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The effect of growth regulators and organic extracts on the growth parameters of Gardinia branches growing under abiotic stress conditions *in vitro*

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Abstract
The experiment was conducted in the Tissue Culture Laboratory/Department of Horticulture and Landscape Architecture/College of Agriculture, Al-Qasim Green University, from September 2024 to June 2025. This experiment aimed to demonstrate the effect of growth regulators or organic extracts on phenotypic and chemical indicators, enzyme activity, and the content of some active compounds in the branches The experiment was conducted according to a completely randomized design (CRD) with two factors and ten replications. The first factor included three concentrations of sodium chloride (50, 100, 150) mmol $^{L-1}$ in addition to the control treatment. The second factor included two types of growth regulators (Melatonin at a concentration of 1 mg L^{-1}, salicylic acid at a concentration of 10 mg $^{L-1}$, and two types of organic extracts: willow leaf extract and moringa leaf extract at a concentration of 10 ml L^{-1}) in addition to the control treatment. The results of the experiment can be summarized as follows:
The results of the experiment showed a negative effect of sodium chloride added to the nutrient medium on most of the studied traits, as the concentration of 150 mmol L^{-1} recorded a significant decrease in the number of leaves, branch length, chlorophyll content, and the percentage of nitrogen, potassium, phosphorus, and protein Carbohydrates in the branches, while the highest rate was recorded in the percentage of sodium, activity of the enzyme peroxidase and catalase, and proline content in the branches, while the concentration of 100 mmol. L^{-1} recorded a significant decrease in the average number of branches. ϖ The results showed a significant effect of growth regulators or organic extracts added to the nutrient medium on most of the studied traits, as the treatment of adding moringa leaf extract at a concentration of 10 ml L^{-1} recorded the highest rate in the number of leaves, branch length, and percentage of nitrogen, phosphorus, and potassium Protein and carbohydrates in the branches, and also recorded the highest rate in the content of active compounds, while it recorded the lowest rate in the percentage of sodium and proline content in the branches, while the growth regulator recorded Salicylic acid at a concentration of 10 mg L^{-1} significantly affected both the number of branches and chlorophyll content. The growth regulator melatonin at a concentration of 1 mg L^{-1} recorded the lowest activity of the peroxidase and catalase enzymes in the branches. The results of the experiment showed that the interaction between sodium chloride salt and the addition of growth regulators or organic extracts to the nutrient medium had a significant effect on most of the studied indicators. The interaction treatment (without adding sodium chloride with the addition of 10 mg L^{-1} salicylic acid) gave the highest rate in the number of branches. While the treatment (without adding sodium chloride with 10 ml L^{-1} Moringa leaf extract) was superior and gave the highest rate of leaf number, branch length and percentage of phosphorus in the branches, while it recorded the lowest rate of sodium percentage, catalase enzyme activity and proline content in the branches. The treatment (50 mmol L^{-1} of sodium chloride and 10 ml L^{-1} Moringa leaf extract) was superior and gave the highest rate of potassium percentage and the highest content of all active compounds under study, while the treatment (100 mmol L^{-1} and 10 ml L^{-1} willow leaf extract) was superior and gave the highest rate of nitrogen and protein in the branches. While the treatment (150 mmol L^{-1} of sodium chloride without adding growth regulators and organic extracts) was superior and gave the highest percentage of sodium and the highest activity of the peroxidase enzyme and proline content in the branches. While the treatment (without adding sodium chloride and the growth regulator melatonin 1 mg L^{-1}) was superior and gave the highest percentage of carbohydrates and the lowest activity of the peroxidase enzyme.

Keywords: Gardenia, abiotic stress, *in vitro* culture, sodium chloride, growth regulators, melatonin

1. Introduction
Gardenia jasminoides is an evergreen plant that has gained widespread popularity in agriculture and traditional medicine due to its high aesthetic value and diverse therapeutic

uses. The plant is characterized by its fragrant white or cream flowers and glossy leaves, making it a popular choice for decorating gardens and homes, as well as an important source in the perfume and cosmetics industries (Chen *et al*., 2020) [6]. It also contains many compounds, including biochemical compounds, such as bucid and crocin, which are of great importance in supporting the system, reducing the damage caused by cancer, which is important in preventing chronic diseases (Xiao *et al*., 2017) [27]. The growing demand for botanical products with therapeutic and aesthetic benefits, such as gardenia, reflects a shift in consumer interest toward natural and safe ingredients. Studies have shown that the aromatic compounds emitted by its flowers have positive effects on mood and stress reduction, opening the door to its use in aromatherapy and mental health applications (Cai *et al*., 2025) [5].

