Effect of Nano-Silicon Oxide Foliar Application on the Vegetative, chemical and Active compounds parameters of Catharanthus roseus L. under salt-stressed

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Abstract
This study was conducted in the wooden lath house of the Department of Horticulture and Landscape Gardening, College of Agriculture, University of Tikrit, during the 2024 agricultural season. The aim was to study the effect of foliar spraying with different concentrations of nano-silicon oxide on improving the response of periwinkle (*Catharanthus roseus* L.) plants to salt stress, and its impact on some physiological, chemical, and anatomical characteristics of the plant. A factorial experiment was implemented according to a Randomized Complete Block Design (R.C.B.D) with three replications. The study included two factors: the first factor involved the addition of three concentrations of sodium chloride (NaCl) salt to the soil (0, 50, 100 mg L^{-1}), and the second factor involved foliar spraying with a nano-silicon oxide solution at three concentrations (0, 50, 100 mg L^{-1}). The results showed the superiority of the control treatment (0 mg L^{-1} NaCl) over the effect of the 100 mg L^{-1} NaCl concentration and the 100 mg L^{-1} nano-silicon oxide concentration in most vegetative growth traits, including plant height, total number of leaves per plant, and leaf silicon content. Meanwhile, the interaction treatment (0 mg L^{-1} NaCl and 100 mg L^{-1} nano-silicon oxide) gave the highest values compared to the interaction treatment (100 mg L^{-1} NaCl and 0 mg L^{-1} nano-silicon oxide) for the same traits mentioned above. On the other hand, salt stress showed the highest rate in the activity of the antioxidant enzymes Peroxidase (unit mg^{-1} protein) and Catalase (mmol min^{-1} mg^{-1} protein) at the concentration of 100 mg L^{-1}, reaching 11.84 and 10.4, respectively. Meanwhile, the highest values were recorded in the interaction treatments, reaching 15.08 and 13.71, respectively, compared to the control treatment, which gave the lowest values of 4.25 and 3.28, respectively. The study concludes that the periwinkle plant is sensitive to salt stress, which causes deterioration in its growth and anatomical traits. The use of nanotechnology (silicon oxide) is an effective and environmentally safe means of mitigating the damages of salt stress, thereby enhancing plant growth, its chemical content, and anatomical structure under stress conditions.

Key words: Nano-Silicon Oxide, *Catharanthus roseus* L, Salt Stress, HPLC

ISSN 2072-3857

Introduction

The periwinkle plant is a member of the Apocynaceae family, scientifically known as *Catharanthus roseus* L. Its native origin is the African continent, specifically the island of Madagascar (1). This plant is an ancient ornamental species cultivated for centuries in tropical, subtropical, and relatively warm temperate regions، It was introduced to European countries at the beginning of the 18th century and quickly spread to be used as an ornamental plant in landscape gardening due to the beauty of its flowers, which vary in color between white and pink (2). Nano-silicon oxide is one of the most important nanomaterials used in agricultural, industrial, and environmental applications due to its unique properties resulting from its small size and high surface area. This compound consists of nano-silicon oxide particles, typically ranging between 10–100 nanometers in diameter, giving it distinctive physical and chemical behavior compared to the conventional form of silica (3). Salt stress is one of the most significant abiotic factors negatively affecting plant growth and productivity. It occurs due to the accumulation of soluble salts in the soil or irrigation water at levels exceeding the plant's tolerance capacity. Salt stress causes a series of physiological, biochemical, and morphological effects on plants, leading to disrupted growth and reduced productivity (4). The study aimed to determine the tolerance level of the periwinkle plant to different concentrations of sodium chloride and its effect on the plant's physiological and chemical characteristics. It also sought to understand the response of the studied plant to foliar spraying with nano-silicon oxide and its impact on the plant's physiological and chemical characteristics, and to elucidate the effect of the interaction between foliar spraying with nano-silicon oxide and the addition of sodium chloride on the physiological and chemical characteristics of the plant.

Cultivation and Maintenance Operations

1. Seed Planting

Seeds of the periwinkle plant (*Catharanthus roseus*), variety Pacifica X P Mix, provided by the Pan American Seed company, were planted on April 16, 2024. The seeds were sown directly into 20-liter plastic pots filled with a potting soil mixture, with a layer of small gravel placed inside each pot. This layer was added to achieve a weight of 2 kg per pot (including the gravel), which helped effectively drain excess water. The pots were arranged inside the wooden lath house of the Department of Horticulture and Landscape Gardening, College of Agriculture, University of Tikrit, as .The total number of experimental units was 24.

2. Stages of Irrigating Experimental Units with the Study Solutions

The experiment involved two factors:

First Factor: This factor involved the addition of a sodium chloride (NaCl) solution (Factor A) at concentrations of (0, 50, 100) mg L^{-1}. The sodium chloride solution was added to the soil according to the treatments after being dissolved in distilled water. Second Factor: This involved foliar spraying with a nano-silicon oxide solution at three levels (0, 50, 100) mg L^{-1}.

