



**EFFECT OF IRRIGATION INTERVALS AND SALICYLIC ACID ON SOME OF THE PHENOTYPIC PHYSIOLOGICAL TRAITS OF *Pinus brutia* Ten. SEEDLINGS.**

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**ABSTRACT**

Article information  
Article history:  
Received: 14/05/2025  
Accepted: 24/08/2025  
Available: 31/03/2026

**Keywords:**  
*irrigation intervals,*  
*Pinus brutia* , *salicylic acid*

DOI:  
[10.33899/mja.2026.160241.1593](https://doi.org/10.33899/mja.2026.160241.1593)

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The experiment was conducted in the nursery of the Department of Forest Science/ University of Mosul/Faculty of Agriculture and Forestry/ during the period from April to December 2024 to study the impact of three watering periods (1 day, 3 days, 5 days) and three concentrations of salicylic acid (zero, 200, 400) mg. Seedling<sup>-1</sup> in some of the phenotypic and physiological qualities of *Pinus brutia* Seedlings. The results showed that watering every (1) day had a significant impact on most of the vegetative and physiological growth qualities. As for the watering period every (5) days, it gave the highest significant increase in the percentage of proline. As for the effect of salicylic acid, the results showed that the concentration of (400) mg. Seedling<sup>-1</sup> had a significant effect on longitudinal Height seedling, leaf area, total chlorophyll and carbohydrates, while the concentration was (200) mg.L<sup>-1</sup> has a significant impact on the diameter of the leg, while the concentration (200 and 400) mg.L<sup>-1</sup> was given Significant increase in the quality of proline for bilateral interference between both watering periods and salicylic acid. The results showed that watering every (1) day and spraying with salicylic acid at a concentration of (400) mg. Seedling<sup>-1</sup> has given the highest rates in most of the studied qualities, and the overlap between the watering period every 5 days and salicylic acid in concentrations of 200 and 400 mg.L<sup>-1</sup> in the leaf content of proline.

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**INTRODUCTION**

Iraq suffers from frequent waves of drought as a result of high temperatures. Drought in Iraq is classified into three types: climatic drought, which occurs as a result of high temperatures and low rainfall rates. Drought is one of the main factors affecting the growth and productivity of plants, as its effects are exacerbated by climatic changes such as global warming and desertification, and the lack of soil moisture in afforestation yards leads to poor growth and deterioration of the health of seedlings, which may lead to their death and failure of afforestation processes (Hasheminasab *et al.*, 2014). *Pinus brutia* trees are a naturally occurring species in Iraq, especially in Dohuk Governorate. This species belongs to the coniferous family (Pinaceae). The height of 20-meter trees reaches about 35 meters and the diameter of the stem ranges between (50-80) cm (Mosa, 2016). and is widely used in reforestation projects. *Pinus brutia* reproduces sexually only by seed, as their production begins at the age of seven years, and increases with the age of the trees until they reach

maturity, after which production decreases (Alan *et al.*, 2018). To ensure the success of afforestation, researchers focus on hardening seedlings in nurseries before transporting them by treating them with chemical compounds to enhance their drought tolerance. One of the most important of these treatments is the use of salicylic acid, which is one of the organic plant hormones that modern research has been addressing in research and study for its great role in many physiological processes that occur in plants. It is also one of the plant hormones that possess the nature of phenolic, which regulates many physiological processes, inducing vegetative growth, regulating the absorption of ions, the movement of vacuoles and hormonal balance. It is also one of the many phenolic compounds that contain an aromatic ring with the hydroxyl group or its derivatives that are found in plants. It has been proven that salicylic acid affects a wide range of physiological processes, stomatal closure, plant productivity, and glycolysis. It is a water-soluble antioxidant compound that can regulate plant growth. It also has a role in tolerating abiotic stress, such as tolerating drought stress in plants (Muthulakshmi and Lingakumar, 2017). Afforestation projects within cities and forests, despite their importance, lead to a significant depletion of water resources. The selection and cultivation of plants without conducting environmental physiological studies that show the extent to which these plants are able to grow in these environments under many environmental stresses, is scientifically incorrect. Plants with relatively low water needs must be selected, and therefore most suitable for environmental conditions. Due to the exacerbation of the problem of environmental stresses, especially drought stresses, and the lack of success of many afforestation projects in our regions due to the increasing environmental pressure, we thought that our study should be on the possibility of using chemical fertilizers to reduce the effects of drought stresses on *Pinus brutia*. Therefore, our study aims to assess the impact of chemical fertilizers in reducing drought stress on *Pinus brutia* with a focus on improving growth and reducing water consumption to achieve sustainable success of afforestation operations.