Soil salinity is one of the most prominent environmental problems that hinders the normal growth of plants and reduces their productivity, especially in arid and semi-arid regions. This phenomenon leads to a significant decrease in the overall growth of the plant as a result of an imbalance in nutritional, hormonal and enzyme balance, in addition to the direct toxic effect of some ions accumulated in the soil, such as sodium and chloride (Pradhan *et al*., 2025) [20]. Understanding these effects is essential for developing agricultural adaptation strategies in environments suffering from soil salinization. The gardenia plant is an excellent model for studying plant adaptation to different environmental conditions. It is capable of growing in temperate and subtropical regions, yet requires precise conditions in terms of growth medium, humidity, and temperature, making it suitable for environmental adaptation studies (Coyago-Cruz *et al*., 2023) [7].

In vitro culture techniques are vital tools for studying the mechanics and physiology of plant tolerance to abiotic stresses such as salinity. These methods provide a homogeneous growth environment in terms of salt content and environmental factors, facilitating evaluation and analysis processes away from the complexities and interferences of field conditions (Taha *et al*., 2020) [23]. It has been observed that conducting such studies using plant tissue culture techniques, which are carried out under controlled conditions, helps in the rapid production of active substances and compounds at high concentrations and purity, under sterile and disease-free conditions, in addition to continuous production throughout the year without being restricted by the planting season (Georgiev *et al*., 2009) [8]. Several systems have been used to evaluate plant tolerance to abiotic stresses *in vitro*, including the cultivation of embryos, callus, shoots, and branches in culture media containing different concentrations of sodium chloride. Shoot cultivation is considered a good option compared to callus cultivation, as it maintains the genetic stability of the resulting plants (Smetanska, 2018) [22]. The cultivated branches exhibit a response similar to that of the whole plant because they represent a miniature version of the plant in terms of genetic and anatomical composition, which enhances the reliability of physiological assessments within salt-enriched nutrient media.

Soil salinization is widespread in Iraq and poses a major challenge to agriculture. It has become imperative to find strategies that mitigate the negative effects of this problem. Recent studies have focused on the use of plant growth regulators and organic extracts, which play an effective role in increasing growth rates and improving performance under salinity conditions. It also contributes to regulating physiological, chemical, and biological processes within the plant. These approaches are part of sustainable efforts to enhance the resistance of local plants, such as gardenia, to the challenges of a saline environment. Therefore, this study aims to:

- Study the effect of salt stress on the morphological and biochemical characteristics and content of active compounds in the gardenia plant outside the living body.
- Study the effect of organic extracts and growth regulators on the morphological and biochemical characteristics and content of active compounds in the gardenia plant outside the living body
- To know the extent to which organic extracts and growth regulators reduce the negative effects of salt stress on Gardenia plants *in vitro*.

3. Materials and Methods

3.1. Experimental Site

The experiment was conducted in the Plant Tissue Culture Laboratory of the Department of Horticulture and Landscape Engineering at the College of Agriculture, Al-Qasim Green University, from September 2024 to June 2025. The experiment was conducted to study the effects of organic extracts and growth regulators on phenotypic and chemical parameters and the content of active substances in gardenia plants *in vitro*.

3.2. Sterilization of work tools

Tweezers and blade holders were sterilized by placing them in an electric oven at 160-180 °C for 2-4 hours. They were then transferred to a sterilization table and placed in glass tubes containing 99% alcohol. These tubes were sterilized and then exposed to a flame to remove the alcohol. The glass dishes used for cutting plant parts were sterilized in an electric oven at 160 °C for 90 minutes.

