



Effects of Chitosan and Silica Treatments on Sweet Basil Growth and Oxidative Defense System Under Salinity Stress

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ABSTRACT

Global climatic change is a major issue that drastically reduces agricultural productivity. Knowing the increasing prevalence of salty soils worldwide, emphasizing the need for innovative strategies to mitigate salt adverse effects. Prior studies have demonstrated the distinct abilities of chitosan and silica to augment plant tolerance against salt stress. However, little is known about how they work together or the specific mechanisms by which they increase salinity stress tolerance. So, the aim of this investigation was to study how chitosan and silica chitosan with 1.5 and 3 g l⁻¹ and silica (0.25, 0.50 and 1.0 g l⁻¹) either chitosan individually or in combination with silica, affected the performance of sweet basil plant growth and bioactive compounds grown under salt stress. Foliar treatment of chitosan with 1.5 and 3 g l⁻¹ and/or silica (0.25, 0.50 and 1.0 g l⁻¹) significantly improved plant growth (plant height, number of branches/plant as well as herb fresh and dry weight) comparing to untreated seedlings under salinity stress conditions. This enhancement might have resulted from significant increases in endogenous indole acetic acid (IAA), antioxidant compounds such as phenols, flavonoids and ascorbic acid contents and antioxidant activity (DPPH) in addition to antioxidant enzymes (superoxide dismutase SOD, peroxidase POD, catalase CAT and nitrate reductase NR), and some osmotic compounds (such as total soluble sugars TSS, proline, total soluble protein) compared to untreated control plants. Meanwhile, different treatments reduced significantly oxidative stress markers such as hydrogen peroxide H₂O₂, superoxide radicals (O⁻₂) which caused oxidative damages to cell walls expressed as malonaldehyde MDA. Furthermore, different treatments of chitosan and/or silica increased significantly essential oil percentages. The GC/MS analysis of the essential oils shows that Eucalyptol was the majors in all cases, while Methyl cinnamate was the second major component. Moreover, the synergistic effectiveness of chitosan and silica in improving salinity tolerance was demonstrated by the enhanced growth parameters and oil percentages that resulted from their combined application.

Keywords: Sweet basil, salinity stress, growth, essential oil, antioxidant enzymes. Oxidative stress markers.

1. Introduction

Within the Labiatae family, sweet basil (*Ocimum basilicum* L.) is among significant medicinal herbs it is sub-bush herb that can be cultivated in annual or perennial systems (Maggioni *et al.*, 2014). Sweet basil has traditionally been used as a medicine to treat renal problems, warts, headaches, diarrhoea, and coughing (Labra *et al.*, 2004). Numerous aromatic compounds with various properties, including anti-parasite, anti-virus, anti-fungal, and antioxidant activity, are present in this plant (Labra *et al.*, 2004, Juliani and Simon 2002). Furthermore, basil leaves have glands that secretes essential oils,

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the principal constituents of which is linalool, a highly valuable chemical for the perfumery, pharmaceutical and cosmetics industries. Additionally, it's employed in the aromatization of meals, drinks and surroundings (Blank *et al.*, 2004). Abiotic stressors often influence and regulate the formation of secondary metabolites in medicinal plants (Wang *et al.*, 2016).

Globally observed and predicted climate change for the twenty-first century undoubtedly presents a severe threat to food security, particularly in African and Asian countries, and has adverse implications for agricultural productivity (Zhang *et al.*, 2023). Extreme temperatures, unpredictable and heavy rainfall patterns, salinization, pollution, and newly developing insect pests are just a few of the challenges brought on by climate change that have a negative impact on farmers' livelihoods (Cisneros-Zevallos *et al.*, 2023; Bakhoun *et al.*, 2023, Sadak., 2022). Soil salinization ranks among the most significant threats to agriculture in the contemporary world (Sadak, 2023). About 32 million hectares of the 1.5 billion hectares of cultivated land worldwide; are affected by soil salinization, which lowers agricultural productivity by about 20% (Harper *et al.*, 2021). The world's biggest problem is water availability due to the annual depletion of fresh water supplies (Minhas *et al.*, 2020), which exacerbates soil salinization and periodically causes the saline area to rise at a pace of roughly 10% (Negacz *et al.*, 2022). Increasing levels of salt in soil disturb the ionic equilibrium of plants, impeding various cellular, biochemical and physiological functions such as cell division and elongation, protein biosynthesis, transpiration, photosynthesis, and lipid metabolism (Lalarukh *et al.*, 2023; Riaz *et al.*, 2022; Yan *et al.*, 2023). Salt stress hinders the uptake of essential nutrients due to higher accumulation of Na^+ and Cl^- in the rhizosphere (Huapeng *et al.* 2024) and upset the steadiness between antioxidants and reactive oxygen species (ROS) which damages cellular organelles, membranes and proteins (Sadak and Dawood2023, Manzoor *et al.*, 2023). There are three known mechanisms which are involved in salinity tolerance i) osmotic tolerance, events involving the sensing and intertwined signaling pathways that occur prior to Na^+ buildup in shoot part; ii) ion exclusion, ion sequestration in the roots, which prevents ion accumulation in the shoot; iii) vacuolar compartmentalization of excessive Na^+ concentrations in the shoot to lessen the harmful effects (Hernández, 2019; Munns and Tester, 2008). Moreover, the plant also orchestrates an array of physiological pathways that decelerate the distressing effect of salt stress and increase plant productivity (Ragaey *et al.*, 2022). Also, salinity causes some medicinal plant's secondary metabolites to increase. According to (Wang *et al.*, 2016) plant's intricate tolerance to salinity involves coordinating the activity of several internal factors involved metabolic regulation, osmotic balance and antioxidant defenses.

Choosing species that can withstand salt is among the most important strategies in saline soil cultivation. Furthermore, applying chemicals to increase stress tolerance is a potential strategy for sustainable farming in a salinity-stressed location (Bahcesular *et al.*, 2020; Souri *et al.*, 2020). Among them, it has been shown that different elicitors, such as chitosan, and micro-nutrient as silicon (Si), regulate plant metabolic pathways when plants are under stress. Dependent on the formation of secondary metabolites, an elicitor can function as an environmental factor, or a signaling molecule transducing a signal or employing biotechnology in expression of genes (Rashidi *et al.*, 2020). Chitosan is a deacetylated chitin molecule that is generated from prime and is obtained through the deacetylation of alkaline chitin. Furthermore, it is naturally created by certain fungi, the external skeleton of crustaceans, the cuticle of insects, and certain algae. In addition to being biodegradable and environmentally safe, it is inexpensive, low toxic, and has a variety of uses in agriculture (Falcón-Rodríguez *et al.*, 2009). Chitosan is a biopolymer, belonging to carbohydrate family. It is primarily derived from a glucose ring and has a free amino group at carbon number-2 (Qazizadah *et al.*, 2021). Furthermore, due to its cationic characters, it exhibits a wide range of physiological and biochemical functions in plant cells (Sadak *et al.*, 2022). Chitosan is used in plants under abiotic and biotic stress as a growth bio stimulant and to trigger defense responses. It is known as a new plant growth promoter as gibberellins (GA_3) that can used to boost growth, yield and its attributes (Bakhoun *et al.*, 2022). The amine and -OH groups present in the chemical composition of chitosan molecule give it a unique feature making it more applicable in many areas and easier for biochemical reactions (Zayed, *et al.*, 2017).

