Effect of Salt Stress and Nano Silicon Oxide on the Physiological characteristics of *Mandevilla Sanderi(Hemels)*Propagated in vitro culture

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Abstract
The experiments were conducted in the Plant Tissue Agriculture Laboratory of the Department of Horticulture and Landscape Engineering/College of Agriculture - Tikrit University for the period from 10/1/2023 to 3/23/2024. With the aim of propagating the *Mandevilla Sanderi* plant using tissue culture and knowing the extent of the plant's tolerance to salt stress and increasing its resistance to stress. After the success of the sterilization process, the plant parts, the growing shoot tips, were grown on MS prepared BA(0-0.5-1-1.5-2mg/l). After 4 weeks, and after 8 weeks, it was called the multiplication stage. The concentration exceeded 2 mg/l over the rest of the concentrations ,The shoots resulting from the multiplication stage were rooted on half (MS)with the prepared concentration IBA (0-0.5-1-1.5-2mg/l),The media with half the salt was significantly superior to the rest of the concentrations, as the concentration of 1 mg/l gave a rooting rate of 80%. After analyze results using design CRD factorial experiment first Si(0-50-100-200mg/l)and other Nacl(50-100mM) and Sio2 with Nacl in concentrations (50:50), (100:50), (200:50), (50:100), (100:100), and (200:100) respectively. The amino acid proline, and the enzymes CAT, SOD, and Peox were estimated. concentration of 100 mM, gave the highest percentage of proline. It reached 97.00 ppm. When using Sio_2 , a concentration of 200 mg/l , it gave a higher SOD of 12.62 units per minute per mg. When using Sio_2 , a concentration of 100 mg/l , it gave a higher SOD of 17.90 mM per minute per mg. While using Sio_2 concentration of 50 mg/Lgave a higher rate of 3.77 enzyme units/mg for the enzyme Pero.

Key words: Sundafil Red , Physiological , silica , stress salt

Introduction

The Brazilian jasmine plant *Mandevilla sanderi* (Hemels) Evergreen shrubs belong to the family Apocynacea [3] It contains large, brightly colored flowers with five red, pink, or yellow lobes, and sometimes white. "Sundafil Red" variety has a dark red color due to the strong anthocyanin pigment in the epidermis cells [4]It is also known as the scented vine[3] Known as Deplanina [6]Plants of the Apocynaceae family are known for their therapeutic properties including antioxidant, anti-inflammatory,and antimicrobial activities [5][6][7] . Soil salinization represents one of the major environmental constraints on world agriculture [8] The physiological and metabolic activities of plants are damaged under salty conditions due to osmotic stress or nutritional imbalances[9] [10] Osmotic imbalance leads to water deficiency, decreased leaf expansion, and closure of stomata, which causes decreased photosynthesis and growth [11] Ionic stress causes the accumulation of excess Na+ in old leaves, which leads to premature senescence of old leaves[12] [13] [14] .*Sio_2*Treating the Amsonia orientalis plant with sodium chloride

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salt at different concentrations (25-125 mM). The results showed that the lengths of shoots and roots, the number of roots, total protein, chlorophyll a and carotenoid content were negatively affected at concentrations higher than 25 mM. The proline content gradually increased with increasing sodium chloride concentration. The activity levels of CAT, POD, and SOD enzymes were all at their maximum levels in plants grown in plants grown on a nutrient medium containing 50 mM sodium chloride SOD and CAT levels increased at lower concentrations but were limited at higher concentrations of Nacl. The result showed that *A.orientalis* can tolerate Nacl concentrations up to 50 mM[15]. Silicon is the second most common element in soil. The soil generally contains from 50 to 400 kg [16] . Although silicon is abundant in the soil, there is no form available to plants and it is always combined with other elements [16] . Recently, advances have been made in elucidating the dilution effects of Si in salt-induced osmotic stress [17] [18] . and oxidative stress [19][20] . It leads to the accumulation of sodium ions (Na+) and chloride (Cl-) in the cytosol, which harms the cell [21].The use of exogenous Si has proven to be an environmentally friendly approach that maintains optimal K+/Na+ ratio, ionic concentration, nutrient balance, and reactive oxygen species (ROS) production [22] .The rule of use for Si is based on the primary origin of conservative K+, the optimal K+/Na+ ratio, ionic balance, nutrient balance, and production of reactive oxygen species (ROS)[23] Silicone application enhances H+-ATPase activity in the plasma membrane and tonoplast. Enhanced H+-ATPase activity upon Si application facilitates Na+ export out of the cell[]the importance of plant tissue farming technology was demonstrated as an alternative method to traditional methods of propagation and genetic modification of plants, through which it was possible to obtain new varieties and strains that are tolerant or resistant to some environmental stresses and were included in genetic improvement programs[24] .Objective of the study Knowing the effect of sodium chloride and silicon oxide on the physiological characteristics of the *Mandevilla sanderi* plant.