3.3. Source of Plants

Tender branches 5-10 cm long were collected from a gardenia tree growing in a private garden. In the preparation room of the tissue culture laboratory, the leaves were removed and cut into lengths of approximately 1.5-2 cm. They were then washed in running water for 30 minutes and transferred to a stratified air chamber for sterilization. The prepared MS medium was used as recommended. 30 g L^{-1} sucrose, 100 mg L^{-1} myoinositol, and 7 g L^{-1} inert material (agar) were added to solidify the medium. The volume was completed with one liter of distilled water. The pH of the medium was then adjusted to 5.7 ± 0.1 using drops of 1 M hydrochloric acid (HCl) solution. Or a 1-molar solution of sodium hydroxide (NAOH). The medium was then placed on a hot plate magnetic stirrer to dissolve the inert material (agar) and homogenize the medium components. The medium was then distributed into previously sterilized glass vials, 10 ml in each vial. The glass vials containing the medium were then transferred to an Autoclave for sterilization. At a temperature of 121 °C and a pressure of 1.04 kg/cm² for 20 minutes. After the sterilization process was completed, the glass containers were left to cool and the culture medium solidified at room temperature, making them ready for cultivation.

3.4. Sterilization of plant parts

The plant parts were transferred to a laminar air flow cabinet and sterilized by immersing them in 70% ethyl alcohol for 10 seconds, followed by sterilization in a 1% sodium hypochlorite (NaOCl) solution From the commercial Clorax disinfectant, the active ingredient concentration is 5.25%, with the addition of two drops of the spreading agent Tween-20 to reduce surface tension and increase the efficiency of the sterilization process for 20 minutes with continuous stirring. After that, the plant parts were washed three times with sterile distilled water to remove the harmful effects of the sterilizing agent

3.5. Planting the plant parts

The plant parts were cut to a length of 1 cm using sterile scalpels and tweezers, with each part containing a single node after removing the ends exposed to the sterile material. The plant parts were then planted vertically in glass containers containing the MS culture medium, previously prepared for cultivation, using special planting tools. Then the glass bottles containing the cultures were incubated inside the growth chamber at a temperature of 25 + 2 and a light intensity of 1000 lux for 16 hours followed by 8 hours of darkness.

Then, the resulting cultures from the first stage (the formation) were cut into stem nodes and each node was planted in a glass container containing MS nutrient medium containing the same components of the medium in the first stage, with the addition of 2 mg L^{-1} BA + 0.1 mg L^{-1} NAA. The glass containers were incubated in the growth chamber for four weeks under the same previous conditions. This process was repeated several times until the required number of growths needed for the study was reached.

3.6. Experimental Procedure

A laboratory experiment was conducted to study the effect of three concentrations of sodium chloride (50, 100, and 150 mmol L^{-1}) on the growth of plants. The control treatment, which represented the first experiment, included two types of growth regulators (melatonin at a concentration of 1 mg L^{-1} and salicylic acid at a concentration of 10 mg/L), and two types of organic extracts (willow leaf extract and moringa leaf extract at a concentration of 10 ml L^{-1}) In addition to the comparison treatment to know the effect of salt stress on growth indicators and production of active compounds. By cultivating the vegetative branches on MS medium containing sucrose 30 g L^{-1} , myoacetol 100 mg L^{-1} , and 0.1 mg L^{-1} growth regulators 2 mg L^{-1} of BA + 0.1 mg L^{-1} NAA mg L^{-1} with the addition of 7 g L^{-1} of the inert material (agar) in addition to the experimental factors The above, except for the organic extracts, were added after the sterilization of the culture medium in an Autoclave using fine filters inside a laminar air flow cabinet. Then, the medium was distributed into glass containers at a rate of 10 ml per glass container. The plant parts were then cultured on the nutrient medium, each part containing one nodule. One nodule was cultured in each glass container, with 10 replicates per experimental unit. The plants were incubated in a growth chamber at 25 °C with a light source for 16 hours of light followed by 8 hours of darkness for a period of four weeks. Data related to the studied parameters were then collected.

3.7 Indicators studied

3.7.1 Number of branches (branch): The number of branches was calculated for five plants randomly and then averaged.

2.7.3 Branch length (cm): Plant length was measured using a ruler for five plants randomly and unspecified, and the average was calculated.

3.7.3 Number of leaves (leaf/plant): The number of leaves was counted for five plants randomly.

Chlorophyll content of branches (100 g fresh weight): Chlorophyll content was calculated according to the method mentioned in (Goodwin 1976). The amount of chlorophyll present in the plant branches was estimated by taking 0.5 g of the dry plant sample and crushing it in a ceramic mortar with acetone concentration 85% until chlorophyll is separated from the plant tissue, then the solution containing the dye is filtered through filter paper, where the tissue is separated from the dye and the volume is completed to 10 ml with acetone. The chlorophyll content is determined using a spectrophotometer at wavelengths of 645 and 663 nanometers and according to the following equation:

Total chlorophyl =20.2 ×D (645) + 8.02 xD (663) (V \W x1000) x 100

Where D(633): = absorbance reading at wavelength 633 nm.
D(645) = absorbance reading at wavelength 645 nm.
V = final volume of extract.
W = weight of plant tissue.