Silicon is not a vital element for plants, when absorbed, it can alter flexibility of cells that may alter plant morphology and mitigate the adverse impacts of salinity. Due to its proven ability to boost plant tolerance to both biotic and abiotic stressors, silicon is gaining more focus (Kalteh *et al.*, 2014). There is growing proof that Si treatment has improved plant fitness in response to a variety of abiotic stressors, including drought (Ahire *et al.*, 2021 and Mir *et al.*, 2022). Silicon (Si) serves as signaling

molecule important for the activation of plant defense mechanisms. After oxygen, Si is thought to be the second most abundant element in the earth's crust. Despite not being regarded as a necessary component for plants, (Epstein, 1999), Si is recognized as a multifunctional micronutrient due to its positive benefits for various plants, especially those that are stressed (Zargar *et al.*, 2012).

Silicon or silica treatment increases nutrient absorption, water contents, disease and pest resistance in plants thus promotes plant growth (Tubana *et al.*, 2016). Silicon functions as stress reliever in abiotic stress conditions by improving antioxidant defense and membrane integrity through modifications to cellular and metabolic processes. Many potential mechanisms have been suggested by which Si could increase plant's ability to withstand salinity, including enhancing water status (Romero *et al.*, 2006), increasing the ultrastructure and photosynthetic activity of leaf organelles (Shu and Lui, 2001), stimulating the antioxidant system (Zhu *et al.*, 2004), and reducing the effects of specific ions by either increasing K in shoots through H-ATPase dependent enhancement (Liang *et al.*, 2005) or by reducing Na uptake (Liang *et al.*, 2003). Since Si deficiency causes a number of dysfunctions related to plant growth and development, silicon is known as a quasi-essential element for plants (Hasanuzzaman *et al.*, 2013). Silicon serves as a plant protectant, and is essential for promoting plant growth and productivity particularly under stressful conditions (Sawas and Ntatsi 2015).

Although numerous researches have examined the effects of chitosan and silica separately on salinity tolerance in a variety of plant species, there is still a lack of information regarding their combined use to improve salinity tolerance in sweet basil plants in field conditions with an emphasis on yield. Our hypothesis is that the combined use of silica and chitosan will have a synergistic impact that will improve the plants' ability to withstand salinity. When compared to the individual treatment of either silica or chitosan, this synergistic impact is anticipated to result in enhanced physiological responses, including less oxidative stress and, crucially, greater yield. We also expect that the dual treatment will strengthen the plants' tolerance to salinity by helping to regulate antioxidant processes.

Our main goal of this study is to examine how chitosan and silica treatments work together to mitigate salinity in sweet basil plants, with an emphasis on how these treatments affect basic physiological processes and yield quality.

2. Materials and Methods

Two pot trails were carried out during two successive seasons 2021 and 2022 under the natural conditions of the greenhouse of the National Research Center (NRC), Dokki, Giza, Egypt. The experiment was conducted in completely randomized Block design (RCBD) with three replications, each replicate contained five pots. Nine treatments combinations of Chitosan at rates of (1.5, 3 g/l, and the mixture of Chitosan levels with Silica levels at rates of (0.25, 0.5 and 1 g/l), were applied. The seeds of basil were sown in the nursery on 1st March of both seasons. After 45 days (In April) from seed sowing, the seedlings were immediately transplanted into pots (30 cm diameter, 50 cm depth). Each pot contained three seedlings and was placed in full sun light. It filled with 10 kg of air-dried soil.

Mechanical and chemical properties of the soil used in this study were determined according to Jackson (1973) and Cottenie *et al.* (1982) and where, the soil texture was sandy, having the following physical composition: 81.50% sand, 13.230% silt, 5.2% clay and 0.45% organic matter (OM). The results of soil chemical analysis were: pH= 7.92; E. C (mmohs/cm)= 0.72; and total nitrogen= 0.09 %; available phosphorus= 2.0 mg/100g; potassium= 20.8 mg/100g and iron= 21.0 ppm. The seedlings were irrigated, by tap water for one month after transplanting, after that the pots irrigated by saline water (NaCl solution 3000 ppm). The nine foliar treatments as follows:

T1: Control.

T2: Chitosan at rate of 1.5 g/l.

T3: Chitosan at rate of 1.5 g/l + Silica 0.25 g/l.

T4: Chitosan at rate of 1.5 g/l + Silica 0.5 g/l.

T5: Chitosan at rate of 1.5 g/l + Silica 1.0 g/l.

T6: Chitosan at rate of 3.0 g/l.

T7: Chitosan at rate of 3.0 g/l + Silica 0.25 g/l.

T8: Chitosan at rate of 3.0 g/l + Silica 0.5 g/l.

T9: Chitosan at rate of 3.0 g/l + Silica 1.0 g/l.

Chitosan and amino acid which were used in this study were supplied from Sigma-Aldrich Co. The purity, 98 wt. %. These nine treatments were applied as a foliar after 60, 90 and 120 days from transplanting. After each applied the plants were irrigated by tap water for one week in order to protect plants from severe salinity. All other agriculture practices operations than experimental treatments were done. Samples were taken for growth characters and chemical constituent's determinations after 150 days from transplanting. The following data were recorded.

Plant height (cm), number of branches/ plant, fresh and dry weights of herb / plant (g) and essential oil percentage of the air fresh herb.

Chemical determinations

Photosynthetic pigments: The approaches of Li and Chen, (2015) were used to estimate and quantify the levels of carotenoids, chlorophyll a, and chlorophyll b. using a mortar and pestle, the fresh tissue was mashed with 80% acetone. Concentrations of photosynthetic pigment are measured in mg g⁻¹ fresh weight (FW).

Indole acetic acid content was extracted and spectrophotometrically measured as (Gusmiaty *et al.*, 2019).

Enzyme extractions were collected following the method described by Chen and Wang, (2006). Superoxide dismutase (SOD) (EC 1.12.1.1) activity was assayed using spectrophotometer (VEB Carl Zeiss) (Chen and Wang, 2006). Peroxidase (POX) (EC 1.11.1.7) activity was determined by (Kumar and Khan, 1982). Catalase (CAT) (EC 1.11.1.6) activity was determined spectrophotometrically by the method of (Chen and Wang, 2006). The enzyme activities were calculated by Kong *et al.* (1999). Nitrate reductase (NR) (EC 1. 7. 1. 1) was extracted and determined as described by Foyer *et al.* (1998).

Phenolic contents were assayed as described by Siddiqui *et al.* (2017).

