* genus, known since antiquity for their toxicity, have been an essential plant resource for the production of plant extracts with medicinal properties (anti-inflammatory, antifungal, and antibacterial) and, more recently, for the study of their allelopathic properties with significant implications in organic agriculture (bioherbicidal potential). With a view to the development of this new direction of research, the present work aims: to present a qualitative evaluation of two types of plant extracts (aqueous and alcoholic) of different concentrations (1% and 5%) obtained from various organs (bark, leaves, arils, and seeds) belonging to three *Taxus* taxa: a spontaneous taxa - *Taxus baccata* L. (**T1**) and two cultivated taxa - *Taxus baccata* (**T2**) and *Taxus baccata 'Robusta'* (**T3**) at different times of the phenological cycle by determining their absorption spectra (of both types of extracts), total amounts of polyphenols and flavonoids, and by evaluating their antioxidant capacity (alcoholic extracts); to investigate, under experimental conditions of cultivation the possible allelopathic effects induced by the aqueous extracts on the germination, growth, and development of the seedlings in two test plant species: *Amaranthus retroflexus* L. (ruderal species) and *Lycopersicon esculentum* Mill, variety Silvia (crop species). The data indicates the presence of phenolic and compounds, alkaloids, and carotenoid pigments in the alcoholic extracts prepared from the different organs of the studied yew taxa, showing higher amounts of polyphenols and flavonoids in the extracts obtained from the leaves of taxon **T2** compared to taxon **T1.** The effect of 1% and 5% aqueous extracts obtained from the bark of the three investigated yew taxa, as well as 1% aqueous extracts prepared from the leaves of taxon **T3** on seed germination, seedling growth, and development was more pronounced in the crop species *Lycopersicon esculentum* Mill, variety Silvia, compared to the ruderal broadleaf weed *Amaranthus retroflexus* L., both species having rapid germination stimulated by ambient light. Aqueous extracts of 5% concentration obtained from the arils of plants belonging to the three yew taxa stimulated the elongation of the seedlings' hypocotyls of both test species.
Keywords: *Taxus*, plant extracts, seed germination

Introduction

Germination and plant growth are highly complex phenomena that result in the development of new plant individuals. These processes can be influenced by a relatively large number of both abiotic and biotic factors. The former includes humidity temperature, the intensity of light radiation present in the plant's growing environment, etc. At the same time, plant development can be affected by interactions with other organisms of microbial, plant, or animal origin. Interactions between plants and biotic and abiotic factors have led to the synthesis of large numbers of secondary metabolites, which are products with specific biological properties.

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These properties are of great interest to the pharmaceutical and agricultural industries, currently the subject of intensely local, national, and international research.

The effort put into weed control has created a strong dependence on the synthesis and application of a wide range of herbicides, which has led to significant changes in weed flora and the selection of herbicide-resistant crop plant biotypes (Kudsk and Streibig 2003). In Romania, the areas in which herbicides were applied in 2007-2020 increased by 31.1%, compared to the previous period, with herbicides becoming the main tool for weed control in most areas of the country. Today, the reliance on herbicides continues to grow internationally as the global population migrates from rural areas to cities and the agricultural labor force declines. Consequently, the use of these synthetic chemicals has risen significantly, increasing the selection pressure induced by their intensive application on plants of agricultural interest, which has invariably led to an increase in multiple weed resistance in crops. Today's efforts to research new weed control technologies and integrated weed management systems aim to counteract the effects of their herbicide resistance (Peterson et al. 2018).

In this context, allelopathy has emerged as a pragmatic approach to solving problems in modern agriculture. Multiple crop approaches such as crop rotation, cover crops, intercropping, mulching, incorporation of crop residues, and application of aqueous extracts are 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 crop production (Farooq et al. 2013). Allelopathy is a naturally occurring ecological phenomenon in which different organisms affect the functioning of other crops. Allelopathy is a natural ecological phenomenon in which different organisms affect the functioning of other organisms in their vicinity, in a negative or positive way (Rice 1984), by releasing secondary metabolites (Farooq et al. 2011a). At the level of plant-plant interactions, the links between partner organisms are generated by the chemicals they produce and release into the environment through various pathways (volatilization, exudation, dissolution, etc.) (Wier et al. 2004).

In a plant's metabolism, the synthesized compounds have various roles: informational, structural, energetic, synthetic, and signaling, some of them with defensive or competitive roles (Soltys et al. 2013). There are many categories of these compounds, such as alkaloids, flavonoids, tannins, organic acids, or volatile compounds, that can induce stimulation or inhibition of seed germination and growth of surrounding plants (Li et al. 2010). Thus, phenolic compounds and terpenoids act differently in different organisms. For example, in plant organisms, they can 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 seed germination, or alter transpiration and respiration rates (Rimando et al. 1998, Abrahim et al. 2003, Dayan and Duke 2006).

The biological properties of secondary metabolites have long attracted the attention of specialists and have been the basis for the development of psychostimulants or phytoinhibitors. The elucidation of the mechanisms that determine the biological properties of secondary metabolites biosynthesized by plants under certain growth conditions requires complex investigations, starting from observations in nature and continuing by deciphering specific molecular aspects. In this sequence, testing the effects of these compounds under various experimental conditions and quantifying them through interdisciplinary analyses using test organisms is an important step. Of particular relevance among these test methods is the testing of aqueous extracts produced by plants, as this method can more accurately reflect the response of test plants to favorable or unfavorable factors in their natural environment (Farooq et al. 2010).

In the present work, various yew taxa were chosen as potential sources of biocontrol compounds (Reigosa et al. 1999), being rich in compounds with phytotoxic activity (phenols, flavonoids, alkaloids) (Das et al. 1998, Parmar et al. 1998). Yew is widely recognized as a source of

paclitaxel, a compound initially known as taxol, patented by Bristol-Myers Squibb under the generic name paclitaxel, used as an antineoplastic agent in the treatment of common cancers, such as ovarian and breast cancer (Onrubia et al. 2010). It also has other biological properties, including anti-inflammatory (Küpeli et al. 2003) and antifungal activity against *Cladosporium oxysporum* Berk. Curt, *Fusarium culmorum* W. G. Smith (Sacc.), *Alternaria alternata* (Fr.) Kiessler) (Baranowska and Wiwart 2003), anti-ulcerogenic (Gurbuz et al. 2004), antioxidant and antibacterial (Prakash et al. 2018), or insecticidal against *Tribolium confusum*, *Trogoderma granarium*, *Sitophilus granarius* (Daniewski et al. 1988).

The present research aims, to characterize the biochemistry of two types of plant extracts (aqueous and alcoholic) of different concentrations (1% and 5%) obtained from different organs (bark, leaves, arils, and seeds) belonging to three yew taxa (one spontaneous and two cultivated), at different times of their phenological cycle, by determining their absorption spectra (for both types of extracts) and the total amount of polyphenols and flavonoids, and their antioxidant capacity (for alcoholic extracts), and to evaluate the possible allelopathic (bioherbicidal) potential of 1% and 5% aqueous extracts obtained from various organs of the respective taxa, interpreting their effects on the germination, growth, and seedling development of two test plant species under experimental laboratory conditions: *Amaranthus retroflexus* L. (broad-leaved ruderal species) and *Lycopersicon esculentum* Mill, variety Silvia (crop species).

Materials and Methods

Study area, plant sample collection, and authentication

The biological material 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**), procured from the nursery S.C. Doropad S.R.L. Suceava, based in Dorohoi and cultivated in Vorniceni commune, Botoșani county (lat. 47.986328° N, long. 26.663299° E, alt. 185 m).