Materials and methods

This experiment was carried out in the plant cell and tissue culture laboratories of the College of Agriculture, Department of Horticulture and Landscape Engineering, Tikrit University for a period from(23/3/2024) to(1/10/2023) for the *Mandevilla sanderi* plant .

Sterilization:A plant sterilization experiment was conducted, represented by the tips of branches from plants growing in a greenhouse. The plant parts are immersed in a solution containing 100 mg/L of ascorbic acid and 150 mg/L of citric acid for a one hour. Then the plant parts are transferred to the cultivation table. The plant parts were treated with 3% sodium hypo chlorate for 30 minutes, after that were washed with distilled and sterile water 3-4 times to remove the harmful effect of the sterile substance.

The stage of establishment and multiplication and rooting : The establishment stage is a first stage of tissue culture that lasts for 4 weeks by using BA (0_0.5_1_1.5_2) mg/L. then plant parts were re-planted (re_culture) for another 8 weeks in the same media "multiplication stage" . The average number of branches (branch of a plant part), average number of leaves leaf (Plant part), average branch length (cm) in this experiment. The percentage, number, and long of roots were studied in rooting stage on MS

medium with growth regulator IBA (0_0.5_1_1.5_2 mg /L) after 4 weeks of planting. The plants from multiplication were grouped for 5 weeks in silicon oxide (200-100-50-0 mg/L)m, NaCl (100-50 mM) and Sio2 with Nacl in concentrations (50:50), (100:50), (200:50), (50:100), (100:100), and (200:100) respectively for stress salt stage study depending estimation of proline ratio, estimation of the enzyme SOD, Catalase and Peroxdase ratio criteria. **Estimation of catalase activity:** 2 grams of fresh leaves were mashed with a potassium phosphate buffer solution (0.1M) pH = 7.8 at a ratio of (1:2 w/v). The extract was filtered using gauze and centrifugation was performed at 10,000g for 30 minutes [24]. The effectiveness of the catalase enzyme was estimated in the leaves. 0.2 ml of the extract was taken and incubated with 1 ml of the mixture containing H2O2 (5mM 6) with phosphate buffer (60mM) pH = 7.4 at 25°C for 4 minutes. Then the enzyme was stopped by adding 1 ml of ammonium molybdate (32.4 mM). Readings are taken to estimate the activity of the enzyme at a wavelength of 405 nm. The activity is estimated according to the following equation :

$$\text{Catalase activity} = \frac{\text{Sample} - \text{Blank 1}}{\text{Blank 2} - \text{Blank 3}} \times 1.171$$

Which :**1-** Blank: It contains 1 ml of the base material (H2O2 with the buffer solution), 1 ml of molybdate, and 0.2 ml of the sample,**2-** Blank: It contains 1 ml of base material (H2O2 with buffer solution), 1 ml of molybdate, and 0.2 ml of buffer solution.

Contains 1 ml of buffer solution, 1 ml of molybdate, and 0.2 ml of buffer solution. **Peroxidase Activity Extraction:** Weigh a certain amount of plant sample and add PBS to it at 4-6°C, the product was filtered and centrifuged at 9000 r/min for 15°/min, and the supernatant was collected as crude enzyme solution. **Measurement of enzyme activity based on colorimetric assay:** Guaiacol was used as the reaction substrate in the colorimetric assay. The reaction system consisted of 2.95 mL of 18 mM guaiacol and 1 mM H2O2 (pH 5 PBS). Add 0.05 ml of enzyme solution, cover the measuring tube with a cap and mix quickly, measure the absorbance value at 470 nm at 30°C, count once every 10 degrees, and use 0.01 change in absorbance value per minute as one unit of enzyme activity. **Estimation of superoxide dismutase activity:** The enzymatic activity of the enzyme was estimated using Pyrogallol as a base material according to the spectrophotometric method. The Spectrophotometer zeroed using an EDTA-Tris pH buffer. The rate of increase in absorbance at a wavelength of 461 nm was estimated at 0.6 at time zero and a minute after adding Pyrogallol. Enzymatic activity was determined according to the method described by [25].

