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THE EFFECT OF THE PLANT PART STERILIZATION METHODS, AND ANTIOXIDANTS TO IMPROVE THE GROWTH AND HEALTHY SAMPLES IN THE INITIAL STAGES OF *Populus euphratica* oliv PLANTS IN VITRO*

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ABSTRACT

The research aimed to study the effect of sterilization methods, adding antioxidants, and the source of the plant part on the rate of growing and healthy samples in the initial stage of *Populus euphratica* oliv. Different sterilization methods were employed using various concentrations of disinfectants. Sodium hypochlorite, for instance, was utilized at concentrations of 10% and 20% for a duration of 10 to 20 minutes. Similarly, calcium hypochlorite was employed at concentrations of 10% and 20% for the same time. Mercury chloride was used at concentrations of 0.1% and 0.2% for a duration of 2 to 4 minutes. The results showed that the use of as for mercury chloride, a concentration of 0.1% for 2 minutes helped reduce microbial contamination and resulted in a percentage of developing samples (46.67%).

In addition, the specimens underwent a treatment involving the combination of Ascorbic acid at a concentration of 100 mg/L and citric acid at 100 mg/L. Polyvinyl peroxide (PVP) was utilized at a concentration of 100 mg/L, while activated charcoal (AC) was introduced at 200 mg/L. The environment was deliberately darkened for a duration of one week.

Remarkably, the treatment with Ascorbic acid at 100 mg/L + citric acid at 100 mg/L, along with the addition of activated charcoal (AC) at a concentration of 200 mg/L, exhibited the most favorable outcomes in reducing oxidized phenol levels in the environment compared to other treatment methods. and the results of the study on the effect of the source of the parts showed that the best treatments were in parts taken from the buds in the order (5-2) on the branch or parts of the top of Suckers (bud 2) compared to the source obtained from Suckers.

Keywords: *Populus euphratica* oliv, mercury chloride, sodium hypochlorite, antioxidant, source of explant, percentage of survivals.

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INTRODUCTION

Trees *Populus euphratica oliv* (*Populus euphratica oliv*) belong to family Willow (Salicaceae), it is distributed in the hemisphere from north of latitude 45 to the equator. There are in Europe, Asia, North Africa and some Regions that under tropical. The *Populus euphratica oliv* is a medium to large tree with deciduous leaves. It often forms a shrub, but it can reach length the tree to 15 meters and branch out in a way middle to severe, fast-growing. Poplar grows in harsh conditions, tolerating temperatures between (-5 °C and 52 °C) and living in well-ventilated clay soil [4]. Poplar species are considered one of the important wood species, especially in hot regions, due to their importance in the environmental, economic, and afforestation fields. They are characterized by rapid growth and an increase in the biomass of wood [41]. The uses of *Populus euphratica oliv* are many and varied. It can be used in the field of landscaping, stabilizing soil on the banks of rivers, and reforestation, in addition to using its leaves for fodder, tree bark for medicinal drugs, the suitability of the wood for many wood industries, and paper pulp [32].

The production of *Populus euphratica oliv* seedlings can be done sexually by seed or vegetative. In practice, propagation of *Populus euphratica oliv* by seed is considered undesirable and useless due to the emergence of genetic isolations in new individuals. In addition, the seed's vital period is very short (days to weeks), and seedling growth requires high humidity, periods of strong rays, long lighting until germination and seedling growth take place [6, 11]. It is considered excessive the vegetative for *Populus euphratica oliv* it is the common method as production can often be done through cuttings or stem cuttings, but the problem with these methods is that the rooting rate of *Populus euphratica oliv* is low and varies from one type to another [42], in this regard Fatemeh *et al.* [13] confirmed that the *Populus euphratica oliv* is characterized by low rooting rates despite all the treatments to improve and encourage the rooting of the cuttings. Given the difficulties and problems of traditional vegetative propagation methods, a tissue culture propagation technique can open wide horizons in the production of *Populus euphratica oliv* to suit the urgent need for this species in Syria.

The forests *Populus euphratica oliv* in Syria impressed by cutting and transformation to lands agricultural, which considered as threatened currently from systems forestry. *Populus euphratica oliv* cultivation in Syria has been known since ancient times, especially on the banks of the rivers in the Euphrates, Khabur, Orontes Barada, and others, and *Populus euphratica oliv* forests were organized into forest types Dense Same Pure [3] references indicate that the areas covered by *Populus euphratica oliv* forests are much larger than they are now. This is due to various reasons, the most important of which is the random and irregular cutting of trees, and the conversion of these forests into irrigated agricultural lands. According to the country report on the state of biodiversity in Syria [7], the *Populus euphratica oliv* forests on the banks of the Euphrates River are considered one of the threatened forest systems at the present time. In the last two decades, tissue culture has become one of the important methods for producing plants vegetative, due to its many features, the most important of which is the production of plants free of maternal diseases, especially fungal, bacterial, and to some extent viral diseases. The new plants are genetically identical and similar to the mother plant, in addition to higher rates of high multiplication, possibility of multiplication throughout the year, and guaranteed total ensuring a root mass proportional to the vegetative total [21]. Many studies show that tissue culture

