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Physiological and Anatomical Responses of Snap Bean Plant to Foliar Application of Clove Fruit Extract Under Salinity Stress

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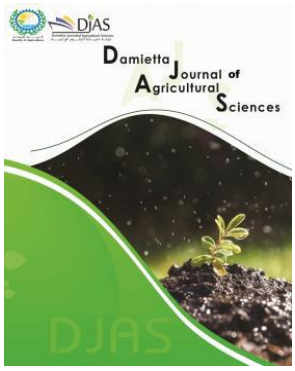

ABSTRACT

In the 2022 and 2023 growing seasons, two pot experiments were conducted at the Faculty of Agriculture, Zagazig University, Egypt. The goal of this study was to investigate the potential of clove fruit extract (CFE) in reducing the negative impacts of salt stress on snap bean plants (Phaseolus vulgaris L.). The salinity treatments included three concentrations: a control with no salt, 100 mM NaCl, and 150 mM NaCl. Foliar treatments included a control (distilled water) and CFE spray prepared by dissolving 10 grams of dried clove fruits in 1 liter of distilled water. The experiments followed a split-plot arrangement within a randomized complete block design with three replicates. Salinity levels were allocated to the main plots, while CFE treatments were assigned to the subplots. Snap bean seeds were sown in plastic pots filled with 8 kg of air-dried clay soil on October 15th, 2022, and October 17th, 2023. Salinity stress significantly elevated oxidative stress indicators such as malondialdehyde (MDA), electrolyte leakage (EL), superoxide (O^{2-}) and hydrogen peroxide (H_2O_2). Additionally, salinity enhanced the accumulation of non-enzymatic antioxidants including alpha tocopherol (α-TOC), glutathione (GSH), and glycine betaine (GB), along with increased activities of antioxidant enzymes like Catalase (CAT), Peroxidase (POD), Ascorbate peroxidase (APX), Superoxide dismutase (SOD), and Glutathione reductase (GR). Severe anatomical damage was observed under high salinity levels. However, the application of clove fruit extract mitigated these harmful effects by reducing oxidative damage, enhancing both enzymatic and non-enzymatic antioxidant defenses, and preserving anatomical structure. Generally, clove fruit extract demonstrated promising effectiveness in alleviating salinity stress in snap bean plants, suggesting its potential use as a natural bio-stimulant to improve crop performance under saline conditions.

Keywords: snap bean, salinity stress, CFE, bio-stimulant

INTRODUCTION

Salinity stress represents a significant obstacle in agriculture, adversely affecting crop yield by exerting both direct and indirect limitations on plant development **(Rady *et al.*, 2013)**. High salt concentrations in the root zone create conditions resembling physiological drought, thereby impairing water absorption and interfering with essential metabolic activities. Furthermore, salinity induces osmotic stress and ion toxicity—mainly due to the excessive buildup of sodium (Na^+) and chloride (Cl^-) ions—which disturbs key physiological and biochemical pathways in plants. This disruption frequently leads to the overproduction of reactive oxygen species (ROS), such as (O_2^-), (H_2O_2), and (OH^-) **(ElSayed *et al.*, 2020; Taha *et al.*, 2020).** Moreover, salt accumulation in the leaf apoplast can trigger cellular dehydration, tissue shrinkage, and eventually lead to cell death. Salinity stress also compromises photosynthesis by reducing chlorophyll levels, promoting stomatal closure, and impairing overall photosynthetic performance (**Desoky *et al.*, 2020).**

Sitohy *et al.* (2020) observed that salinity stress, caused by irrigation water with an electrical conductivity of 7.8 dS m^{-1}, significantly increased sodium (Na^+) accumulation in *Phaseolus vulgaris* plants. This was associated with elevated levels of MDA, alongside enhanced concentrations of proline, soluble sugars, glutathione, and increased activity of antioxidant enzymes. Similarly, **Rady *et al.* (2021)** investigated *Phaseolus vulgaris* L. cultivated in saline soils (EC ranging between 7.55 and 7.61 dS m^{-1}), where they observed detrimental effects including reductions in RWC, MSI, and the levels of free proline, soluble sugars, and endogenous selenium. In contrast, oxidative stress indicators such as O_2^-, H_2O_2, EL, and MDA were significantly elevated. The study also recorded a decline in the activities of key antioxidant enzymes, including CAT,

POX, APX, SOD, and GR. Additionally, **Nobre and Kondo (2022)** emphasized that salinity is a major abiotic factor that disrupts numerous physiological and biochemical functions in plants.

