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Research Article

Comparative chromatographic analysis of phenolic compounds of *Liquidambar orientalis* plant cultivated under *in vitro* salt stress

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Abstract: In this study, the effects of boron salt stress on in vitro cultivated *Liquidambar orientalis* (*L. orientalis*), a relict-endemic plant species, and the resulting changes in its phenolic appearance were investigated. Salt stress can cause negative impact on plant growth and production, especially in species with low salinity and drought tolerance, affecting metabolite expression and somaclonal execution. To evaluate the effects of different boron salts on meristem regeneration and progression, clonal in vitro *L. orientalis* meristems were exposed to boric acid, sodium perborate, sodium metaborate, and disodium octaborate salts. When compared with the control group examples where salt application was not performed, the highest regeneration percentage was determined to be 100% with the application of 1 mg/L disodium octaborate. In terms of the shoot formation capacity index, it was determined to be 5 mg/L. With a value of 4.94, the application of sodium perborate yielded the best result. In *L. orientalis* plants, the greatest change in phenolic compounds due to boron salt applications was observed in the concentration of Quercetin with the sodium perborate salt application at 1 mg/L concentration.

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1. INTRODUCTION

Liquidambar orientalis Miller, called Amber-i Sail in ancient times, is a member of the Altingiaceae family (Figure 1a). The plant, distributed in the southwestern regions of Türkiye and also known as Anatolian sweetgum tree, is a relict endemic species with medicinal-aromatic properties (Alan *et al*., 2018). Anatolian sweetgum, which was included in the noble hardwood group by EUFORGEN (European Forest Genetic Resources Program) in 2001 and is accepted as a species that needs to be protected throughout Europe, is also included in the "Highly Threatened in the Medium-Term Future in Nature" category in the IUCN danger categories list (Ekim *et al*., 2000). The natural distribution area of individuals belonging to the population is only in Fethiye, Marmaris and Köyceğiz districts. However, the natural forests of this species

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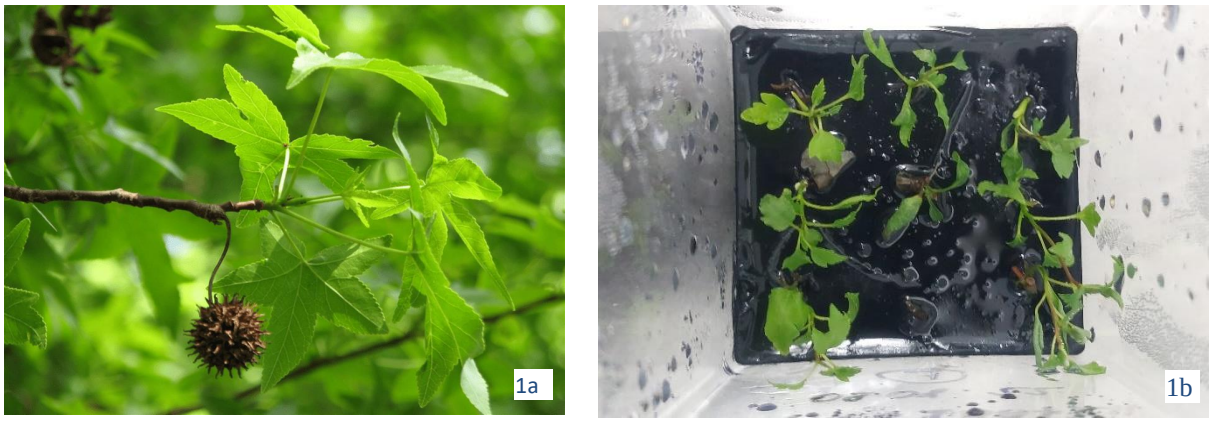
are in danger of extinction due to some reasons such as changing climatic conditions and destruction to create agricultural areas (Alan & Kaya, 2003). The oil with medicinal-aromatic properties obtained from the resin channels of the Anatolian sweetgum tree is an important raw material source for the cosmetics, pharmaceutical and chemical industries, which has been used and traded for thousands of years (Acar, 1989; Bozkurt *et al*., 1989).

Figure 1. *L. orientalis* plant in the natural population area (1a); clonal propagated seedlings *in vitro* (1b).



In vitro propagation techniques are the processes of producing more than one plant with the same genetic characteristics as that plant from organs such as shoots, roots, and stems, which can form a new *in vitro* micro-shoot (Figure 1b). It requires less material and is a faster production method than seed or steel production method (Ozudogru *et al.*, 2011; Kaya *et al.*, 2021). This technique, which is also used in the commercial sector, is used in many plant species in park-garden plants, agriculture, and forestry (Kaya *et al.*, 2018). Sometimes seed germination can take up to two years. Regeneration percentages are generally low. In addition, seed planting should be done seasonally. For these reasons, mass production is preferred commercially. Obtaining propagation material from tall trees is very difficult, thus it limits the success of clonal propagation using semi-mature shoot cuttings (Ozudogru *et al.*, 2013). Micropropagation offers an alternative method for producing Anatolian sweetgum for both commercial and conservation purposes (Bayraktar *et al*., 2015).