The nano-silicon oxide solution was sprayed on the vegetative parts of the plants according to the treatments after being dissolved in distilled water.

3. Experimental Design

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The experiment was designed according to a Randomized Complete Block Design (RCBD) in a factorial experiment system with two factors

4. Studied Traits

Plant height was measured at the end of the experiment using a graduated ruler by measuring the distance from the soil surface

4.1.2 Number of Branches (branch plant^{-1})

The number of branches formed on a single plant was counted manually at the end of the experiment, and the arithmetic mean for each treatment was recorded (6).

4.1.3 Total Number of Leaves (leaf plant^{-1})

The activity of the Peroxidase enzyme was estimated using a spectrophotometric method based on the oxidation of the

4.2.2 Estimation of Catalase Enzyme Activity (Catalase) (mmol min^{-1} mg^{-1} protein)

The activity of the Catalase enzyme was estimated by measuring the rate of hydrogen

4.2.3 Estimation of Proline Content (µg g^{-1} fresh weight)

According to the method proposed by (10).

4.3 Chemical Traits

4.3.1 Estimation of Sodium Element (mg g^{-1})

Sodium was estimated using a flame photometer according to standard methods (11).

4.3.2 Estimation of Chlorine Element (mg g^{-1})

The alkaloids were estimated using The conditions of the HPLC device, the concentration calculation equation, the measurement process, sample preparation, and measurement information such as beam

Results and Discussion

4.1 Vegetative Growth Traits

At the end of the experiment on December 1, 2024, the following traits were studied for all plants in each experimental unit:

4.1.1 Plant Height (cm)

to the growing tip. The arithmetic mean of several representative plants per treatment was recorded (5).

All leaves formed on a single plant were counted, excluding fallen leaves. The arithmetic mean of plants within each experimental unit was recorded (7).

4.2 Physiological Traits of Periwinkle Plant

4.2.1 Estimation of Peroxidase Enzyme Activity (Peroxidase) (unit mg^{-1} protein)

substrate and measuring the change in absorbance (8).

peroxide decomposition using a spectrophotometer (9).

Chlorine was estimated using the silver nitrate titration method or other approved standard methods (12)

4.3.3 Estimation of Silicon Element (mg g^{-1})

Silicon was estimated after alkaline digestion and measured using an appropriate spectrophotometric method (13)

4.4 Estimation of Active Compounds

4.4.1 Catharanthine, Vindoline, Vinblastine, Vincristine

area and sample concentration, etc., must be stated. HPLC technique after extraction with a suitable solvent and comparison with standard solutions (14).

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4.1. Effect of Sodium Chloride Addition, Foliar Spraying with Nano-Silicon Oxide, and Their Interaction on Vegetative Growth Traits of Periwinkle Plant

4.1.1. Plant Height (cm)

The results in Table (1) show that plant height was affected by different concentrations of sodium chloride. The highest value was observed at the 0 mg L^{-1} concentration, with an average height of 63.761 cm, compared to the lowest value recorded at the 100 mg L^{-1} concentration, with a height of 43.233 cm. Regarding the effect of nano-silicon oxide, its concentrations had a clear impact on plant height, as height values increased with increasing concentration. The highest value was recorded at the 100 mg L^{-1} concentration, with an average of 59.383 cm, while the lowest value was at the 0 mg L^{-1} concentration, with an average of 47.556 cm. The interaction between the two factors, sodium chloride and nano-silicon oxide, showed a significant effect. Height values varied markedly according to the dual treatments. The highest plant height appeared in the interaction treatment of sodium chloride at 0 mg L^{-1} with nano-silicon oxide at 100 mg L^{-1}, where the height reached 74.45 cm, compared to the lowest height, which appeared in the interaction treatment of sodium chloride at 100 mg L^{-1} with nano-silicon oxide at 0 mg L^{-1}, with a height of 37.167 cm.

Table (1) Effect of Sodium Chloride Addition, Foliar Spraying with Nano-Silicon Oxide, and Their Interaction on Plant Height (cm) of Periwinkle.

Sodium Chloride (mg L^{-1})	Nano-Silicon Oxide (mg L^{-1})			Mean Effect of Sodium Chloride
	0	50	100	
0	55.66 c	61.16 b	74.45 a	63.76 a
50	49.83 d	51.16 cd	55.66 c	52.22 b
100	37.167 f	44.5 e	48.03 d e	43.23 c
	47.55 c	52.27 b	59.38 a	

4.1.2. Number of Branches per Plant (branch plant^{-1}):

The data presented in Table (2) show that sodium chloride had a clear effect on the number of branches per plant. The concentration of 0 mg L^{-1} recorded the highest number of branches, with an average of 6.78 branches, while the concentration of 100 mg L^{-1} recorded the lowest number of branches, at 3.33 branches plant^{-1}. Meanwhile, the results of foliar spraying with nano-silicon oxide showed a gradual increase in the number of branches with increasing concentration. The highest number of branches was 7.55 branches at the concentration of 100 mg L^{-1}, while the concentration of 0 mg L^{-1} recorded the lowest average number of branches, at 3.22 branches plant^{-1}. Furthermore, the interaction treatments showed a highly significant effect; the values for the number of branches varied depending on the combination of the two factor levels. The highest number of branches appeared in the interaction treatment between sodium chloride at 0 mg L^{-1} and nano-silicon oxide

ISSN 2072-3857

at 100 mg L^{-1}, reaching 11.33 branches $plant^{-1}$. In contrast, the lowest number of branches was recorded in the treatment of sodium chloride at 100 mg L^{-1} and nano-silicon oxide at 0 mg L^{-1}, reaching 2.66 branches $plant^{-1}$.

