JPMB | Journal of Plant Molecular Breeding
Published by Genetics and Agricultural Biotechnology Institute of Tabarestan

2024 | Volume 12 | Issue 1

OPEN ACCESS

**Edited by**
Dr. Ali Dehestani,
Genetics and Agricultural Biotechnology Institute of Tabarestan (GABIT), Sari Agricultural Sciences and Natural Resources University,Sari,Iran (SANRU)

**Date**
Received: 10 July 2024
Accepted: 24 July 2024
Published: 28 October 2024

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**Citation**
Jondoaghleboob, A., Ghasemnezhad, A., Sarmast, M.K. and Rahnama, K. (2024). Callus induction and growth, as well as metabolite variations, of two *Taxus* spp. under in vitro conditions. *J Plant Mol Breed 12* (1): 13-.27. doi: 10.22058/JPMB.2024.2035204.1303.



Original paper

# Callus induction and growth, as well as metabolite variations, of two *Taxus* spp. under in vitro conditions

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**Abstract:** The present study investigated the effect of growth regulators on the quantity and quality of calli of two yew tree species (*Taxus* spp). A factorial experiment based on a completely randomized design was performed using 2,4-D and Kinetin on leaf and stem explants of *T. baccata* and *T. brevifolia*. Calli traits, including fresh weight, total phenol, total alkaloids, and paclitaxel content, were investigated. The simple effect of hormonal treatments on total phenolic content, total alkaloids, and fresh weight of calli was significant. Total phenol content and fresh weight were not affected by the interaction of hormonal treatments, whereas total alkaloid content was. Paclitaxel content did not significantly differ between explants. The highest paclitaxel content was found in the leaf explant of *T. baccata* at 30 µg/g, compared to 5 µg/g and 10 µg/g in *T. baccata* stem, *T. brevifolia* stem, and *T. brevifolia* leaf, respectively. The fresh weight and total alkaloid content of stem calli of both species were higher than the leaves. Yew is a valuable endangered medicinal plant that responds well to in vitro treatments. Therefore, it is possible to manage the production of its valuable metabolites under in vitro conditions, and more research is recommended on the production of paclitaxel using *T. baccata*.

**Keywords:** Alkaloid, metabolite, paclitaxel, taxol, tissue culture.

## Introduction

Plants have been an important source of medicine for humans since ancient times, and their importance in modern medicine has only increased. The World Health Organization estimates that more than 80 percent of people use plants in traditional and/or modern medicine, and many synthetic drugs are formulated based on plant chemicals (Tripathi and Tripathi, 2003).

The yew tree (*Taxus* spp.) is a valuable and rare endangered conifer species found in the ancient Hircanian forests of northern Iran (Alavi et al., 2020). Paclitaxel, which has been approved for the treatment of uterine and breast cancer by the USFDA since 1997, is considered the most important anticancer drug (Abbasi Kajani et al., 2012). However, ten tons of skin and wood from yew trees are required to produce one kilogram of paclitaxel. This means that a large number of trees are needed to provide this amount, and periodic consumption of a patient requires the cutting of around 8 60-year-old trees. This puts the endangered species at risk of complete destruction (Malik et al., 2011).

In vitro cell and tissue culture of yew organs is one alternative method for paclitaxel supplementation. Callus, a parenchymal cell that grows in situ and in vitro conditions, can be used for this purpose. Previous studies have shown that the B5 media containing 4 mg/l of 2.4-D and 0.5 mg/l of Kin provides the most suitable conditions for callus formation in *T. baccata* explants (Zhiri et al., 1995; Ashrafi et al., 2010; Toulabi et al., 2015).

The aim of this study is to determine the yield and yield components of paclitaxel in *T. baccata* and *T. brevifolia* under different media conditions and explant types. By exploring alternative methods for paclitaxel production, we can help protect endangered species like the yew tree and ensure that the production of this valuable drug is sustainable in the long run.

## Materials and Methods

### *Explant preparation and treatment*

The present study was conducted in the tissue culture laboratory of the Department of Horticultural Sciences, Gorgan University of Agricultural Sciences and Natural Resources. The young, non-polluting stems of *T. baccata* and *T. brevifolia* were collected from botany garden of university and the national botanic garden of Noshahr, respectively. Around 5 cm terminal section of suitable stems were separated from the original sample and rinsed in water containing a few drops of dishwashing liquid for 10 minutes. In order to completely remove the residue, several washes were performed in running water and the samples were immediately transferred under a laminar flow hood. The plant materials were treated with 70% alcohol for 15 seconds. Immediately, the samples were disinfected with sodium hypochlorite containing 5% activated chlorine and a drop of dishwashing liquid for 25 minutes. Finally, one-centimeter explants were prepared from disinfected plant organs. Explant were cultured in the B5 media containing 6 levels of a combination of 2,4-D and Kin including concentrations of 1, 2 and 3 mg/l 2.4-D and 0.2 and 0.5 mg/l Kin in 3 replications. The cultures were kept in growth room with the temperature and day light of 24 to 26°C and 16:8 hours of light and dark conditions, respectively. Four weeks after culture, subculture was performed. The resulting callus samples were then used to measure morphological traits such as color intensity, tissue thickness, and growth rate and biochemical characters such as total phenol, total alkaloids and the amount of paclitaxel.

### *Total phenol measurement*

The total phenolic content of the calli was measured using the Slinkard and Singleton (1977) method to assess the potential bioactive properties of the calli. Phenolic compounds are known for their antioxidant and antimicrobial activities, as well as their potential in treating various diseases. Moreover, previous studies have reported that callus cultures of *Taxus* species have high levels of phenolic compounds, including flavonoids and tannins, which have been associated with the biosynthesis of paclitaxel, a well-known anticancer drug. Therefore, measuring the total phenolic content of the calli in this study can provide insight into the potential of these cultures as a source of bioactive compounds and anticancer agents. The obtained data were reported as mg/g of fresh weight, which enables comparison with other studies and facilitates the identification of callus lines with higher phenolic contents for further investigation.