The use of *in vitro* techniques can reinforce *ex situ* and *in situ* conservation techniques for preserving plant genetic resources (Kaya *et al*, 2013). Axillary and/or apical shoot meristem-based *in vitro* propagation is one of the useful technologies used in plant biodiversity conservation (Souza *et al.*, 2017). Although boron salts and other salinity stresses and their effects on seed germination, shoot growth and development have been investigated in the literature (Munns & Termaat, 1986; Nable et al., 1997), there is still some uncertainty about the response of plants to combined stress caused by boron salts and other substances. Salinity stresses are usually found in waterlogged soils with high concentrations of boron and other salts in grains (Nable *et al*., 1997). In addition, naturally occurring high salinity can cause salinity stress in arid and semi-arid areas. In the current literature, there are limited studies on the effects of boron toxicity and salt stress on plant growth and development, and the response of plants to these stresses. There is no consensus on the interrelationships between boron toxicity and salinity stress (Keren & Bingham, 1958).

Boric acid is a crucial micronutrient for plants, influences various fundamental processes such as cell wall formation, flowering, fruit development, and nutrient transport, while also it regulates nitrogen metabolism and enhancing stress tolerance, highlighting the vital role of its proper use in fertilizers for plant health and productivity (Mercan *et al.*, 2022).

Sodium perborate is a chemical compound, which is widely employed across diverse applications, ranging from laundry detergents to teeth whitening products. Renowned for its bleaching and stain-removing capabilities, it finds utility in cleaning agents (Mercan *et al.*, 2022). Sodium metaborate is a versatile chemical compound, which is extensively employed as a buffering agent, pH regulator, and corrosion inhibitor in industrial processes. Additionally, it is utilized in the formulation of cleaning products, detergents, and serves as a boron fertilizer in agriculture, thus these underscore its significance as a valuable compound offering diverse contributions to various industries. Disodium octaborate is a chemically significant compound with diverse applications; it is utilized for wood preservation in various industrial sectors, exhibits antifungal properties, and has been investigated under *in vitro* conditions in various plant species (Mercan *et al.*, 2022).

In this study, the aim of this study is to examine the effects of growing the *Liquidambar orientalis* plant under boron salt stress *in vitro* on the plant and the changes in its phenolic compounds. Phenolic compounds are important components due to their remarkable properties in terms of consumption of the plant and their important health effects as natural antioxidants in human life. In addition, phenolic compounds can also be used as natural sweeteners. Due to the carcinogenic and toxic effects of artificial antioxidants in terms of health, phenolic compounds as natural antioxidants can cause cataracts, senile diseases, cancer, etc. It is said to be good for many diseases. For these reasons, consuming products with a high amount of phenolic substances can reduce the risk of catching diseases and positively affect health.

2. MATERIAL and METHODS

2.1. Preparation of Woody Plant Medium (WPM)

2.462 g/L WPM ready-made nutrient medium and 30 g/L Sucrose were weighed and transferred to a beaker. Sterile distilled water was added to the beaker and mixed homogeneously with a magnetic stirrer. The pH was adjusted to 5.8 and transferred to an autoclave bottle containing 7g/L agar. Autoclaving was performed at 121.6 °C for 15 minutes, and then 1 L of WPM medium was transferred in a laminar flow cabinet with 50 mL of medium in each culture dish. After about 30 minutes, the solidified media were stored in a cooler at +4 °C

2.2 Seed Surface Sterilization and Planting

The seeds of sweetgum were washed under running water for 20 minutes and kept in distilled water at 23 °C for 2 hours, and the floating seeds on the water were removed. Seeds that settled to the bottom in water were used for the sterilization process. Seeds were shaken in falcon (50 mL) for 10 minutes in 70% Ethanol (C_2H_5OH), 10 minutes in 10% Hydrogen Peroxide (H_2O_2), and 10 minutes in 10% commercial Sodium Hypochlorite (NaClO) (Domestos®), respectively (Kaya *et al*, 2017; 2020). After each step, the seeds were rinsed with sterile distilled water and transferred to blotting paper. The dried seeds were transferred to the nutrient media in a laminar flow cabinet. Sowing was carried out in 20 culture vessels with 5 seeds in each medium. The sown culture pots were left to germinate in the climate chamber at 23 °C. The sweetgum seedlings that developed during the germination process were micro propagated 3 times at 6-8 weeks intervals (Mercan *et al.*, 2022). All tools and equipment used in the experimental stages were autoclaved at 121.6 °C for 15 minutes in an autoclave device (Daihan Scientific-MaXterile60).

2.3 Boron Salt Application

In the experiment, four different boron salts, disodium octaborate ($Na_2B_8O_{13}.4H_2O$), sodium metaborate ($NaBO_2$), boric acid (H_3BO_3) and sodium perborate ($NaBO_3.nH_2O$) compounds were prepared at three different concentrations (1, 3 and 5 mg/L) and 1 mg/L benzyl was combined in adenine (BA) based WPM medium. The media adjusted to pH 5.8 were

autoclaved. The meristems extracted *in vitro* conditions were transferred to the prepared media and 10 meristems were planted on each medium. The parameters were repeated 3 times. Culture dishes were incubated under standard culture conditions (16/8 h photoperiod, 25±2 °C, 50 μmol^{-1} m^{-2} sec^{-1}, white cold fluorescent light) for 4 weeks. Each salt treatment was evaluated separately. Within the growth medium, plant specimens were subjected to a triple replication, with ten plant samples utilized for each experimental condition. The vitality status of these plant samples was examined, and the average vitality ratio was calculated, followed by the determination of the regeneration rate. To evaluate shoot counts, the post-growth shoot numbers of the plant samples were carefully analysed, and the mean of the three control groups was computed for comparative analysis (Mercan *et al.*, 2022).