## **MATERIALS AND METHODS**

### **1\_ The location of the experiment.**

This experiment was carried out at the University of Mosul/Faculty of Agriculture and Forestry/Forestry Science Department/Nineveh Governorate/on *Pinus brutia* Seedlings. The research took a period of time from the beginning of April to the end of December of 2024, and laboratory studies were conducted in the Development Laboratory of the Forestry Science Department.

### **2-Preparing the trial site.**

The site was selected inside the wooden canopy in the nursery of the Forest Science Department, with an area of (10\*10)m, and then it was cleaned of bushes and

gravel for the purpose of placing seedlings in it, and the factor coefficients were distributed according to the scheme of the experiment according to the design of the complete randomized sectors (R.C.B.D).

3. The source of obtaining seedlings.

(270 seedlings) were selected from *Pinus brutia* Ten. It is located in the forest and pasture nursery in the Zakho district. It is one of the government nurseries of Dohuk Governorate. It is two years old, has consistent lengths and diameters, and is grown in polyethylene bags.

4- Transferring seedlings to a plastic pot.

The seedlings were transferred to a black plastic pot with dimensions of ( 21\*22) cm and a size of approximately (5)L and filled with loam soil. Then the seedlings were distributed to the experimental units, leaving a space between each treatment. (10) cm. The height, diameter, and number of branches for each seedling were measured before the Treatments were conducted and recorded in the field record of the experiment.

5. Seedling Service Operations.

Service operations were performed symmetrically on all experiment units of watering and weeding operations.

6. Experimental treatments.

The experiment included studying two factors.

The first factor: Seedling watering periods (every 1day, every 3 days, every 5days), and each watering was 1 liter. Sapling<sup>-1</sup> and for all seedlings.

Second factor: Salicylic acid (0, 200, and 400) mg.L<sup>-1</sup> Spraying on the vegetative parts. Concentrations of Salicylic Acid I (0) mg.L<sup>-1</sup> is spraying comparison seedlings with distilled water only, second concentration (200)mg.L<sup>-1</sup>, and was prepared by weighting (200) mg.L<sup>-1</sup> of salicylic acid powder by an electric sensor balance <sup>1</sup>Using a digital precision balance (0.000) g, the required amount was weighed and placed in a (1)L beaker. A small amount of sodium hydroxide and a small amount of distilled water were added, and the mixture was shaken until the turbidity of the solution disappeared, then the volume was completed with distilled water. The third concentration (400) mg L<sup>-1</sup> was prepared by weighing (400) mg of salicylic acid powder and following the same steps used to prepare the second concentration. In mid-April 2024, *Pinus brutia* seedlings were sprayed with the three concentrations of salicylic acid until complete wetting.

7\_ Experiment design and statistical analysis.

The number of treatments was 9 per coefficient of 10 seedlings by three Replicates according to the design of the complete random sectors (RCBD), where the statistical analysis was carried out using the program (SAS) system V 9.0, and a comparison of the averages of treatment according to the Duncan polynomial test at a probability level of 0.05.

8. Field measurements.

1. Increase in seedling height (cm): The height of the main stem of the seedling was measured at the end of the experiment by measuring the height from (0.5) cm above the junction of the stem with the root to the apical tip of the seedling using a measuring ruler. The initial height of the same seedling at the beginning of the experiment was then subtracted, and the result represented the growth in seedling height. This procedure was repeated for five seedlings, and the average seedling height for the treatment in the first block was calculated. The same calculations were repeated for the second and third blocks. The mean seedling height for the three blocks was then obtained, and this mean value was used in the statistical analysis of the experiment.

2- Main stem diameter (mm.Seedling<sup>-1</sup>): At the beginning of the experiment, the stem diameter of each seedling was measured using a Vernier caliper from a point 0.5 cm above the junction of the stem with the root. At the end of the experiment, the diameter of the main stem of each seedling was measured again. The stem diameter growth was then calculated by subtracting the initial diameter of the same seedling from its final diameter, and the result represented the increase in the main stem diameter. This procedure was repeated for five seedlings, and the average value was calculated, representing the stem diameter growth for the seedlings in the first block. The same calculations were repeated for the second and third blocks, and then the overall mean for the three blocks was obtained. This mean value represents the average growth of the main stem diameter and was used in the statistical analysis of the experiment.