Table (2) Effect of Sodium Chloride Addition, Foliar Spraying with Nano-Silicon Oxide Solution, and Their Interaction on the Number of Branches in Periwinkle Plants (branch $plant^{-1}$).

Sodium Chloride (mg L^{-1})	Nano-Silicon Oxide (mg L^{-1})			Mean Effect of Sodium Chloride
	0	50	100	
0	3.66 d	5.33 c	11.33 a	6.77 a
50	3.33 d	5.66 c	7.66 b	5.55 b
100	2.66 d	3.66 d	3.66 d	3.33 c
	3.22 c	4.88 b	7.55 a	

4.1.3. **Total Number of Leaves per Plant (leaf $plant^{-1}$):**

The results in Table (3) The values varied according to the increase in sodium chloride concentration. The highest value for the number of leaves appeared at the 0 mg L^{-1} concentration, with an average of 91.222 leaves $plant^{-1}$. The lowest value appeared at the 100 mg L^{-1} concentration, with an average of 50.66 leaves $plant^{-1}$.

Meanwhile, the results for nano-silicon oxide showed a gradual increase in the number of leaves with an increase in its concentration. The highest average number of leaves was recorded at the 100 mg L^{-1} concentration, with an average of 97.667 leaves $plant^{-1}$. The lowest average was at the 0 mg L^{-1} concentration, with an average of 53 leaves $plant^{-1}$. The concentration of 50 mg L^{-1} gave an intermediate value of 76.444 leaves $plant^{-1}$. As for the interaction treatments between sodium chloride and nano-silicon oxide, a clear difference in the number of leaves was observed. The highest value was recorded in the interaction treatment of sodium chloride at 0 mg L^{-1} and nano-silicon oxide at 100 mg L^{-1}, reaching 140.333 leaves $plant^{-1}$. In contrast, the lowest value appeared in the treatment of sodium chloride at 100 mg L^{-1} and nano-silicon oxide at 0 mg L^{-1}, which reached 38.33 leaves $plant^{-1}$.

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Table (3) Effect of Sodium Chloride Addition, Foliar Spraying with Nano-Silicon Oxide Solution, and Their Interaction on the Total Number of Leaves in Periwinkle Plants (leaf plant^{-1}).

Sodium Chloride (mg L^{-1})	Nano-Silicon Oxide (mg L^{-1})			Mean Effect of Sodium Chloride
	0	50	100	
0	60.33 de	73 c	140.33 a	91.222 a
50	60.33 de	94 b	101.33 b	85.22 b
100	38.33 f	62.33 d	51.33 e	50.66 c
	53 c	76.44 b	97.67 a	

4.2.1. **Peroxidase Enzyme Activity (unit mg^{-1} protein).**

The results indicate that increasing the concentration of sodium chloride led to a significant increase in peroxidase enzyme activity. The highest mean activity was recorded at the 100 mg L^{-1} concentration of sodium chloride, reaching 11.84 units mg^{-1}, compared to the lowest value recorded at the control treatment (0 mg L^{-1}), which was 6.56 units mg^{-1}. This reflects the role of moderate salinity in stimulating the plant's defense system by increasing the activity of antioxidant enzymes, including peroxidase.

Regarding the effect of nano-silicon oxide, the results showed that the 100 mg L^{-1} concentration significantly outperformed in increasing enzyme efficacy, with mean activity reaching 11.60 units mg^{-1}, compared to the 50 and 0 mg L^{-1} concentrations, which recorded lower values of 8.49 and 6.52 units mg^{-1}, respectively.

Concerning the interaction of the two study factors, the interaction treatment between 100 mg L^{-1} sodium chloride and 100 mg L^{-1} nano-silicon oxide recorded the highest value for peroxidase enzyme activity, reaching 15.08 units mg^{-1} plant, significantly surpassing the other interaction treatments. In contrast, the lowest value (4.25 units mg^{-1}) was recorded at the control treatment.

Table (4) Effect of Sodium Chloride Addition, Foliar Spraying with Nano-Silicon Oxide Solution, and Their Interaction on Peroxidase Enzyme Activity.