Flavonoid content: Chang *et al.* (2002), used the aluminum chloride colorimetric technique to determine the crude extract's flavonoid concentration. To summarize, 4 ml of distilled water, 50 µl of crude extract (1 mg/mL ethanol), and 0.3 mL of 5% NaNO₂ solution were combined. After incubating for 5 minutes, 0.3 ml of 10% AlCl₃ solution was added, and the mixture was left to stand for 6 minutes. After that, 2 mL of a 1 mol/l NaOH solution was added, and double-distilled water was used to bring the mixture's final volume to 10 ml. After the combination had stood for 15 minutes, the absorbance at 510 nm was determined. A calibration curve was used to determine the total flavonoid content, which was then reported as mg of rutin equivalent per g of dry weight.

Vitamin C content: was extracted and assayed using the method presented in (Kamofenkel *et al.*, 1995).

Determination of antioxidant activity as 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was carried out according to (Gyamfi *et al.*, 2002).

Total soluble sugars (TSS) were extracted and determined by the method described by Mecozzi, (2005).

Proline content: The proline was measured by Kalsoom *et al.* (2016). After ground of 0.5 g leaves using 10 ml of 3% sulphosalicylic acid, the leaves were centrifuged at 10,000×g for 10 minutes. 2 milliliters of the supernatant and 2 milliliters of recently made acid ninhydrin reagent were combined. For thirty minutes, the mixture was incubated at 90°C in a water bath. Cooling in the ice bath stopped the reaction. To extract the reaction, 5 milliliters of toluene were added, and the mixture was vortexed for 15 seconds. The toluene and aqueous phases were then separated by 20 minutes in darkness. After gathering the toluene phase, the color's absorbance was measured at 520 nm using proline as a reference, and the result was stated as µg g⁻¹ fresh weight.

The total free amino acids (FAA) were determined according to the method described by Sorrequieta *et al.* (2010).

Total soluble nitrogen (TSN) content was measured by the method of Horwitz (2002).

The Hydrogen peroxide level was determined calorimetrically according to Yu *et al.*, (2003).

Superoxide anion radicals were determined by using nitro blue tetrazolium (NBT) according to the method described by Doke (1983).

The level of lipid peroxidation was measured by determining the malondialdehyde (MDA) content using the method of Hodges *et al.* (1999).

Essential oil (EO) isolation of the different fresh samples of basil plants was carried out according to Clevenger, (1928).

Gas chromatography-mass spectrometry (GC-MS)

The samples of all treatments were analyzed using an Agilent 8890 GC System gas chromatography system, which was connected to an Agilent 5977B GC/MSD mass spectrometer and fitted with an HP-5MS fused silica capillary column (30 m, 0.25 mm i.d., 0.25 mm film thickness). The temperature of the oven was kept at 50 °C at first, then programmed to rise to 200 °C at a rate of 5 °C/min then to rise to 280 °C at a rate of 10 °C/min. Finally, it was held at 280 °C for 7 minutes. The carrier gas, helium, was employed at a flow rate of 1.0 mL/min. A split ratio of 1:50 was used to inject 1 µL of the dissolved essential oil (20 µL essential oil / mL diethyl ether) into the gas chromatograph. The injection temperature was 230 °C. Mass spectra were acquired at 70 eV in the electron impact mode (EI) scanning a range of 39 to 500 amu in m/z. By comparing the isolated peaks with information from the mass spectra library (National Institute of Standard and Technology, NIST), the isolated peaks were found

Statistical analysis

Analysis of variance (ANOVA) of complete randomized block design (RCBD) (Gomez and Gomez 1984) was used to statistically analyze the collected data of the field experiments, using MSTAT-C software package (Freed *et al.*, 1989). Bartlett's homogeneity test was carried out prior to conducting combined ANOVA analysis (Steel *et al.*, 1997). Least significant differences test (LSD) was applied to detect the significant differences between tested treatments means (Steel *et al.*, 1997).

3. Results

3.1. Impact of chitosan and/or silica on growth criteria of salinity stressed sweet basil plants

The analysis of variance of the obtained data revealed marked increases in growth parameters of sweet basil plant height (cm), number of branches/plant, herb fresh and dry weight (g) in response to foliar treatment of chitosan with 1.5 g/l and 3.0 g/l (Table 1) under salinity stress. The mechanism of salt tolerance in sweet basil plant was induced by chitosan in a concentration dependent way, higher concentration of chitosan (3.0 g/l) foliar treatment was more effective than lower one (1.5 g/l) in increasing the above mentioned parameters as compared with untreated control plants. The increases of plant height and branches number/plant were non-significant in response to 1.5 g/l and branches number/plant in response to 3.0 mg/l while herb fresh and dry weights were significant in response to both treatments under salinity stress conditions. Furthermore, the interaction effect of different concentrations of chitosan (1.5 & 3.0 g/l) and silica (0.25, 0.50 & 1.00 g/l) caused significant increases of the studied growth parameters as compared with untreated control under salt stress conditions.

However, the application of chitosan 3.0 g/l + 1.00 g/l silica resulted in highest increases in different growth parameters (the percentage of increase were 58.5%, 70.3%, 108.4%, 109.9% and 83.3% in plant height, branches number/plant, herb fresh and dry weight) followed by chitosan 1.5 g/l + 1.0 g/l silica the increases were 55.9%, 50.2%, 73.8%, 76.3% and 58.3% in plant height, branches number/plant, herb fresh and dry weight, respectively as compared with untreated control and the other treatments. (Table 1).

With respect to the effect of different foliar treatments of chitosan and/or silica, Table (1) show that, chitosan caused significant increases of essential oil percentages of sweet basil plants as compared with control untreated plants under salinity stress. Data also, show that higher chitosan level was more

significant than lower one. Furthermore, treating sweet basil plant with mixture of chitosan and silica with different concentrations caused more significant increases (Table 1).

Table 1: Impact of chitosan (1.5 & 3.0 g/l) and Silica (0.25, 0.50 &1.00 g/l) on growth criteria of sweet basil under salinity stress conditions (Data are means of two seasons).

Treatments (g/l)	plant height (cm)	Branches no/plant	herb fresh weight (g)	herb dry weight (g)	Essential oil (%)
T1	39.33±0.67	3.33±0.33	79.45±2.91	16.13±0.52	0.12±0.01
T2	42.33±1.20	3.67±0.33	114.7±8.66	23.13±1.58	0.15±0.01
T3	50.00±0.58	4.67±0.33	122.3±3.22	24.97±0.70	0.16±0.01
T4	57.00±1.53	4.67±0.88	138.0±4.67	28.50±1.00	0.18±0.01
T5	61.33±1.33	5.00±0.58	138.1±0.58	28.43±0.09	0.19±0.01
T6	46.00±1.53	3.67±0.33	130.9±2.33	26.57±0.77	0.18±0.01
T7	52.67±1.45	4.67±0.33	134.2±2.62	26.93±0.64	0.20±0.01
T8	57.00±2.08	5.00±0.58	146.75±1.53	30.30±0.42	0.21±0.02
T9	62.33±0.67	5.67±0.33	165.55±2.62	33.87±0.43	0.22±0.02
LSD 0.05	4.18	1.45	12.4	2.44	0.03

Error bars represent ± SE values derived from three biological replicates.