3- leaf surface area (cm<sup>2</sup>. seedling<sup>-1</sup>): The leaf area of the seedlings was estimated based on the assumption that the needle leaves of pine trees have a cylindrical shape (Zivanovic and Jokanovic, 2023). Measurements were carried out on 100 randomly collected leaves from seedlings across three replicates encompassing all treatments. The fresh weight of the leaves was measured immediately in the field and stored in tightly sealed bags to maintain their moisture and prevent loss before the drying process, ensuring the accuracy of the results and their representation of the studied plant population. The dimensions of the leaves were measured using a digital Vernier caliper, recording the diameter and length of each leaf. To estimate the area of a single needle leaf, it was assumed to be half of a geometric cylinder. By applying the corresponding mathematical formula for this shape, the surface area of each needle of *Pinus brutia* seedlings was calculated. The formula used is as follows:

Surface area of half-cylinder (needle surface area) =  $(2\pi * r^2 + 2\pi * r * h) / 2$

$\pi = 3.14$

r = radius of the needle (cm)

h = length of the needle (cm)

Afterward, the total surface area of all leaves was calculated, giving the total surface area and weight of 100 leaves. From this, the leaf area for each seedling in the experiment was determined by calculating the leaf weight per seedling and applying a proportional relationship to obtain the leaf area for each seedling.

4- shoot dry weight of vegetative total (g.Seedling<sup>-1</sup>): The vegetative total of the selected seedlings was dried to calculate the dry weight and the vegetative total was placed in a paper punched bag and dried using an electric oven at a temperature of (70° ± 1) until the weight was stable, then the samples were weighed with a sensitive balance of (0.001g for three seedlings from each transaction and for three sectors.

5- Estimation of the total chlorophyll content of the leaves (mg.g<sup>-1</sup>) and total chlorophyll was estimated based on the (Mackinney method,1941) and modified by (Arnon,1949). Then, a weight of 0.2 g of healthy soft green leaves was taken. The leaves were crushed by a ceramic mortar, and then acetone (12) ml concentration (80%) was added, then placed in the Centrifuge for (5 minutes) at a speed (3000 cycles/minute) for the purpose of separating the leachate from the precipitate. The leachate was then taken, and absorbency was read at wavelengths (645, 663 nm) by the Spectrophotometer. The following equations were used to calculate the amount of chlorophyll in the leaves, calculated on the basis of mg. g<sup>-1</sup> wet weight.

$$\text{Total Chlorophyll} = [20.2(D645) + 8.02(D663)] \times V / 1000 \times W$$

6- Estimation of the carbohydrate content of the leaves (mg.g<sup>-1</sup> g dry weight): The carbohydrate content in the leaves was estimated according to the method of (Dubois *et al.*, 1956) by taking a weight of (0.1) g of dry leaves where they were crushed in a ceramic mortar and adding (10) ml of distilled water, then the solution is taken and placed in a test tube and placed in a centrifuge for (15) minutes at a speed of (3000) cycles. Minute<sup>-1</sup>, then take the leachate and add distilled water for the purpose of completing the volume to (10) ml, then take (1) ml of the solution and add (1) ml of phenol at a concentration of (5%), then add (5) ml of concentrated sulfuric acid H<sub>2</sub>SO<sub>4</sub> and leave the sample for (15) minutes, then put in a water bath at a temperature of (25-30) ° C for a period of (20) minutes, then the absorbance is read using a spectrophotometer at a wavelength of (488) nm, and the readings fall on the standard curve of glucose sugar to obtain the concentration of carbohydrate content as mentioned by.

$$\text{Carbohydrates (mg/g)} = \frac{\text{Volume of extract used} \times \text{Total extract volume} \times \text{Read device}}{\text{Sample weight} \times 1000}$$

7. Estimation of Proline Amino Acid Content in Leaves (mg.NM-1 Dry Weight): The amino acid content of proline in the leaves was estimated according to the method reported by (Mahamdkhan and Heidri, 2008), where 0.4 g of dry leaves were taken and crushed with Sulfosalicylic acid at a concentration of 3% homogenization limit. After that, it was filtered through filter paper, and then (2) ml of the filtrate was added to it (2 ml of Ninhydrin acid and (2) ml of ice acetic acid and placed in a test tube in a water bath at a temperature of (100) degrees Celsius. Then the samples

were transferred to an ice bath for 30 minutes. After that, each sample was added (4) ml of toluene, where it was well mixed in each test tube by a vibrator for a period of (20) minutes. After that, the samples were heated to normal room temperature; i.e., the upper two layers would be pink, containing Prolene, and the lower layer would be bright. Then, the absorption was read at a wavelength of 520 nm by the spectrophotometer.

## **RESULTS AND DISCUSSION**

### **4-1 Increase Height Growth (cm):**

The results of Table (1) regarding the increase in the height growth of the stem showed that there are significant differences between the watering periods according to the Duncan test, where the treatment of watering every (1) day recorded the highest significant increase in length of (10.58) cm, while the treatment of watering every (5) day recorded the lowest value of (5.23) cm, with a significant difference of (5.35) cm, representing an increase of (102.29%).