$$\text{SOD activity (Unit / mg)} = \frac{\text{\% inhibition of pyrogallol auto-oxidant}}{\text{\% 50}}$$

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Extraction and determination of the amino acid (proline): The method [26] was used, where the free (unbound) proline was extracted by adding 10 ml of 30% aqueous sulfosalicylic acid to the fresh sample (5.0 g). The sample was ground and then filtered. 2 ml was then taken From the filtrate, 2 ml of ninhydrin reagent solution was added to it, which was prepared by dissolving ninhydrin in glacial acetic acid and phosphoric acid, and then 2 ml of glacial acetic acid was added to the mixture. Then the sample was heated with the detector in a water bath for an hour, and after cooling the sample, 4 ml of toluene was added to it and shaken well. Then the aqueous phase was separated and the upper part (the toluene layer) was used at a wavelength of 520 (O.D.) to measure the UV/Visible optical density.) nanometer using a spectrophotometer (LKB-Biochrom4050), and the proline standard curve was used to estimate the amount of proline in the sample.

Preparation of the standard curve for proline: Different concentrations of the amino acid proline were prepared. So that each tube contains 2 ml as follows: 1.0 mg/ml, 2.0 mg/ml, 3.0 mg/ml, 4.0 mg/ml, 5.0 mg/ml, 6.0 mg/ml. 7.0 mg/ml, 8.0 mg/ml with a control note (distilled water only). The amount of proline was estimated by the previously mentioned method for estimating proline, and the result was represented in Figure (3), which shows the standard curve for proline.

Results and Discussion

The results in Table (1) showed that adding BA at different concentrations to the MS nutrient medium had a significant effect on the cultivation of the growing tip , as the concentration of 2 mg/L gave the highest number of branches, reaching 1,400 branches, plant part^{-1}, which was significantly superior to concentration 0 (control).Which gave the least number of branches, amounting to 1,000 branches, plant part^{-1}**.** The characteristic of plant length was revealed, as the concentration of 1.5 mg/L gave the highest plant length of 5,000 cm, which was significantly superior to the rest of the concentrations, while the unprocessed medium (the comparison treatment) gave the lowest branch length of 2,000 cm. The results in Table(1):Multiplication results after 8 weeks of cultivation showed that adding BA at different concentrations to the MS nutrient medium had a significant effect on cultivation, as the concentration of 2 mg/L gave the highest number of branches, reaching 3,800 branches, plant part^{-1}, which is significantly different from The rest of the concentration, while the unprocessed medium (Control) gave the lowest branch length, reaching 1,000 cm. The plant height characteristic was significant as the medium supplied with BA at a concentration of 1.5 mg/l showed the highest plant height reaching 5,600 cm, which is significantly superior to the rest of the concentrations of 0, 0.5, 1 and 2 mg/l , while the medium not prepared with BA (control) was given. The minimum plant height reached 2,600 cm. The reason for the increase in the number of branches and the number of leaves with the increase in the addition of BA to the medium may be attributed to the role of cytokines in

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their stimulating effect on the speed of cell division as they are unspecialized and undifferentiated cells in an initial developmental stage of cell division[1]And the formation of chlorophyll and preventing the loss of leaves, thus keeping their green leaves as long as possible, breaking the apical increases when using the comparison factor in the establishment stage due to the dominance, and also increasing the number of branches. High percentages of the number of branches were obtained due to achieving a state of balance between internal hormones and growth regulators added to the nutrient medium [27] [28] The characteristic of plant height

internal tissue content of the cultivated plant part of plant hormones[29] .