T1-Control, T2- chitosan at rate of 1.5 g/l, T3- chitosan at rate of 1.5 g/l + Silica (0.25 g/l), T4- chitosan at rate of 1.5 g/l + Silica (0.5 g/l), T5- chitosan at rate of 1.5 g/l + Silica (1.0 g/l), T6- chitosan at rate of 3.0 g/l, T7- chitosan at rate of 3.0 g/l + Silica (0.25 g/l), T8- chitosan at rate of 3.0 g/l + Silica (0.5 g/l) and T9- chitosan at rate of 3.0 g/l + Silica (1.0 g/l)

3.2. Impact of chitosan and/or silica on photosynthetic pigments of salinity stressed sweet basil plants

The obtained data in Table 2 demonstrated that foliar treatment of sweet basil with different concentrations of the chitosan (1.5 and 3.0 g/l⁻¹), and/or silica (0.25, 0.50 and 1.0 mg/l⁻¹) increased chlorophyll a, chlorophyll b, carotenoids and consequently total pigments contents as compared with the untreated control plants under salinity stress conditions. Foliar treatment of higher level of chitosan (3.0 g/l⁻¹) was more effective than lower one (1.5 g/l⁻¹) in increasing the above mentioned parameters, furthermore addition of chitosan (with the two used concentrations) with silica (with the three used concentrations) as foliar treatments was more effective than chitosan treatments alone.

Table 2: Impact of chitosan (1.5 & 3.0 g/l) and Silica (0.25, 0.50 &1.00 g/l) on photosynthetic pigments (mg/g fresh weight) and endogenous indole acetic acid (IAA (mg/100 g fresh wt.)) of sweet basil under salinity stress conditions (Data are means of two seasons).

Treatments (g/l)	Chlorophyll a (mg g ⁻¹ f. wt.)	Chlorophyll b (mg g ⁻¹ f. wt.)	Carotenoids (mg g ⁻¹ f. wt.)	Total pigments (mg g ⁻¹ f. wt.)	IAA (mg/100 g f. wt.)
T1	733.53±1.78	536.24±0.87	291.26±2.62	1561.03±2.96	19.81±0.17
T2	864.80±0.89	611.79±2.21	315.85±6.06	1792.45±7.38	24.38±0.37
T3	893.25±1.39	627.52±1.25	340.42±1.00	1861.18±0.86	30.24±0.64
T4	914.25±0.92	638.61±1.11	354.53±0.09	1907.38±2.13	37.06±0.06
T5	920.76±0.62	648.92±1.69	364.66±0.17	1934.33±2.49	41.24±0.23
T6	887.45±0.23	640.72±2.54	339.11±0.58	1867.27±2.32	31.51±0.00
T7	909.04±0.90	684.43±1.76	370.62±0.55	1964.08±0.50	40.15±0.28
T8	928.43±2.07	708.56±2.06	391.35±1.15	2028.34±1.96	44.40±0.38
T9	957.36±3.50	712.89±1.36	409.62±1.68	2079.87±1.61	49.00±0.34
LSD 0.05	5.25	5.2	7.11	9.83	1.04

Error bars represent ± SE values derived from three biological replicates.

The highest increases of different photosynthetic pigments constituents were obtained by foliar treatment of chitosan (3.0 g/l⁻¹) and silica (1.0 g/l⁻¹), the increases were from 733.53 to 957.36 in chlorophyll

a, from 536.24 to 712.89 in chlorophyll b, from 291.26 to 409.62 in carotenoids and 1561.03 to 2028.34 in total pigments with percentages of increases 30.5%, 32.9%, 40.6% and 33.2% of chlo a, chlo b, carotenoids and total pigments respectively as compared with untreated control plant (Table 2)

3.3. Impact of chitosan and/or silica on endogenous indole acetic acid (IAA) of salinity stressed sweet basil plants

The analysis of variance of the obtained data revealed significant increases in endogenous indole acetic acid content (IAA) of sweet basil due to foliar treatment of chitosan with 1.5 g/l and 3.0 g/l (Table 2) under salinity stress condition. The increase in endogenous IAA is a concentration dependent this means that, higher concentration of chitosan (3.0 g/l) foliar treatment was more effective than lower one (1.5 g/l) in increasing the above mentioned parameters as compared with untreated control plants. IAA content increased from 19.81 to 24.38 under 1.5 g/l and to 31.51 with 3.0 g/l chitosan (the percentages of increases were 23.06% and 59.06% with 1.5 and 3.0 g/l chitosan treatment. The Furthermore, the interaction effect of different concentrations of chitosan (1.5 & 3.0 g/l) and silica with different concentrations (0.25, 0.50 & 1.00 g/l) caused more significant increases of the endogenous IAA content as compared with untreated control under salt stress conditions. However, the application of chitosan 3.0 g/l + 1.00 g/l silica resulted in highest increases in different growth parameters (the percentage of increase were 147%) as compared with untreated control and the other treatments. (Table 2)

3.4. Impact of chitosan and/or silica on antioxidants enzyme activities of salinity stressed sweet basil plants

The main goal of this study was to investigate the efficiency of chitosan, silica and their combinations in alleviating the harmful impacts of salinity stress on sweet basil. Results in this study showed that superoxide dismutase SOD, peroxidase POX, catalase CAT, and nitrate reductase NR activities were significantly ($P \leq 0.05$) increased in foliar treated basil plants of chitosan and/or silica with different concentrations as compared with untreated plants (Table 3). These stimulating effects were positively related to the concentrations of the investigated compounds, higher concentrations of chitosan increased SOD, POX, CAT and NR increased by 63.1%, 1.2%, 21.5 and 22.5% as compared with control plants. The maximum activities of the investigated antioxidant enzymes were assayed in high-dose chitosan (3.0g/l) + silica (1.0 g/l) -treated sweet basil plants exposed to salt stress, it caused increases by 128.8%, 171.4%, 56.7% and 56.1% in SOD, POX, CAT and NR respectively.

Table 3: Impact of chitosan (1.5 & 3.0 g/l) and Silica (0.25, 0.50 & 1.00 g/l) on antioxidant enzyme activities (superoxide dismutase SOD, peroxidase POD, catalase CAT and nitrate reductase NR) (nmole/g fresh wt)) of sweet basil under salinity stress conditions (Data are means of two seasons).