As for the effect of salicylic acid concentrations, Duncan's test showed that the concentration (400)mg. L<sup>-1</sup> the highest significant increase in stem length (9.61 cm) compared to the control treatment (0mg.L<sup>-1</sup>), which recorded (6.61 cm), with a moral difference of (3 cm) and a relative increase of (45.38%).

From the bilateral overlaps between the watering and salicylic acid periods, the results of the Duncan test showed significant differences between the different overlaps, as the overlap between the daily watering every (1)day and the salicylic acid was recorded at a concentration of (400)mg. L<sup>-1</sup> the highest rate of increase in leg length was (11.74 cm), compared to the overlap between watering every (5) days and salicylic acid at a concentration of (0)mg.L<sup>-1</sup>, which recorded the lowest rate of (3.28 cm), with a significant difference of (8.46 cm) and a relative increase of (257.92%).

### **4-2 Increase Diameter Growth (mm):**

Table (2) of the Duncan test shows a significant superiority of the impact of irrigation periods on the character of the increase in the diameter of the stem. It was noted from the table that there are significant differences between the periods. Watering every (1) day gave the highest rate of significant increase of (3.75 mm) compared to watering every (5 days), which gave the lowest average value of ( 2.33 mm) with a significant difference of ( 1.42 mm), which is equivalent to an increase rate of ( 60.94% ).

As for the effect of salicylic acid concentrations, the results of the Duncan test table showed that the concentration (200) mg. L<sup>-1</sup> resulted in the highest significant increase in stem diameter, (3.35) mm compared to the control treatment (0) m<sup>-1</sup>(2.53) mm), with a significant difference of (0.82) mm and an increase of (32.41% ).

**Table (1) the effect of watering periods and salicylic acid and the overlaps between them in the characteristic of the increase in the stem length (cm) of *Pinus brutia* seedlings.**

	Watering intervals			Effect of watering intervals
	Watering 1 day	Watering 3 days	Watering 5 days	
Salicylic acid concentrations (Zero mg.L <sup>-1</sup> )	9.2 d	5.73 f	3.28 h	6.61 c
Salicylic acid concentrations (200 mg.L <sup>-1</sup> )	10.76 b	7.58 e	5.03 g	7.79 b
Salicylic acid concentrations (400 mg.L <sup>-1</sup> )	11.74 a	9.70 c	7.40 e	9.61a
Effect of Salicylic Acid	10.58 a	7.47 b	5.23 c	

\*Numbers with similar letters for single factors and their overlaps do not differ significantly from each other according to the Duncan polynomial test at a probability level of 0.05.

The bilateral overlap between irrigation periods and salicylic acid also showed significant differences, as the overlap between daily watering (1 day) and salicylic acid (200 mg. Liter<sup>1</sup>) The highest rate of significant increase was (3.47) mm, compared to the lowest value recorded at the overlap between the watering periods every (5) days and salicylic acid ( zero mg. liters), which amounted to (1.76) mm with a significant difference of (2.61) mm and an increase of (148.29%).

**Table (2) the effect of watering periods and concentrations of salicylic acid and the overlaps between them on the characteristic of the increase in stem diameter (mm) of *Pinus brutia* seedlings.**

	Watering intervals			Effect of watering intervals
	Watering 1 day	Watering 3 days	Watering 5 days	
Salicylic acid concentrations (Zero mg.L <sup>-1</sup> )	3.41 b	2.43 f	1.76 g	2.53 c
Salicylic acid concentrations (200 mg.L <sup>-1</sup> )	3.47b	3.36d	2.47f	3.35 a
Salicylic acid concentrations (400 mg.L <sup>-1</sup> )	3.47b	2.56e	2.89d	2.97 b
Effect of Salicylic Acid	3.75 a	2.78 b	2.33 c	

\*Numbers with similar letters for single factors and their overlaps do not differ significantly from each other according to the Duncan polynomial test at a probability level of 0.05.

### 4.3 Leaf Surface Area (cm<sup>2</sup>):

Table (3) showed that the three watering periods differed significantly in the impact on the leaf area, where watering every (1) day was morally superior to other watering periods, as it had the highest rate of increase in this characteristic (1185)cm<sup>2</sup> with a significant difference of (501)cm<sup>2</sup>, which is equivalent to an increase rate of (73.25%) compared to the watering period every (5) days, which gave the lowest rate of (684 )cm<sup>2</sup>.

As for the effect of salicylic acid on the leaf surface area, Duncan's test indicates that there are significant differences between the concentrations in their effect in this characteristic, the concentration exceeded (400)mg.L<sup>-1</sup> and gave the highest average

of (1126)cm<sup>2</sup> with a significant difference (397)cm<sup>2</sup>, which is equivalent to an increase of (54 %) compared to the comparison treatment (729)cm<sup>2</sup>.