technology is one of the most important means to help improve plant production, and that it has enormous potential, especially since it has overcome many of the obstacles that limit increasing plant production using traditional vegetative methods of propagation [23]. This technique has been used successfully in recent years to propagate many types and origins of woody plants, especially fruit types (5) as the possibility has been pointed out to increase some species and Hajun Poplar a farmer tissues [33]. Whitehead and Giles [40] for the first time, lateral shoots of several types of Poplar were grown on different nutritional environments. It was found that after (6 - 8 weeks) new growths are formed. According to references, the response of plants to tissue culture depends on several factors, including Genotypes, Cultivar, source of explants incubation conditions, components of the nutrient medium, the sterilization process, and others [8].

According to studies, the surface of plants is usually contaminated with microorganisms from the air, and therefore surface sterilization of the explant is a critical, precise, and necessary process because the process of controlling microbial contamination in the field for woody plants is very difficult [37]. According to various references, the tissue culture technique consists of several successive stages, including the Initiation stage, Multiplication, rooting, and acclimatization, but the success of the method depends on the success of the initial stage in securing healthy and growing explants. One of the problems of the initial stage is the microorganisms section vegetarian and especially pollution with objects minute [16], the danger of explants contaminated with microbes increases significantly when grown in a medium that contains sugar and various organic materials that support the growth of microorganisms (such as fungi and bacteria). These microbes grow much faster than the planted explant and ultimately can lead to the death of this part because they are excreted. Many substances that are toxic to the explant, in addition to the part's participation in nutrients in the medium or the destruction and damage of plant tissue cells, reduce the possibility of the part branching and rooting and causing tissue yellowing and abnormal growth, and treat microbial contamination in tissue culture there are many methods and mechanisms, but the important condition regardless of the methods and mechanisms is to finally obtain samples. An uncontaminated plant that can grow, develop, and form organs (of cultures the continuation of the other stages of agriculture). The surface of the explant is naturally contaminated with microbes from the surrounding environment, and surface disinfection of the explant with chemicals is a very critical step. Usually, there are many disinfectant materials, including sodium hypochloride, calcium, ethyl alcohol, mercuric chloride, silver nitrate, hydrogen peroxide, and others, used for surface disinfection of explants in tissue culture technology. These substances are toxic to tissues, so disinfection must ensure the elimination of microbes without harm or death to tissues plant [20].

Media Source

To prepare tissue culture, many explants can be used, such as meristematic tissue, shoot tips, cambium tissue, leaves, and others, but in the field of propagation, subsidiary, and lateral buds are considered most widely used in the field, especially in woody plants [39]. Regarding the effect of the explant (Explanate) Ziad *et al.* [43] found in a study of olive propagation indicated that the success of the results of the initial stage and the multiplication stage differed depending on the type and source of the explant. One of the factors affecting the success of the growth and development of the explant is the composition of the

nutrient medium, as it was found that the success of growing the explant and its development depends primarily on the composition of the nutritional environment, which plays an essential role [8]. In this regard, several studies have indicated the success of growing *Populus euphratica* Oliv in the Murashig and Skoog (MS) medium [20, 24] surface sterilization usually uses materials such as sodium or calcium hypochloride, mercury chloride, silver nitrate, ethyl alcohol, hydrogenperoxide, and others [15]. In addition to pollutants, tissue culture also faces the problem of browning in the initial stage, which is considered a decisive factor in the success of the initial stage. Compared to herbaceous species, browning is considered one of the most important difficulties in growing woody plants in the laboratory. All species secrete phenolic compounds and accumulate in their tissues, and this is what causes browning (the phenomenon of brown color) in the environment, often when the explant is taken and the tissue is injured. When they are damaged, the phenolic compounds are oxidized, which inhibits the active enzymes. This leads, as a result, to the inhibition of growth and development, and ultimately to the death of the explant [27]. Various studies were conducted to study the effect of types of sterilizers and treatment periods on sterilizing explants. In the cultivation of pear buds, it was tested [12] effect of sodium hypochloride, mercury chloride, and silver nitrate b different coefficients, found the best results for reducing the percentage of contamination while maintaining a good percentage of samples were with the use of sodium hypochloride (2 for 15% minutes) and mercury chloride 0.1 for 7% minutes).