Treatments (g/l)	SOD	POX	CAT	NR
		U/min/g FW		nM NO ₂ /g FW
T1	22.94±0.43	41.16±0.27	295.08±0.64	309.13±1.85
T2	31.81±0.42	64.24±0.35	332.07±0.32	322.64±0.01
T3	35.88±0.13	69.81±0.26	341.44±0.69	350.62±1.15
T4	40.75±0.05	77.09±0.35	362.73±0.85	373.60±0.72
T5	49.25±0.23	85.22±0.30	393.99±0.21	376.91±0.38
T6	37.43±0.27	89.08±0.35	358.58±2.2	378.64±0.59
T7	44.27±0.34	94.25±0.35	388.00±2.51	396.13±0.87
T8	49.75±0.58	102.8±0.43	424.94±0.79	454.32±1.05
T9	52.49±0.62	111.7±0.58	462.44±0.49	482.46±0.66
LSD 0.05	1.22	1.05	3.86	2.98

Error bars represent ± SE values derived from three biological replicates.

3.5. Impact of chitosan and/or silica on antioxidant compounds of salinity stressed sweet basil plants

Results in this investigation showed that, phenols, flavonoids and ascorbic acid contents in addition to DPPH activity were increased significantly ($P \leq 0.05$) in sweet basil plants treated with chitosan (1.5 and 3.0 g/l), and chitosan and silica (0.25, 0.50 and 1.0 g/l) as compared with untreated plants (Table 4). These stimulating effects were positively related to the concentrations of the investigated compounds, higher concentrations of chitosan increased phenols, flavonoids, ascorbic acid contents and DPPH activity increased by 89.0%, 102.5%, 32.6 and 32.6% as compared with control plants. The maximum activities of the investigated antioxidant compounds were assayed in high-dose chitosan (3.0g/l) + silica (1.0 g/l) -treated sweet basil plants exposed to salt stress, it caused increases by 150.9%, 357.7%, 75.0% and 54.7% in phenols, flavonoids, ascorbic acid contents and DPPH activity respectively (Table 5).

Table 4: Impact of chitosan (1.5 &3.0 g/l) and Silica (0.25, 0.50 &1.00 g/l) on antioxidant compounds phenols, flavonoids and ascorbic acid (nmole/g fresh wt) and DPPH% of sweet basil under salinity stress conditions (Data are means of two seasons).

Treatments (g/l)	Phenols	Flavonoids	Ascorbic acid	DPPH%
	mg/100 g fresh wt		(μ mole/100 g fresh wt)	
T1	22.03 $\pm$ 0.34	15.7 $\pm$ 0.07	1.44 $\pm$ 0.01	50.57 $\pm$ 0.23
T2	33.16 $\pm$ 0.49	18.77 $\pm$ 0.29	1.67 $\pm$ 0.02	54.02 $\pm$ 0.41
T3	36.9 $\pm$ 0.27	28.23 $\pm$ 0.94	1.86 $\pm$ 0.01	59.84 $\pm$ 0.25
T4	41.76 $\pm$ 0.24	40.59 $\pm$ 0.62	2.06 $\pm$ 0.02	66.84 $\pm$ 0.24
T5	46.77 $\pm$ 0.22	51.00 $\pm$ 0.35	2.23 $\pm$ 0.02	72.43 $\pm$ 0.30
T6	41.64 $\pm$ 0.34	31.80 $\pm$ 0.18	1.91 $\pm$ 0.01	63.90 $\pm$ 0.13
T7	48.92 $\pm$ 0.39	42.81 $\pm$ 0.43	2.05 $\pm$ 0.01	67.98 $\pm$ 0.43
T8	51.41 $\pm$ 0.47	60.88 $\pm$ 0.26	2.41 $\pm$ 0.01	75.84 $\pm$ 0.26
T9	55.28 $\pm$ 0.64	71.75 $\pm$ 0.33	2.52 $\pm$ 0.01	78.25 $\pm$ 0.32
LSD 0.05	1.25	1.43	0.04	0.86

Error bars represent $\pm$ SE values derived from three biological replicates.

3.6. Impact of chitosan and/or silica on some osmotica of salinity stressed sweet basil plants

Chitosan foliar treatment (1.5 and 3.0 g/l) increased total soluble sugars TSS, proline, free amino acids and total soluble protein content of sweet basil under salinity stress as compared with untreated control (Table 5). Higher levels caused more significant increases than lower one, by 27.1%, 38.9, 28.8 and 18.5% in higher concentration compared with 11.7%, 10.3%, 18.2% and 13.1% of TSS, proline, FAA and TSN. Furthermore, chitosan +silica applications with different levels caused more significant $P \leq 0.05$ increases in the above mentioned osmoprotectant compounds compared with untreated controls. Chitosan with 3.0 g/l + silica with 1.0 g/l was the most effective treatment it increased TSS by 54.0%, proline by 85.4%, FAA by 58.1% and TSN by 48.1% compared to control plants

3.7. Impact of chitosan and/or silica on oxidative stress markers of salinity stressed sweet basil plants

The oxidative stress markers such as hydrogen peroxide H_2O_2 , superoxide radicles ($O^{\cdot}_2$) of sweet basil in response to foliar treatment of chitosan and silica foliar treatment under salinity stress conditions are presented in Table 6. The application of chitosan alone or the combination of chitosan and silica with different concentrations significantly reduced these oxidative stress markers compared to the non-treated control plants. Specifically, the application of higher concentration of chitosan 3.0 g/l was more effective than lower one (1.5 g/l) which caused a decrease in H_2O_2 by 26.9% than lower one 21.3% and in $O^{\cdot}_2$ 23.7% in the higher concentration and by 17.0% in the lower concentration, compared to the untreated control plant. Moreover, the interaction effect of both chitosan and silica with different used concentrations were more effective than chitosan alone in reducing hydrogen peroxide H_2O_2 and superoxide radicles ($O^{\cdot}_2$). Chitosan 3.0 g/l + 1.0 g/l silica treatment was superior over all other

treatments in decreasing oxidative stress markers (H_2O_2 and $O^{\bullet}_2$), the percentages of decreases were 42.9% and 43.2% in H_2O_2 and $O^{\bullet}_2$, respectively (Table 6).

Table 5: Impact of chitosan (1.5 & 3.0 g/l) and Silica (0.25, 0.50 & 1.00 g/l) on osmotica proline, Total soluble sugars (TSS), total free amino acids (FAA) and Total soluble nitrogen (TSN) (mg/100g dry wt) of sweet basil under salinity stress conditions (Data are means of two seasons).

Treatments (g/l)	Proline	TSS	FAA	TSN
T1	28.48±1.08	104.62±0.91	222.32±0.94	423.11±1.46
T2	31.40±0.52	116.88±1.74	262.77±1.18	478.46±1.96
T3	36.16±0.12	124.90±0.80	292.95±1.34	496.18±1.41
T4	44.13±0.29	136.48±2.22	315.57±1.85	526.37±2.16
T5	47.56±0.61	151.35±0.57	323.41±0.77	568.59±0.34
T6	39.56±0.05	132.92±0.85	286.45±0.65	501.47±0.55
T7	44.03±0.21	145.27±0.84	305.64±2.09	531.01±0.87
T8	50.71±0.36	155.15±0.97	324.64±1.03	590.58±1.74
T9	52.82±0.43	161.15±1.12	351.47±0.56	626.67±2.46
LSD 0.05	1.57	3.85	3.86	4.86

Error bars represent ± SE values derived from three biological replicates.