As for the overlap between the periods of watering and salicylic acid, the Duncan test for comparison between the computational media of the impact of these overlaps in the leaf space indicates that there are significant differences between these overlaps. Watering every (1) day and salicylic acid with a concentration of (400)mg.L<sup>-1</sup> and gave the highest rate of this trait (1261) cm<sup>2</sup> with a significant difference of (863.61) cm<sup>2</sup>, which is equivalent to (174.96%) if compared with watering every (5) days and salicylic acid with a concentration of (0) mg.L<sup>-1</sup> which gave the lowest rates (493.60) cm<sup>2</sup>.

**Table (3) the effect of watering periods and concentrations of salicylic acid, and the overlaps between them in the character of the paper surface area (cm.mm<sup>-2</sup>).**

	Watering intervals			Effect of watering intervals
	Watering 1 day	Watering 3 days	Watering 5 days	
Salicylic acid concentrations (Zero mg.L <sup>-1</sup> )	1036 c	656 g	493 h	729 c
Salicylic acid concentrations (200 mg.L <sup>-1</sup> )	1162 b	905 d	702 f	923 b
Salicylic acid concentrations (400 mg.L <sup>-1</sup> )	1261a	990d	340 f	1126 a
Effect of Salicylic Acid	1158 a	909 b	684 c	

\*Numbers with similar letters for single factors and their overlaps do not differ significantly from each other according to the Duncan polynomial test at a probability level of 0.05.

#### **4-4 Total dry shoot (g):**

The Duncan test Table (4) showed that the watering periods had a significant impact on the dry weight of the shoot, as the highest rate every (1) day was (23.46)g with a significant difference of (11.6)g, which is equivalent to (97.80%) if compared with the longest watering period every (5) days, which recorded the lowest rates for this trait of (11.86)g.

As for the effect of salicylic acid concentrations on the dry weight of the shoot Duncan's test indicates that there are significant differences between the concentrations of salicylic acid in their effect on this trait, the concentration exceeded (400)mg.L<sup>-1</sup> and gave the highest average dry weight of the vegetative total (21.08)g with a significant difference of (7.51)g, which is equivalent to (54.85%) when compared with the comparison treatment, which recorded the lowest average weight of(13.69)g.

As for the effect of the bilateral overlap between the periods of watering and salicylic acid in the dry weight of the shoot, the Duncan test shows that there are significant differences between the overlaps of these two factors, as the overlap between the period of watering every(1) day and salicylic acid with a concentration of (400)mg differed and surpassed morally.liter<sup>-1</sup>), where it reached the highest dry

weight of the vegetative total of (25.66)g and led to a moral increase of (17.86)g, which is equivalent to (228.97%) for the overlap between the watering period every(5) days and salicylic acid with a concentration of (0mg)L<sup>-1</sup>), which recorded the lowest rates (7.80)g.

**Table (4) Effect of watering periods, salicylic acid, and their interaction on the dry weight (g) of *Pinus brutia* seedlings.**

	Watering intervals			Effect of watering intervals
	Watering 1 day	Watering 3 days	Watering 5 days	
Salicylic acid concentrations (Zero mg.L <sup>-1</sup> )	20.76 d	12.52 f	7.80 h	13.69 c
Salicylic acid concentrations (200 mg.L <sup>-1</sup> )	23.97 b	16.83 e	11.32 g	17.37 b
Salicylic acid concentrations (400 mg.L <sup>-1</sup> )	25.66 a	21.15 c	16.45 e	9.89 a
Effect of Salicylic Acid	23.46 a	19.95 b	11.86 c	

\*Numbers with similar letters for single factors and their overlaps do not differ significantly from each other according to the Duncan polynomial test at a probability level of 0.05.

**4-5 Estimation of the total chlorophyll content in leaves (mg.g<sup>-1</sup> MW):**

The results of Table (5) of the impact of periods showed that the watering periods differed significantly by affecting the total chlorophyll content of the leaves, as the highest rate at the shortest watering period every (1) day was (1.049) mg.g<sup>-1</sup> fresh weight with a significant difference (0.595) mg.g<sup>-1</sup>, which is equivalent to (131.05%) if compared with the every (5) days, which recorded the lowest rates for this trait of (0.454) mg.g<sup>-1</sup> fresh weight).

As for the effect of salicylic acid concentrations, Duncan's test indicates that there are significant differences between salicylic acid concentrations in their effect on the total chlorophyll content of the leaves, the concentration exceeded (400)mg.L<sup>-1</sup> and recorded the highest rate of (0.773) mg.g<sup>-1</sup> fresh weight with a significant difference of (0.101) mg.g<sup>-1</sup> fresh weight), which is equivalent to (15.02)% when compared with the zero salicylic treatment, which recorded the lowest rate of(0.672) mg.g<sup>-1</sup> fresh weight.