Cowpea (*Vigna unguiculata*) plants exposed to elevated salinity levels (EC = 7 dS m^{-1}) experienced notable declines in several stem anatomical features. These included reduced thickness of the stem epidermis, cortex, phloem, and xylem, as well as a decrease in stem and vessel diameters and a lower number of vascular bundles. Similarly, leaf anatomical traits were significantly impacted under saline conditions. Reductions were recorded in leaf blade thickness, palisade and spongy mesophyll layers, and phloem and xylem thicknesses, along with diminished length and width of the midvein and vessel diameter. These alterations were evident when compared to control plants irrigated with tap water (EC = 0.7 dS m^{-1}) **(Desoky *et al.*, 2020).** In line with these findings, **Sitohy *et al*. (2020)** also reported that salinity stress negatively influenced the anatomical structure of *Phaseolus vulgaris* plants.

Snap beans are considered one of the most important vegetable crops within the Fabaceae family. However, it is known for its sensitivity to salinity stress **(Mass and Hoffman, 1997).**

Considering the detrimental impacts of salinity stress, it becomes essential to implement effective approaches that enhance plant resilience and minimize growth and yield losses. One promising strategy involves the foliar application of plant-based bio-stimulants, such as clove fruit extract (CFE) from *Syzygium aromaticum*, either alone or in conjunction with antioxidants like salicylic acid, owing to their beneficial effects on plant physiological processes **(Calvo *et al.*, 2014; Rady and Mohamed, 2015).** In potato (*Solanum tuberosum* L.) plants subjected to drought conditions in arid environments, foliar treatments with CFE and/or SA resulted in significant improvements in photosynthetic traits, including increased levels of chlorophyll, carotenoids, net photosynthetic rate, transpiration rate, and stomatal conductance. These treatments also enhanced antioxidant defense mechanisms by boosting concentrations of glutathione, proline, ascorbate, soluble sugars, and α-tocopherol, in addition to upregulating the activities of key antioxidant enzymes such as glutathione reductase, peroxidase, superoxide dismutase, catalase, and ascorbate peroxidase. Furthermore, both relative RWC and MSI were significantly enhanced, while markers of oxidative stress—such as EL, MDA, O_2^-, and H_2O_2—were notably decreased under drought stress **(Desoky *et al.*, 2021).**

Foliar spraying with *Ammi visnaga* seed extract (ASE) notably improved anatomical features in cowpea (*Vigna unguiculata*) plants grown under high salinity conditions (EC = 7 dS m^{-1}), when compared to non-treated plants. In the stem, ASE application led to increased thicknesses of the epidermis, cortex, phloem, and xylem, along with larger stem and vessel diameters and a greater number of vascular bundles. Likewise, in leaf tissues, ASE enhanced the blade thickness, palisade and spongy mesophyll layers, and the dimensions of the phloem and xylem. Additionally, it resulted in significant increases in midvein length and width, as well as vessel diameter **(Desoky *et al.*, 2020).** In contrast, salt stress alone was found to negatively affect anatomical structure, growth performance, and yield in *Phaseolus vulgaris* plants (**Sitohy *et al.*, 2020**).

The current study aimed to investigate the potential role of foliar-applied clove fruit extract in modulating the internal physio-chemical composition of snap bean plants subjected to saline conditions. Additionally, the study sought to explore the link between improved physiological traits and anatomical adaptations. The central hypothesis proposed that exogenous application of CFE would enhance the accumulation of specific antioxidants and osmo-protectants, thereby alleviating the damaging effects associated with salt-induced stress.