3.8. Impact of chitosan and/or silica on lipid peroxidation products of salinity stressed sweet basil plants

The extent of the salt-induced oxidative damage was assessed by measuring the levels of malondialdehyde (MDA) formation on sweet basil plant (Table 6). Exogenous application of either chitosan (1.5 and 3.0 g/l) and/or silica (0.25, 0.50 and 1.00 g/l) significantly reduced the peroxidation product (MDA) compared with untreated control plant. The values of peroxidation products are negatively related to the concentration of either chitosan or silica. Lower levels of MDA were estimated in chitosan-treated plants at high dose (3.0 g/l). The percentage of decrease was 19.6% in response to 3.0 g/l chitosan compared with untreated control. Furthermore, the interaction between chitosan + silica with different concentrations were more effective than chitosan alone, the most effective foliar treatment was 3.0 g/l chitosan + 1.0 g/l silica it caused 31.1% or reduction in comparison to untreated control.

Table 6: Impact of chitosan (1.5 & 3.0 g/l) and Silica (0.25, 0.50 & 1.00 g/l) on hydrogen peroxide H_2O_2 , superoxide radicles ($O^{\bullet}_2$) and malondialdehyde (MDA) (nmole/g fresh wt) of sweet basil under salinity stress conditions (Data are means of two seasons).

Treatments (g/l)	H_2O_2	$O^{\bullet}_2$	MDA
T1	4.05±0.05	5.23±0.06	11.53±0.12
T2	3.19±0.07	4.34±0.01	9.27±0.06
T3	3.04±0.04	4.07±0.03	9.02±0.05
T4	2.98±0.05	3.59±0.03	8.6±0.03
T5	2.65±0.00	3.35±0.03	8.42±0.03
T6	2.96±0.05	3.99±0.05	8.95±0.08
T7	2.71±0.02	3.63±0.01	8.59±0.12
T8	2.53±0.06	3.19±0.03	8.19±0.08
T9	2.31±0.00	2.97±0.02	7.94±0.04
LSD 0.05	0.13	0.11	0.24

Error bars represent ± SE values derived from three biological replicates.

3.9. Impact of chitosan and/or silica on essential oil constituents of salinity stressed sweet basil plants

The essential oil components of sweet basil plant, which grew under the saline conditions, were identified by GC-MS. Twenty- eight components were identified in the essential oil extracted from sweet basil plant. The effect of different foliar treatments was studied on the essential oil components of sweet basil plant. The GC/MS analysis of the essential oils in table (7) shows that Eucalyptol was the majors in all cases that recorded (25.83% and 25.02%) with (chitosan 3.0 g/l and silica 1.0 g/l) and

(chitosan 1.5 g/l and silica 1.0 g/l), respectively compared with untreated plants that recorded the lowest value (16.88%). The same result was recorded with β -Pinene (4.98 % and 3.12 %), respectively. While Methyl cinnamate was the second major component that recorded (15.5 % and 15.19%) with (chitosan 3.0 g/l) and (chitosan 1.5 g/l and silica 0.5 g/l), respectively. The high rate of foliar treatment with (chitosan 3 g/l and silica 1.0 g/l) recorded the highest value of (+)-2-Bornanone (13.8%), trans-Methyl cinnamate (8.16%) and β -Myrcene (3.45%). while foliar treatment with (chitosan 1.5 g/l and silica 0.5 g/l) record the highest value of β -Caryophyllene (9.22 %). Linalool (6.55 %) record the highest value with (chitosan 3 g/L and silica 0.5 g/l).

Table 7: Impact of chitosan (1.5 &3.0 g/l) and Silica (0.25, 0.50 &1.00 g/l) on essential oil constituents of sweet basil under salinity stress conditions during the second season.

Peak	RT	Compounds	T1	T2	T3	T4	T5	T6	T7	T8	T9
1	6.296	α -pinene	1.56	1.41	0.88	1.04	1.87	1.92		2.66	2.74
2	6.708	Camphene	2.19	2.95	1.96	1.05	3.06	2.56	1.99	3.4	2.65
3	7.4	β -Pinene	2.63	2.04	2.02	1.91	3.12	2.75	2.16	3.11	4.98
4	7.721	β -Myrcene	2.2	1.59	1.38	1.75	1.62	1.54		1.72	3.45
5	8.648	p-Cymene	3.49	2.22	1.87	2.25	3.16	2.31	1.7		
6	8.962	Eucalyptol	16.88	18.39	19.78	20.1	25.02	23.45	21.5	23.2	25.83
7	9.054	β -Ocimene	1.07	1.11	1.48	1.55	1.4	0.91		1.17	4.97
8	9.569	γ -Terpinene	1.06	1.23	1.36	1.3	1.06	1.28		1.35	1.57
9	9.832	Sabinene hydrate	2.77	2.46	1.94	1.32	1.94	2.21	1.36	2.72	1.91
10	10.387	Terpinolene	1.11		1.15		0.99	1		1.5	1.68
11	10.788	Linalool	4.6	5.07	5.99	5.73	3.27	5.96	4.98	6.55	5.39
12	12.281	(+)-2-Bornanone	11.12	12.14	12.44	11.52	13.29	12.67	11.9	13.7	13.81
13	13.059	Terpinen-4-ol	4.29	4.32	3.7	3.66	3.55	3.77	3.17	3.93	2.45
14	13.282	α -terpineol	1.49	1.06	1.71	1.05			0.69	0.93	
15	14.627	Pulegone	1.37		0.82			0.67	0.98	0.83	
16	14.776	(-)-Carvone	1.23	1.3	0.84		0.77		1.25	1.52	
17	16.659	trans-Methyl cinnamate	6.13	7.63	5.97	4.99	7.29	7.28	8.06	6.46	8.16
18	18.341	Copaene	0.99	1.47	1.1	1.25	1.55	1.02	1.8	1.49	0.94
19	18.604	Methyl cinnamate	8.92	9.77	13.63	15.19	13.67	15.5	15.1	7.53	7.3
20	18.776	β -Elemene	7.39	4.16	2.13	2.46	3.74	2.96	5.67	5.5	2.07
21	19.296	β -Caryophyllene	6.82	8.8	6.99	9.22	5.05	4.89	6.61	4.87	5.47
22	19.743	β -Copaene	1.09	1.49	0.76		1.2	0.71	1.45	0.92	
23	19.892	α -Guaiene	0.81	1.27	0.89	1.08			0.85	0.64	0.82
24	20.046	α -Humulene	1.1	1.06	1.31	1.56			1.09	0.82	0.93
25	20.452	β -Cubebene	1.02	1.07	0.8	1.02			0.84		
26	20.67	Germacrene D	2.22	1.37	3.05	3.53	1.01	1.65	1.83	1.48	1.4
27	21.785	γ -Cadinene	2.78	3.62	3.05	4.09	2.34	2.29	4.05	2.1	1.46
28	21.934	δ -Cadinene	0.76	0.99	0.97	1.38		0.66	1.05		