### *Total alkaloid measurement*

To measure the total alkaloids, three grams of callus were extracted using a modified method based on Zarezadeh et al. (2000). First, the calli were mixed with 15-20 ml of ammonia and left for 20 minutes. Then, 30 ml of chloroform was added and the solution was mixed on a shaker at a rate of 50-60 rpm for 2.5 hours. The samples were filtered using filter paper under vacuum conditions in a chemical hood. After filtration, the solution volume was reduced to 15 ml using a 50-60°C rotary evaporator for 20 minutes. If necessary, chloroform was added to adjust the volume. Next, 15 ml of 2% tartaric acid was added to the mixture and the aqueous solution was immediately separated. The pH of the aqueous solution was then adjusted to 9 using 25% ammonia. This was followed by the addition of 20 ml of chloroform twice and 10 ml of glacial acetic acid to each sample. After 10 minutes and until the alkaloids were completely dissolved, 3 drops of crystal violet reagent were added to each sample. Finally, the resulting compound was titrated with 1N perchloric acid. At the end of the titration, the color of the crystal violet changed from purple to blue to green.

### *Extraction and measurement of paclitaxel*

Paclitaxel is a well-known anticancer drug that is mainly extracted from the bark of the Pacific yew tree (*Taxus brevifolia*). However, callus cultures of *Taxus* species have been reported to produce paclitaxel, making them a potential alternative source of this important drug. Therefore, measuring the amount of paclitaxel in the calli of this study can provide insight into the potential of these cultures as a source of paclitaxel and contribute to the development of a sustainable and cost-effective production method. The obtained data will be reported as the amount of paclitaxel per gram of fresh weight and can be compared with other studies to identify callus lines with higher paclitaxel contents for further investigation. To extract and determine the amount of paclitaxel, two grams of three-month-old callus were crushed and soaked in 100 ml of methanol for 16-24 hours at room temperature on a shaker to ensure complete solvent permeability. To improve extraction efficiency, the sample was then subjected to ultrasonication for 10 minutes at 25°C. The resulting mixture was filtered using filter paper and equal volume of water was added. To further purify the sample, it was rinsed twice with a solvent (20-30 ml per step) and repeated three times with dichloromethane (Ghassempour et al., 2009). The methanolic extract was then subjected to rotary evaporation to remove dichloromethane, resulting in a dry matter that was dissolved in 5 ml of high-purity acetonitrile and stored at -20°C until analysis (Malik et al., 2011).

### *Determination of the of paclitaxel using HPLC*

The amount of paclitaxel in the stem and leaf of *T. bacata* and *T. brevifolia*, as well as in the callus obtained from these species, was determined using high-performance liquid chromatography (HPLC) with a Merck-Hitachi system (Germany). The measurements were carried out using a C68 column with dimensions of 4.6 × 250 mm. The mobile phase consisted of methanol, acetonitrile, and water at a ratio of 40:40:20 (v/v/v), with a flow rate of 1 mL/min. The UV detection wavelength was set at 230 nm, and the injection rate for both the standards and samples was 20 µL. Prior to injection, the sample was filtered twice using a 0.42 µm filter. The amount of paclitaxel in each sample was calculated by injecting a Paclitaxel-USA standard. The resulting data were reported as mg paclitaxel/g fresh weight of callus. HPLC is a widely used analytical technique for the analysis of phytochemicals, including paclitaxel. The use of HPLC in this study enables the accurate and precise quantification of paclitaxel in the samples from *T. bacata* and *T. brevifolia*, as well as in the callus cultures derived from these species. The obtained data provide insight into the potential of these cultures as a sustainable and cost-effective source of paclitaxel for pharmaceutical applications.

### *Data analysis*

The study was conducted based on a completely randomized design with a factorial arrangement of 6 treatments and 3 replications. The treatments included three levels of 2,4-D (1, 2, and 3 mg/L) and two levels of Kin (0.2 and 0.5 mg/L), as well as two

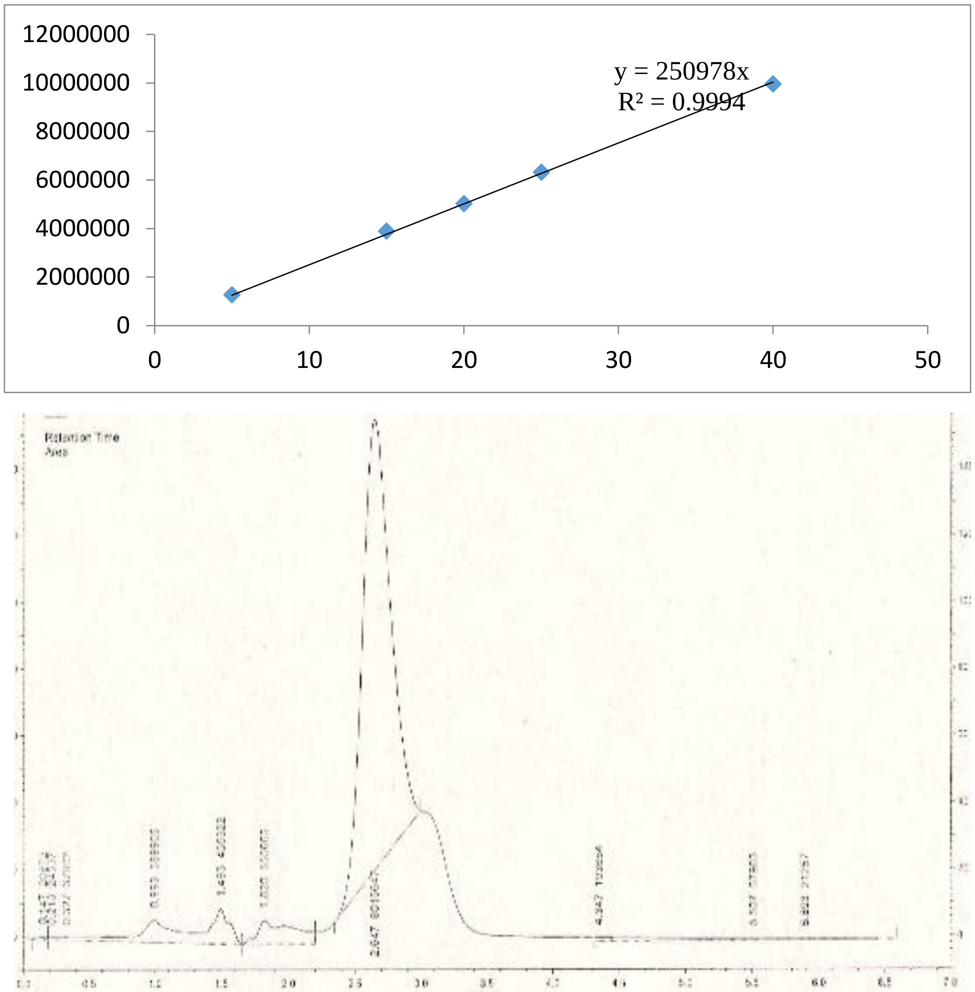


**Figure 1.** Calibration curve (A) and chromatogram (B) of Paclitaxel.

species of *T. baccata* and *T. brevifolia* and two organ types (stems and leaves). The data were analyzed using SPSS software version 16, and the mean values were compared using the least significant difference (LSD) test at a 5% probability level. Graphs were generated using Microsoft Excel version 2010. The use of a factorial design allows for the investigation of the effects of multiple factors and their interactions on the response variable. In this study, the aim was to optimize the production of callus cultures from *T. baccata* and *T. brevifolia* through the manipulation of growth regulators. The statistical analysis and LSD test enable the determination of significant differences between treatments, while the use of graphs facilitates the visualization of the results. Overall, the experimental design and statistical analysis used in this study enhance the rigor and reproducibility of the results.