MATERIALS AND METHODS

Over two consecutive growing seasons (2022 and 2023), two pot experiments were carried out under greenhouse conditions at the Experimental Station of the Faculty of Agriculture, Zagazig University, located in Sharkia Governorate, Egypt. The purpose of the study was to assess the effectiveness of clove fruit extract (CFE) in alleviating salinity-induced stress in snap bean (*Phaseolus vulgaris* L.) plants. The salinity treatments consisted of three levels: non-saline control, 100 mM NaCl, and 150 mM NaCl. CFE treatments (At 20-, 35-, and 50-days post-sowing, three foliar applications were performed during the early morning) included a control (distilled water spray) and a foliar application of CFE prepared by soaking 10 grams of dried clove fruits in 1 liter of distilled water. The experimental layout followed a split-plot design within a completely randomized block structure with three replications. Salinity levels were designated to the main plots, while CFE treatments were applied to the subplots. Snap bean seeds were sown on October 15th, 2022, and October 17th, 2023, in plastic pots filled with 8 kg of air-dried clay soil. The cultivar used, *Phaseolus vulgaris* L. *cv.* Bronco, was obtained from the Vegetative Research Section of the Horticulture Research Institute at the Agricultural Research Center, Giza. Bronco was chosen due to its known sensitivity to salinity and its widespread cultivation across Egypt. Prior to sowing, seeds underwent surface sterilization using 0.1% $HgCl_2$ and were rinsed thoroughly with deionized water. Eight seeds were planted per pot, and thinning was conducted after emergence to maintain four healthy plants in each pot. Soil physical and chemical properties were analyzed for each season based on the methods outlined by **Jackson (1967) and Black *et al*. (1965),** with the results presented in Table 1. All recommended agronomic practices for snap bean cultivation were followed throughout the experiments

A total of 10 grams of dried clove fruits were soaked in one liter of distilled water at 50°C for 24 hours. After the extraction period, the mixture was filtered, and the volume was adjusted to one liter using distilled water. The extract's chemical constituents were then analyzed, and the results are displayed in Table 2.

Table 1: Mechanical and chemical analysis of the soil used

Mechanical analysis			Chemical analysis										
Coarse Sand (%)	Silt (%)	Clay (%)	Cations (mg/100g soil)				Anion (mg/100g soil)				E.C 25°C (ds m^{-1})	pH	W. H. C.
			Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{-}	HCO_3^{-}	Cl^{-}	SO_4^{--}			
52.95	27.95	19.3	3.0	1.8	2.5	0.1	0.00	0.5	1.18	5.72	2.96	7.71	34.64

Table 2. Selected chemical constituents of CFE on a dry weight basis.

Component	Unit	Value
1- Total phenolic compounds (TPC)	**mg GAE/g CFE**	**323.79**
2- Total flavonoids (TF)	**mg QE/g CFE**	**34.65**
3- Phenolic compounds		
3,4-Dihydroxybenzoic-acid	**mg/ g CFE**	**0.74**
Ellagic-acid		**0.62**
Eugenol		**104.7**
Eugenyl-acetate		**86.39**
Gallic-acid		**18.33**
Naphthalene		**0.21**
Tannic acid		**0.78**
Vanillin		**1.49**
4- Antioxidants and osmoprotectants:		
Total free amino acid	**g/Kg DW**	**70.2**
Free Proline	**mg/Kg DW**	**19**
Soluble sugars	**mg/Kg DW**	**55.6**
5- Mineral nutrients		
Mg	**g/Kg DW**	**3.2**
Ca	**g/Kg DW**	**12.3**
Fe	**g/Kg DW**	**1.3**
P	**g/Kg DW**	**11.8**
K	**g/Kg DW**	**16.5**
N		**16.9**
6- Vitamins		
Vitamin A	**mg/Kg DW**	**25.6**
Vitamin E	**mg/Kg DW**	**55.2**
Vitamin D	**mg/Kg DW**	**32.4**
Vitamin C	**mg/Kg DW**	**36.9**

Foliar applications of CFE were conducted at 20, 35, and 50 days after sowing. Three applications were made during the early morning using a 20-liter dorsal sprayer, ensuring thorough coverage to the point of runoff. The treatments included distilled water (control) and a 5% CFE solution. To improve the absorption of the spray solution by the leaf tissues, 0.1% (v/v) Tween-20 was added as a surfactant to each solution. At 55 days of post-sowing, samples of snap bean plants collected from each experimental pot. To preserve the integrity of the root system the soil on roots carefully removed using a gentle stream of water. then plants separated into stems and leaves. The following data were recorded:

1. Assessment of electrolyte leakage (EL).

Total content of inorganic ions leached from the leaves was determined using the method outlined by **Sullivan and Ross (1979).**