Sodium Chloride (mg L^{-1})	Nano-Silicon Oxide (mg L^{-1})			Mean Effect of Sodium Chloride
	0	50	100	
0	4.25 i	6.24 h	9.19 d	6.56 c
50	7.23 f	6.87 g	10.53 c	8.21 b
100	8.09 e	12.36 b	15.08 a	11.84 a
	6.52 c	8.49 b	11.60 a	

4.2.2. **Catalase Enzyme Activity (mmol min^{-1} mg^{-1} protein).**

The results indicate that increasing the sodium chloride concentration led to a noticeable rise in catalase enzyme activity. The 100 mg L^{-1} concentration recorded the highest activity rate at 10.40 mmol min^{-1} mg^{-1}, compared to the 0 and 50 mg L^{-1}

ISSN 2072-3857

concentrations, which recorded 4.97 and 6.81 mmol min^{-1} mg^{-1}, respectively. Regarding nano-silicon oxide, the results showed that its addition led to a gradual increase in enzyme activity with increasing concentration. The 100 mg L^{-1} concentration achieved the highest value at 9.22 mmol min^{-1} mg^{-1}, followed by the 50 mg L^{-1} concentration at 7.53, and the control treatment at 5.43 mmol min^{-1} mg^{-1}. On the other hand, the interactive effect between sodium chloride and nano-silicon showed a clear and significant superiority. The treatment combining the highest concentration of sodium chloride (100 mg L^{-1}) and the highest concentration of nano-silicon oxide (100 mg L^{-1}) recorded the highest value for catalase enzyme activity at 13.71 mmol min^{-1} mg^{-1}, significantly outperforming the other treatments.

Table (5) Effect of Sodium Chloride Addition, Foliar Spraying with Nano-Silicon Oxide Solution, and Their Interaction on Catalase Enzyme Activity (mmol min^{-1} mg^{-1}).

Sodium Chloride (mg L^{-1})	**Nano-Silicon Oxide (mg L^{-1})**			**Mean Effect of Sodium Chloride**
	0	**50**	**100**	
0	3.28 h	5.37 g	6.27 e	4.97 c
50	5.656 f	7.116d	7.67 c	6.81 b
100	7.36 d	10.12 b	13.71 a	10.40 a
	5.43 c	7.53 b	9.22a	

4.3.1. **Sodium Content (mg g^{-1}).**

The results in Table (6) show that the addition of sodium chloride and the nano-silicon solution had a significant effect on the sodium concentration in the plant. Looking at the effect of sodium chloride, it is clear that the sodium concentration gradually increased with rising salinity levels. It was 0.17 mg g^{-1} at a concentration of (0 mg L^{-1}), then increased to 1.63 mg g^{-1} with the addition of 50 mg L^{-1}, and further rose to 1.88 mg g^{-1} at a concentration of 100 mg L^{-1}. Regarding the effect of nano-silicon, it can be observed that the sodium concentration gradually decreased in treatments where nano-silicon was used (50 and 100 mg L^{-1}) compared to treatments without silicon. The concentration was 1.31 mg g^{-1} in the control treatment, decreased to 1.27 mg g^{-1} at 50 mg L^{-1}, and reached 1.10 mg g^{-1} at the highest salt concentration of 100 mg L^{-1}. As for the interaction effect between nano-silicon and sodium chloride, the results showed that the best treatment for reducing sodium accumulation within the plant was at the interaction between the 100 mg L^{-1} salt concentration and 0 mg L^{-1} nano-silicon, where the concentration reached 1.97 mg g^{-1}, which is higher than the other salt treatments. This indicates a protective interactive role of silicon under moderate salt stress. The highest sodium concentration was in the treatment of 0 mg L^{-1} salt with the addition of 100 mg L^{-1} silicon, reaching 0.12 mg g^{-1}.

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Table (6) Effect of Sodium Chloride Addition, Foliar Spraying with Nano-Silicon Oxide Solution, and Their Interaction on Sodium Content (mg g^{-1}).

Sodium Chloride (mg L^{-1})	Nano-Silicon Oxide (mg L^{-1})			Mean Effectof Sodium Chloride
	0	50	100	
0	0.25 d	0.13 e	0.12 e	0.17 c
50	1.72 b	1.74 b	1.45 c	1.63 b
100	1.97 a	1.95 a	1.72 b	1.88 a
	1.31 a	1.27 a	1.10 b	

4.3.2. **Chlorine Content (mg g^{-1}).**

Table (7) shows that the addition of the sodium chloride solution and the nano-silicon solution led to significant differences in the chlorine rate, with statistical differences appearing at a significant probability level. Regarding the effect of sodium chloride, the concentration of 100 mg L^{-1} achieved the highest value for the chlorine rate, reaching 143.00 mg g^{-1}, a value significantly higher than the other treatments. This indicates that increasing the concentration of sodium chloride raises the alkalinity of the solution. In contrast, the concentration of 0 mg L^{-1} recorded the lowest value of 112.55 mg g^{-1}, indicating that the absence of sodium chloride clearly reduces acidity compared to other treatments. Concerning the effect of nano-silicon, the treatment with 0 mg L^{-1} recorded the highest value (0.45 mg g^{-1}), then decreased to 0.37 mg g^{-1} at a concentration of 50 mg L^{-1}, while the lowest value was at a concentration of 100 mg L^{-1}, around (0.29 mg g^{-1}). This points to the role of silicon in enhancing the alkalinity of the medium. As for the interactive effect between sodium chloride and nano-silicon, the interaction between the two factors showed significant differences. The highest value was in the combined treatment (sodium chloride 100 mg L^{-1} + nano-silicon 100 mg L^{-1}) with an average of 57.67 mg g^{-1}, while the lowest value was recorded in the treatment (0 mg L^{-1} sodium chloride + 100 mg L^{-1} silicon) with an average of 0.13 mg g^{-1}.