2.4 Identification of Phenolic Compounds by UPLC-MS/MS

Approximately 3g of each plant sample was taken and they were decomposed with 200mL of liquid nitrogen. A mixture of 30mL of acetone: water (80:20) was added and left to extract at -86 °C for 6 hours. After the mixture removed from the cooler was kept in an ultrasonic bath for 15 minutes, the extract was centrifuged at 4000rpm at 20°C for 10 minutes. It was filtered through Whatman No 4 filter paper and then the residue was extracted 2 more times with 30 mL of acetone: water mixtures. The acetone in the combined extracts was evaporated under low vacuum at 40°C (Rotary Evaporator Heidolph Basis Hei-VAP ML). The aqueous phase was washed 3 times with 30mL of n-hexane followed by diethyl ether followed by liquid-liquid extraction 3 times with 30mL of ethyl acetate. The organic phases were combined. It was dried by evaporation at 40°C and redissolved in water: methanol (80:20) mixture. The solution was passed through Macherey-Nagel Chromafil Xtra PTFE-20/25 0.20µm filters and analyzed by UPLC-MS/MS (Waters Acquity Ultra Performance LC, Xevo TQ-S MS-MS) (Kıvrak & Kıvrak, 2017).

3. RESULTS and DISCUSSION

3.1. Effects of Boron Salts on Meristem Regeneration and Shoot Growth

When the samples incubated in WPM medium containing four different boron salts at different concentrations were compared with the control group, the best results were obtained from 5 mg/L sodium perborate (Figure 2). However, meristems cut from *L. orientalis* micro shoots (Figure 3a) gave the best regeneration percentage (Figure 3b, Figure 4) in the medium containing 1 mg/L disodium octaborate, while maximum shoot numbers per meristem were obtained without application 3 mg/L boric acid (Figure 5).

The shoot-forming capacity of *L. orientalis* plants grown with 4 different boron salts was measured. As a result of the measurements, the control group showed a shoot count of 4, while plants grown with boric acid application had shoot counts sequentially from low to high concentrations as (4, 5, 3 mg/L). Plants grown with sodium perborate application had shoot counts sequentially from low to high concentrations as (4, 4, 5mg/L). For plants treated with sodium metaborate, shoot counts were sequentially from low to high concentrations as (4, 4, 2 mg/L). Finally, for plants treated with disodium octaborate, shoot counts were sequentially from low to high concentrations as (3, 3, 2 mg/L) (Figure 2).

Figure 2. The shoot-forming capacities index calculated after salt application (Mercan *et al.*, 2022).