In propagating salt wheat [35] sodium hypochloride and mercury chloride are used at different concentrations and treatment periods, and it was concluded that the percentage of contamination and the rate of samples growing and free of contamination differed according to the disinfection materials, their concentration, and the duration of treatment with them. In a study of the effect of different concentrations of sodium hypochloride and chlorine [25] the best results are for the rate of developing and healthy parts when sodium hypochloride was used at a concentration of (12%), as Teixeira et al. [36] noted in sterilizing parts of bananas with a concentration of sodium hypochloride, the concentration (10%) was better than all other treatments as it gave the highest rate for uncontaminated samples and all samples were not damaged. In testing six types of sterilizers: calcium hypochloride, sodium hypochloride, sodium chloride dicyanoate, mercury chloride, silver nitrate, and hydrogen peroxane, it was found [15] in sterilizing sour cherry buds, showed that the sterilization results varied depending on the disinfectant, its concentration, and the duration of treatment. In studying the effect of sterilization duration [5] researched the effect of different time periods (10 - 15 - 20 – 25 minutes) for treatment with sodium hypochloride (2%) parts of pineapple, found that increasing the duration of treatment causes a significant decrease in the contamination rate, but coincides with an increase in the proportion of dead specimens. It was confirmed in the results that the important factor in the effect of the duration of sterilization treatment is achieving the lowest contamination rate while ensuring the least damage to the cells tissue. For the same goal [19] in comparing several sterilization materials and different, time periods in carob cultivation, found that the best sterilization results were with mercury chloride (0.1%) for a minute. Studies also show that the sterilization process is related to the physiological stages of the mother plant, and in this regard, Papafotiou and Martini [31] indicated in a study of several sampling dates and different sterilization methods, found that the best date was March, as it

showed the lowest percentage of contamination with microorganisms. In addition to microbial contamination, browning is considered one of the greatest difficulties in tissue culture, especially woody plants, which often causes the death of explants in the initial stage. The color change due to browning in the environment begins quickly from the cut surface of the explant after planting then it spreads and covers the entire medium. According to the studies, this discoloration (browning) is linked to phenolic compounds. Healthy plant cells contain phenolic compounds in the cell membranes and oxidation enzymes in the cytoplasm. When the cell walls are damaged by wounding or shearing, the phenolic compounds and oxidation enzymes are released from the site of the wound into the surrounding nutritional environment, where the phenolic compounds are oxidized and give a dark color. Without damage or injury, the cells cannot dark color and oxidation of phenols appear naturally [10]. Spread Oxidized phenols (browning) in the environment are due to yellowing of the explant and inhibition of growth and development [26]. Jones et al. confirmed [14] the oxidation of phenols leads to the cessation of cell division and then death and loss of tissue added. According to studies, the problem of tanning can be regulated and controlled through various mechanisms, especially by using antioxidants such as polyvinyl lpyrrolidine (PVP). Activated charcoal, ascorbic acid, citric acid, environmental darkening, etc. [18, 29]. Abdelwahd et al. confirmed [2] the use of antioxidants can effectively reduce the oxidation of phenols and avoid browning in the medium thus preserving the explant as Nazim et al. [28] found that adding antioxidants Oxidation to the medium can significantly and significantly help in reducing the phenomenon of browning. Studies confirm that darkness in the medium can reduce the oxidation of phenol under lighting, as darkness helps impede the activities of enzymes that oxidize phenolic compounds [30].

MATERIALS AND METHODS

The research was conducted in the tissue culture laboratory, Faculty of Agriculture, Al-Furat University, Deir ez-Zor Governorate, during the period of 2021-2022. The study focused on *Populus euphratica* oliv trees obtained from the banks of the Euphrates River. The explants were prepared and disinfected by removing leaves, cutting them into sections, and rinsing them under running water. They were then soaked in distilled water with a fungicide. The buds were washed with distilled water and dipped in ethyl alcohol. Different sterilization methods were used using various concentrations of disinfectants. Sodium hypochlorite, for instance, was utilized in concentrations of 10% and 20% for a duration of 10 to 20 minutes. Similarly, calcium hypochlorite was employed in concentrations of 10% and 20% for the same time frame was employed mercury chloride in concentrations of 0.1% and 0.2% for a duration of 2 to 4 minutes. The results showed that the use of as for mercury chloride, in the concentration of 0.1 for 2 minutes, the second factor, the specimens underwent a treatment involving the combination of Ascorbic acid in a concentration of 100 mg/L and citric acid at 100 mg/L. Polyvinyl peroxide (PVP) was utilized at a concentration of 100 mg/L, while activated charcoal (AC) was introduced at 200 mg/L. Incubated in darkness without antibiotics for a week.

The third factors was the source of Explant (contain 2-5 buds) followed by washing the parts with distilled. They were grown in vassal tubes containing MS medium [20] supplemented with BAP (1000 ppm), IBA (100 ppm), GA3 (100 ppm) with. PH 5.6. The cultures were incubated at a temperature of 25 ± 2 and 16 hours

day⁻¹ under a growth chamber for 30 days. The data were taken on the percentage of contamination (bacteria – fungi), survival, tanning plants, and healthy plants)

Statistical analysis

Experiments were conducted using a completely randomized design (C.R.D) with 10 replications (Explant per replicate). The data were statistically analyzed, and the means were compared using the Least Significant Difference (LSD) test at a probability level of 5%.