Table (7) Effect of Sodium Chloride Addition, Foliar Spraying with Nano-Silicon Oxide Solution, and Their Interaction on Chlorine Content (mg g^{-1}).

Sodium Chloride (mg L^{-1})	Nano-Silicon Oxide (mg L^{-1})			Mean Effect of Sodium Chloride
	0	50	100	
0	0.34 d	0.33 d	0.13 f	0.27 ab
50	0.44 c	0.27e	0.28 e	0.33 ab
100	0.57 a	0.50 b	0.47b c	0.27 b
	0.45 a	0.37 b	0.29 c	

4.3.3. **Silicon Content (mg g^{-1}).**

The table illustrates the effect of salinity on silicon concentration. Exposing plants to salinity (50 and 100 mg L^{-1}) led to a decrease in internal silicon concentration,

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reaching 17.6 mg g^{-1} at 50 mg L^{-1} and 15.91 mg g^{-1} at 100 mg L^{-1}. The effect of nano-silicon is clear from the salt-free treatment (0 mg L^{-1}); increasing the nano-silicon concentration from (0 to 100 mg L^{-1}) raised the plant silicon concentration from 4.82 mg g^{-1} (without silicon) to 29.76 mg g^{-1} at the highest concentration (100 mg L^{-1}). The interaction effect between silicon and salinity, as shown in the same table, indicated that the addition of nano-silicon clearly mitigated the effect of salinity. The silicon concentration increased in the salinity + silicon treatments, especially at 100 mg L^{-1} salt and 100 mg L^{-1} silicon, where the concentration reached 31.7667 mg g^{-1}, the highest among all treatments. It also increased at 50 mg L^{-1} salt and 100 mg L^{-1} silicon to 30.43 mg g^{-1}.

Table (8) Effect of Sodium Chloride Addition, Foliar Spraying with Nano-Silicon Oxide Solution, and Their Interaction on Silicon Content (mg g^{-1}).

Sodium Chloride (mg L^{-1})	Nano-Silicon Oxide (mg L^{-1})			Mean Effect of Sodium Chloride
	0	50	100	
0	4.90 g	15.73 f	27.10 c	15.91 a
50	4.56 g	17.80 d	30.43 b	17.60 b
100	5.00g	19.13 d	31.76 a	15.91 c
	4.82 c	17.55 b	29.76 a	

4.2.3. **Proline Content (µg g^{-1} fresh weight).**

The results indicate that increasing the sodium chloride concentration led to a significant rise in proline content. The highest rate of salinity effect was recorded at the 100 mg L^{-1} concentration (0.56 µg g^{-1} fresh weight), compared to the lowest value when no sodium chloride was added (0.30 µg g^{-1} fresh weight). Regarding the effect of nano-silicon oxide, the results showed that the control (0 mg L^{-1}) achieved the highest overall mean for proline content (0.44 µg g^{-1} fresh weight). Meanwhile, the values decreased with increasing silicon concentration to 50 and 100 mg L^{-1}, reaching 0.38 and 0.39 µg g^{-1} fresh weight, respectively. When studying the interaction effect between the two factors, the highest proline content (0.60 µg g^{-1} fresh weight) was recorded in the treatment of 100 mg L^{-1} sodium chloride without the addition of nano-silicon, and this treatment was significantly superior to the other treatments.

Table (8) Effect of Sodium Chloride Addition, Foliar Spraying with Nano-Silicon Oxide Solution, and Their Interaction on Proline Content (µg g^{-1} fresh weight).

Sodium Chloride (mg L^{-1})	Nano-Silicon Oxide (mg L^{-1})			Mean Effect of Sodium Chloride
	0	50	100	
0	0.33 d	0.31 d	0.26 e	0.30 c
50	0.40 c	0.33 d	0.35 d	0.36 b
100	0.60 a	0.52 b	0.57 a	0.56 a
	0.44 a	0.38 b	0.39 b	

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4.4. **Effect of Sodium Chloride Addition and Foliar Spraying with Nano-Silicon Oxide on Active Compounds**.