Biological sampling was carried out in April (IV), June (VI), and September (IX) of the year 2021. According to the literature (Robakowski et al. 2018), the selected harvesting times correspond to the specific seasons of the phenological cycle of the analyzed species: strobili production in spring, intense vegetative growth in summer, and biomass allocation to roots in autumn. No plant material was collected for the winter period, as the weather conditions recorded during that period did not allow field access to the Yew Reserve in Tudora Forest. Vouchers of the taxa taken and used in the study were deposited, after identification (by Dr. Irina Irimia) at the Herbarium of the Faculty of Biology of "Alexandru Ioan Cuza" University of Iasi, with the corresponding identification numbers: 186539 - taxon **T1**, 186537 - taxon **T2**, 186538 - taxon **T3**. The processing of the plant material was carried out in the research facilities of the Faculty of Biology of "Alexandru Ioan Cuza" University of Iași, using the equipment from 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.

Experimental design

The biological material collected for analysis (bark, leaves, arils, and seeds) was subjected to heat treatment at 60°C in a ventilated oven for 60 minutes for enzyme inactivation. Subsequently, the material was dried at room temperature in adequately ventilated areas away from direct sunlight. The dried material was ground using an electric grinder and used for

alcoholic and aqueous extracts preparation. In parallel, the determination of the water and dry matter content of the samples was carried out to report the results of the laboratory analyses (Boldor et al. 1983). Alcoholic extracts of 1% and 5% concentration, respectively, were prepared from the harvested plant material for a qualitative evaluation, and aqueous extracts of the same concentrations were prepared and used to investigate, under experimental laboratory conditions, their potential allelopathic effects on the germination, growth, and development processes of the seedlings, using seeds belonging to the test species *Amaranthus retroflexus* L. - (ruderal broad-leaved weed) and *Lycopersicon esculentum* Mill. Silvia variety - (crop plants), species with rapid germination stimulated by ambient light (Boldor et al. 1983, Asaad et al. 2017).

Preparation of alcoholic extracts

Alcoholic extracts were prepared using 70% ethyl alcohol (Chemical Company S.A., Iasi, Romania) 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 the compounds of interest from the plant material (Naczk 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. Thus, 5 ml of 70% ethyl alcohol was added to 1 g of powder (rhytidome, leaves, seeds), and the tubes were shaken on a mechanical shaker at 300 RPM for 24 hours. Afterward, the extracts were centrifuged for 15 minutes at 4000 RPM and the supernatant was collected in separate tubes. Over the residue 5ml of 70% ethyl alcohol were added, repeating the extraction procedure for another 24 hours. The final (combined) extracts were centrifuged for 15 minutes at 4000 RPM with the supernatant used for further analysis. Aril extracts were obtained according to the method used by Tabaszewska et al. (2021), with 10 g of fresh material extracted in 150 ml of 80% ethyl alcohol. Alcoholic extracts of 5% concentration were obtained at the following dilutions: 1:4 for qualitative spectrophotometric evaluation and 1:9 for quantitative biochemical analysis.

Preparation of aqueous extracts

Aqueous extracts of bark, leaves, arils, and seeds of 1% and 5% concentrations 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 a 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).

Qualitative evaluation of absorption spectra of aqueous and alcoholic extracts

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

Determination of total polyphenol content in alcoholic extracts

A 1:9 dilution was made from the initial 5% alcoholic extracts using the Folin-Ciocalteu method (Herald et al. 2012). Absorbance readings were performed at $\lambda = 760$ nm against distilled water on a Shimadzu UV - mini spectrophotometer. The calibration curve was generated using gallic acid of 0-400 µg/ml concentrations. Polyphenol concentrations in extracts were calculated using a calibration curve with $R^2=0.9977$ and expressed as mg gallic acid/g dry matter.

Determination of flavonoid content in alcoholic extracts

Determination of flavonoid compound content was carried out on 1:9 diluted alcoholic extracts using the method of Jia et al. (1999) and Herald et al. (2012). The analysis was performed at λ = 510 nm against distilled water. Standard solutions were prepared for the calibration curve using quercetin in 0-500 µg/ml concentrations. The flavonoid content in the extracts was calculated from the calibration curve with R^2=0.9879 and expressed as mg quercetin/g dry matter.

Determination of the antioxidant activity of alcoholic extracts

The antioxidant activity of alcoholic extracts was determined according to the method of Thaipong et al. (2006) and Herald et al. (2012). A 60 µM DPPH alcohol solution was prepared. The samples were obtained by mixing 0.1 ml diluted plant extract (1:9) with 2.9 ml DPPH solution. The resulting mixture was incubated in the dark at room temperature for 3 hours. The analysis was performed at λ= 515 nm against ethanol. Standard solutions were prepared to obtain the calibration curve, using ascorbic acid concentrations of 0-200 µg/ml. Antioxidant activity was calculated using the formula obtained from the calibration curve (R^2=0.9936) and expressed as mg ascorbic acid equivalent per gram of dry matter.

Experimental conditions for testing aqueous extracts

Seeds of *Lycopersicon esculentum* Mill., variety Silvia - tomato, crop species - purchased from Agrosel, and of redroot pigweed - *Amaranthus retroflexus* L., a broad-leaved ruderal weed, were used to test the effects of aqueous extracts on seed germination. Seed sterilization was carried out in two stages: a first stage with 2.5% sodium hypochlorite for 3 minutes, followed by three successive rinses with sterile distilled water, and a second stage with 3% hydrogen peroxide for 3 minutes, followed by three rinses with distilled water. Two sets of experimental variants were set up for each species; each variant consisted of 6 tubes with equal volumes of 5 ml aqueous extracts of 1% and 5% concentrations of bark, leaves, arils, and seeds, respectively, and one tube with distilled water (control variant). The aqueous extracts prepared from the plant material corresponded to the specific times of harvesting (IV-April, VI-June, IX-September for leaves and bark, respectively, and IX-September for arils and seeds). After sterilization, test plants seeds (15 *Amaranthus* and 10 *Lycopersicon* seeds for each experimental variant, respectively) were subjected to imbibition for 24 hours. Afterwards, the seeds were placed on filter paper in Petri dishes (previously sterilized in an autoclave sterilizer at 180°C for 2 hours to avoid microbial contamination); the handling of the biological material was 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, and the filter paper was moistened with 1 ml of distilled water as needed several times a day during the duration of the study. 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 (26±2°C). The experiment lasted 14 days (336 hours), including the 24 hours of imbibition.

Germination index analysis

The effects of aqueous extracts on the germination process in the test species were evaluated by recording the total number of germinated seeds in each variant at 24-hour intervals throughout the experiment. The values were used to calculate four germination indices (Table 1) according to the formulas described by Boldor et al., (1983) and Mominul Islam and Kato-Noguchi, (2014), with the results being calculated as the average of the values obtained in triplicate for each experimental variant.

Table 1. The equations used to calculate different germination indices

Germination parameters	Equations	References
Germination percentage (**GP**)	$\mathbf{GP} = \left[\frac{\text{Number of germinated seeds at final count}}{\text{Total number of seeds sets for bioassay}}\right] \text{x } 100$	Mominul Islam and Kato-Noguchi 2014.
Speed of emergence (**SE**)	$\mathbf{SE} = \left(\frac{\text{Number of germinated seeds at the starting day for germination}}{\text{Number of germinated seeds at the final days of measuremnet}}\right)$	Mominul Islam and Kto-Noguchi 2014
Germination energy (**GE**)	$\mathbf{GE} = \left(\frac{\text{Number of germinated seeds at the 120 h}}{\text{Total number of seeds sets for bioassay}}\right) \text{x}$	Boldor et al. 1983, Mominul Islam and Kato-Noguchi 2014
Seedling vigour index (**SVI**)	$\mathbf{SVI} = \left(\frac{\text{Seedling length (mm)x Germination percent}}{100}\right)$	Mominul Islam and Kato-Noguchi 2014

Biometric Determinations of newly formed seedlings

To assess the influence of aqueous extracts on the initial growth of newly formed seedlings, the following seedling parameters were measured: root 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 ImageJ software.