Estimation of Nutrient Content in Branches

Nutrient content in gardenia branches was determined after drying them in an electric oven at 70 °C until the weight was constant. The sample was placed in tightly sealed plastic containers and stored in a dry place until digestion was carried out. 0.1 g of the plant sample was taken and digested according to the method proposed by Cresser and Parsons (1979) [10]. The elements were determined as follows:

Estimation of proline content in branches: The analysis was conducted at the Ministry of Science and Technology / Department of Environment and Water, where proline was extracted according to the method of the scientist (Lassen-Rasmus Dahl, 2018) [21], where (3 g) of the plant sample was taken and placed in a bottle with a capacity of (25 ml) and (25 ml) of hydrochloric acid (1 M) was added to it at a temperature of 55 C for three hours, after which the sample was dried by Rotary evaporator and add (5 ml) of sodium citrate pH 2.2. Then the sample is filtered using a plastic filter and then taken to the device for injection. Take (1 ml) of the extract and add (200 µl) of
5% orthophthalein aldehyde (OPA) and shake the sample for two minutes after which take 100 µl of the final mixture and inject it into the HPLC device.

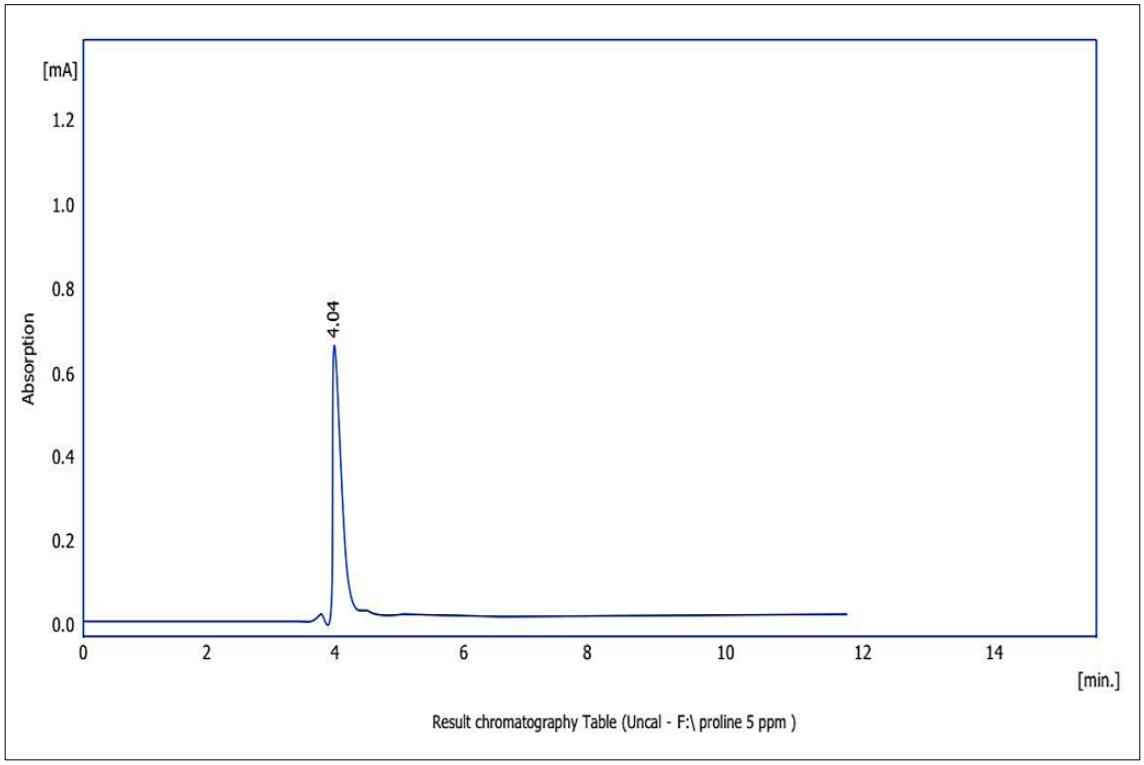

Fig 1: Standard curve of Prolin

Experimental Design and Statistical Analysis

The experiment was implemented as a factorial experiment using a Complete Randomized Design (CRD) with two factors and ten replicates for each experiment. The data were subjected to analysis of variance using the GenStat statistical program, and the means of the coefficients were compared using the least significant difference (LSD) test at a probability level of 0.05 to test for significant differences between the means.