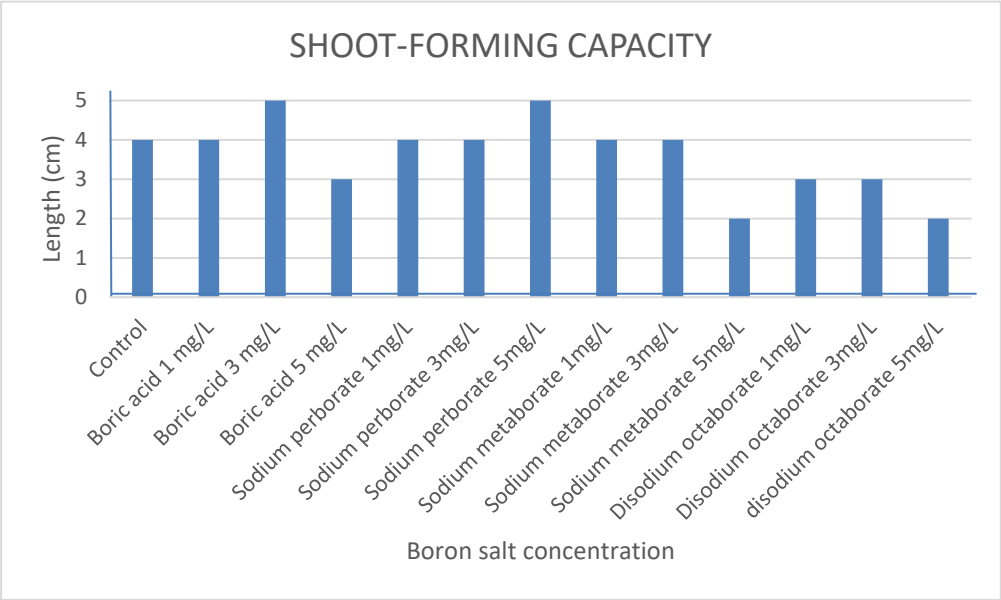
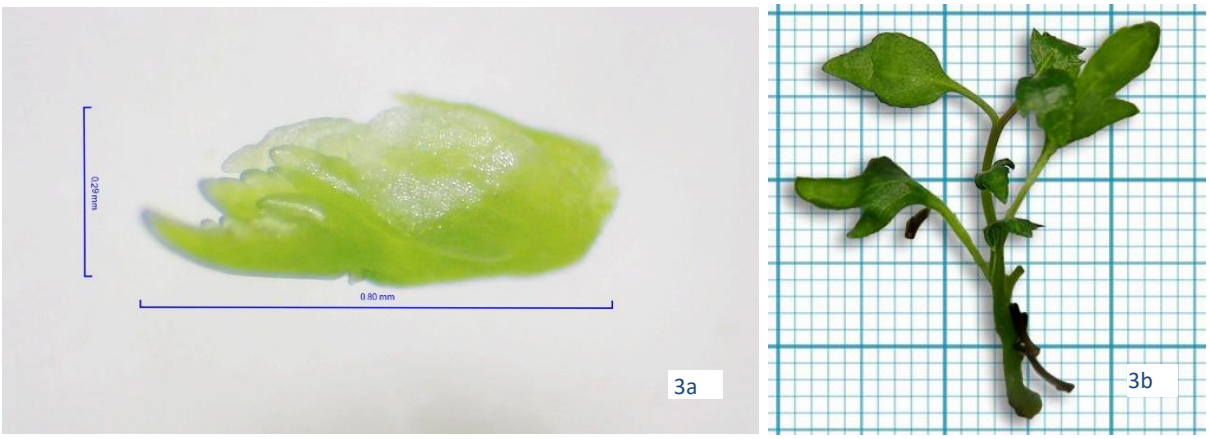
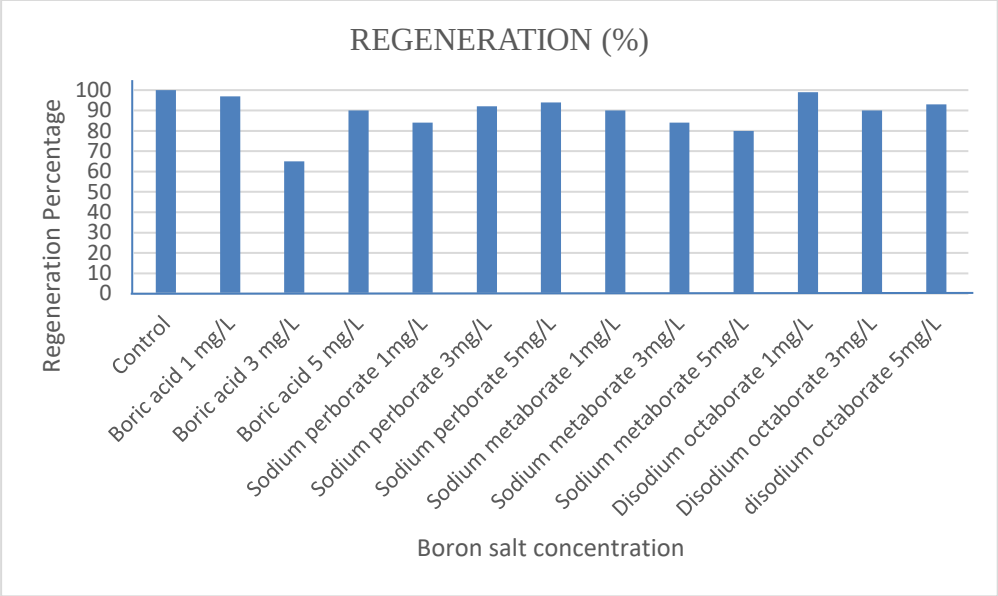


Figure 3. Meristem (3a) cut from *L. orientalis* micro-shoots, *L.orientalis* plant grown in WPM medium containing 1mg/L Disodium octaborate (3b) (Mercan *et al.*, 2022).