2. Measurement of hydrogen peroxide and superoxide concentrations.

Superoxide (O_2^-) levels were measured by incubating leaf fragments for one hour in a solution containing 0.05% NBT, 10 mM NaN_3, and 10 mM potassium phosphate buffer (pH 7.8). Following incubation, mixture heated at 85°C for 15 minutes and then rapidly cooled. Absorbance subsequently measured at 580 nm **(Kubiś, 2008).**

3. Malondialdehyde determination (MDA).

The concentration of malondialdehyde (MDA) (μmol g^{-1} fresh weight) was quantified by

first crushing 0.1g leaf material in a sodium phosphate buffer. The mixture then subjected to centrifugation at 20,000×g for 25 minutes under chilling conditions. The optical density of clear supernatant was measured at 532nm, any background interference corrected by deducting the absorbance at 600 nm, according to **Heath and Packer (1968).**

4. Quantitative assessment of non-enzymatic antioxidant compounds.

High-performance liquid chromatography (HPLC), employing a methanol–water solvent system and ultraviolet detection at 292 nm, utilized to measure α-tocopherol (α-TOC) levels, in accordance with the protocols **of Konings *et al.* (1996) and Ching and Mohamed (2001).** Dtermination of ascorbic acid (AsA) content followed the procedure outlined by **Kampfenkel *et al.* (1995),** involving a specific reagent blend and measuring the absorbance at 525 nm. Glutathione concentration was evaluated using the approach introduced by **Griffith (1980),** which involves the reaction with DTNB and absorbance detection at 412 nm. Glycine betaine amounts estimated using the technique reported by **Di Martino *et al*. (2003).**

5.Determination of enzymatic antioxidant compounds.

5.1 Catalase (CAT) Assay.

Catalase (CAT) enzymatic activity was assessed through spectrophotometric analysis, following the methodology established by **Chance and Maehly (1955).**

5.2 Peroxidase Activity Assay.

Peroxidase activity in *Phaseolus vulgaris* foliage evaluated using the method described by **Thomas *et al.* (1982).**

5.3 Ascorbate Peroxidase (APX) Activity Assay.

APX activity determined through spectrophotometric evaluation, employing the protocol developed by **Fielding and Hall (1978).**

5.4 Superoxide Dismutase (SOD) Activity Assay

Superoxide dismutase activity measured by observing the decrease in absorbance of the superoxide-nitro blue tetrazolium (NBT) complex, following the method outlined by **Sairam *et al*. (2002).**

5.5 Glutathione reductase (GR) Activity Assay

GR activity assessed by monitoring the NADPH oxidation, with absorbance measurements recorded at 340 nm. Enzyme activity was reported as A564 min^{-1} mg^{-1} protein, based on the procedure outlined by **Rao *et al*. (1996).**

6. Anatomical studies.

Histological examinations conducted during the booting phase in the second growing season, using two plants from each treatment. Samples collected from leaflet blade of the fourth upper compound leaf on the main stem and cross-sectional slices of the fourth internode of the main stem of snap bean plants subjected to various treatments. These samples fixed in FAA solution, dehydrated with butyl alcohol, and embedded in paraffin wax. Thin slices, approximately 14 microns (µm) in thickness, were obtained using a rotary microtome. Paraffin sections were placed on glass slides, stained with safranin and light green; then. cleared in xylene and mounted in Canada balsam, following the procedure described by **Nassar and El-Sahhar (1998).** The prepared slides were observed under a light microscope, and images were captured using a Canon PowerShot S80 digital camera connected to a computer with the Zoom Browser EX program. The prepared sections examined to evaluate histological alterations and photomicrographs were captured. The following parameters were measured in microns as follows: 1) the leaf: thickness of blade, mesophyll tissue, midrib, and sclerenchyma, as well as the width and thickness of the midrib vascular bundle and sclerenchyma tissue; 2) the stem: the thickness of the epidermis , cortex, phloem, and xylem, the stem diameter, vessel diameter, and the count of vascular bundles.