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 the graphs as follows: **** = $p<0.0001$, *** = $p<0.001$, ** = $p<0.01$, * = $p<0.05$.

Results and discussions

Summary qualitative evaluation of aqueous and alcoholic extracts

The obtained values indicate the presence of several types of compounds, with variations according to a specific taxon, the season/time of investigation, type of extract, organ, and extract concentration (Plates I and II). According to the literature, the presence of alkaloids was indicated by the existence of maxima in the 220 nm region and in the 280 nm region of the analysis spectrum, maxima that correspond to groups with nitrogen atoms (Porto 2016), being known that in the case of alkaloids synthesized by *Taxus* species, the absorption maxima are for taxine A - 220 and 255 nm, for 2-deacetyltaxine A - 224 and 264 nm, for taxine B - 210 and 277 nm, and for isotaxine B - 282 nm (Wilson and Hooser in Gupta 2018).

From our investigations, it can be observed that during the period of the formation of the strobiles (April), both aqueous and alcoholic extracts of 1% and 5% concentration of bark and leaf show the same absorption maxima in the 220 and 250 nm region for all taxa. Differences appear in June (intense growth stage) when it is observed, for example, that the alcoholic extracts of bark and leaf of 1% concentration in the case of spontaneous taxon **T1** show the lowest absorbances, compared to the other two cultivated taxa, in the 220-250 nm region, and

that the highest values were recorded for the bark of taxon **T2** and the leaves of taxon **T3**. In aqueous extracts of the same concentration, only the bark of the three investigated taxa shows compounds in the range mentioned above, where once a new spontaneous taxon **T1** records lower values, and taxon **T2,** the highest value. In September, when the allocation of newly formed biomass to the roots of yew plants takes place, it is observed that in the 220-250 nm range of the spectrum, all alcoholic extracts of 1% concentration from bark, leaves, and seeds show approximately the same absorbance, which leads us to consider that all these organs contain substantially similar amounts of compounds. The higher concentrations of compounds, by the intensity of absorbance, were recorded in extracts of 5% concentration belonging to the same variants. Generally, aqueous extracts, as opposed to alcoholic extracts, have higher amounts of compounds, with the highest concentrations observed in seed extracts, which show the highest absorbance for all taxa.

According to data presented in the literature (Kajdžanoska et al. 2010, Baciu et al. 2013, Bunghez et al. 2013, Butnariu 2014), the main classes of compounds identified with absorption maxima in the 260-280 nm region are phenolic compounds. Consistent with this information, from our analyses, we can consider that during June, alcoholic extracts are higher in phenolic compounds compared to aqueous extracts; thus, for 1% leaf extracts, cultivated taxa show higher concentrations compared to the spontaneous species, and at 5% concentration, alcoholic extracts display approximately the same absorbance for all organs and taxa. Differences between taxa occur for aqueous extracts from bark and leaf at 5% concentration, where the spontaneous taxon **T1** shows the lowest absorbances. The 1% and 5% alcoholic extracts obtained during June and September show approximately the same absorbance/concentration of phenolic compounds, with slight differences between taxa at 1% concentration. Aqueous extracts show the highest absorbances for leaf extracts, where **T2** and **T3** taxa are richer in phenolic compounds. The maxima recorded in the 440 nm region of the absorption spectrum, according to the literature, indicates the presence of carotenoid pigments (Horváth et al. 2010, Zăvoi et al. 2011), which have appeared in the experiments carried out on yew in aqueous extracts from arils of 5% concentration in taxa **T1** and **T3**. The results obtained after the experiments are in agreement with the literature, which mentions the presence of phenolic compounds in different yew organs (Das et al. 1993, Das et al. 1998), as well as alkaloids (Parmar et Jha 1998), the synthesis of the latter in plants of this taxonomic group and their pharmacological activity (Malik et al. 2011) being the reason why yew specimens have been intensively exploited over time (Dhar et al. 2013).

The total polyphenol content of alcoholic extracts

Phenolic compounds are the major groups of allelochemical compounds present in plants. These are a group of organic compounds that have in their structure an aromatic nucleus onto which one or more hydroxyl (-OH) groups are grafted. Phenolic compounds with allelopathic effect include (Inderjit 1996) simple aromatic phenols, hydroxylated and substituted benzoic acids, aldehydes, hydroxylated cinnamic acids, as well as coumarins, tannins, and some flavonoids (Liu et al. 2008) in agreement with John et Sarada, (2012). Phenols are known to be produced by plants in response to stress caused by pollutants or pathogens, functioning as a defense mechanism in their metabolism (Smolders et al., 2000). Higher values of this parameter may indicate a non-enzymatic antioxidant response of plants, and lower values at lower concentrations may suggest that the stress level exceeds the metabolic capacity of the synthesis of these compounds by those organisms. The practical results obtained during the present experiment on the total polyphenol content of the alcoholic extracts showed variations between taxa, organs, and time of analysis (Figure 1).

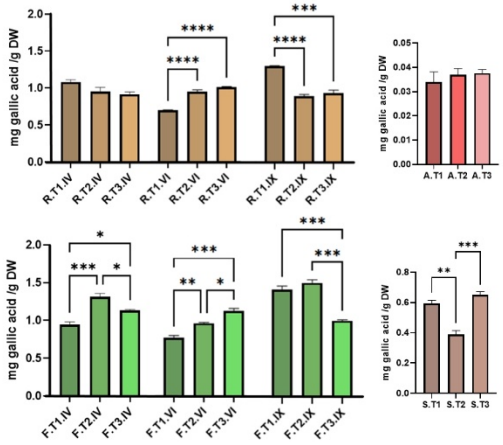

Figure 1. Total polyphenol content

R = bark; **F** = leaf; **A** = aril; **S** = seed; **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)

Leaf extracts had the highest content of polyphenols; this biochemical parameter showed statistically significant differences between taxa and analysis times, with spontaneous taxon **T1** and cultivated taxon **T2** showing the highest levels of these compounds during September. The extracts obtained from bark show the highest level of polyphenols at the time of biomass allocation to roots (September) in spontaneous taxon **T1,** and the lowest during June, when cultivated taxa **T2** and **T3** show statistically significant higher levels of phenolic compounds, compared to taxon **T1**. In the case of extracts prepared from seeds, the highest level of polyphenols is recorded in taxon **T3**, followed by taxa **T1** and **T2**. The literature data indicates that shaded *Taxus* individuals have a higher total content of phenolic compounds than those growing in the light. While the activity of phenylpropanoid enzymes (phenylalanine, ammonia-lyase, and chalcone synthase) is light-dependent, the accumulation of polyphenolic compounds is probably due to specific functional adaptations of these trees to shaded conditions. Thus, a high level of secondary metabolites in yew specimens and, in addition, a higher level of specific biochemical compounds in their young leaves represent important metabolic properties of these trees; similar tendencies were also observed in Scot's pine (Giertych 2001) in agreement with Brzezińska et Kozłowska (2008).

The data obtained in our research confirm the information presented in the literature for the specimens belonging to the analyzed spontaneous species (taxon **T1**), which has the highest concentration of polyphenols, given the specific vegetation conditions, namely the deciduous forest in the area of the Yew Reserve in Tudora, with a rich canopy, which does not easily allow sunlight to reach the yew trees. In addition, physiological research carried out in the field and the laboratory during the same study period revealed that specimens of the spontaneous taxon **T1** in that reserve, which does not benefit from optimal illumination, recorded the lowest photosynthetic and transpiration rate values under conditions of maximum leaf assimilatory pigment content (Hageneder 2013, Perrin et Mitchell 2013, Zarek 2016).