## Results

### *Explant treatments*

In the present study, due to the high levels of surface contamination observed, different methods were used for surface disinfection of plant organs. As shown in Table 1, for both species of woody perennials tested, treating the stem samples with ethanol 70% for 15 seconds and then sodium hypochlorite (5% active chlorine) for 25 minutes was found to be the best method of surface disinfection. For leaves, 70% ethanol for 5 seconds and sodium hypochlorite (5% active chlorine) for 20 minutes resulted in the best surface sterilization (Table 1).

### *Effect 2,4-D and Kin on morphological characteristics*

While callus formation from stem explants in both species began 15 days after culture initiation, callus formation from leaf explants in *T. baccata* began after only 12 days. In the leaf explant of *T. brevifolia*, callus formation occurred slightly later than in *T. baccata* (Figure 2). Callus formation in *T. baccata* leaves occurred throughout the explant, whereas in *T. brevifolia*, callus formation was observed only at the base of the petiole.

The results showed that among the used treatments, the highest rate of callus growth was observed in stem explants, followed by petioles and leaves (Figure 2). The callus color remained green for the first four weeks after formation. To prevent color change and promote continued growth, the calli were subcultured after 4 weeks in media with the same nutrient and hormonal composition, but with the addition of 200 mg/L of activated charcoal to refresh the media. The color of the subcultured calli returned to orang-brown. The resulting calli had a soft and friable texture at all levels of treatments.

### *The effect of 2,4-D and Kinetin on callus fresh weight*

In this study, we investigated the effect of two plant growth regulators, 2,4-dichlorophenoxyacetic acid (2,4-D) and kinetin, on callus formation in stem and leaf explants of two species of the genus Taxus, *T. baccata* and *T. brevifolia*.

Callus formation is a crucial step in plant tissue culture and can be used for the mass propagation of plants, the production of secondary metabolites, and the study of plant cell growth and differentiation. The fresh weight of callus was used as a measure of callus formation after four weeks of culture on Murashige and Skoog (MS) medium supplemented with different concentrations of 2,4-D and kinetin. Our results showed that the combination of 2,4-D and kinetin had a significant effect on callus fresh weight in both species.

**Table 1.** Effect of surface sterilization on microbial contamination and burning of stem and leaf explant of two Yew species in invitro conditions

| Plant species | Treatment | Burning% | Contamination% |
|---|---|---|---|
| *T.baccata* | | | |
| Stem | 30 s ethanol 70% + 20 m sodium hypochlorite 5% | 35.5 | 86.1 |
| | 50 s ethanol 70% + 25 m sodium hypochlorite 5% | 62 | 5.5 |
| | 15 s ethanol 70% + 25 m sodium hypochlorite 5% | 4.7 | 0 |
| Leaf | 20 s ethanol 70% + 10 m sodium hypochlorite 5% | 0 | 100 |
| | 20 s ethanol 70% + 15 m sodium hypochlorite 5% | 60 | 61.1 |
| | 5 s ethanol 70% + 20 m sodium hypochlorite 5% | 10 | 0 |
| *T.brevifolia* | | | |
| Stem | 30 s ethanol 70% + 20 m sodium hypochlorite 5% | 0 | 100 |
| | 50 s ethanol 70% + 25 m sodium hypochlorite 5% | 66.6 | 11.1 |
| | 15 s ethanol 70% + 25 m sodium hypochlorite 5% | 11.6 | 11.1 |
| Leaf | 20 s ethanol 70% + 10 m sodium hypochlorite 5% | 0 | 100 |
| | 20 s ethanol 70% + 15 m sodium hypochlorite 5% | 36.11 | 100 |
| | 5 s ethanol 70% + 20 m sodium hypochlorite 5% | 0 | 44.4 |