4.4.1. Catharanthine Alkaloid Content (µg g⁻ ¹ dry weight)

The results indicate that increasing the sodium chloride concentration led to a gradual rise in Catharanthine content. The highest rate of salinity effect was recorded at the 100 mg L⁻ ¹ concentration (70.21 µg g⁻ ¹ dry weight), compared to the lowest value when no sodium chloride was added (24.40 µg g⁻ ¹ dry weight). Regarding the effect of nano-silicon oxide, the results showed that spraying with silicon led to a clear improvement in Catharanthine content compared to no addition. The 100 mg L⁻ ¹ concentration achieved the highest overall mean (51.88 µg g⁻ ¹ dry weight), followed by the 50 mg L⁻ ¹ concentration (51.73 µg g⁻ ¹ dry weight), while the value decreased in the control treatment (38.73 µg g⁻ ¹ dry weight). The highest Catharanthine content (81.39 µg g⁻ ¹ dry weight) was recorded in the treatment of 100 mg L⁻ ¹ sodium chloride combined with 100 mg L⁻ ¹ nano-silicon oxide. This indicates the presence of a synergistic effect between salinity and nano-silicon in stimulating the production of this alkaloid. In contrast, the lowest value (16.72 µg g⁻ ¹ dry weight) was recorded in the treatment with no sodium chloride addition and no silicon spraying.

Table (9) Effect of Sodium Chloride Addition, Foliar Spraying with Nano-Silicon Oxide, and Their Interaction on the Leaf Content of Periwinkle Plants of the Catharanthine Alkaloid (µg g⁻ ¹ dry weight).

Sodium Chloride (mg L⁻ ¹)	Nano-Silicon Oxide (mg L⁻ ¹)			Mean Effect of Sodium Chloride
	0	50	100	
0	16.72	27.97	28.50	24.40
50	43.42	54.06	45.76	47.75
100	56.06	73.18	81.39	70.21
	38.73	51.73	51.88	

4.4.2. Vindoline Alkaloid Content (µg g⁻ ¹ dry weight)

The results showed that increasing the sodium chloride concentration from (0 to 100 mg L⁻ ¹) led to a gradual increase in Vindoline content. The highest value was at the 100 mg L⁻ ¹ concentration, reaching 93.50 µg g⁻ ¹ dry weight, compared to the lowest Vindoline ratio recorded in the control treatment, which was 47.69 µg g⁻ ¹ dry weight. Regarding the effect of nano-silicon oxide, spraying with 100 mg L⁻ ¹ nano-silicon contributed to raising Vindoline content more compared to no addition. The highest values were 77.92 µg g⁻ ¹ dry weight, while the control treatment recorded the lowest Vindoline value at 64.08 µg g⁻ ¹ dry weight. Concerning the interaction between silicon and sodium chloride, the results show that the interaction effect was most evident at the highest concentrations of the two treatments. The combination of 100 mg L⁻ ¹ NaCl and 100 mg L⁻ ¹ silicon resulted in the highest Vindoline value in the

ISSN 2072-3857

table (95.30 µg g^{-1} dry weight). This confirms that the interaction between salt stress and nano-silicon is the most influential factor in enhancing Vindoline accumulation, compared to the lowest values of 47.0 – 47.4 µg g^{-1} dry weight in treatments not exposed to any salt stress or silicon addition.

Table (10) Effect of Sodium Chloride Addition, Foliar Spraying with Nano-Silicon Oxide, and Their Interaction on the Leaf Content of Periwinkle Plants of the Vindoline Alkaloid (µg g^{-1} dry weight).

Sodium Chloride (mg L^{-1})	**Nano-Silicon Oxide (mg L^{-1})**			**Mean Effect of Sodium Chloride**
	0	**50**	**100**	
0	48.8	47.0	47.4	47.69
50	59.8	64.0	81.4	68.42
100	83.7	91.9	104.9	93.50
	64.08	67.61	77.92	

4.4.3. Vinblastine Alkaloid Content (µg g^{-1} dry weight)

The table shows that increasing the sodium chloride concentration (from 0 to 50 and then 100 mg L^{-1}) led to an increase in Vinblastine content in the leaves. The highest concentration value was recorded at 100 mg L^{-1} (70.21 µg g^{-1} dry weight) compared to the lowest value of 24.40 µg g^{-1} dry weight per plant at the 0 mg L^{-1} concentration. The data in the table indicate that foliar spraying with nano-silicon at concentrations (0, 50, 100 mg L^{-1}) generally led to a decrease in Vinblastine alkaloid concentration. The control treatment recorded 51.88 µg g^{-1} dry weight, which decreased to 38.73 µg g^{-1} dry weight at the 50 mg L^{-1} concentration, and then further decreased to 38.73 µg g^{-1} dry weight at the 100 mg L^{-1} concentration. Meanwhile, the interactive effect between sodium chloride and nano-silicon reveals that the interaction between nano-silicon and salinity was the most effective. The highest interaction value reached 81.39 µg g^{-1} dry weight in the treatment of 100 mg L^{-1} sodium chloride and 50 mg L^{-1} nano-silicon oxide, compared to the lowest value recorded in the control treatment, which was 24.40 µg g^{-1} dry weight.

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Table (11) Effect of Sodium Chloride Addition, Foliar Spraying with Nano-Silicon Oxide, and Their Interaction on the Leaf Content of Periwinkle Plants of the Vinblastine Alkaloid (µg g⁻ ¹ dry weight).