4. Results and Discussion

4.1. Average number of branches (plant branch-1)

The results shown in Table (4-1) showed significant differences in the average number of branches of gardenia plants grown *in vitro*. The treatment with 100 mmol L^{-1} sodium chloride recorded the lowest average, reaching 2.29, which did not significantly differ from the treatment with sodium chloride 150 mmol. L^{-1}, which recorded (2.45), while they differed significantly with the comparison treatment and the treatment of adding 50 mmol. L^{-1}, which gave the highest rate in the number of branches, reaching (3.37 and 3.17), respectively.

Table (4-1) shows a significant effect on the average number of branches growing in media prepared with different concentrations of organic extracts or growth regulators. Salicylic acid 10 mg/ L^{-1}recorded the highest rate of branch numbers, reaching (3.01), compared to the control treatment, which recorded the lowest rate, reaching (2.44). The interaction between sodium chloride salt and the addition of organic extracts or growth regulators had an effect Significant difference in the average number of branches for gardenia plants, as the interaction treatment (0 mmol. L^{-1} sodium chloride and 10 mg. L^{-1} salicylic acid) gave the highest average number of branches, reaching (4.10), compared to the interaction treatment (100 mmol. L^{-1} sodium chloride without adding organic extracts or growth regulators), which gave the lowest average number of branches, reaching (2.10).

Table 4.1: The effect of organic extracts or growth regulators on the rate of number of growing gardenia branches under salt stress conditions outside the living body (plant branch-1)

NaCL (S) (mmol L^{-1})	Organic extracts and growth regulators (T)					effect (S)
	Control 0	Melatonin 1 mg L^{-1}	Moringa 10 ml L^{-1}	Salicylic 10 mg L^{-1}	Willow 10 ml L^{-1}	
0	2.47	3.10	3.47	4.10	3.73	3.37
50	2.73	3.43	3.13	3.40	3.13	3.17
100	2.10	2.13	2.43	2.40	2.37	2.29
150	2.47	2.50	2.40	2.13	2.77	2.45
effect (T)	2.44	2.79	2.86	3.01	3.00	
L.S.D	S : 0.578		T : 0.646		S*T : 1.292	

4.2 Average number of leaves (leaf per plant)

The results shown in Table (4-2) showed significant differences in the average number of leaves of gardenia plants grown *in vitro*. The treatment with 150 mmol L^{-1} sodium chloride recorded the lowest average of 17.00, which did not differ significantly from the treatment with sodium chloride 100 mmol. L^{-1}, which recorded (17.53), while they differed significantly with the comparison treatment and the treatment of adding 50 mmol. L^{-1}, which

gave the highest rate in the number of leaves, reaching (23.60 and 21.40), respectively.

It is noted from Table (4-2) that there is a significant effect on the average number of growing leaves in media equipped with different concentrations of organic extracts or growth regulators, as the 10 mg. L^{-1} Moringa extract recorded the highest average number of leaves, reaching (24.17), compared to the comparison treatment, which recorded the lowest average, reaching (14.92).

The interaction between sodium chloride salt and the addition of organic extracts or growth regulators had a significant effect on the average number of leaves for gardenia plants, as the interaction treatment (0 mmol. L^{-1} sodium chloride and 10 mg. L^{-1} of moringa extract) gave the highest average number of leaves, reaching (32.67) Compared to the interaction treatment (100 mmol L^{-1} sodium chloride without adding organic extracts or growth regulators), which gave the lowest rate in the number of leaves, reaching (11.33).