Table (1) shows the effect of different concentrations of BA on the establishment and multiplication of *Mandevilla sanderi* plant apices resulting from in vitro culture cultivation on MS media after 4 weeks and 8 weeks

Multiplication stage after 8 weeks for cultivation			Establishment stage after 4 weeks for cultivation			BA
plant height/cm	Number of leaf	Number of branch	plant height/cm	Number of leaf	Number of branch	Mg.L^{-1}
2.600 e	3.800 e	1.000 e	2.000 e	2.000 d	1.000 b	**0**
4.500 c	5.400 d	1.400 d	4.000 c	3.000 c	1.400 a	**0.5**
4.800 b	7.800 c	1.600 c	4.2000 b	5.000 b	1.200 ab	**1**
5.600 a	9.800 b	1.800 b	5.000 a	5.400 b	1.400 a	**1.5**
4.000 d	13.200 a	3.800 a	3.500 d	10.000 a	1.400 a	**2**

***Numbers with similar letters are not significantly different from each other according to Duncan's multinomial test at a probability level of 0.05.**

Rooting the shoots on MS medium with half the concentration of salts prepared with

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different concentrations of IBA

The growth regulator indole butyric acid (IBA) is one of the best growth regulators for the purpose of rooting because of its slow decomposition in the plant and its slow transport within the plant, and most of it remains in the treatment area and is safe for the plant[30]. As well as the efficiency of IBA in the rooting process, due to its relatively high stability, it is known that it is not affected by auxin-degrading enzymes[31]. Adding growth regulators leads to an increase in the number of roots and their root lengths, reaching the optimal concentration, and increasing it leads to adverse effects[32] The growth regulator IBA plays a major role in the development of adventitious roots[33] . The results of Table (2) showed the effect of different concentrations of IBA on the rooting of the branches resulting from the tissue culture technique of the *Mandevilla sanderi* plant, by growing them on MS nutrient medium with half the concentration of salts. After four weeks of planting, the different rooting percentages were obtained, as the medium gave half the concentration. Salts prepared with IBA at a concentration of 1 mg/l had the highest percentage of rooting, reaching 80%, which differs significantly from the rest of the concentrations. As for the number of roots, the 1 mg/l treatment excelled by giving it the highest number of roots, amounting to 7,180 roots per plant part^{-1}, which was significantly superior to the rest of the treatments, while the 1.5 and 2 mg/l treatments had the lowest number of roots, amounting to 1,056 roots per plant part^{-1} and 1.186 Root plant part^{-1}. The increase in the percentage of rooting, number of roots, and root length when adding different concentrations of IBA to the MS nutrient medium may be due to the fact that IBA achieves a good hormonal balance for the rooting process[34]

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Table (2) shows the effect of different concentrations of IBA on the rooting of *Mandevilla sanderi* plants resulting from in vitro culture cultivation on MS nutrient medium, half the concentration of solid salts after 4 weeks of tissue culture

root length /cm	Number of roots	Percentage of rooting %	IBA Mg/l
1.740 c	1.740 c	70 %	**0**
2.400 b	2.400 b	70 %	**0.5**
7.080 a	7.180 a	80 %	**1**
0.800 d	1.056 d	30 %	**1.5**
0.7000 e	1.186 d	30 %	**2**