RESULTS AND DISCUSSION

Effect of sterilization methods

The data in Table 1 show that all transactions are cleared, it was effective in determining the rate of contaminated, developing, and healthy samples in the initial stage of Laboratory propagation of *Populus euphratica* Oliv. Euphrates. The results indicate that the effect of treating explants with sodium hypochloride in a concentration of (10%) for (10) minutes showed a high percentage of contaminated parts, reaching (86.67%) with a decrease in the percentage of dead parts (6.67%) and the percentage of growing and healthy parts. It is also noted that increasing the duration of treatment with sodium hypochloride to (10%) to (20) minutes did not show any significant effect in increasing the percentage of dead samples compared to the duration (10) minutes, while it led to a decrease in the percentage of (10) contaminated parts (80%). The results also indicate that increasing the concentration of sodium hypochloride to (20%) for (10) minutes led to a significant decrease in the percentage of contaminated parts, which amounted to (43.33%) with an increase in the percentage of dead parts (23.33%) and the percentage of growing parts (33.33%) compared to the concentration (10%) at the same treatment period. The results show that increasing the duration of treatment with sodium hypochloride (20%) to (20) minutes showed a decrease in the percentage of contaminated parts, reaching (36.67%) and an increase in the percentage of dead parts (30%) while the percentage of growing parts was not significantly affected by the increase duration (36.37%). The results of Table 1 indicate that the effect of treating the parts with calcium hypochloride at a concentration of (10%) for (10) minutes showed a high percentage of contaminated parts (80%) and a low percentage of dead parts (13.33%) and growing parts (6.67%). As the duration of treatment with calcium hypochloride concentrations increase (10%) to (20) minutes, a significant decrease in the number of contaminated (10%) parts is observed, and an increase in the percentage of growing parts (66.67%), while the percentage of dead parts (16.67%) was not significantly affected compared to the duration of treatment (10) minutes. As for increasing the concentration of calcium hypochloride to (20%) for (10) minutes, no significant differences appeared compared to the concentration (10) except for an increase in the percentage of dead parts (16.67%). In terms of the effect of using mercury chloride in sterilization, it is noted that treatment with mercury chloride concentration for (10%) minutes led to a percentage of contaminated parts (43.67%) a percentage of dead parts (43.33%) (10%) and a percentage of growing parts (46.67) When the duration of treatment with mercury chloride (0.1%) is increased to (4) minutes, a significant decrease in the percentage of contaminated parts (26.26%) and the percentage of growing parts (20%) is observed, and a significant increase in the percentage of dead parts (53.33%) compared to a period of (2) minutes. The results also showed that increasing the concentration of mercury chloride to (0.2%) for (2) minutes led to a significant

decrease in the percentage of contaminated parts (30%) and growing parts (36.67%) and an increase in the percentage of dead parts (33.33%).

Table 1: The effect of different sterilization methods on the initial stage of laboratory propagation of *Populus euphratica* oliv.

Average percentage of parts, developing and healthy parts	Average percentage of dead parts	Average percentage of contaminated parts	Sterilization treatment	
			Transaction duration	Disinfection material
6.67%	6.67%	86.67%	10minute	Sodium hypochloride concentration 10%
10.00%	10.00%	80.00%	20minutes	Sodium hypochloride concentration 10%
33.33%	23.33%	43.33%	10minute	Sodium hypochloride concentration 20%
36.67%	30.00%	36.67%	20minutes	Sodium hypochloride concentration 20%
6.67%	13.33%	80.00%	10minute	Calcium hypochloride concentration 10%
16.67%	16.67%	66.67%	20minutes	Calcium hypochloride concentration 10%
13.33%	20.00%	66.67%	10minute	Calcium hypochloride concentration 20%
10.00%	33.33%	56.67%	20minutes	Calcium hypochloride concentration 20%
46.67%	10.00%	43.33%	2minutes	Mercury chloride concentration 0.1%
20.00%	53.33%	26.67%	4minutes	Mercury chloride concentration 0.1%
36.67%	33.33%	30.00%	2minutes	Mercury chloride concentration 0.2%
13.33%	70.00%	16.67%	4minutes	Mercury chloride concentration 0.2%
1.2869	1.5382	1.685	LSD 0.05	
*The number of explants grown for each treatment is 30 parts				

As the duration of treatment with mercury chloride (0.2%) increases to (4) minutes, a significant decrease is observed in the percentage of contaminated parts (16.67%) and the percentage of developing parts (13.33%) an increase in the percentage of dead parts (70%) compared to treatment for (2) minutes at the same concentration. Through the results of experiments on the effect of different sterilization methods, it is noted that mercury chloride gave reducing in contamination rates in general compared to sodium and calcium hypochloride. The lowest contamination rate was when treated with mercury chloride concentration (0.2%) for (4) minutes, while the highest contamination value was with the treatment of Sodium hypochloride (10%) for (10) minutes. It is also noted that increasing the concentration in the three materials and for the same working period led to a significant decrease in the percentage of pollution also, increasing the duration of treatment at the same concentration of the three materials showed a decrease in the percentage of pollution. Comparing the results of the effect of the three sterilizers on the percentages of dead parts showed that the highest