Table 4.2: The effect of organic extracts or growth regulators on the average number of growing gardenia leaves under salt stress conditions outside the living body (plant leaf-1)

NaCL (S) (mmol L^{-1})	Organic extracts and growth regulators (T)					Effect (S)
	Control 0	Melatonin 1 mg L^{-1}	Moringa 10 ml L^{-1}	Salicylic 10 mg L^{-1}	Willow 10 ml L^{-1}	
0	19.00	21.67	32.67	25.33	19.33	23.60
50	17.00	25.67	28.33	19.33	16.67	21.40
100	11.33	18.00	18.33	20.33	19.67	17.53
150	12.33	17.00	17.33	19.00	19.33	17.00
effect (T)	14.92	20.58	24.17	21.00	18.75	
L.S.D	S : 1.082 T : 1.210 S*T : 2.420					

4.3 Average branch length (cm/plant-1)

The results shown in Table (4-3) showed significant differences in the average branch length of gardenia plants grown *in vitro*. The treatment with 150 mmol/L-1 sodium chloride recorded the lowest average, reaching 6.293, which did not significantly differ from the treatment with sodium chloride 100 mmol.L-1 , which recorded (6.360), while they differed significantly with the comparison treatment and the treatment of adding 50 mmol.L-1 , which gave the highest rate in branch length, reaching (7.613 and 7.227), respectively.

It is noted from Table (4-3) that there is a significant effect on the average length of growing branches in media equipped with different concentrations of organic extracts or growth regulators, as the 10 mg.L-1 Moringa extract recorded the highest average in branch length, reaching (7.267), compared to the comparison treatment, which recorded the lowest average, reaching (6.358).

The interaction between sodium chloride salt and the addition of organic extracts or growth regulators had a significant effect on the average branch length of the gardenia plants, as the interaction treatment (0 mmol.L-1 sodium chloride and 10 mg.L-1 of moringa extract) gave the highest average branch length of (8,000) Compared to the interaction treatment (150 mmol L-1 sodium chloride without adding organic extracts or growth regulators), which gave the lowest rate of branch length, reaching (5.433).

Table 4.3: The effect of organic extracts or growth regulators on the branch length rate in the gardenia plant growing under salt stress conditions outside the living body (plant poison-1)

NaCL (S) (mmol L^{-1})	Organic extracts and growth regulators (T)					Effect (S)
	Control 0	Melatonin 1 mg L^{-1}	Moringa 10ml L^{-1}	Salicylic 10 mg L^{-1}	Willow 10ml L^{-1}	
0	7.167	7.833	8.000	7.167	7.900	7.613
50	7.367	7.600	7.400	7.333	6.433	7.227
100	5.467	5.667	6.833	7.000	6.833	6.360
150	5.433	6.333	6.833	6.500	6.367	6.293
effect (T)	6.358	6.858	7.267	7.000	6.883	
L.S.D	S : 0.2823		T : 0.3156		S*T : 0.6313	

4.4 Chlorophyll content (mg.g-1 fresh weight)

The results shown in Table (4-4) showed significant differences in the chlorophyll content of ex vivo-grown gardenia cultures due to sodium chloride. The treatment with 150 mmol.L^{-1} sodium chloride added recorded the lowest level of (53.2) compared to the control treatment, which produced the highest level of chlorophyll content of (69.7).

It is noted from Table (4-4) that there is a significant effect on the content of chlorophyll branches growing in media equipped with different concentrations of organic extracts or growth regulators, as salicylic acid 10 mg. L^{-1} recorded the highest rate in chlorophyll content, reaching (69.3), compared to the comparison treatment, which recorded the lowest rate, reaching (42.2).

The interaction between sodium chloride salt and the addition of organic extracts or growth regulators had a significant effect on the chlorophyll content of gardenia plants, as the interaction treatment (50 mmol. L^{-1} sodium chloride and 10 mg. L^{-1} of willow extract) gave the highest content rate Chlorophyll content reached (82.2), compared to the interaction treatment (150 mmol L^{-1} sodium chloride without adding organic extracts or growth regulators), which gave the lowest rate of chlorophyll content, reaching (29.4).