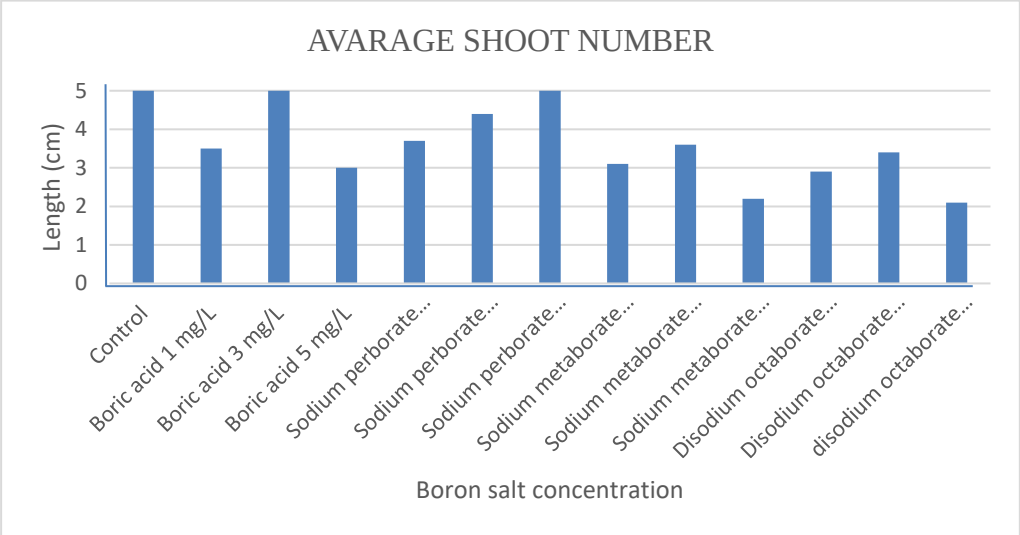
As a result of the cultivation of the control group, which was grown with 4 different boron salt stress and no salt application, the regeneration percentages were 100% in the control group. Regeneration percentages of plants grown with boric acid were measured from low concentration to high concentration (95%, 64%, 90%), respectively. The regeneration percentages of the plants grown with sodium perborate were measured from low concentration to high concentration (85%, 92%, 94%), respectively. Regeneration percentages of plants grown with sodium metaborate were measured from low concentration to high concentration (90%, 85%, 80%), respectively. The regeneration percentages of plants grown with disodium octaborate were measured from low concentration to high concentration (99%, 90%, 94%), respectively (Figure 4).

Figure 4. Meristem regeneration rates after different boron salt application (Mercan *et al.*, 2022).



The average numbers of meristems were counted for plants grown with 4 different boron salts at various concentrations. In the control group, the average meristem count for the grown plants was observed to be 5. However, for plants treated with boric acid salt, the average meristem counts were sequentially as (3.4, 5, 3cm) from low to high concentrations. Similarly, for plants treated with sodium perborate salt, the average meristem counts were sequentially as (3.7, 4.4, 5cm) from low to high concentrations. For plants treated with sodium metaborate salt, the average meristem counts were sequentially as (3.1, 3.6, 2.2cm) from low to high concentrations. Lastly, for plants treated with disodium octaborate salt, the average meristem counts were sequentially as (2.9, 3.4, 2.1cm) from low to high concentrations (Figure 5).

Figure 5. Meristem shoot number after different boron salt application (Mercan *et al.*, 2022).



In Table 1, the phenolic compound concentrations of the plants grown with 4 different boron salts and the control group, which were not subjected to salt stress, are given. As a result of the application of each salt at 3 different concentrations, the concentrations of plant phenolic compounds were measured in µg/kg. In Figure 6 and Figure 7, MRM chromatograms of quercetin and catechin hydrate phenolic compounds of major phenolic compounds of *L. orientalis* plant grown with 4 different boron salts applied at the same concentration are given.

Table 1. The effect of boron salts applied at different concentrations on the phenolic compounds (µg/kg) of the plant *Liquidambar orientalis*.

Phenolic compounds	Control	Boric acid			Sodium perborate			Sodium metaborate			Disodium octaborate		
		BA-1	BA-3	BA-5	SP- 1	SP- 3	SP- 5	SM- 1	SM- 3	SM-5	DO-1	DO-3	DO-5
Pyrogallol	6464.1	4479.6	4322.4	3986.5	6606.9	6444.5	5365.3	6865.1	5862.7	5273.9	6426.0	4781.7	5272.6
Gallic acid	4632.8	3541.6	3695.8	2961.2	5121.6	4760.6	4044.7	5034.4	4276.8	3878.4	4008.9	3191.4	3585.7
3-4-Dihydroxy benzoic acid	614.9	570.7	378.5	448.2	633.3	731.1	900.3	709.3	798.8	1022.1	686.5	653.9	516.3
Gentisic acid	900.8	872.2	607.4	746.0	915.2	1111.7	1294.7	1120.9	1111.2	1519.8	1025.2	1131.1	832.4
4-Hydroxy benzoic acid	315.9	408.1	194.4	449.9	198.1	278.6	273.0	172.8	207.6	320.4	287.3	387.9	322.6
Catechin hydrate	13742.1	16557.6	9383.5	5790.8	21813.6	17654.5	15312.0	15825.5	10008.4	13166.5	17653.7	20633.5	9424.1
Caffeic acid	574.1	330.5	438.9	542.7	375.6	513.8	624.3	482.8	529.9	568.5	272.5	387.5	407.3
p-Coumaric acid	1879.0	1284.1	467.1	1429.2	458.2	1078.8	894.9	1102.5	848.7	1837.8	533.2	981.2	497.1
Catechin gallate	2458.5	1077.2	1738.4	2376.7	471.6	568.1	537.1	739.9	790.7	901.8	1032.0	1069.5	704.0
Myricetin	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Naringenin	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Kaempferol	17.7	22.6	25.6	17.3	10.0	21.0	13.9	10.1	12.7	22.5	11.5	30.8	25.1
Epicatechin	26670.6	31374.1	12604.6	9878.7	44766.0	34950.0	27439.0	34081.8	24141.7	24798.7	34675.8	43448.1	17046.2
3,4-Dihydroxybenzaldehyde	131.2	146.5	103.3	120.9	172.0	171.4	145.9	178.5	161.5	152.6	144.3	135.9	132.8
Quercetin	12177.8	38407.6	9654.9	11367.8	42193.3	40304.6	45732.4	11197.5	21135.2	27701.1	16967.4	9447.6	6537.1