percentage of dead parts was in the mercuric chloride treatments, and the highest rates were with the treatment with mercuric chloride (1-2% for a period of 4 minutes). The results of the work confirm the importance of mercury chloride in reducing the percentage of pollution and maintaining the percentage of developing samples, consistent with what was indicated by Ahmad et al. [1]. The results confirm that increasing the concentration of the three substances (sodium hydroxide, calcium, and mercury chloride) or increasing the duration of treatment with them led to reduce the percentage of pollution on the one hand, but increasing the percentage of dead parts on the other hand, and this result is consistent with Zaid study [35]. The increase in the percentage of dead parts with increasing concentration or duration of treatment of disinfectant materials can be attributed to the indirect effect of the chemical composition of these disinfectant materials, which leads to the death of active tissue in explants [17].

Source of the plant

From the data in Table 2, it can be concluded that the location of the explants played an important role in determining the percentage of pollution and growing samples. It is noted from the table that the location of the vegetative part on the branch affected the averages of the dead and contaminated parts, while it did not affect the averages of the growing and healthy parts. Bud (2) from the top of the branch gave the lowest percentage of pollution (36.66%) and the highest percentage of dead parts (20%) Compared to the bud (5) from the base of the branch. Comparing the locations of the crab buds showed a significant effect on all sterilization indicators, as it was noted that bud (2) from the top of the crab gave the lowest percentage of contamination (36.66%) and the lowest percentage of dead parts and the highest percentage of growing parts (46.66%) compared to the buds (5). From the base of Suckers. When comparing the results of the shoots of the branch and Suckers, the conclusion was achieved that the highest percentage of contamination was in the shoots from the base of Suckers, with significant differences compared to all other sites, while the lowest percentage of contamination was from the shoots (2) of the branch or Suckers. It also turns out that the highest percentage of dead parts is in the buds (5) of the base of Suckers, and the lowest percentage of contamination is in the buds (2) of the branch or Suckers. From the results, it is noted that the lowest average number of growing parts is in the buds (5) from the base of Suckers with significant differences compared to all other sites, while the branch, buds, and bud (2) of Suckers gave the highest percentage of healthy growing parts, without significant differences between the three sites. And the differences in sites that the explants' pollution rate is related to the type, location, and age of the explant. The place where the plant sample was taken played an important role in determining the percentage of pollution and growing parts, and this is due to the surrounding environmental condition, and to the variation in tissues in different sites in terms of nutritional and hormonal content. The same observation is confirmed by Nazim *et al.* [28].

Table 2: effect of different methods of sterilization of *Populus euphratica* oliv in vitro propagated

Average percentage of healthy plants	Average percentage of survivals	Average percentage of contaminated	Location of the explants	
			Bud sequence	source of the explants
43.33 %	20.00%	36.67 %	2	Source of the vegetative part of the branch
43.33 %	13.33 %	43.33 %	5	
46.67 %	20.00 %	36.67 %	2	Source of the explant of Suckers
20.00 %	26.67 %	53.33 %	5	
1.9594	1.6303	1.8826	LSD 0.05	

*The number of explants grown for each treatment is 30 parts

Antioxidants

Data from Table 3, indicate that all treatments and darkening helped reduce the oxidation of phenols in the medium which those Phenolic substances increases the percentage of pollution and the use of antioxidants reduces the proportion of phenolic substances. It is noted that adding ascorbic acid, citric acid, PV, activated charcoal, or exposure to darkness significantly reduced the percentages of parts with phenol compared to the control. The results of this work regarding the superiority of ascorbic acid and citric acid treatment in reducing the percentage of phenol and improving the percentage of growing samples are consistent with the results of George [9] on bananas, Mante and Tepper [22] on bananas and Nazim et al. [28] on grapes. Ascorbic acid and citric acid treatments were also used successfully to reduce browning (phenol oxidation) in cherry cultivation [34]. To clarify the role of ascorbic acid and citric acid in reducing phenol oxidation in the medium [38]. This indicates the importance of the two acids in inhibiting oxidation enzymes and preventing the release of ions necessary for the oxidation group. The same observation is confirmed by George [9] regarding the role of ascorbic acid and citric acid in the ability to bind and bind the ions necessary to activate phenol oxidation enzymes and the role of acid Ascorbic acid reduces the rate of phenol oxidation due to its role in stopping the production of free oxygen radicals (ROS) at the site of a wound on the explant and protecting cells from damage by oxidative enzymes, in addition to its role in encouraging cell division and elongation [42]. Regarding the role of activated charcoal in addition to the role of ascorbic acid and citric acid in reducing phenol oxidation in this work, (2) the treatment of activated charcoal or the treatment of citric acid and ascorbic acid was effective and significantly in reduced the rate of phenol oxidation in faba bean cultivation, and the results of the activated charcoal treatment in reducing phenol oxidation are consistent with previous results that was indicated by Sharada *et al.* [33].