Total flavonoid content of alcoholic extracts

Flavonoids are an important group of compounds, widely studied due to their possible beneficial effects on human health (Luo et al. 2014, Karak et al. 2019). These biologically active natural compounds are currently an essential phytochemical substrate for a wide variety of applications aimed at obtaining medicinal, pharmaceutical, and cosmetic preparations (Panche et al. 2016, Karak et al. 2019), due to their anti-inflammatory, antioxidant, anticarcinogenic, and antimutagenic properties, along with their ability to modulate their cellular key enzyme

function (Panche et al. 2016, Alseekh et al. 2020). Flavonoids are diverse bioactive compounds that can be classified into different classes as flavones, flavonols, flavan-3-ols, flavanonols, isoflavones, and bioflavonoids (Pietta et al. 2000, Andrade et al. 2018) and can be extracted from a diverse range of sources, from microorganisms to higher plants (Verma et al. 2020) according to Bekhouche et al. (2022).

The practical results obtained during our research (Figure 2) show that the bark and leaves of *Taxus* are the organs richest in such compounds. Significant amounts of such compounds are found in the plant material analyzed during autumn (IX - 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 amounts of flavonoids 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**.

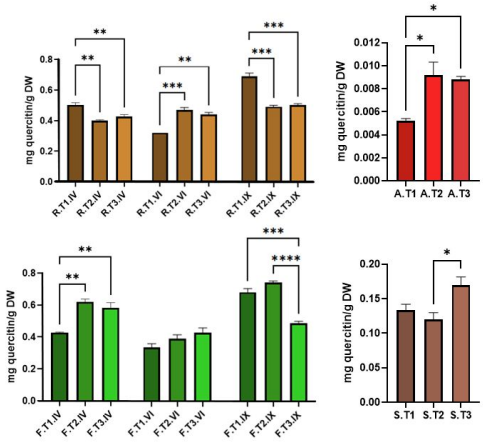

Figure 2. Total flavonoid content

R = bark; **F** = leaf; **A** = aril; **S** = seed; **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)

Little information is known on the chemical composition and biological activity of extracts prepared from various organs belonging to the species *Taxus baccata*. For example, Milutinović et al. (2015) reported that the total flavonoid content in a methanolic extract prepared from leaves and seeds of *Taxus baccata* growing in Serbia was, at the time of conducting experiments, 161.98 ± 1.02 mg rutin equivalent/g dry extract, while Senol et al. (2015) reported a total flavonoid content of 48.89 ± 0.76 mg quercetin equivalent/g in an ethanolic extract from leaves and shoots of *Taxus baccata* growing in Turkey. According to the literature, the differences in total flavonoid content can be attributed to genetic variations, geographical origin, climatic growing conditions, and yew tree populations investigated by Bekhouche et al. (2022).

Antioxidant activity of alcoholic extracts

The antioxidant activity and its intensity in plants depend on the existence of different compounds in the plant species. The antioxidant and radical scavenging activities of flavonoid compounds in plant organisms are well studied and presented in the literature (Das et al. 1992). Some of the phenolic compounds isolated from plants (anthocyanidin, catechins, flavones, flavonols, and isoflavones), tannins (ellagic acid, gallic acid), phenyl isopropanol (caffeic acid, coumaric acids, ferulic acid), lignans, catechol, and many others are compounds with significant antioxidant properties (Rice-Evans et al. 1996 according to Emami et al. 2007). There is very little information in the literature on the antioxidant activity of extracts prepared from different organs of *Taxus baccata*; among these, most data discuss antioxidant activities,

along with other biological activities of individual components derived from this taxon (Erdemoglu et al. 2004, Kucukboyaci et al. 2010 cf. Milutinović et al. 2015.) For example, taxifolin present in several plant species, including *T. baccata*, is thought to scavenge DPPH free radicals (Topal et al. 2016), and other flavonoid compounds, such as proanthocyanidins, amentoflavone, kaempferol, myricetin, isorhamnetin, and apigenin, have been reported to scavenge free radicals (Bekhouche et al. 2022).

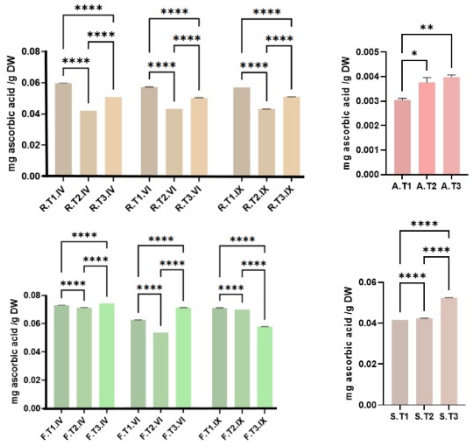

Figure 3. Antioxidant activity of alcoholic extracts

R = bark; **F** = leaf; **A** = aril; **S** = seed; **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)

The data obtained in our research using alcoholic extracts from various organs of the studied *Taxus* taxa (Figure 3) show the highest values of antioxidant activity for extracts obtained from leaves, followed by those from bark, seeds, and arils. Statistically significant differences appear between all taxa at all time points of analysis (IV, VI, IX) as spontaneous species (**T1**) show the highest antioxidant activity in ethanolic extracts from the bark. Extracts obtained from leaves, arils, and seeds, taken from the cultivated taxon **T3** show high antioxidant activity at the timing of strobila formation (IV-April) and intense vegetative growth (VI-June).

Germination indexes

The aqueous extracts obtained from the organs of the studied *Taxus* taxa had different effects on the germination indices (germination percentage, germination rate, germination energy, and vigor index) in the two test species. The effects were more pronounced in the case of seeds of tomato - *Lycopersicon esculentum* Mill., variety Silvia (crop plant), compared to those of *Amaranthus retroflexus* L. (ruderal weed). For both test species analyzed, seed germination was monitored over 336 hours (14 days), following the working protocol for this type of determination presented in the literature (Yarnia et al. 2009, Singh et al. 2012). The application of 5% aqueous extracts obtained from the bark of the cultivated taxon **T3** induced significantly reduced germination percentage values in *Amaranthus* seeds during the period of biomass allocation to roots (IX), (Plate III, A); the other types of extracts (prepared from leaves, arils, and seeds of all studied taxa) resulted in slightly lower values of this germination index, close to those obtained in the control variant (distilled water treatment). In the case of the second germination index - germination speed, it was found that following the application of the 5% aqueous leaf extract treatment, taxon **T3** showed statistically significant differences compared to the control variant at all times of analysis (IV, VI, IX); this treatment is followed by the treatments with 5% and 1% arils extracts, respectively, in all taxa tested, as well as the 1% extracts from the seeds of the spontaneous taxon **T1** and the cultivated taxon **T3** (Plate III, B). Germination energy showed significantly lower values, compared to the control variant,

following treatments with 5% aqueous extract obtained from the bark of cultivated taxon **T3** at the time of biomass allocation to the roots (IX). At the same time, the 5% aqueous extract prepared from the arils of spontaneous taxon **T1** resulted in significantly lower mean values of this germination index compared to the mean values recorded with the control variant (Plate III, C). Significantly reduced vigour index values compared to the control variant were recorded following treatments with 5% aqueous extracts obtained from the bark of spontaneous taxon **T1** (VI) and cultivated taxon **T3** (IX). In the case of the application of treatments with 5% extracts obtained from bark (the cultivated taxon **T2)** and leaves (all three taxa) in April (strobili formation), it can be observed that this time, the extracts have a stimulating effect on the respective physiological parameter (Plate III, D), due to the significantly increased values obtained.