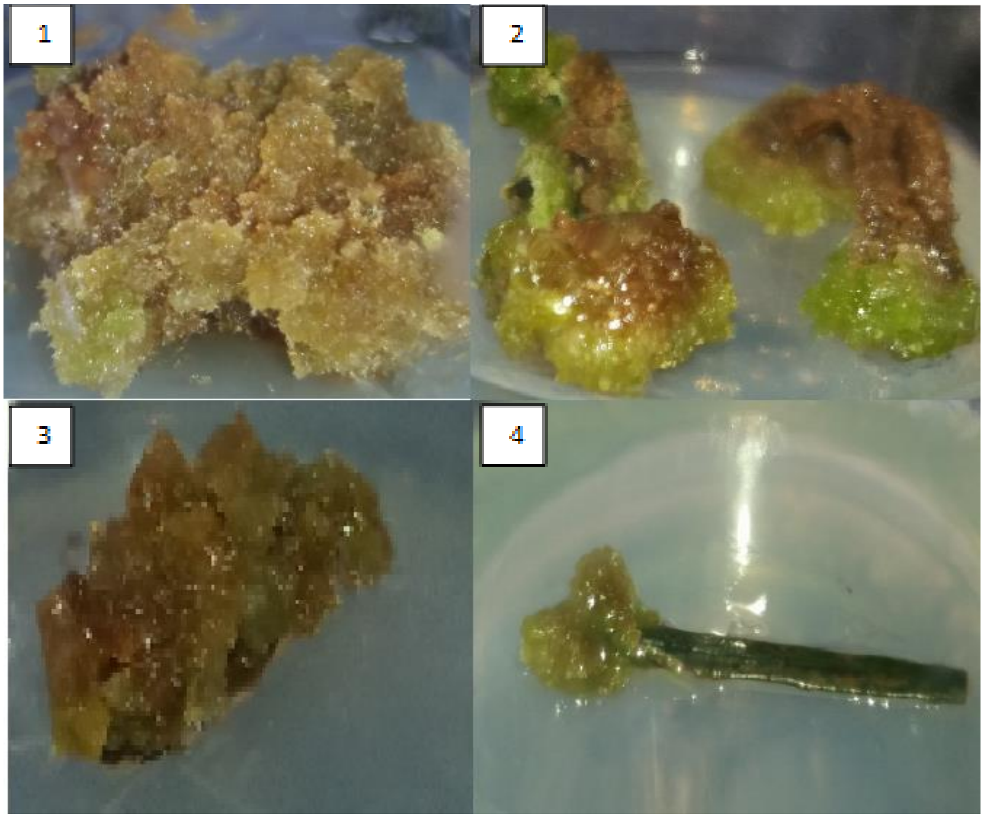


**Figure 2.** Callus formation in *T. baccata* and *T. brevifolia* stem and leaf explants(1) *T. baccata* stem, (2) *T. baccata* leaf, (3) *T. brevifolia* stem, (4) *T. brevifolia* leaf.

**Table 2.** Analysis of variance of the effect of 2,4-D, Kin and their interaction on phenol, total alkaloids and total fresh weight of callus.

| Source of variation | df | Total phenol | Total alkaloid | Total fresh total sugar weight |
|---|---|---|---|---|
| 2,4-D | 3 | 50.119** | 13.74** | 12.318** 2.845$^{ns}$ |
| Kin | 2 | 0.001$^{ns}$ | 13.74** | 36.739* 0.319$^{ns}$ |
| Species | 1 | 58.75** | 8.15** | 20.119** 32.86** |
| 2,4-D×kin | 6 | 8.261** | 9.275** | 3.996* 0.102$^{ns}$ |
| Kin× Species | 2 | 164.905** | 26.494** | 24.307** 94.293** |
| 2,4-D×Species | 3 | 4.142** | 8.13** | 5.685** 0.168$^{ns}$ |
| Species×2,4-D× Kin | 6 | 4.078** | 3.289* | 0.991$^{ns}$ 1.047$^{ns}$ |
| Erro | 42 | 0.001 | 0.003 | 0.058 0.002 |

ns, *, ** : non-significance and significance at the level of 5% and 1%, respectively.

Specifically, it has been found that the optimal combination of 2,4-D and kinetin for stem explants of *T. baccata* was 2 mg/L 2,4-D and 0.1 mg/L kinetin, while the optimal combination for leaf explants of *T. brevifolia* was 1 mg/L 2,4-D and 0.1 mg/L kinetin. These findings suggest that the use of appropriate concentrations of 2,4-D and kinetin can enhance callus formation in these species, which may have important implications for their propagation and the production of secondary metabolites.

The results of the analysis of variance showed that the effect of 2,4-D and kinetin individually, as well as their interaction, on callus fresh weight was significant, as shown in Table 2. Increasing the concentration of both hormonal agents individually resulted in an increase in callus fresh weight, as shown in Figures 3(b) and 3(a). The interaction effect of treatments was also found to be significant, as shown in Figure 3(d). Although increasing the concentration of kinetin did not have a significant effect on callus growth compared to increasing the concentration of 2.4-D, under the hormonal combination of 3 mg/L 2.4-D and 0.5 mg/L kin, and 2 mg/L 2.4-D and 0.5 mg/L kin, no significant difference was observed, as shown in Figure 3(d).

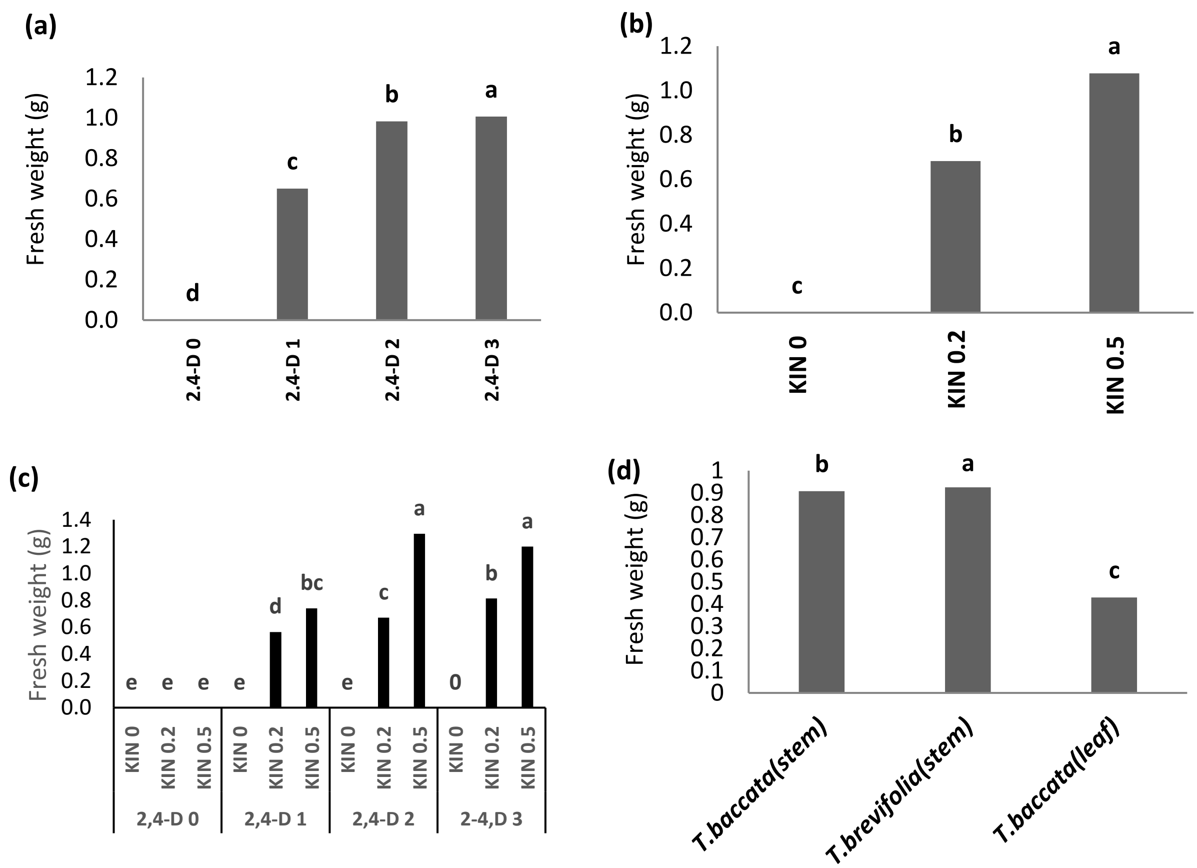


**Figure 3.** Effect of kin on yew callus FW (a), effect of 2,4-D on yew callus FW (b), callus FW of different yew species (c), interaction effect of treatments on callus FW(d).