Data represent four independent experiments.
LOQ: Limit of Quantification
ND: Not detected

BA-1: 1 mg/L boric acid; BA-3: 3 mg/L boric acid; BA-5: 5 mg/L boric acid; SP-1: 1 mg/L sodium perborate; SP-3: 3 mg/L sodium perborate in; SP-5: 5 mg/L sodium perborate; SM-1: 1 mg/L sodium metaborate; SM-3: 3 mg/L sodium metaborate; SM-5: 5 mg/L sodium metaborate; DO-1: 1 mg/L disodium octaborate; DO-3: 3 mg/L disodium octaborate; DO-5: 5 mg/L disodium octaborate; Control: Plants without boron salt stress.

Figure 6. MRM chromatograms of Catechin hydrate phenolic in *L. orientalis* plant in which four different boron salts were applied at 3 mg/L concentration in UPLC-MS/MS device.

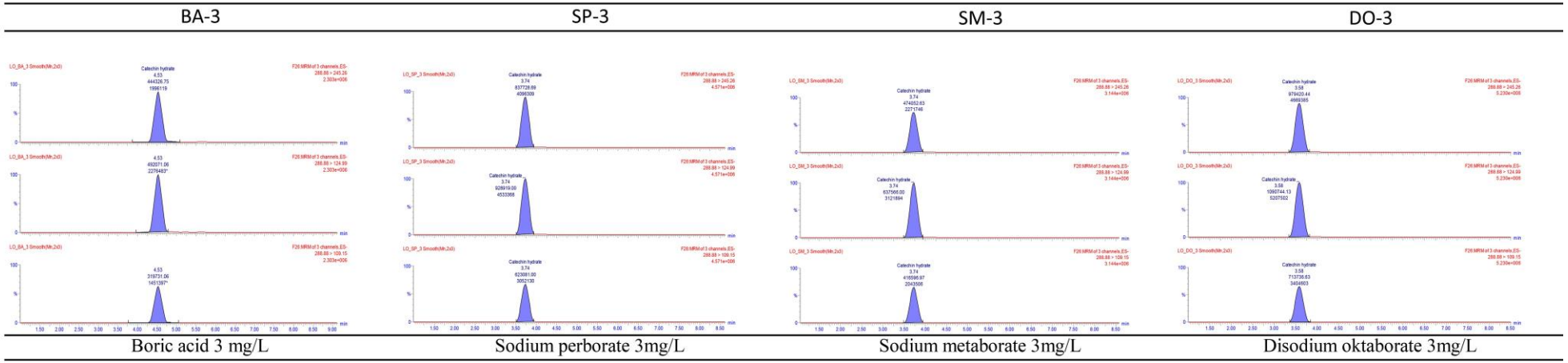
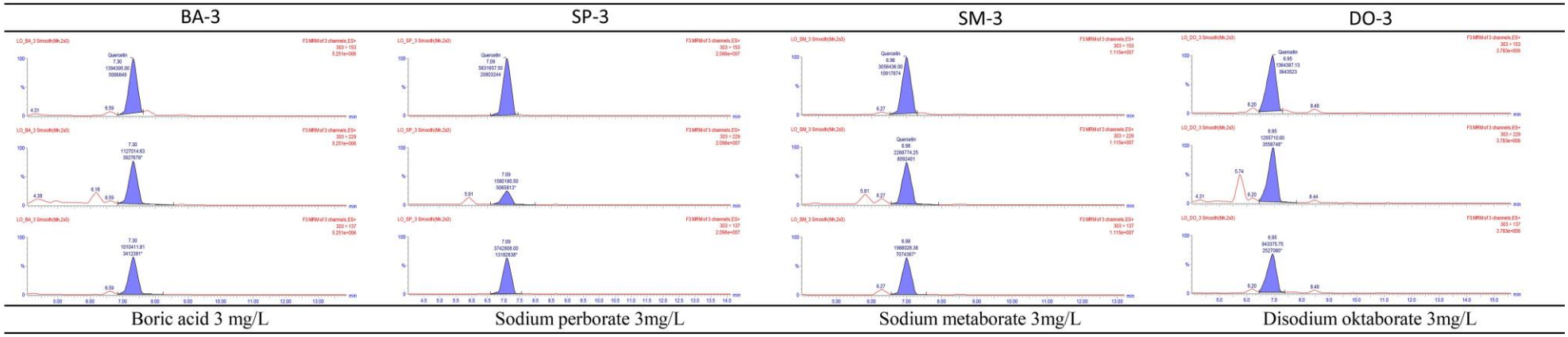


Figure 7. MRM chromatograms of Quarcetin phenolic in *L. orientalis* plant in which four different boron salts were applied at 3 mg/L concentration in UPLC-MS/MS device.