The cumulative analysis of the obtained data shows that the most obvious effects of aqueous extracts prepared from bark, leaves, arils, and seeds from specimens belonging to the three *Taxus* taxa on the germination process in *Amaranthus retroflexus* were produced by extracts of 5% concentration from bark and leaves of cultivated yew taxa specimens, especially taxon **T3**. Among the analyzed germination indices, the emergence rate recorded the lowest values in response to the application of the treatments, compared to the control variant, being the least influenced parameter of the germination percentage.

The germination percentage for the crop species *Lycopersicon esculentum* Mill., variety Silvia showed significantly reduced values following the application of aqueous extracts treatments obtained from the 1% concentration bark at the times of intense vegetative growth (VI) and biomass allocation to roots (IX), but also when applying extracts from the 5% concentration bark in June (time of intense vegetative growth). The other aqueous extracts from leaves, arils, and seeds applied to *Lycopersicon* seeds resulted in slightly reduced values of this germination index compared to the control, but statistically insignificant (Plate III, a). As a result of treatments with 1% aqueous extracts prepared from the bark of cultivated taxa **T2** and **T3**, the germination rate shows significantly reduced values compared to the control variant in April, when strobili formation occurs. In the period of vegetative growth (June), treatments applied with 5% aqueous extracts obtained from the leaves of the investigated taxa show significantly lower values when compared to the control sample (Plate III, b). The highest effects on germination energy were recorded for this test species following treatments with aqueous bark extracts of 5% concentration (VI - intense vegetative growth stage) and 1% (IX - stage of biomass allocation to roots), respectively, in which case significantly lower values are observed, compared to the control, for all three investigated taxa. The other aqueous extracts (leaf, arils, and seed preparations) applied to *Lycopersicon* seeds resulted in slightly reduced values of this germination parameter compared to the control, but not significant (Plate III, c). The vigour index is the germination parameter which, in the case of this test species, for each time of analysis (IV - April, VI - June, IX - September), recorded statistically significantly lower values than the control variant, in the case of treatments with aqueous extracts of concentration 1 and 5%, respectively, obtained from the rhytidome. Significantly lower values were also recorded when applying treatments with aqueous extracts of 1% concentration obtained from the leaves of taxa **T1** - spontaneous taxon and **T3** - cultivated taxa during the formation of strobila (Plate III, d). Overall, in the case of the test species *Lycopersicon esculentum*, variety Silvia the germination indexes considered showed different values, allowing their ranking, in descending order, in the following sequence: vigour index, germination energy, emergence speed, germination percentage; the treatments applied with aqueous extracts of 1 and 5% concentration obtained from the bark of the three *Taxus* taxa induced the most pronounced effects on the seeds of the test species plants during the germination process under experimental conditions.

Biometric determinations

In the experiments with the test species of the ruderal redroot pigweed - *Amaranthus retroflexus* L. - some of the aqueous extracts of *Taxus* tested generally inhibited the analyzed biometric parameters. In this species, stronger influences were recorded when treatments with the extracts obtained from the bark and leaves of all three yew taxa were applied. The mass of newly formed seedlings by germination was generally reduced, but statistically insignificant. In the case of the application of 5% aqueous extracts obtained from leaves of individuals belonging to all three yew taxa under consideration in the period of strobila formation (IV), a stimulation of mass accumulation in newly formed seedlings was observed (Plate IV, E). The mean root length of newly formed seedlings was statistically significantly reduced following treatments with 5% aqueous extracts obtained from the bark of taxa **T1** harvested in June (stage of intense vegetative growth) and **T3** harvested in September (biomass allocation to roots stage). The application of treatments with aqueous extracts obtained from leaves belonging to the cultivated taxon **T3** also had a slightly stimulatory effect on this parameter, in which case statistically significantly higher values of the mean root length of newly formed seedlings were recorded compared to the control variant (Plate IV, F). The same slightly stimulatory effect was observed for the mean hypocotyl length of newly formed seedlings when applying 5% aqueous extracts obtained from the bark of taxa **T2** and **T3**, as well as from the leaves of all three yew taxa studied (**T1**, **T2**, **T3**) - extracts prepared from material collected in April when the strobiles are formed. Similar results, but with significantly higher values than the control sample, were also obtained in June (stage of intense vegetative growth), following treatment with the aqueous extract obtained from the leaves of the cultivated taxon **T2** (Plate IV, G).

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 rhytidome, leaves, arils, and seeds obtained from the three yew taxa allows us to consider that they were, in general, significantly reduced compared to the control sample. The mass of fresh newly formed seedlings was significantly statistically reduced following treatments with 1% aqueous extracts obtained from the bark of all three yew taxa studied (VI - June) (Plate IV, e). The other aqueous extracts from leaves, arils, and seeds applied to *Lycopersicon* seeds showed slightly reduced or increased values of this parameter compared to the control, but statistically insignificant. The mean root length of newly formed seedlings generally showed significantly reduced values after treatments with aqueous extracts obtained from the three yew taxa studied. The lowest values were recorded for seedlings from seeds treated with aqueous extracts of 1 and 5% concentration obtained from the rhytidome of the three yew taxa investigated, obtained at two moments of their phenological cycle, namely intense vegetative growth (VI) and biomass allocation to roots (IX). For the time of strobila formation (IV), the 1% aqueous extracts prepared from the leaves of taxa **T1** (spontaneous taxon) and **T3** (cultivated taxon) showed statistically significantly lower values of this parameter compared to the control (Plate IV, f). The most significantly reduced effects in the mean hypocotyl length of newly formed seedlings (Plate IV, g) were recorded following the application of 1 and 5% aqueous bark extracts obtained from the three yew taxa in June (stage of intense vegetative growth) and September (biomass allocation to roots stage). Also, the 5% aqueous leaf extract obtained from the cultivated taxon **T3** affected the hypocotyl elongation of newly formed seedlings during September (biomass allocation to roots stage), with significantly lower values than the control. For the aqueous extracts obtained from the arils of the three yew taxa investigated, it can be observed that the elongation of the hypocotyl of the seedlings is significantly higher after the treatments compared to the control.

The inhibitory effects of aqueous extracts of yew plants on seed germination and growth parameters of newly formed seedlings of the test species may be due to specific allelochemicals (including phenolic compounds) in their composition. Furthermore, the toxicity of aqueous

extracts might be due to the interactions of the allelochemicals present in them rather than the effects of a single allelochemical (Dadkhah 2012). The toxicity of *Taxus baccata* species has been known since antiquity, and yew leaf extracts have been used for both homicides and suicides. Intoxication with parts of the yew plant (seeds, bark, leaves) is well described in the literature, but cases of suicide by yew ingestion are rarely reported. *Taxus baccata* species contains a complex mixture of compounds, including phenolic constituents (for example, 3,5-dimethoxyphenol), non-alkaloid diterpenoids (for instance, 10-deacteylbaccatin III), alkaloid diterpenoids (for example, paclitaxel, taxin B) or flavonoids (ex. myricetin) and bioflavonoids (bilobetin) (Gupta 2005), compounds that could be responsible for such a severe toxic effect.
Germination is now known to be inhibited by phenolic compounds (Li et al. 2014) or alkaloids (Lovett and Hoult 1998), which can reduce mitotic activity in roots and hypocotyls, suppress hormonal activity, reduce nutrient uptake rates, inhibit photosynthesis and respiration as well as enzymatic action, reduce cell membrane permeability (Rice 1984, Dadkhah 2012), elongate plant roots, cell division, alter cell ultrastructure and subsequently interfere with the growth and normal development of the whole plant (John and Sarada 2012).
Comparative interpretation of the data from the present experiments allows us to consider that the inhibition of germination of the test plants was most strongly manifested in the case of application to their seeds of aqueous extracts of 1% and 5% concentration, respectively, prepared in descending order of their effectiveness from the bark, leaves, seeds, and arils of the three yew taxa. The literature discusses the allelopathic effect of extracts obtained from the integument and endosperm of Chinese yew (*Taxus chinensis* (Rehder & E.H.Wilson) Rehder var. mairei) seeds which, according to the authors, inhibited the germination of cabbage (*Brassica oleracea* L.) seeds and the growth of newly formed seedlings (Zhang et al. 2010). Although there is information in the literature that several gymnosperm genera have demonstrated allelopathic effects on other plants in either *in vitro* tests or *in situ* community studies, studies using such plant material, with the exception perhaps of some members of the genus *Pinus*, are still in their infancy, leaving room for much future research. The possible application of plant extracts or chemicals extracted from these plant species as possible bioherbicides are research topics of genuine interest for the future, with the idea of founding technologies to obtain from this group of plants biologically active compounds of broad interest for organic agriculture (Teixeira da Silva et al. 2015).