Table 4.4: Effect of organic extracts or growth regulators on the chlorophyll content of Cardinia branches growing under salt stress conditions outside the body (mg.g-1 fresh weight)

NaCL (S) (mmol L^{-1})	Organic extracts and growth regulators (T)					effect (S)
	Control 0	Melatonin 1 mg L^{-1}	Moringa 10 ml L^{-1}	Salicylic 10 mg L^{-1}	Willow 10 ml L^{-1}	
0	58.9	69.0	80.8	59.4	80.5	69.7
50	39.8	37.9	59.7	72.3	82.2	58.4
100	40.6	71.8	59.9	72.9	40.3	57.1
150	29.4	69.4	52.5	72.5	42.4	53.2
effect (T)	42.2	62.0	63.2	69.3	61.3	
L.S.D	S : 6.890		T : 7.700		S*T : 15.400	

The results of the indicators shown in Tables (4-1), (4-2), (4-3) and (4-4) indicate a significant decrease in the average number of leaves, number of branches, branch length and total chlorophyll content in the branches of the gardenia plant growing under salt stress conditions outside the living body. This decrease in the number of leaves, number of branches, and length of branches is due to a disruption in the plant's physiological processes and nutritional balance, due to an increase in osmotic pressure and a decrease in the amount of water and nutritional ions entering the cells, as well as ionic toxicity resulting from the accumulation of toxic chloride and sodium ions (Wani and Hossain, 2015) [26]. Also, increasing the concentration of sodium chloride (NaCl) in the nutrient medium slows the growth rate due to inhibiting the action of growth-promoting hormones, especially cytokinins CK and gibberellins (GA), in addition to activating growth-inhibiting hormones such as abscisic acid (ABA) (Taiz and Zeiger, 2006) [24]. The increased release of free radicals (ROS) resulting from salt stress in the nutritional medium causes the breakdown of proteins and cell organelles and the oxidation of lipids in cell membranes, thus declining cell division and reducing photosynthesis rates (Hu and Schmidhalter, 2005) [15]. The decrease in total chlorophyll content in gardenia branches exposed to salt stress is due to an imbalance in the ionic balance within the plant, which negatively affects the absorption of ions that make up chlorophyll, such as nitrogen, magnesium, and iron. These negative effects are also due to the competition of sodium and chlorine ions with the ions that make up the chlorophyll molecule for absorption sites The membranes of the crana on which the pigment is located are distorted or the enzyme activities responsible for building this pigment are inhibited (Mohammed, 2007, Hamidi-Moghaddam *et al*., 2019) [19, 11].

The results shown in the above tables showed a significant role for adding growth regulators or organic extracts in the average number of leaves, number of branches, branch length, and total chlorophyll content of the branches of the gardenia plant growing in the nutrient medium prepared with growth regulators or organic extracts, The reason for the increase in the number of branches may be due to the role of the growth regulator salicylic acid in improving the growth of plants grown under salt stress conditions through its ability to maintain enzymes and the permeability of plasma membranes and increase photosynthetic pigments in plants exposed to salt stress, Or by regulating physiological processes and reducing oxidation of cell membranes, which is reflected in improving the permeability of nutrients and supporting the antioxidant system, such as increasing the effectiveness of the peroxidase enzyme and the content of the amino acid proline, thus improving branch growth indicators. (Hayat *et al*., 2005) [12].

The increased chlorophyll content in the branches of gardenia may be due to the ability of salicylic acid to protect plastids from destruction as a result of increased production of free radicals due to salt stress and increased levels of antioxidant enzymes such as peroxidase and catalase, which work to protect plastids and pigments from decomposition due to environmental stress (Joseph *et al*., 2010) [16], The increase in the average number of branches and the length of the plant growing in media prepared with moringa extract is due to the fact that this extract contains high levels of growth regulators, especially zeatin and gibberellins, as well as high levels of amino acids, vitamins, proteins, and carbohydrates (Xiong *et al*., 2021) [28] Which was effectively reflected in achieving a state of nutritional balance within the plant and thus improving growth, or the increase in growth rates is due to the content of moringa extract of plant hormones, which led to an increase in cell division and elongation and thus an increase in the number and length of branches (Youssef, 2022) [29].

4.5 Percentage of Sodium in the Branches

The results shown in Table (4-5) showed significant differences in the percentage of sodium in the branches of ex vivo-grown gardenia plants, with the control treatment recording the lowest rate of (4.890) compared to the 150 mmol L^{-1} sodium chloride treatment, which recorded the highest percentage of sodium in the branches, (5.6.038).

It is noted from Table (4-5) that there is a significant effect on the percentage of sodium in the growing branches in media equipped with different concentrations of organic extracts or growth regulators, as the 10 mg. L^{-1} Moringa extract recorded the lowest rate in the percentage of sodium in the branches, reaching (5.129), compared to the comparison treatment, which recorded the highest percentage, reaching (5.883).