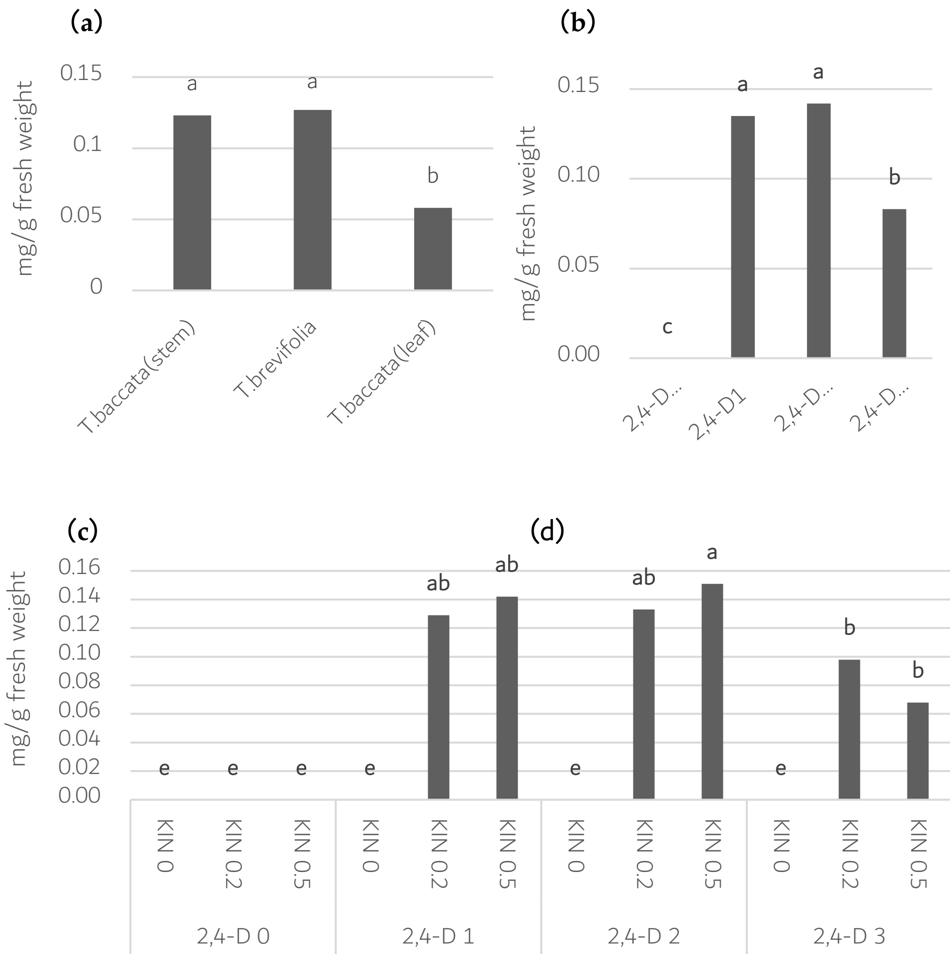


**Figure 4.** Effect of plant species on phenol content of callus (a), Effect of 2,4-D on phenol content of callus(b), Interaction effect of 2,4-D and kinetin on the phenol content of yew callus(c).

***The effect of different levels of 2,4-D and Kin on the amount of total phenol of yew callus in two different species***

In this study, the effect of different levels of 2,4-D and kinetin on the total phenol content of callus cultures from two yew species, *Taxus baccata* and *Taxus brevifolia* was investigated. The results showed that the effect of 2,4-D, the interaction effect of 2,4-D × kinetin, and the triple effect of 2,4-D × kinetin × species was significant on the amount of total phenol of callus in both species (Table 2).However, total phenol decreased with increasing 2,4-D concentration (Figure 4(b)). Phenol accumulation of leaf explants was found to be less than stems explants in both species. However, there was no significant difference between the phenol content of calli produced on stem explants (Figure 4(a)). Under the interaction of 2,4-D and kinetin, the amount of phenol decreased with increasing the concentration of treatment (Figure 4(c)). On the other hand, between the treatment of 2 mg/l of 2,4-D and 0.5 mg kinetin and 2 mg/l 2,4-D and 0.2 mg/l kinetin, no significant difference was observed. A similar trend was observed between 3 mg/l 2,4-D and 0.2 mg/l kinetin, with 3 mg/l 2,4-D and 0.5 mg/l kinetin. Unlike 2,4-D, kinetin does not appear to play an effective role in tissue phenolic compounds accumulation.