Conclusions

Following the investigations carried out on the *Taxus* taxa under study, at three defining moments of the phenological cycle, the chemical characterization of the plant extracts obtained and the testing of their effects on the germination, growth, and development of the seedlings of the test species chosen for analysis, we can conclude the following: in the composition of alcoholic and aqueous extracts of the investigated taxa, the presence of phenolic, alkaloid and carotenoid compounds was revealed; according to the literature, the analysis and discussion of the absorption spectra of these extracts constitutes a novelty for science; the amount of polyphenolic and flavonoids, and the antioxidant activity of alcoholic extracts obtained from rhytidome, leaves, arils and seeds in the analyzed yew taxa showed slight quantitative variations between taxa and times of analysis; in this respect, the cultivated taxon **T2** shows higher amounts of polyphenolic and flavonoid compounds than the spontaneous taxon **T1**; aqueous extracts, especially those obtained from bark, exert inhibitory effects on the analyzed germination parameters (germination percentage, germination speed, germination energy, vigour index); the application of 5% aqueous extracts obtained from the rhytidome of spontaneous taxon **T1** and cultivated taxon **T3** significantly inhibits root elongation 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 the three yew taxa and extracts of 1% concentration prepared from the leaves of the cultivated taxon **T3** inhibited root elongation of newly formed seedlings; hypocotyl elongation of newly formed seedlings in both test plant species was stimulated by the application of 5% aqueous extracts obtained from arils harvested from all three yew taxa; seeds of *Lycopersicon esculentum* Mill. , variety Silvia showed higher sensitivity to the applied aqueous extracts compared to seeds of *Amaranthus retroflexus* L. The allelopathic effects produced by the aqueous extracts obtained from the three yew taxa on the test plants can be continued and completed by increasing the number and diversifying the physiological and biochemical analyses on a wider range of target plants of agricultural interest, as an important scientific research step in the work of identifying, isolating and testing compounds with implications in organic agriculture (with bioherbicidal potential).

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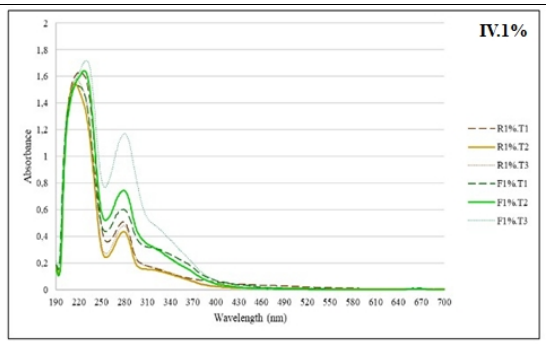
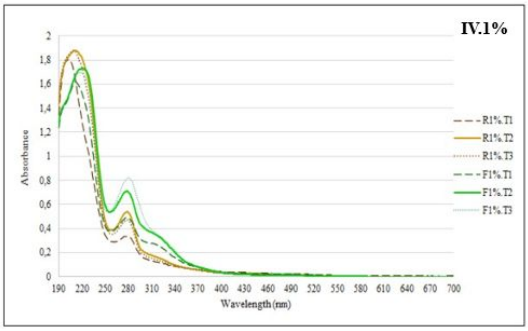
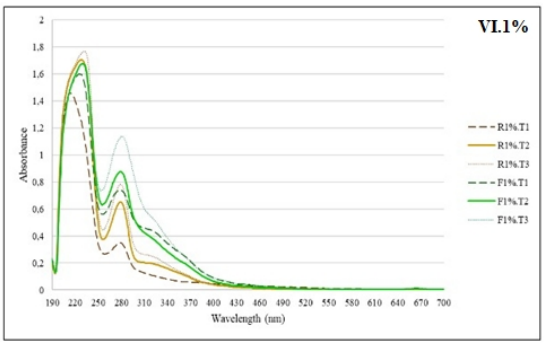
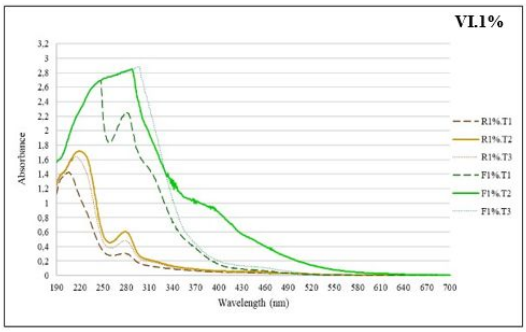
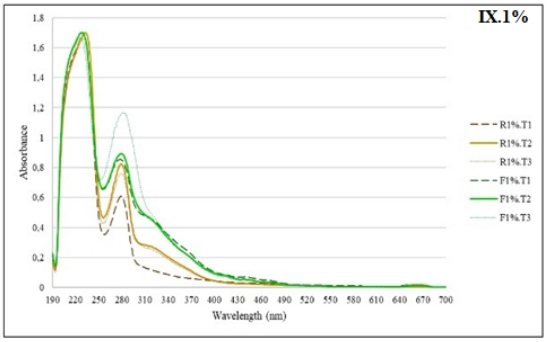
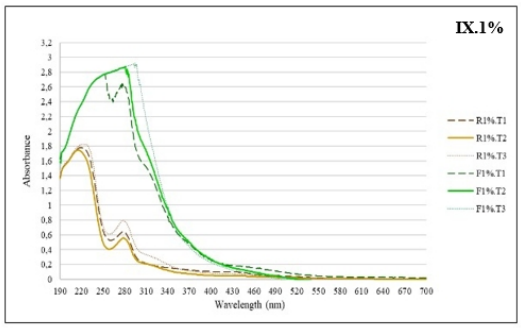
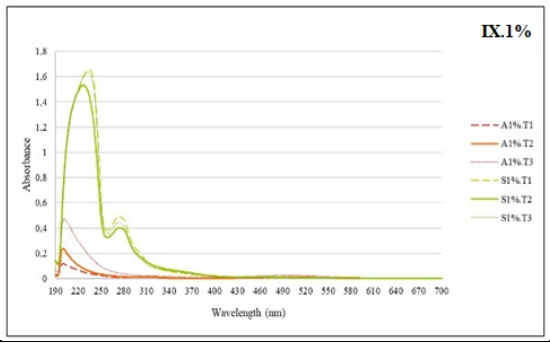
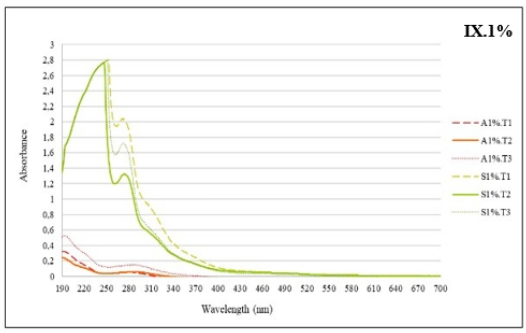
PLATE I