Sodium Chloride (mg L⁻ ¹)	Nano-Silicon Oxide (mg L⁻ ¹)			Mean Effect of Sodium Chloride
	0	50	100	
0	16.72	27.97	28.50	24.40
50	43.42	54.06	45.76	47.75
100	56.06	73.18	81.39	70.21
	38.73	51.73	51.88	

4.4.4. Vincristine Alkaloid Content (µg g⁻ ¹ dry weight)

The results show that both factors had a significant effect in stimulating the production of the alkaloid compared to the control treatment. It is evident from the results that increasing the sodium chloride concentration from 0 to 100 mg L⁻ ¹ led to a gradual and clear rise in Vincristine content. At the 0 mg L⁻ ¹ concentration, plants recorded the lowest value (25.36 µg g⁻ ¹ dry weight), while the highest value was at 100 mg L⁻ ¹ (65.07 µg g⁻ ¹ dry weight). Regarding the individual effect of nano-silicon oxide, it is clear from the values that spraying with nano-silicon, especially at the 100 mg L⁻ ¹ concentration, achieved a clear increase in Vincristine concentration compared to no addition. The content increased from 25.36 µg g⁻ ¹ dry weight (without silicon) to 30.60 µg g⁻ ¹ dry weight (at 100 mg L⁻ ¹ nano-silicon oxide) under the same salinity conditions. Regarding the interactive effect between salt and silicon, the results reveal that the interaction between nano-silicon and salinity was the most effective, yielding the highest Vincristine content. The highest value, 87.08 µg g⁻ ¹ dry weight, was observed in the treatment with 100 mg L⁻ ¹ NaCl + 100 mg L⁻ ¹ nano-silicon oxide, compared to the lowest value, 25.36 µg g⁻ ¹ dry weight, in the control treatment.

Table (12) Effect of Sodium Chloride Addition, Foliar Spraying with Nano-Silicon Oxide, and Their Interaction on the Leaf Content of Periwinkle Plants of the Vincristine Alkaloid (µg g⁻ ¹ dry weight).

Sodium Chloride (mg L⁻ ¹)	Nano-Silicon Oxide (mg L⁻ ¹)			Mean Effect of Sodium Chloride
	0	50	100	
0	25.36	34.16	32.29	30.60
50	41.67	49.33	46.90	45.97
100	65.07	70.05	87.08	74.07
	44.03	51.18	55.42	

ISSN 2072-3857

Discussion

The results in Tables (
1-3) explain the role played by sodium chloride and nano-silicon oxide and their importance in the vegetative growth of the periwinkle plant. The concept of stress refers to a force that impedes the vital and natural functions of the plant or disrupts the metabolic processes of living organisms (15). Salinity is considered one of the most critical environmental challenges negatively affecting agricultural production, leading to increasing economic losses on a global scale (16). Increasing the concentration of dissolved salts in the growth environment exposes the plant to numerous stresses, such as osmotic stress, ionic toxicity, ionic imbalance, enzymatic stress, oxidative stress, and hormonal imbalances (17).
Sodium chloride causes a clear effect on the morphological characteristics of the plant during its growth period. The reduction in plant vegetative growth is attributed to the presence of sodium ions in the growth medium, which leads to a decrease in growth regulators, such as gibberellins, which play a vital role in plant growth. This also contributes to increased osmotic pressure on the plant (18; 19). The results indicate that the effect of nano-silicon oxide on plant vegetative growth varies with the salinity levels in the soil. When nano-silicon oxide was applied to plants exposed to saline concentrations, clear improvements were observed in growth rate and plant performance indicators compared to growth under saline conditions without nanomaterial intervention. The spraying of nano-silicon oxide helped reduce the negative impact of salinity on root and leaf expression, while enhancing nutrient uptake and improving cell membrane stability. It was observed that plants treated with nano-silicon oxide were able to tolerate high levels of sodium chloride, which is attributed to increased vascular and cellular tissue capacity for water absorption and reduced accumulation of harmful ions in the tissues. At the performance level, the spraying of this nanomaterial led to increased root and stem length, balanced leaf growth, and accelerated photosynthetic processes, positively reflecting on the quality of the plants. In contrast, a gradual decline in growth rates was observed with increasing levels of sodium chloride in the soil. However, the use of nano-silicon oxide reduced the performance gap between treated plants and those grown under severe salinity conditions, reflecting its effectiveness as an assisting agent in salinity resistance. This is attributed to its properties in enhancing the stimulation of metabolic processes and improving the activation of plant defense mechanisms against salt-induced stress (3). Interpretation of Physiological Results The reason for the increase in the activity of antioxidant enzymes, including catalase and peroxidase, is attributed to the presence of sodium chloride in the growth medium. In saline environments, the presence of reactive oxygen species within plant cells is stimulated by the osmotic and ionic effects of salt (20). The activity of many enzymes is affected by H_2O_2, which directly or indirectly oxidizes antioxidant stores such as ASA and GSH, leading to the accumulation of oxidized redox pools within cells for various cellular processes (21). High salinity conditions stimulate the plasma membrane, producing highly toxic superoxide radicals ($O_2^{\bullet -}$) with a short lifespan, which are generated by the enzyme NADPH oxidase (22). Interestingly, the observed increase in