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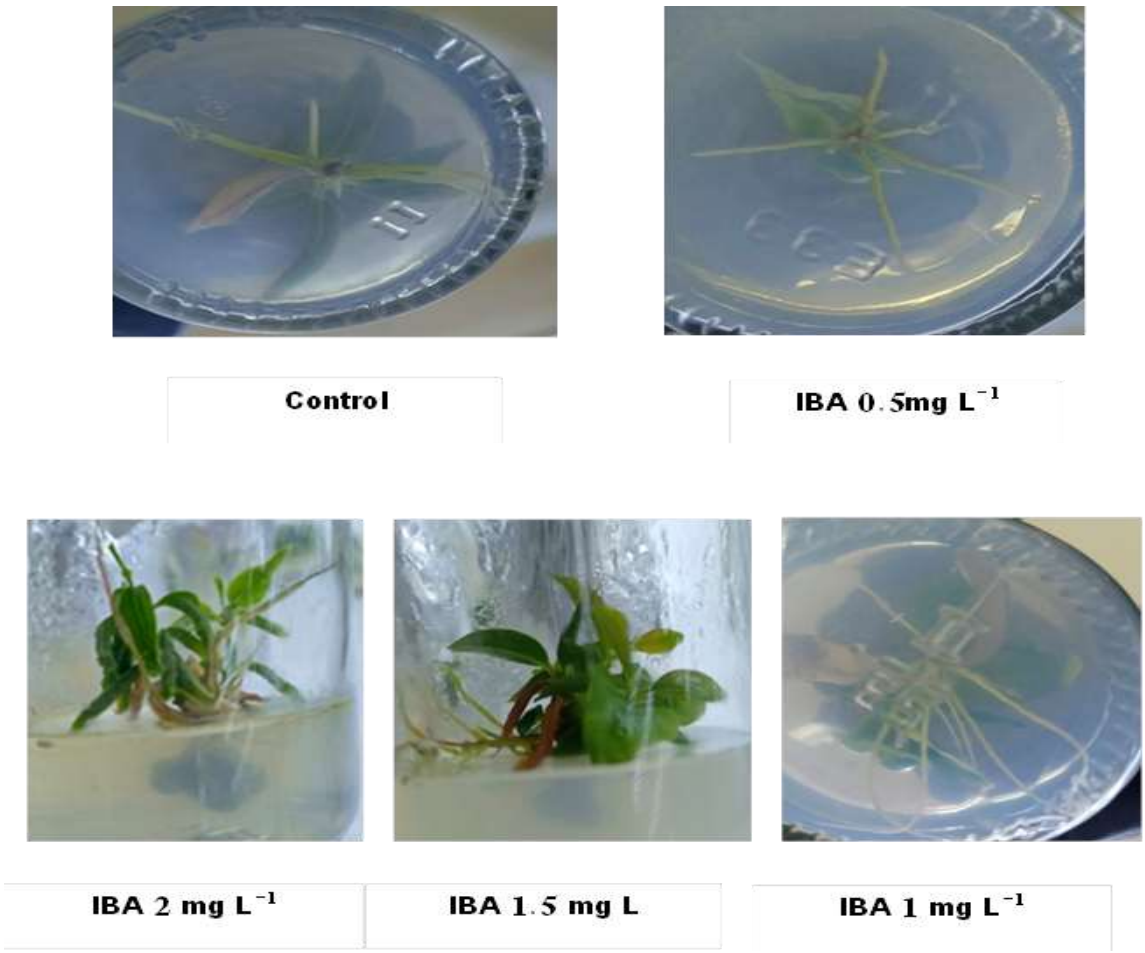

Figure(3) the effect of different concentrations of the IBA on shoots resulting from tissue culture of *Mandevilla sanderi* on half-salt MS media after 4 weeks of cultivation

Effect of different concentrations of Sodium Chloride and Silicon Oxide and their interaction on the Proline content (ppm) of the leaves of the *Mandevilla sanderi* plant

The results of Table(8) The addition of Sio_2 has a significant effect on the proline content of the leaves. The treatment equipped with Sio_2 at a concentration of 200 mg/L was distinguished by giving the highest percentage of proline, reaching 35.00 ppm, which differed significantly from the treatment with 50 mg/l, reaching a percentage of 31.00 ppm. A treatment with a concentration of 100 mg/L gave the lowest rate of 28.00 ppm. While the addition of sodium chloride salt has a significant effect on the potassium content of the leaves, the treatment equipped with sodium chloride at a concentration of 100 mM was distinguished by giving the highest percentage of proline, reaching 97.00 ppm, which differed significantly from the treatment of 50 mM, which gave a percentage of 85.00 ppm. The treatment with concentration 0 gave the lowest percentage of 29.00ppm. As for the interaction, it had a significant effect and was distinguished by the treatment, the interaction of sodium chloride salt at a concentration of 100 mM with silicon oxide nanoparticles at a concentration of 50 mg/l, by giving it the highest percentage of proline, amounting to 71.00 ppm, which was significantly superior to the treatment, the interaction of 50 Mm . Of sodium chloride salt with silicon oxide

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nanoparticles 200 mg/L , giving it the lowest proline percentage of 39.00 ppm. showed that the proline content increases with increasing sodium chloride levels, that is, with increasing salinity, which indicates the occurrence of metabolic transformations of some amino acids into proline[35].If there is an increase in the accumulation of osmotic regulators such as amino acids, including proline, in tissue due to salt stress, it will be one of the mechanisms of resistance to salt stress [2] [36].