Table 3: Antioxidant effect the percentage of phenol in the medium in the initial stage of laboratory propagation of *Populus euphratica* oliv

Average percentage of parts, developing and healthy parts	Average percentage of phenol (browning)	Average percentage of dead parts	Average percentage of contaminated parts	Transaction
6.67%	60.00%	6.67%	26.67%	Control
50.00%	16.67%	10.00%	23.33%	Ascorbic acid 100 mg/l + citric acid 100mg/L
26.67%	26.67%	20.00%	26.67%	Polyvinyl peroxide (PVP) 100 mg/L
46.67%	16.67%	13.33%	23.33%	Activated charcoal (AC) 200 mg/L
10.00%	43.33%	20.00%	26.67%	Darken the environment for a week
1.8826	1.9594	1.6303	1.8826	LSD 0.05

*The number of explants grown for each treatment is 30 shear

Conclusions and recommendations:

Based on experiments studying sterilization methods, it is recommended to follow certain procedures to ensure the best growth of *Populus euphratica* oliv specimens during the initial stage while minimizing pollutants and phenol content in the environment. These procedures include treating the explants with 0.1% mercuric chloride for 2 minutes, and either adding B Ascorbic acid (100 mg/L) and citric acid (100 mg/L) or using activated charcoal (AC) at a concentration of 200 mg/L to reduce phenol percentages in the surrounding environment. Additionally, selecting explants from the top of Suckers and arranging buds (2-5) on the branches is advised.

REFERENCES

- 1- Ahmad, I.; M. J. Jaskani; M. Nafees; I. Ashraf and R. Qure (2016). Control of media browning in micro propagation of guava (psidium guajava l.). Pak. J. Bot., 48(2):713-716.
- 2- Abdelwahd, R.; N. Hakamu; M. Labhilil and S. M. Udupa (2008). Use of an adsorbent and antioxidants to reduce the effects of leached phenolics in in vitro plantlet regeneration of faba bean. Afr. J. Biotechnol. 7(8):997-1002.
- 3- Amer, M. A. (2002). *Populus euphratica* oliv forests in the Lower Euphrates Basin region - an integrated ecosystem in need of protection, September 1 - 6, Al-Furat University Aleppo -Arab Conference for Biologists.
- 4- Browicz. K. (1977). Chorology of *Populus euphratica* oliv. Arboretum kornichie (22):5 -72.
- 5- Buah, J. N.; A. A. Paul and R. A. Junior (2015). In vitro Growth and Multiplication of Pineapple under Different Duration of Sterilization and Different Concentrations of Benzy laminopurine and Sucrose. Biotechnology, 14: 35-.04

- 6- Cao, D.; Li. J. Huang; Z. Baskin; C. C. Baskin; J. M. Hao; P. Zhou and J. Li (2012). Reproductive characteristics of a *Populus euphratica* population and prospects for its restoration in China. *Plos One* 7(7): e39121
- 7- Country report on the status of biodiversity in Syria.
- 8- Doo, H.; kim. J. Gopal and I. Sivanesan (2017). Nanomaterials in plant tissue culture: the disclosed and undisclosed: *RSC Advances Journal*. <https://doi.org/10.1039/C7RA07025J>.
- 9- George E. F. (1996). "Plant Propagation by Tissue Culture," Parts 1 and 2, Edition, Wilts, Exegetics Ltd., Eversley.
- 10- El-Gloushy, S. F.; R. Liu and H. K. Fan (2020). A complete protocol to reduce browning during coconut (*Cocos nucifera* L.) tissue culture through shoot tips and inflorescence explants. *Plant archives*, 20:2196-2204.
- 11- Eduardo Gonza ´lez • Francisco Antonio Comi ´n Etienne Muller (2010). Seed dispersal, germination and early seedling establishment of *Populus alba* L. under simulated water table declines in different substrates. *Trees* 24:151– 163.
- 12- Eyob Kassaye Wolella (2017). Surface sterilization and in vitro propagation of *Prunus domestica* L. cv. Stanley using axillary buds as explants. *Journal of Biotech Research*. 8:18-26.
- 13- Fatemeh alhmadloo. Mohsen CALAGARI, Azadeh SALEHI, Gholam Reza GOODARZI (2018). Investigation of rooting and growth characteristics of poplar clones in hydroponic and soil cultures. *JOURNAL OF FOREST SCIENCE*, 64(5): 207–215.
- 14- Jones, A.M.; Saxena, P.K. Inhibition of phenylpropanoid biosynthesis in *Artemisia annua* L.)2013(: A novel approach to reduce oxidative browning in plant tissue culture. *Plos One* 8: 76802.
- 15- Ines Mihaljević1, Krunoslav Dugalić, Vesna Tomaš1, Marija Viljevac, Ankica Pranjić, Zlatko Čmelik2, Boris Puškar and Zorica Jurković (2013). In vitro sterilization procedures for micropropagation of 'oblačinska' sour cherry. *Journal of Agricultural Sciences* 58(2): 117-126
- 16- Gulzar Akhtar, Muhammad Jafar Jaskani, .Yasar Sajjad, and Ahsan Akram (2016). Effect of Antioxidants, Amino Acids and Plant Growth Regulators on in vitro Propagation of *Rosa centifolia* . *Iran J Biotechnol*. Mar 14(1):51–55.
- 17- Hossa`m Saad El-Din Mohamed (2011). Micropropagation of isianthus (*Eustomagrandiflorum* (Raf. Shinn.)ex vivo), Al-Nahrain University *Journal* September,14(3): 28-39
- 18- Komalavalli, N. and M.V. Rao (2000). In vitro micropropagation of *Gymnemasyvestre*- A multipurpose medicinal plant. *Plant Cell, Tissue and Organ Culture* 61(2):97-105
- 19- Al- sayed, L.; S. Zaid, and M. Al-Sabbagh (2014). Study of the effect of some growth regulators on the propagation and rooting stages of the carob plant *Ceratonia siliqua*L. In *Glass, Damascus University Journal for Basic Sciences* 38(1):389 - 406
- 20- Murashige, T. and F. Skoog (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15:473-.794