As for the effect of the bilateral overlap between the periods of watering and salicylic acid in the total chlorophyll content of the leaves, Duncan's test shows that there are significant differences between the overlaps of these two factors, as the overlap between the period of watering every (1) day and salicylic acid with a concentration of (400)mg differed and surpassed significantly.L<sup>-1</sup>) over the rest of the other interventions, it had the highest average of (1.111) mg. g<sup>-1</sup> of fresh weight and led to a significantly increase of (0.683) mm.g<sup>-1</sup> frsh weight, which is equivalent to (159.57%) compared to the overlap between the watering period every (5) days and salicylic acid with a concentration of (0)mg.L<sup>-1</sup>, which recorded the lowest rates of this trait (0.428) mg.g<sup>-1</sup> fresh weight.

**Table (5) the effect of watering periods and concentrations of salicylic acid and the overlaps between them in the character of the total chlorophyll content of the leaves (mg.g<sup>-1</sup> fresh weight) for *Pinus brutia* Seedlings.**

	Watering intervals			Effect of watering intervals
	Watering 1day	Watering 3 days	Watering 5 days	
Salicylic acid concentrations (Zero mg.L <sup>-1</sup> )	0.731 c	0.634 f	0.428 i	0.625 c
Salicylic acid concentrations (200 mg.L <sup>-1</sup> )	1.079 b	0.698 e	0.457 h	0.745 b
Salicylic acid concentrations (400 mg.L <sup>-1</sup> )	1.111 a	0.731 d	0.467 h	0.773 a
Effect of Salicylic Acid	1.049 a	0.687 b	0.454 c	

\*Numbers with similar letters for single factors and their overlaps do not differ significantly from each other according to the Duncan polynomial test at a probability level of 0.05.

#### 4-6 Estimation of the carbohydrate content in the leaves (mg.g<sup>-1</sup> dry weight):

The results of Table (6) showed that there are significant differences between the periods, where the treatment of watering every (1) day recorded the highest rate of significant increase in the content of leaves of carbohydrates amounted to (15.91) mg.g<sup>-1</sup> dry weight, compared to the treatment of watering every (5) days, which recorded the lowest rate of (11.85) mg.g<sup>-1</sup> dry weight, with a significant difference of (4.06) mg.g<sup>-1</sup> dry weight, which is equivalent to an increase of (34.26%).

Regarding the effect of the concentration of salicylic acid, Duncan's test showed significant differences and gave a concentration of 400 mg. L<sup>-1</sup> The highest average was (15.20 mg. g<sup>-1</sup> dry weight with a significant difference (1.79) mg.g<sup>-1</sup> dry weight and an increase of (13.34%) compared to the comparison factor, which gave the lowest rate (13.41)mg.g<sup>-1</sup> dry weight.

The bilateral overlap between the watering and salicylic acid periods The table data for estimating the carbohydrate content of the leaves showed that the watering period was every (1) day and the concentration of salicylic acid (400)mg.L<sup>-1</sup> recorded the highest rate of (17.03)mg.g<sup>-1</sup> and differed morally from the overlap between the irrigation period (1) day and salicylic acid (0) mg.L<sup>-1</sup> dry weight, which recorded the lowest rate of (11.18 mg.g<sup>-1</sup> dry weight) and a significant difference of (5.85) mg.g<sup>-1</sup> dry weight and by an increase (52.32%).

**Table (6) the effect of watering periods and concentrations of salicylic acid and the overlaps between them in the characterization of the leaf content of carbohydrates (mg.g<sup>-1</sup> dry weight) for *Pinus brutia* Seedlings.**

	Watering intervals			Effect of watering intervals
	Watering 1day	Watering 3 days	Watering 5 days	
Salicylic acid concentrations (Zero mg.L <sup>-1</sup> )	14.92 d	14.13 f	11.18 i	13.41 c
Salicylic acid concentrations (200 mg.L <sup>-1</sup> )	15.77 c	14.60 e	11.63 h	14.00 b
Salicylic acid concentrations (400 mg.L <sup>-1</sup> )	17.03 a	15.85 b	12.73 g	15.20 a
Effect of Salicylic Acid	15.91 a	14.86 b	11.85 c	

\*Numbers with similar letters for single factors and their overlaps do not differ significantly from each other according to the Duncan polynomial test at a probability level of 0.05.

#### 4-7 Estimation of the amino acid (prolene) content in leaves (mg.g<sup>-1</sup> dry weight):

The results of Table (7) for the effect of watering periods showed that there are significant differences between the periods, as the irrigation treatment every (5)days recorded the highest rate of significant increase in the leaf content of proline, which amounted to (47.614)mg.g<sup>-1</sup> dry weight, compared to a watering treatment every (1) day that recorded the lowest rate of (45.725)mg.g<sup>-1</sup> dry weight, with a significant difference of (1.889)mg.g<sup>-1</sup> dry weight and a relative increase of (4.13%).