ISSN 2072-3857

specific superoxide dismutase (SOD) activity in maize leaves is attributed to elevated activity of other enzymes such as catalase (CAT) within these leaves. Interpretation of Nutrient and Proline Results The data in Tables (15-21) explain that increasing the concentration of sodium chloride in the growth medium leads to increased plant uptake, which in turn inhibits the absorption of other ions (23). Increasing ion concentration in the growth environment reduces the selective capacity for mineral element absorption, causing increased sodium ion uptake in plant tissues (24). Consequently, all vital processes necessary for growth and divisions cease optimally due to cell plasmolysis within plant tissue (25). The decrease in plant phosphorus and nitrogen content occurs due to high salinity. Additionally, sodium chloride salt limits the absorption of K^+ ions (26). The importance of silicon in nitrogen and phosphorus absorption has been observed in plants deficient in these two elements when they were supplied at the beginning of the experiment, particularly in their use efficiency. The effect of silicon in increasing the use efficiency of these two elements has drawn attention (27). In addition to the nutritional benefits of silicon in plants deficient in nitrogen and phosphorus, its effectiveness as an antioxidant has emerged through increasing levels of phenols and carotenoids (28). The increase in proline is considered one of the most prominent indicators associated with salt stress, serving as a natural response to resist this type of stress (29). Proline content increases with rising levels of sodium chloride, i.e., with increasing salinity, indicating metabolic shifts of some amino acids to form proline. This has been confirmed (30). Discussion of Alkaloid Results Through the results obtained in our plant stress experiments in Tables (22-25), the varying effects of the two concentrations are related to the degree of stress stimulation. The 50 mg L^{-1} concentration likely provides a "moderate stress" signal, sufficient to stimulate defense mechanisms and maintain plant growth without depleting its resources, thereby improving biomass production. In contrast, the higher 100 mg L^{-1} concentration may induce a "severe stress" state, which, although potentially more detrimental to growth, acts as a stronger stimulus, directing more resources toward the synthesis of secondary defensive metabolites such as alkaloids. The chemical interactions experienced by plant alkaloids like vincrodine, vincristine, catharanthine, and vinblastine are related to changes in their surrounding environments, especially when exposed to saline solutions and nanomaterials. Sodium chloride is a common salt that affects the stability and bioavailability of alkaloids, as it can modify their chemical structure through ionic interactions and changes in acid-base equilibria, directly affecting the biological efficacy and pharmacological properties of these alkaloids. On the other hand, nano-silicon oxide is a material with unique properties, characterized by its large surface area and crystalline network, which facilitates processes of interaction, inhibition, or induction of chemical transformation of plant alkaloids. The combined effect of sodium chloride and nano-silicon oxide on the four alkaloids depends on the individual properties of each substance, but their interaction together may produce synergistic effects leading to noticeable changes in the physical and chemical properties of these alkaloids, including improved stability or modified therapeutic efficacy (31). Moreover, the use of nano-silicon dioxide particles limits the accumulation of malondialdehyde and hydrogen peroxide, alleviates damage

ISSN 2072-3857

caused by induced stress, and enhances plant resistance to stress (28). Studies indicate that the antioxidant defense enhancement facilitated by nano-silicon dioxide particles represents a pivotal mechanism in protecting plants from oxidative stress. Moderate bursts of reactive oxygen species resulting from nanomaterial-induced stress can act as a signal to activate transcription factors responsible for regulating key genes in phenylpropanoid and antioxidant pathways (32), leading to enhanced biosynthesis of the protective alkaloids observed in our study, especially at the 100 mg L^{-1} Concentrationdos5.

Conclusions:

The results demonstrated that salt stress had a significant and comprehensive negative effect on most of the studied traits of the periwinkle plant. The severity of this effect increased with rising concentrations of sodium chloride (NaCl). The study revealed that treatment with nano-silicon oxide effectively contributed to enhancing the plant's ability to tolerate salt stress, reduce ionic toxicity, activate the defensive enzyme system, and increase the accumulation of proline as one of the protective osmolytes. Furthermore, the interaction between the moderate salinity level and the high nano-silicon level (sodium chloride at 50 mg L^{-1} with nano-silicon oxide at 100 mg L^{-1}) showed significant superiority in most of the studied traits compared to the other treatments.

5.2. **Recommendations:**

Adopt nano-silicon oxide at a concentration of 100 mg L^{-1} within agricultural management programs, especially in areas suffering from moderate to high salinity, due to its proven effective role in improving vegetative and reproductive performance, and enhancing the efficiency of physiological processes related to growth and productivity. Avoid exposing plants to high salinity levels without applying mitigating treatments. The results showed a significant decline in vital growth indicators under high salt stress, confirming the necessity of implementing appropriate management or nutritional practices to mitigate its negative effects.

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