- 21- Mosleh, M. S. D. and A. R. Khetam (2010). Effect of different concentration of Kinetin and NAA on micropropagation of *Gardenia jasmenoides*. J. Zank. Sulaimani. 1(13):103-120
- 22- Mante, S. and H.B. Tepper (1983). Production of *Musa textiles* cv. Nee plants from apical meristemslices in vitro. Plant Cell, Tissue and Organ Culture, 2:151-159.
- 23- Maria-Antonietta, G. and C. Benedetta (2013). Microropagation of *Citrus* spp. by organogenesis and somatic. embryogenesis protocols for micropropagation of selected economically-Important horticultural plants Methods in Molecular Biology 994: 99-118
- 24- Matthias Fladung (2013). Efficient in vitro plantlet regeneration in *Populus euphratica* Oliv. African Journal of Biotechnology February 12(8):826-832.
- 25- Martins João Paulo Rodrigues, Moacir Pasqual¹, Adalvan Daniel Martins, Suelen Francisca Ribeira (2015). Effects of salts and sucrose concentrations on in vitro propagation of *Billbergia zebrina* (Herbert) Lindley (Bromeliaceae). AJCS 9(1):85-91.
- 26- Mederos-Molina, S. and Trujillo, M. I. (1999). Elimination of browning exudateand in vitro development of shoots in *Pistacia vera* L. cv. mateur and *Pistacia atlanticadesf* culture. Acta SocietatisBotanicorumPoloniae, 68(1):21-24.
- 27- Confalonieri, M.; A. Balestrazzi; S. Bisoffi and D. Carbonera (2003). In vitro culture and genetic engineering of *Populus* spp.: synergy for forest tree improvement. Plant Cell Tissue and Organ Culture 72: 109–138.
- 28- Nazim Dhanoun Saeed, Ammar Zaki Qassab Bashi, Omar Muzaffar Omar (2013). Precise propagation of black opulus *euphratica* oliv trees *Populus nigra* L. using tissue culture, Al-Rafidain Science Journal, 24(6):1-17.
- 29- Ngomuo, M.; E. Mneney and P. Ndakidemi (2014). Control of lethal browning by using ascorbic acid on shoot tip cultures of a local *Musa* spp. (Banana) cv. Mzuzu in Tanzania. Afr. J. Biotechnol 13(16):1721-1725.
- 30- Ochoa-Alejo, N. and R. Malagon (2001). In vitro chili pepper biotechnology. In Vitro Cell Dev Biol Plant 37:701–729.
- 31- Papafotiou, M. and A. N. Martini (2009). Effect of Season and Sterilization Method on Response of x *Malosorbusflorentina* (Zucc.) Browicz (Rosaceae) Buds to In Vitro Culture Proc. VI th IS on New Floricultural Crops Ed(s). Johnston (et al.) Acta Hort. 813, ISHS
- 32- Polle, A.; D. Janz; T. Teichmann and V. Lipka (2013). Poplar geneticin gineering: promoting desirable wood characteristics and pest resistance. Appl. Microbiol. Biotechnol. 97:5669–9765. (et al.) Acta Hort. 813, ISHS 2009. (508-503)
- 33- Sharada, M.; A. Ahuja and M. K. Kaul (2003). Regeneration of plantlets via callus cultures in *Celastruspaniculatus* Wild-A rare endangered, medicinal plant. J Plant BiochemBiotechnol. 12:65–.96
- 34- Sabooni, N. and A. Shekafandeh (2017). Somatic embryogenesis and plant regeneration of blackberry using the thin cell layer technique. Plant Cell, Tissue and Organ Culture (PCTOC), 130(2):313-321.