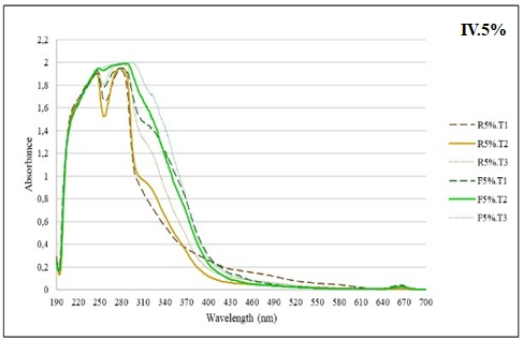
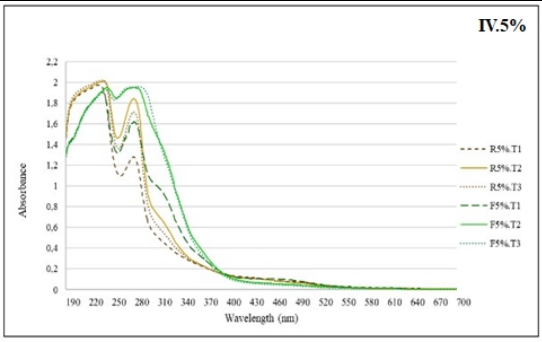
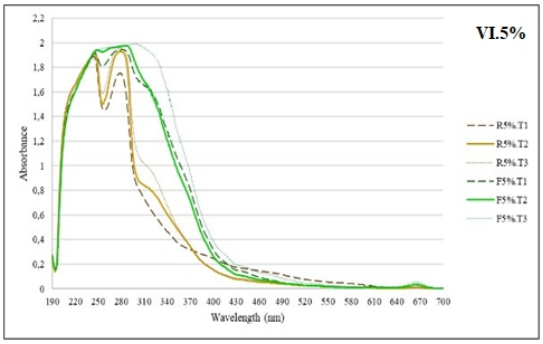
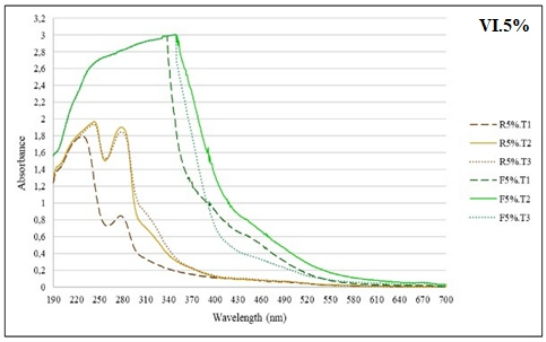
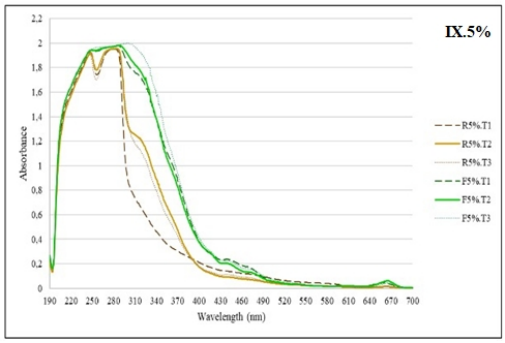
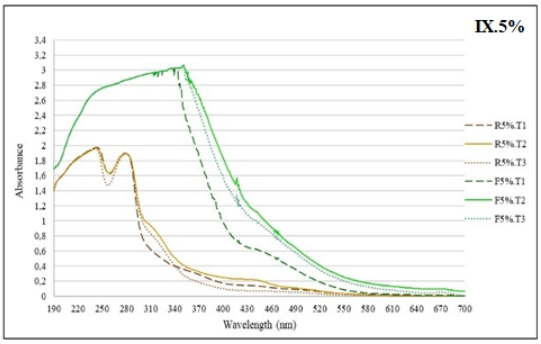
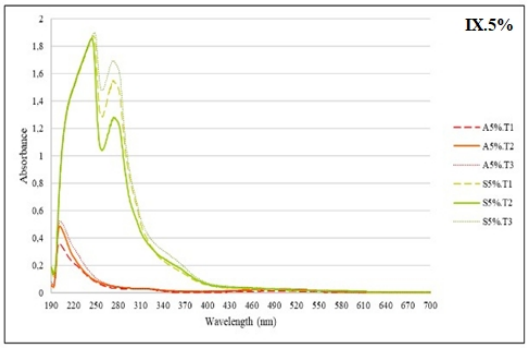
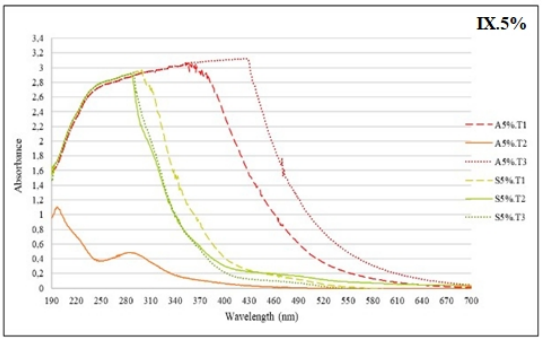
Absorption spectra of alcoholic and aqueous extracts of 1% concentration from *Taxus* bark, leaf, arils, and seeds

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

PLATE II

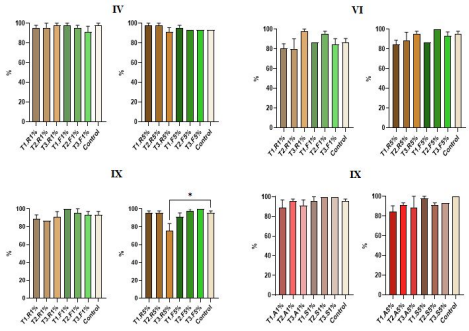
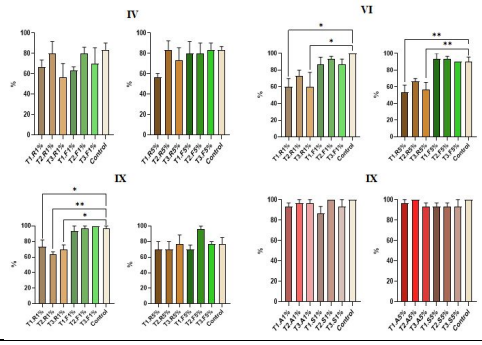
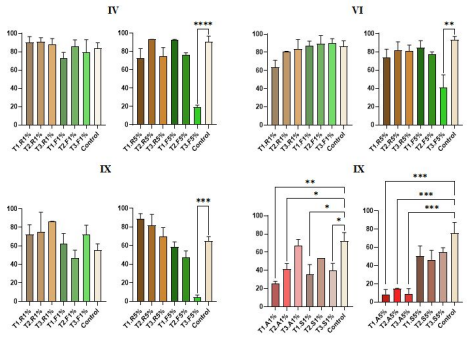
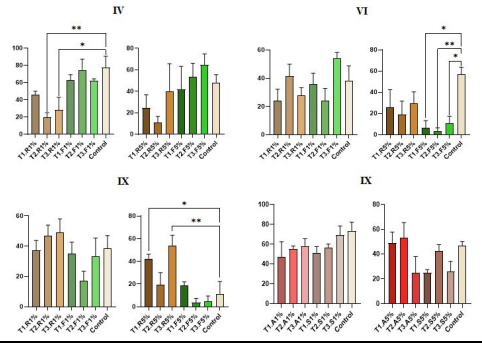
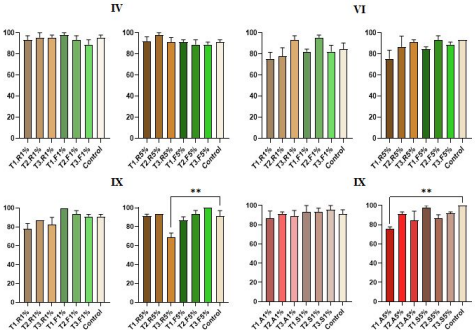
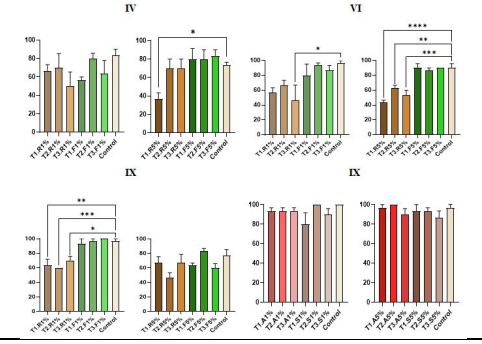
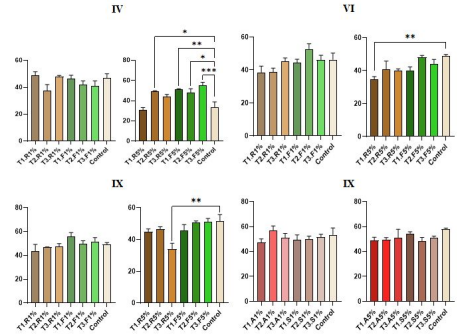
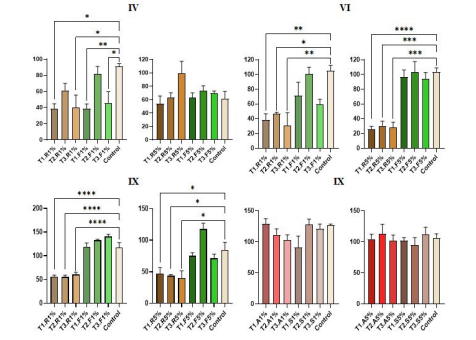
Absorption spectra of alcoholic and aqueous extracts of 5% concentration from *Taxus* bark, leaf, arils, and seeds