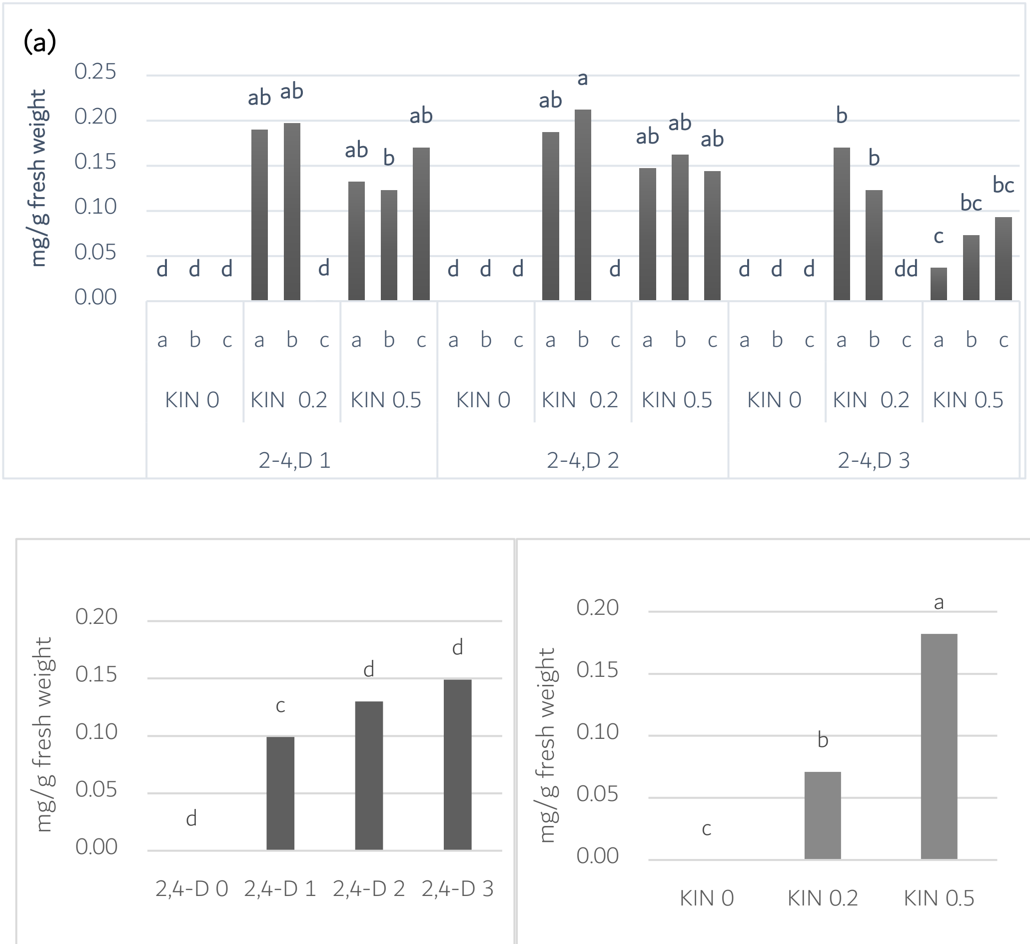


**Figure 5.** Interaction effect of 2, 4-D × Kin × species on the phenol content of yew callus (a = Baccata stem explant, B = *T. baccata* leaf petiole explant. C = *T. brevifolia* stem explant) (a), Effect of kinetin concentration on the total alkaloid (b), Effect of 2,4-D on the total alkaloid(c).

***The effect of different levels of 2,4-D and Kin on the total alkaloid content of two species of yew***

The analysis of variance in Table 3 showed that the total alkaloid content was significantly affected in both *Taxus baccata* and *Taxus brevifolia* when 2,4-D and kinetin were used alone or together. With increasing 2,4-D and kinetin levels, total alkaloid accumulation increased. However, the increase in alkaloid accumulation of stem explants of both species was higher than *T. baccata* leaves (Figure 5). In the interaction effect of 2,4-D×kinetin (Figure 6(a)), the amount of total alkaloids increased by increasing the kinetin concentration from 0.2 to 0.5 mg/l, regardless of the concentration of 2,4-D. It seems that the role of kinetin was more effective than that of 2,4-D in total alkaloids accumulation. Surprisingly, in contrast to total phenolic accumulation, no significant change was observed in the amount of total alkaloids when 2,4-D concentration increased. These findings suggest that the use of appropriate concentrations of 2,4-D and kinetin can enhance the production of alkaloids in yew callus cultures, but the effect of kinetin may be more pronounced than that of 2,4-D. Further studies are needed to investigate the specific alkaloids produced by these cultures and their potential pharmacological properties. Figure 6 shows that under the interaction of 2,4-D, kinetin, and species, the total alkaloid content of calli obtained from *T. baccata* leaf and stem explants was significantly different from that of callus obtained from *T. brevifolia* stem explant.

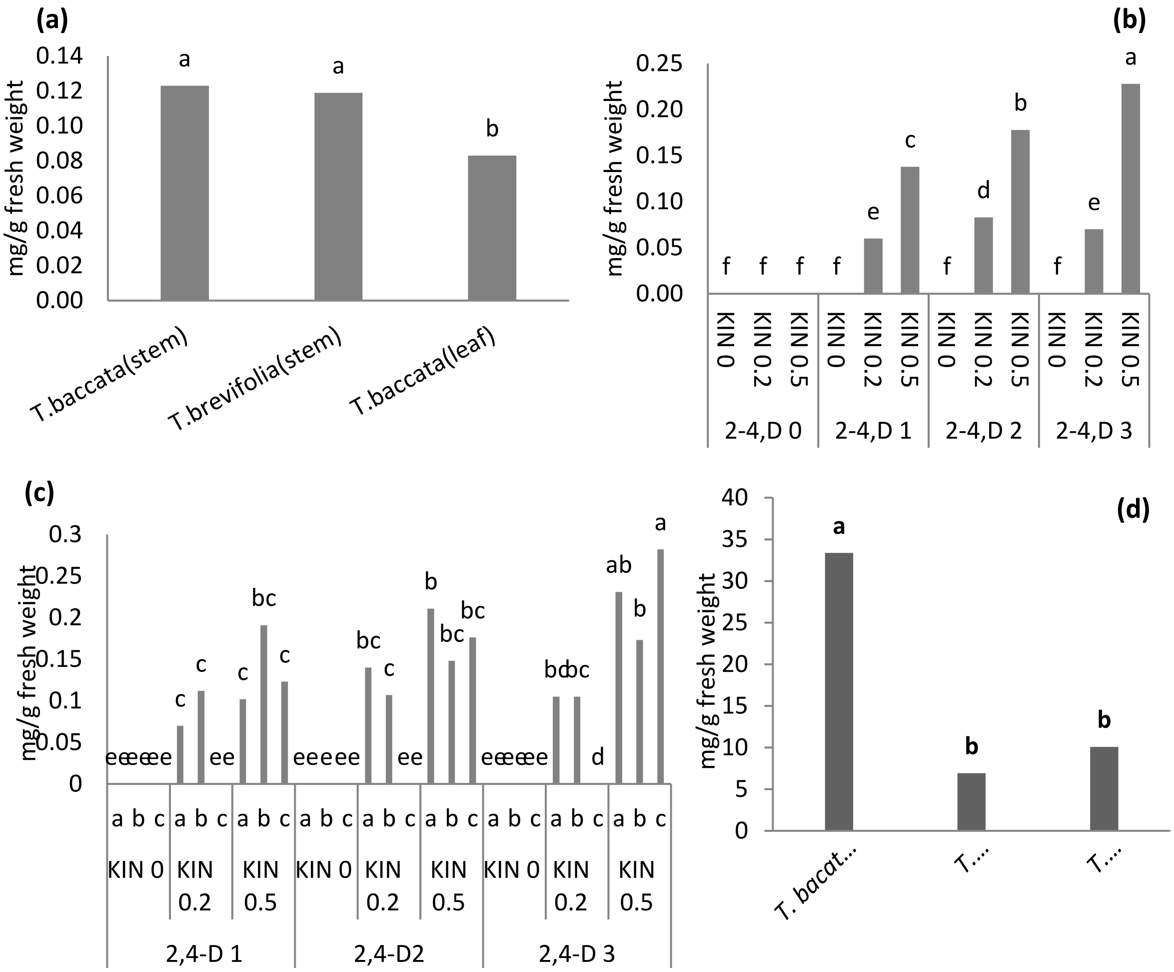


**Figure 6.** Interaction effect of kinetin×2,4-D on the total alkaloid (a), effect of species on the total alkaloid (b**),** total alkaloid content affected by complex effect of treatments (a =stem callus of *T. baccata*, b =leaf callus of *T. baccata*, c =stem callus of *T. brevifolia*) (c), mean value of paclitaxel affected by plant species(d).