- 35- Zaid, S. H. (2011). Using plant tissue culture technology in micropropagation of salt ragweed *Atriplex halimus* L. Tolerant to salt and drought. Tishreen University Journal for Research and Scientific Studies - Biological Sciences Series 33(2).
- 36- Teixeira, S. L.; J. M. Ribeiro and M. T. Teixeira (2006). Influence of NaClO on nutrient medium sterilization and on pineapple (*Ananas comosus* cv Smooth cayenne) behavior. *Plant Cell, Tissue and Organ Culture*, 86:375-378
- 37- Tian, L.; Y. Wen; S. Jayasankar; S. Sibbald (2007). Regeneration of *Prunus salicina* Lindl (Japanese plum) from hypocotyls of mature seeds. *In vitro cell. dev. Biol.* 43: 343–347
- 38- Vasconcelos, C. M.; E. B. Olivira; L. F. Arantes; S. N. Rossi, R. L. Rocha, R. Puschmann, and J. B. P. Chaves (2020). Antibrowning effect of the combination of ascorbic, citric and tartaric acids on quality of minimally processed yacon (*Smallanthus sonchifolius*). *Boletim do Centro de Pesquisa de Processamento de Alimentos*, 36(2).
- 39- Welander M, Jansson E & Lindqvist H (1989): In vitro propagation of *Populus wilsoniana* – a hybrid of ornamental. *Plant Cell Tiss. Org. Cult.* 3(18): 209-219
- 40- H. M. C. Whitehead and K. L. Giles (1977): Rapid propagation of poplars by tissue culture methods. *N.Z. J. For. Sci.* 7:40-43
- 41- Xiyang, Zhao., Huiquan Zheng, Shanwen Li., Chuanping Yang., Jing Jiang (2014): The rooting of poplar cuttings: a review. *New Forests* (2014) 45:21–.43
- 42- Y. He, X. Guo, R. Lu, B. Niu, V. Pasapula and P. Hou (2009): “Changes in Morphology and Biochemical Indices in Browning Callus Derived from *Jatropha curcas* Hypocotyls,” *Plant Cell, Tissue and Organ Culture*, Vol. 98, 2009, pp. 11-17. <http://dx.doi.org/10.1007/s11240-009-9533-y>
- 43- Ziad Al-Hussein, A. Al-Jarad, and R. Al-Beik (2009). The influence of some Parameters on the success of the initial cultivation stage for propagation of olives (*Olea europaea* L.). *Aleppo University Research Journal. Agricultural sciences series. Number (72):155 -175*

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تأثير الجزء النباتي وطرائق التعقيم ومضادات الأكسدة لتحسين نمو وسلامة العينات النامية في المراحل الأولية لأكثار الحور الفراتي خارج الجسم الحي*

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الملخص

استهدف البحث دراسة تأثير طرق التعقيم وإضافة مضادات الأكسدة ومصدر الجزء النباتي في معدل العينات النامية والسليمة في المرحلة التأسيسية لزراعة الحور الفراتي، وقد أجريت اختبارات البحث في مخبر زراعة الأنسجة في كلية الهندسة الزراعية بدير الزور، حيث تم استخدام هيبوكلوريد الصوديوم بتركيزات 10% و 20% لمدة تتراوح من 10 إلى 20 دقيقة. وبالمثل، تم استخدام هيبوكلوريد الكالسيوم بتركيزات 10% و 20% لنفس الإطار الزمني. وتم استخدام كلوريد الزئبق بتركيزين 0.1% و 0.2% لمدة 2 إلى 4 دقائق، أظهرت النتائج أن استخدام كلوريد الزئبق تركيز 0.1% لمدة 2 دقيقة ساعد في تخفيف التلوث الميكروبي و أعطى نسبة من العينات النامية (46.67%)، بالإضافة إلى ذلك، وفي المعاملة الثانية تم استخدام مزيج من حمض الأسكوربيك بتركيز 100 ملغم/لتر وحمض الستريك بتركيز 100 ملغم/لتر. وتم استخدام بيروكسيد البولي فينيل (PVP) بتركيز 100 ملغم/لتر، بينما تم استخدام الفحم المنشط (AC) بتركيز 200 ملغم/لتر. وتم تعقيم البيئة عمداً لمدة أسبوع واحد. وأن المعاملة بـ حمض الأسكوربيك بتركيز 100 ملغم/لتر مع حمض الليمون بتركيز 100 ملغم/لتر. والمعاملة بالفحم المنشط (AC) بتركيز 200 ملغم/لتر تقلل من أكسدة الفينول في البيئة الزراعية، وأعطت نتائج دراسة تأثير مصدر الأجزاء أن أفضل المعاملات كانت في أجزاء اخذت من البراعم بالترتيب (2-5) على الفرع أو أجزاء من قمة السرطان (البرعم 2)، مقارنة بالمصدر المأخوذ من السرطانات.

الكلمات المفتاحية: الحور الفراتي، كلوريد الزئبق، هيبوكلوريد الصوديوم، مضادات الأكسدة، مصدر الجزء النباتي، العينات النامية والسليمة.

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