With regard to the effect salicylic acid, Duncan's test showed that the concentration (200) mg.L<sup>-1</sup> and concentration (400)mg.L<sup>-1</sup> recorded the highest rates, (46.842)mg.g<sup>-1</sup>, 46.853mg.g<sup>-1</sup>, respectively but it did not differ morally among them, but it did not exceed the comparison treatment, which amounted to (246.35)mg.g<sup>-1</sup> dry weight.

The bilateral overlap between the periods of watering and salicylic acid in the leaf content of proline The results of the Duncan test showed that the period of watering every (5) days and the concentration of salicylic acid (400)mg.L<sup>-1</sup> gave the highest rate of (47.883mg.g<sup>-1</sup> dry weight), but it did not differ significantly from the overlap between the period of watering every (5) days and salicylic acid (200)mg.L<sup>-1</sup> while morally superior to the overlap between the watering period every(1) day and the concentration of salicylic acid (0) mg.L<sup>-1</sup>, which recorded the lowest rate of (45.602mg.g<sup>-1</sup> dry weight) with a significant difference of (2.281 mg.g<sup>-1</sup> dry weight) with an increase of ( 5%).

**Table (7) Effect of watering periods and salicylic acid concentrations on proline content in the leaves of *Pinus brutia* seedlings (mg·g<sup>-1</sup> dry weight).**

	Watering intervals			Effect of watering intervals
	Watering 1 day	Watering 3 days	Watering 5 days	
Salicylic acid concentrations (Zero mg.L <sup>-1</sup> )	45.602 g	46.378 d	47.077 b	46.352 c
Salicylic acid concentrations (200 mg.L <sup>-1</sup> )	45.770 f	46.875 c	47.882 a	46.842 a
Salicylic acid concentrations (400 mg.L <sup>-1</sup> )	45.805 e	46.870 c	47.883 a	46.853 a
Effect of Salicylic Acid	45.725 c	46.708 b	47.614 a	

\*Numbers with similar letters for single factors and their overlaps do not differ significantly from each other according to the Duncan polynomial test at a probability level of 0.05.

### **Discussion:**

The results showed that watering periods had a significant impact on the phenotypic qualities of *Pinus brutia* seedlings, as shown in tables (1, 2, 3, 4). Clear differences were recorded between the transactions, indicating that the seedlings were affected by water stress resulting from the variation of watering periods. It was found that reducing water stress through watering by shorter periods (every 1 day or every 3 days) led to a noticeable increase in the rates of the studied qualities (seedling height, stem diameter, leaf surface area, and dry weight of the shoot). The data also showed that watering every (5) days led to a decrease in the rates of the studied traits, as a result of the decrease in cell division and expansion, which limits the growth of

the vegetative parts of the plant (Hasanuzzaman *et al.*, 2022). This is due to the fundamental role of water in stimulating cell division and elongation processes (Taiz *et al.*, 2023 also stressed that the elongation of cells is strongly affected under the influence of water stress, as the growth of cells depends on their ability to maintain their fullness, which is directly affected by the dryness of the soil and the lack of water supply and mineral elements necessary for growing plant tissues, which leads to the deterioration of biological and physiological processes within the plant, and the results of tables(5 and 6) illustrate the singular effect of the watering period factor in the studied physiological qualities of the total chlorophyll and carbohydrate content of leaves for seedlings *Pinus brutia* indicates that there are significant differences between the studied transactions for different watering periods. An increase in the values of physiological growth indicators is observed when watering stress decreases with shorter periods every (1) day, which led to an increase in the studied physiological growth indicators, respectively (the total chlorophyll and carbohydrate content in the leaves) for *Pinus brutia* seedlings compared to watering periods every(3) days and every (5) days. The reason for this may be due to the role of water stress, especially watering every (5) days, which led to negative effects on growth standards. The reason may be that the effective oxine roots are an important and essential source of cell damage under The conditions of water stress, which are highly toxic to plant cells so that they react quickly with the components of the cell and interact with the lipids in the cell membranes, which lead to their rapid damage by creating holes that lead to the leakage of their content and drought and thus their death and cause damage to the membranes, as it affects the respiratory processes in the mitochondria and the destruction of chlorophyll pigment, and then the efficiency of fixing CO<sub>2</sub> carbon dioxide in the chloroplast decreases (Gupta, 2010) pointed out that drought stress causes the stimulation of oxidizing free radicals as a result of the disturbance of the regulatory system in the crana membranes in the chloroplast and thus inhibiting or stopping photosynthesis. (Farooq *et al.*,2009). In their research on three species of Buckthorn (Chaitanya *et al.*,2009) in their study on five species of white berries, *Morus alba* pointed to the important role played by prolin in adapting to water stress. The rapid and direct rise in the level of prolene in plant tissues is an accurate indicator of the extent to which it is affected by and tolerates stress, as its accumulation in the leaves increases as the period of exposure of the plant to drought increases, which was also found by(Shult and Matthews, 1993) in their research on two origins of wild apples, where they observed a remarkable rise in the levels of prolene in the leaves of water-stressed plants. As for the effect of salicylic acid concentrations on some of the vegetative traits studied for *Pinus brutia* seedlings, the results of tables (1, 2, 3,and 4) indicate that there are differences between the concentrations and have a significant impact, especially at the concentration (400mg.L<sup>-1</sup>) Compared to the comparison treatment, the reason for the increase may