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

PLATE III

Effects of *Taxus* extracts on germination indices in test plants

Amaranthus retroflexus L.	*Lycopersicon esculentum* Mill. variety Silvia
A 	**a** 
B 	**b** 
C 	**c** 
D 	**d** 

Legend:

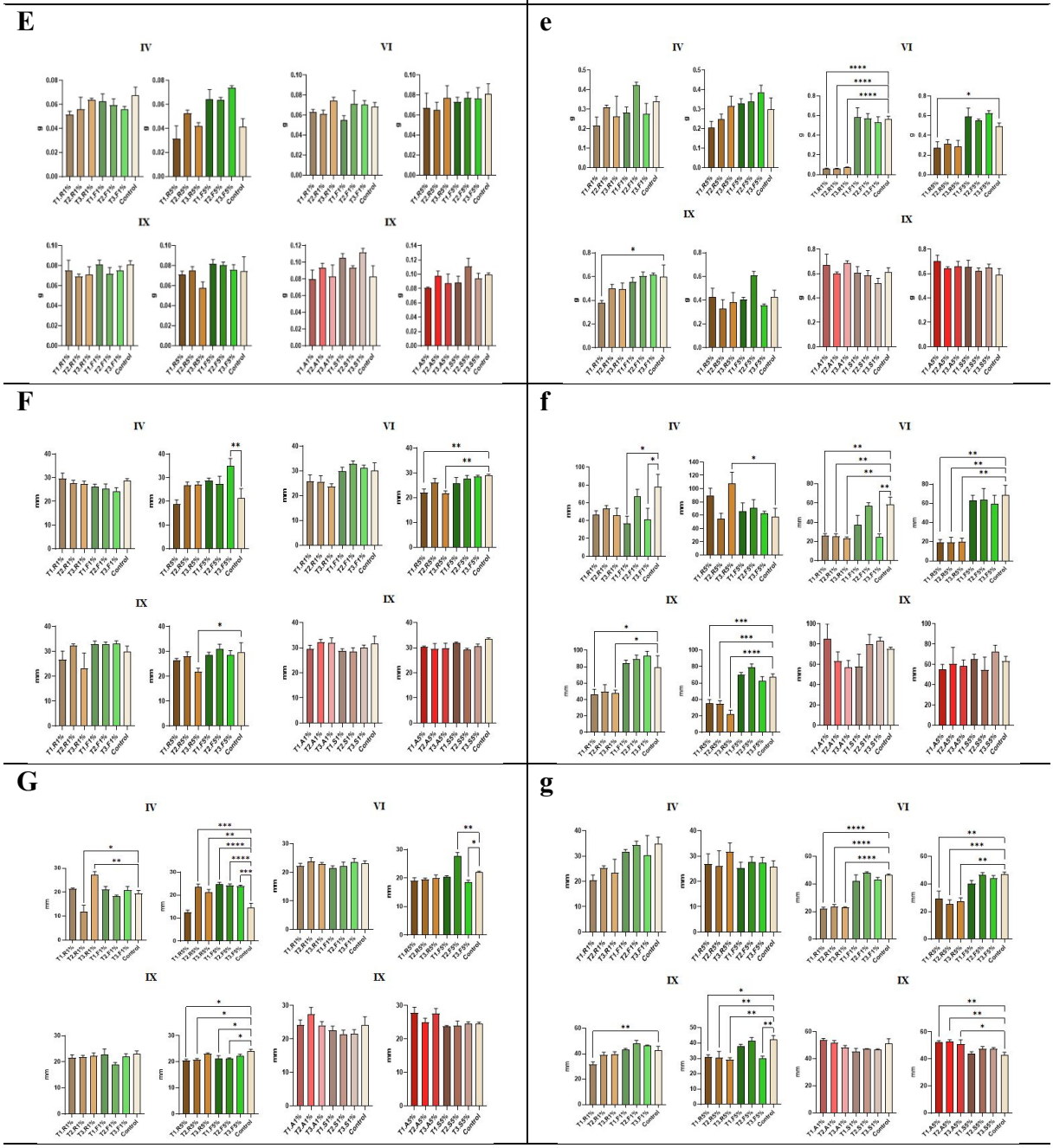
A, a - Germination percentage; **B, b** - Speed of emergence; **C, c** - Germination energy; **D,d** - Seedling vigour index. **IV**-April (strobili production); **VI-**June (intense vegetative growth); **IX-**September (biomass allocation to roots); **T1** = *T. baccata* – spontaneous; **T2** = *T. baccata* –

cultivated; **T3** = *T. baccata 'Robusta'* – cultivated; **R** = bark; **F** = leaf; **A** = aril; **S** = seed; 1%, 5% = extracts of concentration; **** =p<0.0001; *** = p<0.001; ** = p<0.01; * = p<0.05

PLATE IV

Biometric determinations of new seedlings from test plants

Amaranthus retroflexus L.	*Lycopersicon esculentum* Mill. variety Silvia



Legend:

E, e - Fresh seedling mass; **F, f** - Mean root length of seedlings; **G, g** - Mean hypocotyl length of seedlings; **IV**-April (strobili production); **VI**-June (intense vegetative growth); **IX**-September (biomass allocation to roots); **T1** = *T. baccata* – spontaneous; **T2** = *T. baccata* – cultivated; **T3** = *T. baccata 'Robusta'* – cultivated; **R** = bark; **F** = leaf; **A** = aril; **S** = seed; 1%, 5% = extracts of concentration; **** =p<0.0001; *** = p<0.001; ** = p<0.01; * = p<0.05