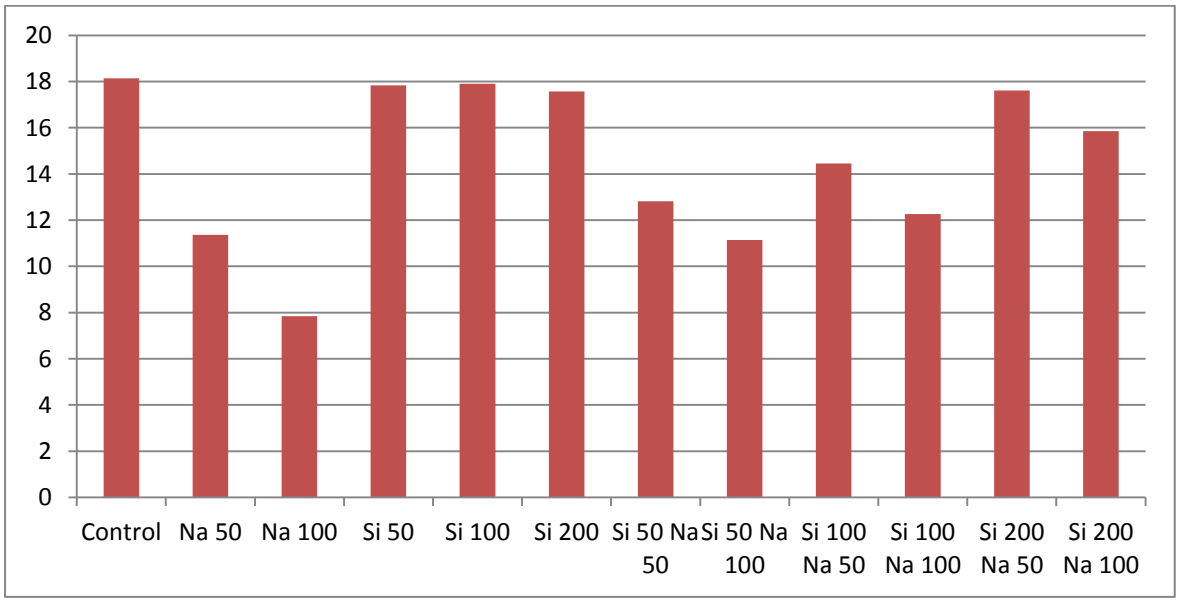
Table(5)shows the effect of different concentrations of sodium chloride and silicon oxide and their interaction on the proline content (ppm) of the leaves of the *Mandevilla sanderi* plant

Nacl mM	Sio_2			Nacl mM
	200	100	50	
29.00 k	35.00 i	28.00 l	31.00 j	**0**
85.00 b	39.00 h	52.00 f	63.00 d	**50**
97.00 a	47.00 g	60.00 e	71.00 c	**100**

***Numbers with similar letters are not significantly different from each other according to Duncan's multinomial test at a probability level of 0.05.**

The effect of different concentrations of silicon oxide and sodium chloride salt and the interaction between them had an effect on the percentage of catalase enzymes.

The results of Figure (1) The control treatment gave the highest percentage of enzymes at 18.14 mM per minute per mg, compared to the Nacl treatment with a concentration of 100 mM , which gave the lowest rate at 7.36 mM per minute per mg.



Figure(1)shows the effect of different concentrations of Nacl and Sio_2 and their interaction on the plant content of the Catalase enzyme of the *Mandevilla sanderi* plant.

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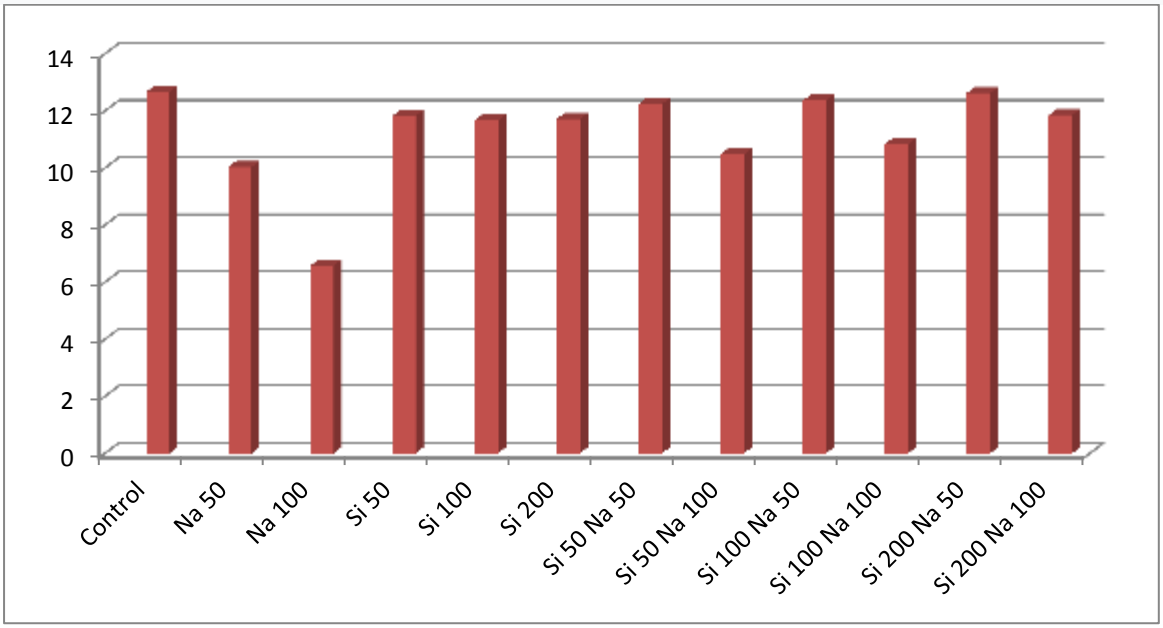
The results of Figure(2)The control treatment gave the highest rate of enzymes at 12.66 units per minute per mg ,compared to the sodium chloride salt treatment with a concentration of 100 mM, which gave the lowest rate at 6.57 units per minute per mg.