4. Discussion

Soil and water salinity pose a huge danger to agricultural crop yield, especially for plants which are salinity intolerant. Salinity is a main factor contributing to serious global problem, especially in arid and semi-arid countries (Rady *et al.*, 2011). Salinity causes “physiological drought” which adversely modifies plant physio-biochemical indicators (e.g., water content, nutrient uptake, meristematic activities, cell enlargements, chlorophylls content, metabolic processes, and photosynthesis efficiency). These negative impacts are accompanied by an excessive rise in reactive oxygen species (ROS) (Sadak *et al.*, 2012, Abdelhamid *et al.*, 2013, Rady *et al.*, 2015) and an increase in respiratory rate brought on by increased energy requirements, which results in oxidative stress (Ragaey *et al.*, 2022, Sadak., 2023).

In addition to oxidative stress, salinity also induces “osmotic stress” which results in an imbalance of water, a reduction in growth stimulus (IAA and GA3), and increase in growth inhibitors (ABA), and stomata closure, ionic imbalance, a loss in photosynthesis, and accumulation of toxic ions. Consequently, inhibiting plant growth and productivity (Sadak and Dawood, 2023). Adopting saline-using agricultural policies that emphasize agronomic management would increase the effectiveness of salty soil or water. Salinity tolerance of sweet basil is moderate especially at higher levels of salinity, because its growth was depressed due to salinity. A reduction in plant growth under salinity is expectable and a great deal of reports have been substantiated this hypothesis (Sadak *et al.*, 2022).

Nevertheless, salinity stress has a number of negative effects on physiological and morphological processes, which ultimately lead to a decrease in the accumulation of dry matter. Thus, the goal of this study was to determine the equilibrium between the salt stress impact and the existing climate changes. Our findings demonstrated that exogenous treatment of chitosan significantly improved growth of sweet basil under salinity stress, via improving different physiological parameters. These findings might be explained by increased water and essential nutrients uptake which adjust cell osmotic pressure, as well as reduced the accumulated harmful reactive oxygen species via increasing antioxidant compounds and improved antioxidant enzyme activities (Malerba and Cerana, 2020). Additionally, the activation of the ROS scavenging system, enhanced stomatal conductance, and stimulated growth of xylem (Hidangmayum *et al.*, 2019, Malerba and Cerana, 2020). In addition, the hydrophilic nature of chitosan may alleviate stress effects by reducing cell water content and accelerating several biological macromolecules’ activities (Chakraborty *et al.*, 2020). Furthermore, the current results showed that at chitosan and/or silica treatment with different concentrations on sweet basil plants fulfills pronounced improvements in growth, under salinity stress conditions. These results were explained by the numerous benefits that chitosan and silica provide. Applying silica is a suitable management approach since it lessened the detrimental effects of salinity stress on growth criteria of sweet basil (Nasrudin, *et al.*, 2022). In this context, Artyszak (2018) ostulated that these increases in growth parameters could be ascribed to the stimulation of photosynthetic activities and ideal growth conditions in silica-treated plants. Furthermore, the enhanced antioxidant capacity and K⁺ absorption, which raise the number of chloroplasts per cell (Taiz and Zeiger, 2010), might be the causes of Si beneficial effects on sweet basil growth (Farouk and Omar, 2020). Furthermore, the beneficial impacts of Si are linked with decreasing Na⁺ and increasing K⁺, thus causing the K⁺/Na⁺ ratio, which eventually improves sweet basil plant growth under salinity and stimulates gene expression (Kim *et al.*, 2014).

Moreover, chitosan treatments improved the photosynthetic pigments of sweet basil plant under salinity stress. These increases might be attributed to the role of chitosan in enhancing cytokinins contents that stimulated chlorophylls synthesis and/or to increase the availability of amino compounds released from chitosan (Chibu and Shibayama 2001). Likewise, chitosan increased chlorophyll and carotenoids of plant by activating the expression of genes responsible of the biosynthesis of photosynthetic pigments (Naderi *et al.*, 2015). Regarding the positive effect of Si treatment on chlorophyll a & b as well as carotenoid contents under salinity it was documented in various plants which found that Si treatment maintained chlorophyll production (Dhiman *et al.*, 2021 and Sadak *et al.*, 2023). Si's attenuating effect on chlorophyll content could be caused by enhanced ROS-scavenging activity, higher chloroplast numbers per cell, improved K⁺ uptake, or preserved chloroplast ultrastructure. Furthermore, after receiving Si treatment under salinity stress, carotenoid production enhanced in sweet basil, maybe as a result of Si's antioxidant properties, which lessened oxidative damage brought on by salinity Farouk and Omar, (2020), who recorded that Si application sustained chlorophyll biosynthesis under control or stress conditions (Zhu & Hong, 2014).

The enhanced effect of chitosan on increased IAA contents of sweet basil plants grown under salt stress might lead to an improvement of enzyme activity and as a result increased growth parameters in treated plants. Furthermore, these increases might be due to the induced effect of chitosan on auxin-related gene expression that accelerate IAA biosynthesis and reduced IAA oxidase activities (Li *et al.*, 2018). Moreover, the increases in IAA contents of sweet basil plants due to foliar spraying with Silica could be explained by the effect of Si element in improving plant tolerance to drought stress via regulating the levels of phytohormones (Yin *et al.*, 2016). In recent years, Si has been reported to enhance phytohormone synthesis to upregulate the physiological process of crop plants under extreme environmental conditions (Mir *et al.*, 2022). Under abiotic stress conditions, Si enhanced the metabolism and synthesis of phytohormones in plants (Kim *et al.*, 2016). The major phytohormones

upregulated by Si under abiotic stress conditions include IAA (Arif *et al.*, 2021). Si-induced phytohormones synergistically regulate the growth, metabolism, and stress tolerance in pepper, as shown by *in silico* data analysis (Gómez-Merino *et al.*, 2020).

Treating sweet basil with chitosan caused significant reductions in H_2O_2 and O as well as MDA contents under salt stress this decreases were correlated with the increased antioxidants as well as improving antioxidant enzymes which scavenge ROS and prevent cell membranes from oxidative stress (Hawrylak-Nowak *et al.*, 2020). In addition, charge-charge interactions between positively

charged chitosan amine groups and negatively charged membrane phospholipids promote a signal that will lead to the octadecanoid pathway (Almeida *et al.*, 2020). Si treatment reduced H_2O_2 , O^{2-} and MDA contents. According to Farouk *et al.*, (2020), this moderating effect was observed in plants that had Si foliar spraying. These findings suggest that Si may be able to mitigate the negative effects of salinity. However, non-enzymatic abilities like osmolyte and antioxidant solute buildup might also be blamed for this relief (Al Murad *et al.*, 2020; Farouk and Omar, 2020), in addition to increased antioxidant enzyme activity. Furthermore, Si application techniques have been employed in this study to demonstrate the benefits of Si treatment for plants exposed to oxidative damage in stressful environmental settings. Plants treated with Si experience reduced cellular dehydration (Agarie *et al.*, 1998). Second, the ROS scavenging system, which includes antioxidant enzymes and solutes, was activated with Si-treatment. Third, Si treatment significantly improved the ultrastructure of chloroplasts while reducing the permeability of cellular membranes (Farouk and Omar, 2020).