On the other hand, in medium containing 2 mg/l 2,4-D and 0.5 mg/l kinetin, no significant difference was observed between *T. brevifolia* and *T. baccata*. However, there was a significant difference between *T. baccata* explants.

### *Effect of treatments on the paclitaxel content of sample*

Results of this study showed that the amount of paclitaxel in *T. baccata* stem callus was significantly higher (33.36 μg/g) than that of *T. brevifolia* stems (26.45 μg/g) and leaves (23.28 μg/g) (Figure 6(d)). However, no significant difference was observed between the paclitaxel content of calli derived from *T. brevifolia* stem and leaf explants.

## Discussion

Surface microbial contamination is one of the most important barriers in in-vitro culture of woody perennials, especially when explants are directly collected from the wild. The first step in tissue culture is the establishment of culture, which means the elimination of surface sterilization of tissue against fungal and bacterial contamination. Plants often contain different sources of microbial contamination, such as microorganisms present on the surface, in small gaps, emerging leaves, and buds. Therefore, proper control of these microbial contaminants is an important and necessary step to reduce the contamination of explants used in tissue culture (Nikvash et al., 2006; Sarmast, 2018). In both stem and leaf explants, increasing the time of sterilization with ethanol resulted in a temporary reduction in the percentage of contamination, but also reduced the viability of explants. It appears that ethanol enhances the penetration of the main disinfectant solution into the tissue by removing the cuticle layer from the tissue surface. However, ethanol should be used for a short time due to its antimicrobial properties and potential to cause plant toxicity (Asareh et al., 2007). Studies have shown that in *Juniperus communis*, fungal contamination can be controlled in most cases by applying ethanol for a short duration and using an appropriate concentration of sodium hypochlorite (Sarmast, 2018).

Ashrafi et al. (2010) reported that longitudinal sections of the stem show better cell proliferation in tissue culture. They suggested that callus formation from the cambium and the outer parenchymal tissues of the stem is likely due to the larger area provided for the absorption of central components. Compared to the stem, the callus formed in leaf explants was smaller and weaker, probably due to the smaller amount of meristematic tissue in the leaf. Based on the type of explant, stem explants were found to be more effective than leaf explants for callus formation in both species, which is consistent with findings reported by other researchers (Ashrafi et al., 2010). The culture media and various growth regulators can affect callus quality, which depends on the type and amount of elements present in the culture medium (macro and micronutrients, vitamins, etc.). It has been reported that culture media containing low concentrations of kinetin (Kin) and high concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) can promote the formation of callus with a rapid growth rate and a soft and loose structure (Davarpanah et al., 2015), which was also observed in the results of the present study. Overall, the callus formation potential of *T. baccata* was found to be greater than that of *T. brevifolia* for both types of explants (Figure 2). This may be due to differences in the environmental and geographical conditions of the habitats of the two species. It should be noted that callus production of different species and even subspecies of the same species might have different responses to culture media and hormonal composition (Bruňáková et al., 2004).

The lowest callus fresh weight was observed in the control treatment. Our observations showed that explants cultured in media containing no hormonal compounds did not exhibit either callogenesis or organogenesis, indicating that the presence of growth regulators is essential for the formation of callus (Brukhin et al., 1996; Bagheri and Saffari, 2011).

We found that media containing 2.4-D at 3 mg/L was recommended for callus production from both stem and leaf explants of *T. baccata*. Karimian et al. (2014) also reported that the role of 2.4-D in callus production of *T. brevifolia* stem explant was stronger than that of kinetin. The results of the mean comparison (Figure 3(c)) showed that the fresh callus weight of *T. baccata* stem and leaf was lower than that of *T. brevifolia* stem explants. However, the callus formation of *T. baccata* leaf samples was less than that of the stem (Figure 3(c)). These results are consistent with those of Razavi et al. (2017) and Ghafoori et al. (2012), who reported that callus formation in *T. baccata* stem explants is significantly higher than that in explants prepared from the leaf.

Application of appropriate concentrations of 2,4-D was found to enhance the production of phenolic compounds in yew callus cultures, which may have important implications for the production of bioactive compounds from yew tissue cultures. However, browning is one of the most important barriers to in vitro culture, which may affect the production of phenolic compounds. Tissues cultured under in vitro conditions may become brown for various reasons, including wounds caused by cutting explants, contamination with pathogens, oxidation of phenolic compounds and tannins, high concentration of sterilizers agents, pH adjustment, ambient heat, etc. (Chee, 1995). Therefore, strategies to reduce browning in tissue cultures should be considered to improve the production of phenolic compounds.

In general, the findings of this study suggest that the manipulation of plant growth regulators can have a significant impact on the production of phenolic compounds in yew callus cultures. Further studies are needed to investigate the specific phenolic compounds produced by these cultures and their potential pharmacological properties.

These results suggest that the genetic differences between the two yew species may play a role in the production of alkaloids in callus cultures. Therefore, optimization of growth conditions and plant growth regulators should be tailored to the specific species being studied. Khataee and Karimi (2010) showed that the simultaneous application of BA (benzyladenine) at 0.5 and 1 mg/l, with different concentrations of NAA (naphthalene acetic acid), significantly increased the alkaloid content of callus compared to the control. These findings suggest that the use of appropriate combinations of plant growth regulators can enhance the production of alkaloids in yew callus cultures. Further studies are needed to investigate the specific alkaloids produced by these cultures and their potential pharmacological properties.