Figure(2)shows the effect of different concentrations of Nacl and Sio_2 and their interaction on the plant content of the enzyme Superoxide dismutase of the *Mandevilla sanderi* plant

The results of Figure(3) The comparison treatment gave the highest enzyme percentage, amounting to 3.82 enzyme units/mg, compared to the Nacl treatment, concentration of 100 mM, which gave the lowest enzyme percentage, amounting to 2.23 enzyme units/mg.

This study showed significant changes in the enzymatic antioxidant systems (CAT, SOD and APX) and in the expression of the genes controlling these enzymes under the influence of salt stress, which indicates the important role that these enzymes play in salt stress tolerance in plants37]

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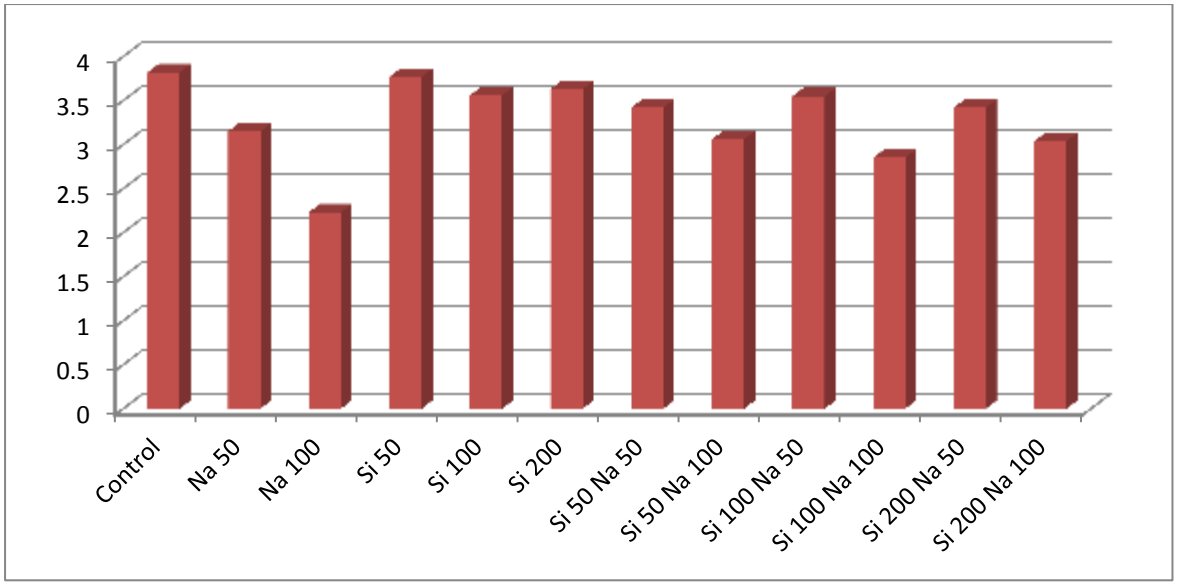

Figure(3)shows the effect of different concentrations of Nacl and Sio_2 and their interaction on the plant content of the Peroxdase enzyme of the *Mandevilla sanderi* plant

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