Regarding the increased activities of different studied antioxidant enzymes, the activities of catalase and peroxidase in milk thistle plant were improved by chitosan treatment (Safikhani *et al.*, 2018). Chitosan elicitation seems to promote the accumulation of catalase, thus corroborating their potential in improving plants' antioxidant defences (da Silva *et al.*, 2021). It is likely that chitosan activates superoxide dismutase and catalase involved in the detoxification of H_2O_2 in plants. Sadak and Talaat (2021) concluded that chitosan enhanced the protective parameters such as antioxidant enzymes and free radical scavengers and consequently helped wheat plants to decrease lipid peroxidation. Furthermore, chitosan was reported to boost the synthesis of secondary metabolites. Chakraborty *et al.*, (2009) observed that in suspension culture of *Cocos nucifera*, chitosan boosted the synthesis of phenylpropanoid derivatives. According to Rashidi *et al.*, (2020) adding chitosan enhanced phenolic content in sweet basil plant under different salt levels. Additionally, Falcón-Rodríguez *et al.*, (2009) stated that treatment tobacco plant with chitosan, as an elicitor, improved the defensive activities of peroxidase, phenylalanine ammonia-lyase (PAL), and Glucan. In addition to acting as a secondary messenger and binding with cellular membranes as a plant elicitor, chitosan also controls the synthesis of some hormones, including IAA (Bakhoum *et al.*, 2022). In addition to controlling gene expression and other plant defense processes, this hormone closes the stomata.

According to a number of studies, Si treatment enhanced antioxidant compounds and enzyme activities, which modified the redox-homeostasis approach (Zhu and Gong, 2014; Farouk and Omar, 2020). Furthermore, Si treatment improved the low concentration of non-enzymatic antioxidant molecules in plants exposed to salt (Soundararajan *et al.*, 2018; Al Murad *et al.*, 2020 and Goyal *et al.*, 2022). Superoxide dismutase and other ROS-scavenging enzymes must remain in balance in order to determine the steady-state concentration of O^{2-} and H_2O_2 (Foyer, 2018). This will help to keep ROS levels under control and improve plant development under salinity stress. Superoxide dismutase starts the process of detoxifying O^{2-} by converting it into H_2O_2 , which is toxic and needs to be removed by passing via particular channels. Intracellular H_2O_2 levels are regulated by a number of enzymes, including peroxidase and catalase (Foyer, 2018). According to the current findings, the coordination of superoxide dismutase and peroxidase and catalase with O^{2-} and H_2O_2 scavenging plays a crucial defensive role. Additionally, the active contribution of these enzymes is marginally associated with salinity-induced oxidative damage in Si-treated sweet basil. Moreover, a good indicator of decreased salinity-induced ROS generation in salt-stressed sweet basil plants is the effective rise in ascorbic acid content brought about by chitosan and Si treatment. According to Sofy *et al.*, (2020) and Farouk and Omar (2020), phenolic compounds are frequently stress-induced metabolites that accumulate in plant tissue when salt and Si supplementation are applied. Because they contain mediators that donate electrons, soluble phenols function as antioxidants.

To maintain the ideal cell water potential and safeguard DNA and macromolecule structures, sweet basil herbs treated with chitosan and Si accumulate extra osmotic adjustment solutes, such as total

soluble sugars, proline and soluble nitrogen as osmoprotectants (Chaves *et al.*, 2009). Since, chitosan application could induce significant difference of organic acids, sugars and amino acids in leaves of wheat seedlings (Zhang *et al.*, 2017) and significantly increased soluble sugar content under severe drought in basil plants (Pirbalouti *et al.*, 2017).

According to the current study's findings as well as those of previous studies, chitosan and Si caused osmoticum accumulation in a variety of plants (Frouk *et al.*, 2022 and Sadak *et al.*, 2023), presumably to improve defense against salt. The physiobio-chemical processes in plants are regulated

by these osmolytes. These pathways include the preservation of membrane integrity, the maintenance of an ideal redox state, the regulation of gene expression that responds to salt stress (Manivannan and Ahn, 2017), and the preservation of plant water status (Abdelaal *et al.*, 2020; Farouk and Omar, 2020). A Si-mediated increase in osmotic might enhanced salt tolerance of sweet basil, due to proline caused reduction in ROS levels, in addition to total soluble sugars as the main soluble solutes which provide energy and carbon backbones needed for cell development and secondary metabolite production (Alamri *et al.*, 2018). Along with better Rubisco and carbonic anhydrase enzyme activities, alterations in proline biosynthesizing and degrading enzymes and/or gene expression may account for the increased osotia buildup under salinity or with Si treatment (Siddiqui *et al.*, 2020). Sustaining elevated levels of indicators related to plant water status improves the metabolic activity sustained by osmotic adjustment.

Moreover, the obtained data demonstrated that the positive effects were increasing gradually by using higher levels of Chitosan and silica increased steadily. These results are consistent with those obtained by (Chibu and Shibayama, 1999; Mondal *et al.*, 2013; Shedeed, 2018) they showed that the morphological and the physiological traits, and yield was improved by using the higher rates of foliar Chitosan and silica. In this regard, (Kamenidou, *et al.*, 2005; Wang *et al.*, 2022) recommended that a number of horticultural plants have been improved through supplementation in Si according to the source, the rate, the Si content, and its deposit in plant tissues which varied from one species to another.

5. Conclusion

With a focus on how it affects the growth, we examined the potential benefits of applying chitosan and silica together to improve salinity tolerance in sweet basil plants. Our results highlight the significant potential of this complementary strategy in reducing the adverse effects of salinity stress imposed by climate change on sweet basil production. Results showed that the application of chitosan and silica together significantly improved a number of physiological markers, such as photosynthetic pigments, endogenous IAA, antioxidant compounds and enzyme activities, osmotic such as TSS, proline and TSN content, Furthermore, in salt-stressed sweet basil plants, the dual treatment showed a significant decrease in hydrogen peroxide, superoxide radicles, and malondialdehyde concentration, This demonstrates how well chitosan and silica work to reduce oxidative stress and improve sweet basil plants' overall tolerance to salt stress. Moreover, critical agronomic variables as essential oil percentages and its most important essential oil constituents, showed a synergistic impact from the combined application of chitosan and silica. Based on these results, it appears that chitosan and silica work together to promote salinity resilience more comprehensively, which in turn improves sweet basil yield.

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