The production of alkaloid tropane in tissue culture is indeed highly dependent on the composition of the culture medium. Previous studies have shown that various factors, such as nutrient sources, growth regulators, and different growth conditions, can significantly affect the production of tropane alkaloids in tissue culture (Iranbakhsh et al., 2007). The type and concentration of growth regulators have been found to be important factors that strongly influence the accumulation of these metabolites. To optimize the production of tropane alkaloids in tissue culture, it is crucial to carefully select and adjust the growth conditions and growth regulators used. The use of appropriate combinations of growth regulators, such as auxins and cytokinins, has been shown to enhance the production of tropane alkaloids in tissue culture . In addition, the use of elicitors, such as methyl jasmonate and salicylic acid, can also stimulate the production of tropane alkaloids in tissue culture .

Overall, understanding the factors that affect the production of tropane alkaloids in tissue culture is essential for the development of efficient and sustainable methods for the production of these important metabolites. Further studies are needed to elucidate the specific mechanisms underlying the regulation of tropane alkaloid biosynthesis in tissue culture and to optimize the production of specific alkaloids with potential pharmacological properties.

Despite the fact that paclitaxel was first identified in *T. brevifolia*, studies have shown that the amount of this compound and its derivatives in some other species of this genus, such as *T. baccata*, is higher than that of *T. brevifolia*. However, it should be noted that the rate of paclitaxel varies from species to species and even from organ to organ, and can vary between 0 and 500 μg/g dry weight (Vidensek et al., 1990). Previous studies by other researchers (Ahadi et al., 2013) have reported that, in general and under natural conditions, the potential of paclitaxel production is higher in *T. baccata* compared to *T. brevifolia*. This tendency was also observed in the in vitro studies of the present study (Figure 6(d)). These findings suggest that tissue culture techniques can be used to produce paclitaxel and other important metabolites in a controlled and sustainable manner. Further studies are needed to optimize the production of specific metabolites with potential pharmacological properties and to elucidate the underlying mechanisms of their biosynthesis in tissue culture.

## Conclusion

The results of the study showed that the disinfection method used for tissue culture depends not only on the type and duration of disinfectant but also on the different plant species and organs being used. Therefore, it is recommended to establish a reliable disinfection method for the specific plant species and organs before conducting the main experiment. The best hormonal treatment for callus induction in yew trees was found to be 3 mg/l of 2,4-D in combination with 0.2 mg/l of kinetin, regardless of the type of treatment. The fresh weight of *T. brevifolia* callus was higher than that of *T. baccata* under equal conditions. However, the callus from *T. baccata* stems had a higher weight than that from its leaves. The study also found that an increase in the concentration of 2,4-D and kinetin in the medium led to an increase in the total alkaloid content of the callus. However, the alkaloid content of the callus induced from the stem of both species was higher than that obtained from the leaves of *T. baccata*. In contrast, an increase in the concentration of 2,4-D led to a decrease in the total phenol content of the callus. Furthermore, the phenolization of leaf explants was found to be less than that of stem explants in both species. However, no significant difference was observed between the stem phenolization rates of the studied species. It is suggested that in future studies, the effect of different concentrations of hormones be evaluated in more detail, and the metabolic concentration in the culture medium should also be investigated to optimize the production of specific metabolites with potential pharmacological properties.

## Supplementary Materials

No supplementary material is available for this article.

## Author Contributions

Laboratory analysis, data curation, writing- original draft preparation A.J.; Idea, supervision methodology, A.Gh.; Advisor, M.K.S., K.R. Authors have read and agreed to the published version of the manuscript.

## Funding

This research received no external funding.

## Acknowledgments

The authors would like to thank the Vice Chancellor for Research of Gorgan University of Agricultural Sciences and Natural Resources for providing facilities essential to the successful implementation of this research.

## Conflict of Interest Statement

The authors declare no conflict of interest.

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JPMB | Journal of Plant Molecular Breeding
Published by Genetics and Agricultural Biotechnology Institute of Tabarestan
2024 | Volume 12 | Issue 1

# القا و رشد کالوس، و همچنین تغییرات متابولیت، دو گونه Taxus تحت شرایط درون شیشه‌ای

مقاله پژوهشی

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**چکیده:** مطالعه حاضر به بررسی اثر تنظیم‌کننده‌های رشد بر کمیت و کیفیت کالوس دو گونه Taxus می‌پردازد. آزمایشی به صورت فاکتوریل در قالب طرح کاملاً تصادفی با استفاده از 2،4-D و کاینتین بر ریزنمونه‌های برگ و ساقه گونه‌های *T. baccata* و *T. brevifolia* انجام شد. صفاتی چون وزن تر، فنل کل، آلکالوئید کل و محتوای پاکلی‌تاکسل مورد بررسی قرار گرفت. اثر ساده تیمارهای هورمونی بر میزان فنل کل، آلکالوئید کل و وزن تر کالوس معنی‌دار بود. محتوای فنل کل و وزن تر تحت تأثیر اثر متقابل تیمارهای هورمونی قرار نگرفت، در حالی که محتوای آلکالوئید کل تحت تأثیر تیمارها قرار داشت. محتوای پاکلی‌تاکسل تحت تاثیر نوع ریزنمونه قرار نداشت. بیشترین مقدار پاکلی تاکسل در ریزنمونه برگ *T.baccata* با ۳۰ میکروگرم بر گرم در مقایسه با ۵ میکروگرم بر گرم و ۱۰ میکروگرم در گرم به ترتیب در ساقه *T.baccata*، ساقه *T. brevifolia* و برگ *T. brevifolia* مشاهده شد. وزن تر و محتوای آلکالوئید کل کالوس ساقه هر دو گونه بیشتر از برگ بود. سرخدار یک گیاه دارویی ارزشمند در حال انقراض بوده که به خوبی به تیمار آزمایشگاهی پاسخ می‌دهد. با توجه به امکان تولید متابولیت‌های ارزشمند این گیاه در شرایط آزمایشگاهی، تحقیقات بیشتر درخصوص تولید پاکلی‌تاکسل درگونه T. baccata در شرایط درون شیشه‌ای توصیه می‌شود.

**کلمات کلیدی:** آلکالوئید، متابولیت، پاکلیتاکسل، تاکسول، کشت بافت.

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**تاریخ**

دریافت: ۲۰ تیر ۱۴۰۳
پذیرش: ۳ مرداد ۱۴۰۳
چاپ: ۷ آبان ۱۴۰۳

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**ارجاع به این مقاله**

Jondoaghleboob, A., Ghasemnezhad, A., Sarmast, M.K. and Rahnama, K. (2024). Callus induction and growth, as well as metabolite variations, of two *Taxus* spp. under in vitro conditions. *J Plant Mol Breed 12* (1): 13-.27.
doi: 10.22058/jpmb.2024.2035204.1303.