The interaction between sodium chloride salt and the addition of organic extracts or growth regulators had a significant effect on the percentage of sodium in the branches of gardenia plants, as the interaction treatment (150 mmol. L^{-1} sodium chloride and without the addition of organic extracts or growth regulators) gave the highest rate of sodium percentage, reaching (6.535), compared to the interaction treatment (without the addition of sodium chloride salt) Adding moringa extract (10 mg/ L^{-1}) gave the lowest sodium percentage in the branches, reaching (4.439).

Table 4.5: Effect of organic extracts or growth regulators on the percentage of sodium in gardenia branches growing under salt stress conditions outside the living body (%)

NaCL (S) (mmol L^{-1})	Organic extracts and growth regulators (T)					Effect (S)
	Control 0	Melatonin 1 mg L^{-1}	Moringa 10ml L^{-1}	Salicylic 10 mg L^{-1}	Willow 10 ml L^{-1}	
0	4.980	4.460	4.439	5.317	5.255	4.890
50	5.597	4.937	4.588	5.660	5.145	5.185
100	6.418	5.447	5.685	5.738	5.605	5.779
150	6.535	5.942	5.805	6.02	5.888	6.038
effect (T)	5.883	5.197	5.129	5.684	5.473	
L.S.D	S : 0.2946		T : 0.3293		S*T : 0.6587	

The reason for the increase in the sodium percentage (Table (4-5)) in the growing branches of gardenia under conditions of salt stress may be due to the increase in the concentration of sodium chloride in the nutritional medium, as the sodium ion works to withdraw Ca from the cell membranes and replaces them, causing damage to the cell membranes, Thus, the membranes shift from a state of selective absorption to a state of complete permeability, which leads to the entry of sodium ions in large quantities and their accumulation in the cytoplasm and cell vacuoles, in addition to the state of competition that occurs between sodium and potassium ions over the absorption sites (Tazi and Zeiger, 2006: Al-Hattab, 2018) [24, 3].

4.6. Proline content in branches (mg kg-1)

The results shown in Table (4-6) show significant differences in the proline content of gardenia cultures grown *in vitro*. The control treatment recorded the lowest proline content in branches, reaching 36.71, compared to the 150 mmol L^{-1} treatment, which recorded the highest, reaching 68.09.

It is noted from Table (4-6) that there is a significant effect on the proline content in the growing branches in media equipped with different concentrations of organic extracts or growth regulators, as the comparison treatment recorded the highest rate of proline content, reaching (90.65), compared to the 10 mg. L^{-1} treatment of Moringa extract, which recorded the lowest rate, reaching (38.34).

The interaction between sodium chloride salt and the addition of organic extracts or growth regulators had a significant effect on the proline content rate in gardenia plants, as the interaction treatment (150 mmol. L^{-1} sodium chloride without adding organic extracts or growth regulators) gave the highest proline content rate of (120.61), compared to the interaction treatment (without adding sodium chloride salt with 10 (mg/ L^{-1} of Moringa extract) yielded the lowest proline content (35.06).

Table 4.6: Effect of organic extracts or growth regulators on proline content in Cardinia branches growing under salt stress conditions *in vitro* (mg/kg-1)

NaCL (S) (mmol L^{-1})	Organic extracts and growth regulators (T)					Effect (S)
	Control 0	Melatonin 1 mg L^{-1}	Moringa 10 ml L^{-1}	Salicylic 10 mg L^{-1}	Willow 10 ml L^{-1}	
0	41.68	35.52	35.06	35.98	35.33	36.71
50	89.76	41.00	35.74	41.32	40.52	49.67
100	110.55	41.62	40.74	42.21	40.89	55.20
150	120.61	65.08	41.80	70.38	42.6	68.09
effect (T)	90.65	45.8	38.34	47.47	39.84	
L.S.D	S : 2.059		T : 2.302		S*T : 4.630	

The results also showed an effective role of Moringa leaf extract in the content of the amino acid proline, as this extract works as a natural growth stimulant when added to the nutritional medium, as it works to increase growth rates due to it containing plant hormones and some antioxidants, which work as defensive means when increased Proline levels and reduces the severity of stress, thus reducing proline accumulation and making its level better and improving physiological processes within plant cells (Vazquez *et al*., 2019) [25].

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