Statistical analysis

Statistical evaluation was performed using analysis of variance (ANOVA), variations comparisons were conducted using Fisher's Least Significant Difference (LSD) test at 0.05 level, as outlined by **Sokal and Rohlf (1980).1984).**

RESULTS AND DISCUSSION

The findings from two successive growing seasons emphasized the impact of three concentrations of CFE on the physiological and structural characteristics of snap bean plants exposed to different degrees of salinity stress. The outcomes observed in both seasons are examined and addressed in the following sections:

1. Physiological and biochemical properties

1.1 Electrolyte Leakage (EL), Malondialdehyde (MDA), Superoxide (O_2^-), and Hydrogen Peroxide (H_2O_2) Content.

As indicated in Table 3, salinity stress led to rise in EL, MDA, $O_2^{\bullet-}$, and H_2O_2 during both growing seasons. The most substantial increases were recorded under high salt stress (150 mM NaCl), with EL rising by 102.4% and 89.7%, MDA by 180.5% and 179.8%, $O_2^{\bullet-}$ by 97.8% and 90.7%, and H_2O_2 by 73.2% and 73%, in the first and second seasons, respectively. It is well-known that reduced stomatal conductance limits CO_2 fixation, while electron transfer and light-dependent photosynthetic reactions remain unaffected. Nevertheless, the shortage of $NADP^+$ (an electron acceptor) compels the use of O_2 as a substitute, which results in generation of reactive oxygen species (ROS), such as hydroxyl radicals (OH^-), hydrogen peroxide (H_2O_2), and the superoxide ($O_2^{\bullet-}$). This subsequently triggers lipid peroxidation in the cellular membranes and an increase in electrolyte leakage (EL) under stressed conditions (**Yadavi *et al.*, 2014).** These findings align with **Sofy *et al.* (2020) and Rady *et al.* (2021),** who also found elevated levels of oxidative stress

markers ($O_2^{\bullet-}$ and H_2O_2), EL, and MDA in plants under salt stress.

As shown in Table 3, CFE foliar application decreased significantly the levels of EL, MDA, $O_2^{\bullet-}$, and H_2O_2 in both growing seasons. The application of CFE reduced EL by 17.8% and 19.1%, MDA by 22.1% and 22.8%, $O_2^{\bullet-}$ by 17.8% and 18.8%, and H_2O_2 by 13.9% and 14.4% respectively, in the first and second seasons. Previous studies have shown that CFE can help mitigate EL and MDA levels, as ROS-induced membrane lipid peroxidation and subsequent EL are common responses to stress conditions **(Allen *et al.*, 1997).** These results align with those of **Desoky *et al*. (2021),** who found that foliar applications of salicylic acid (SA) and/or CFE in potato plants reduced significantly EL, MDA, $O_2^{\bullet-}$, and H_2O_2 levels under stress.

The data presented in Table 3 emphasize combined impact of foliar CFE under varying levels of salinity stress on EL, MDA, $O_2^{\bullet-}$, and H_2O_2 of snap bean plants. The use of CFE successfully mitigated the salinity stress adverse effects, resulting in notable decreases in EL, MDA, $O_2^{\bullet-}$, and H_2O_2 compare to control. These findings consistent with that reported by **Baghizadeh *et al.* (2009) and Desoky *et al*. (2020, 2021).**

1.2 Activity of Antioxidant Enzymes: Superoxide Dismutase (SOD), Peroxidase (POX), Catalase (CAT), Ascorbate Peroxidase (APX), and Glutathione Reductase (GR)

Salinity stress increased significantly the activity of various antioxidant enzymes, (POX, CAT, APX, SOD, and GR),in both growing seasons (Table 4). The greater enzyme activities was recorded under then higher salinity stress (150 mM NaCl), with increases of 87.5% and 92.4% for POX; 14.8% and 14.5% for CAT; 19.8% and 20.1% for APX; 132.1% and 136.4% for SOD; and 62.8% and 62.3% for GR, respectively, in the first and second seasons (Table 4). Plants employ different mechanisms to manage the abiotic stress; one of which includes enhancement of the activity of antioxidant enzymes such as CAT, SOD, and POX, crucial for eliminating ROS **(Parihar *et al.*, 2015).** Moreover, many studies demonstrated that plants mitigate oxidative damage caused by ROS by elevating the antioxidant enzymes activity like SOD, POX, CAT, and APX, which work to neutralize ROS and minimize cellular damage (**Hameed *et al.*, 2021; Imran *et al.*, 2020; Saleem et al., 2020a, 2020b, 2020c).**