be attributed to the role of salicylic acid as a growth stimulator and is classified among plant hormones that encourage vegetative growth. In addition, it works to reduce the abiotic tension inhibiting growth, which in turn leads to an increase in the concentrations of some other plant hormones that have an impact on the process of division and elongation of plant cells, such as oxines and gabralins.(Shahin *et al.*, 2010), Salicylic acid has a positive effect on vegetative growth characteristics and can be interpreted as playing an important role in regulating vital processes and growth in the plant ( Raskin, 1992). The results of tables (5 , 6 and 7) show the effect of salicylic acid on some of the physiological characteristics of *Pinus brutia* to the existence of significant differences between the different studied coefficients, as a significant increase in the values of the physiological growth indicators of each of (the total content of chlorophyll, carbohydrates and proline)compared to the comparison treatment, and the reason is due to the effect of spraying with salicylic acid as it is one of the plant hormones that can encourage the construction of hormone oxine and gabraline in addition to encouraging the construction of chlorophyll and carbohydrates resulting from increased growth here (Métrauxs, 2001) It may also encourage the process of gene expression to build many hydrochloric compounds, including Proline (khan *et al.*, 2015). to exceed spraying with salicylic acid. It is in line with its physiological role as it works to accelerate the formation of chlorophyll pigments, accelerate the process of photosynthesis, and increase the activity of some important enzymes (Metwally *et al.* 2003) .He confirmed (Fariduddin *et al.*, 2003).

## CONCLUSIONS

The results of the study showed that the periods of watering and treatment with salicylic acid have significantly affected some of the phenotypic and physiological traits of the *Pinus brutia* seedlings.

## CONFLICT OF INTEREST

Conflict of interest: the author declares that there is no conflict of interest with regard to the publication of this article.

تأثير فترات الري وحمض الساليسيليك في بعض الصفات المظهرية والفسلجية لشتلات الصنوبر البروتي  
*Pinus brutia* Ten.

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## الخلاصة

اجريت التجربة في مشتل قسم علوم الغابات / جامعة الموصل/ كلية الزراعة والغابات/ خلال الفترة من نيسان والى كانون الاول لعام 2024 لدراسة تأثير ثلاث فترات سقي ( 1 يوم ، 3 يوم ، 5 يوم) وثلاث تراكيز من حمض الساليسيليك ( صفر ، 200 ، 400 ) ملغم. شتلة<sup>1</sup> في بعض الصفات المظهرية والفسلجية لشتلات

السنوبر البروتي، وظهرت النتائج ان السقي كل(1) يوم كان لها تأثير معنوي في معظم صفات النمو الخضري والفسلجي اما فترات السقي كل(5) يوم اعطت اعلى زيادة معنوية في نسبة البرولين، اما بالنسبة لتأثير حامض السالسيليك فأظهرت النتائج ان تركيز (400)ملغم.ثنتل<sup>-1</sup> كان لها تأثير معنوي في النمو الطولي والمساحة الورقية والكلوروفيل الكلي والكاربوهيدرات، اما التركيز (200)ملغم.لتر<sup>-1</sup> اثر معنوياً في قطر الساق بينما اعطى التركيز (200 و400)ملغم.لتر<sup>-1</sup> زيادة معنوية في صفة البرولين اما بالنسبة للتداخل الثنائي بين كل من فترات السقي وحامض السالسيليك فقد بينت النتائج ان السقي كل(1) يوم والرش بحامض السالسيليك بتركيز(400)ملغم.ثنتل<sup>-1</sup> قد اعطى اعلى المعدلات في اغلب الصفات المدروسة وتفوق التداخل بين فترة السقي كل(5)يوم وحامض السالسيليك بالتركيزين (200 و400)ملغم.لتر<sup>-1</sup> في محتوى الاوراق من البرولين.

الكلمات المفتاحية: السنوبر البروتي، فترات السقي، حامض السالسيليك.

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