4. DISCUSSION and CONCLUSION

4.1. Effects of Boron Salts on Meristem Regeneration and Shoot Growth

In plants treated with boric acid salt, the average meristem counts were observed as 3, 4, 5, and 3 cm for low, medium, and high concentrations, respectively. These results indicate that at low concentrations, boric acid could potentially reduce the number of meristems in plants, but at medium concentrations, results similar to the control group were obtained. For plants treated with sodium perborate salt, the average meristem counts were observed as 3.7, 4.4, and 5cm for low, medium, and high concentrations, respectively. These findings suggest that sodium perborate may have the ability to enhance the meristem count in plants, particularly showing a significant increase at high concentrations. In plants treated with sodium metaborate salt, the average meristem counts were observed as 3.1, 3.6, and 2.2 cm for low, medium, and high concentrations, respectively. These results demonstrate that sodium metaborate can potentially decrease the meristem count at low and medium concentrations, with a more pronounced reduction observed at high concentrations. For plants treated with disodium octaborate salt, the average meristem counts were observed as 2.9, 3.4, and 2.1cm for low, medium, and high concentrations, respectively. These outcomes indicate that disodium octaborate might lead to a decrease in meristem count at both low and high concentrations, while results similar to the control group were achieved at medium concentrations (Figure 2).

Upon scrutinizing the effect of boric acid salt on regeneration percentages, it is discerned that at low concentrations, the regenerative abilities of plants exhibit a slight decrement. However, at moderate concentrations, regeneration percentages akin to those of the control group are attained. These outcomes signify that boric acid may endorse the regenerative potential of plants at suitable concentrations. Upon investigating the influence of sodium perborate salt, it is evident that at low concentrations, there is a marginal reduction in regeneration percentages, yet a conspicuous augmentation is observed at both intermediate and high concentrations. These findings postulate that sodium perborate has the potential to amplify the regenerative capacities of plants, particularly under conditions of heightened stress. Analysis of the impact of sodium metaborate salt reveals that at low and moderate concentrations, there is a decline in regeneration percentages, while a more pronounced reduction is evident at elevated concentrations. These outcomes suggest that heightened levels of sodium metaborate could detrimentally affect the regenerative capabilities of plants. Exploring the effect of disodium octaborate salt, it is ascertained that reductions in regeneration percentages manifest at both low and high concentrations, although outcomes closely approximate those of the control group at intermediate concentrations. These results intimate that disodium octaborate has the potential to bolster plant regeneration at appropriate concentrations (Figure 4).

When scrutinizing the effect of boric acid application on shoot counts, it is evident that at low concentrations, there is a mild reduction in shoot formation capacity, yet at medium concentrations, results comparable to those of the control group are attained. These findings suggest that boric acid can support the shoot formation capabilities of plants at suitable concentrations. Analysing the impact of sodium perborate application, it is observed that shoot counts experience a slight decline at low concentrations, while a significant increase is noted at both intermediate and high concentrations. These observations suggest that sodium perborate might enhance the shoot formation capacity of plants, particularly under conditions of elevated stress. Upon examining the effects of sodium metaborate treatment, reductions in shoot counts are seen at both low and medium concentrations, with a more pronounced decrease at high concentrations. These results indicate that high concentrations of sodium metaborate could adversely affect the shoot formation capacity of plants. When evaluating the influence of disodium octaborate treatment, it becomes apparent that reductions in shoot counts occur at

both low and high concentrations, while results closely resemble those of the control group at intermediate concentrations. These findings imply that disodium octaborate has the potential to support plant shoot formation at appropriate concentrations (Figure 5).

In vitro propagation of woody plants has some limitations due to genetic changes in cultures' growth and aging process. *In vitro* cultures are a widely used method to propagate important woody plant species (Kıvrak-Kıran et al., 2021) and cytokinin and/or cytokinin and auxin growth regulators are used at optimized ratios for culture initiation. Nutrient media that do not contain plant growth regulators or contain auxin-like plant growth regulators for rooting (George *et al*., 2007) and indole butyric acid (IBA) are often used to stimulate the roots of woody plants (Kataeva & Butenko, 1987). Micropropagation of woody plant species is crucial for enhancing forest yield by producing high-value commercial seedlings. Furthermore, mineral nutrition is a significant factor in inducing organogenic responses. This study aimed to investigate the impact of various boron salts on the organogenesis of meristems derived from *in vitro* cultured *L. orientalis* micro-shoots. Culture medium supplemented with different concentrations of different boron salts differentially affected the *in vitro* organogenic control of *L. orientalis*. Boron-induced callus formation in explants allows the initiation of well-developed micro-sprouts that can be used for bud development. Similarly, Brondani *et al*. (2012) evaluated the effects of calcium and boron on nodal segment regeneration from Eucalyptus grandis micro-shoots.

Murashige and Skoog (MS) medium supplemented with different concentrations of calcium and boron (Murashige & Skoog, 1962) was modified to induce regenerative responses in 45-day-old E. grandis nodal explants and after 60 days dry weight, fresh weight, fresh and dry weight percentages, relative dry weight, fresh weight, fresh and dry weight accumulated by explants, water content and relative substance content were evaluated. The culture medium supplemented with different concentrations of calcium and boron was found to affect the organogenic control of E. grandis *in vitro*. It is known that the physical and superstructure properties of cell walls are affected by boron deficiency (Findeklee & Goldbach, 1996). Also, when plants are grown with insufficient boron, boron accumulates mainly in the cell wall (Hu *et al*., 1996). Large amounts of boron are not required by plants, but if not supplied in required amounts it can cause critical plant growth problems. Lack of chlorosis due to boron deficiency, however, has similar toxicity effects with other microelements. Boron, together with calcium, is used in plant cell wall formation and required for plant cell division. Other roles of boron include carbohydrate metabolism, sugar translocation, potassium transport to the stoma, nitrogen metabolism, pollen germination, regulation of hormone levels and formation of certain proteins, regular functioning and growth of apical meristems, membrane function and structure, and nucleic acid synthesis (Liang *et al.*, 2011). In this study, four different boron salts (boric acid, sodium metaborate, sodium perborate and disodium octaborate) were used as boron microelement source at three different concentrations and compared with the control group 3 mg/L boric acid and 5 mg/L. While an increase in the number of stems was observed in 1 mg/L sodium perborate application, it was observed that 5 mg/L disodium octaborate application was also effective on stem elongation. In plants, borate salts and boric acid are actively transported into cells as undissociated boric acid when boron levels are insufficient. At higher soil concentrations, passive diffusion takes place. These compounds are then transported to leaves via the xylem, where they are deposited and remain as the water evaporates. However, they show little movement to other parts such as the stem and fruit, and are inert in the phloem (Lank & Wahl, 2014). Boron is an essential microelement for higher plant species with levels of interspecific variation demanded for maximum plant growth (Lovatt & Dugger, 1984). Studies on boron show that boron plays an important role in cell wall crosslinking, including complexing with specific pectin content (Loomis & Durst, 1992). Examination of the functions of borax and boric acid on Vicia faba and other plant species showed the effect of boron in plant

nutrition (Warrington, 1923). The first large-scale study of boron's functions on plant development was carried out where fifty plant species were selected and grown in sand cultures with normal nutrient solutions at different boron concentrations. The best plant growth, low plant damaging concentrations, deficiency symptoms, trace boron levels were recorded for each species. The majority of plants showed best growth up to 5 mg/L boron levels and approximately one-third of the plants grown at trace boron levels showed morphological signs of deficiency. It was concluded that the beneficial and harmful effects of boron salts overlap among plant species, and are therefore divided into three broad categories sensitive, semi-tolerant and tolerant (Eaton, 1944). Tolerant strains withstand high boron concentrations with little effect, and sensitive strains react strongly to too much or too little boron. In light of the results obtained from our study, it can be said that it is in the semi-tolerant category for *L. orientalis*. Many studies in the literature show that various variations are noticed in plants propagated by *in vitro* tissue culture techniques (Barret *et al*., 2006; Baranek *et al*., 2010). These variations, spontaneous in nature or caused by environmental factors, are induced by different stress conditions and biochemical compounds in *in vitro* cultures. Diversity arises from differentiated cells using multiple or single cells during embryogenesis and organogenesis, and *in vitro* cultural condition induces regulation of pre-existing variation expressions. Many of these variations pose a major problem for seedling producers. However, these changes are promising for future studies focusing on plant quality.

4.2. Effect of Boron Salts on Phenolic Substances of *Liquidambar orientalis* Plant

When the samples incubated in WPM medium containing four different boron salts at different concentrations were compared with the control group, it was determined that the best result in the pyrogallol content was in the 1mg/L application of sodium perborate salt. It has been determined that 3-4-Hydroxy benzoic acid, Catechin hydrate, Quercetin, Epicatechin and Gentisic acid are major in *Liquidambar orientalis* plant. As a result of the boron salts applied at different concentrations, it was observed that the salt applications other than the plants applied boric acid increased the amount of Gentisic acid. It was determined that the amount of phenolic substance decreased as the amount of salt applied increased on the changes on catechin hydrate. Among the boron salts applied to the *L. orientalis* plant, it was observed that the phenolic content increased significantly as a result of the application of sodium perborate and disodium octaborate salts at a concentration of 3mg/L. It was observed that the amount of phenolic phenolic substance increased when the amount of Quarcetin, another major phenolic, was applied at low concentration, and this amount decreased significantly as the amount increased. On the other hand, epicatechin, another major phenolic compound, application of boric acid, sodium perborate and sodium metaborate at a concentration of 1 mg/L increased the density of this compound, while the application of higher concentrations decreased the density of epicatechin. As a result of disodium octaborate salt application, it was observed that the concentration amount of 1mg/L and 3mg/L concentration increased the epicatechin density, while the application at 5 mg/L concentration decreased the amount of this compound (Table 1).

As a result of this study, it is seen that the *L. orientalis* plant has half tolerance to boron salt stress, while the applied boron salts concentration of 5mg/L begins to affect the plant negatively. In order to better understand the living conditions of the *L. orientalis* plant, it will be possible to transfer the plant to future generations by doing more studies.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Muhammed Mustafa Polat: Investigation, Formal Analysis and Writing. **Ergun Kaya:** Investigation and Critical Reading. **Ibrahim Kivrak:** Methodology, Supervision, Validation and Writing.

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