Clove fruit extract boosted the antioxidant enzymes activity raising POX by 13.1% and 12.2%; CAT by 3.36% and 3.31%; APX by 3.80% and 3.71%; SOD by 25.3% and 24.2%; and GR by 7.75% and 7.54%, respectively, in the first and second seasons (Table 4). The observed enhancement in the activity of antioxidant enzyme could be due to the increased levels of secondary metabolites (phenolics and flavonoids), which assist plants in managing abiotic stresses (**El-Amier *et al.*, 2019).** For instance, CFE application on drought-stressed potato, resulted in the activation of catalase that helped in reducing oxidative damage by lowering H_2O_2 and MDA concentrations. Furthermore, CFE boosted SOD activity, facilitating O_2^-conversion to H_2O_2, and also increased the activities of POX, APX, and GR (**Desoky *et al.*, 2021).** Additionally, the combination of CFE with silymarin resulted in the highest enzyme activity (**Alharby *et al.*, 2021; Desoky *et al.*, 2021; Ali *et al.*, 2022).**

Table 4 displays information on the interaction between foliar treatment with CFE and different salinity stress levels on activities of SOD, POX, CAT, APX, and GR in snap bean plants. CFE application mitigated the salinity stress adverse impacts, leading to considerable increases in the activities of SOD, POX, CAT, APX, and GR compared to the control in both growing seasons. These findings agree with that reported by **Desoky *et al.* (2020; 2020).**

1.3 Content of non-enzymatic antioxidants (α-tocopherol, α TOC; ascorbic acid, AsA; glutathione, GSH; and glycine betaine, GB).

Salinity stress notably increased α-TOC, AsA, GSH, and GB levels in both growing seasons (Table 5). Under the higher salt stress (150 mM NaCl), the highest accumulation was recorded, with α-TOC increasing by 63.2% and 65%; AsA by 45.2% and 48.4%; GSH by 103.2% and 106.4%, and GB by 36.3% and 35.4%, respectively, in the first and second seasons. Plants have sophisticated defense mechanism against oxidative stress, comprising both the enzymatic and non-enzymatic antioxidants that counteract ROS in different cellular regions. However, when ROS generation surpasses the protective capacity of antioxidants, cellular damage takes place. Non-enzymatic antioxidants like ascorbic acid (AsA), glutathione (GSH), carotenoids, tocopherols, and flavonoids are crucial in mitigating oxidative stress throw scavenging ROS **(Gill and Tuteja, 2010).** These antioxidants, together with enzymatic ones, have a crucial role in the plant's defensive mechanism **(Foyer and Noctor, 2011).**

GSH participates in numerous cellular functions, such as antioxidant protection, detoxification of foreign compounds, regulation of the cell cycle, apoptosis, cysteine storage, maintenance of redox balance, immune response, and fibrogenesis **(Hasanuzzaman *et al.*, 2017)**. Stressful conditions lead to increase in free radicals production, resulting in oxidative damage and alterations in antioxidant activity **(Taha, 2016**). The observed increase in AsA and GSH levels under salinity stress, compared to non-stressed controls, aligns with findings of **Desoky *et al.* (2021**). Furthermore, glycine betaine serves as an important ROS scavenger **(Hasanuzzaman *et al.*, 2014).**

Clove fruit extract increased the levels of α-TOC by 16.1% and 15.7%; AsA by 8.1% and 7.1%;

GSH by 11.9% and 12.3%; and GB by 5.9% and 6.1%, respectively, in the first and second growing seasons. Regarding the influence of phenolic compounds, previous research has shown that extracts from *Carthamus tinctorius*, which contain phenolic components, help reduce the accumulation of $O_2^{\bullet-}$ and H_2O_2 under stress conditions. This reduction is attributed to the ability of phenolic compounds to enhance non-enzymatic antioxidants like AsA, which effectively scavenge $O_2^{\bullet-}$ and H_2O_2 (**Salem *et al.*, 2014).** Additionally, the application of gallic acid (GAL), a key phenolic component of CFE, has been demonstrated to increase AsA and GSH levels in rice plants under stress, offering protection against excessive ROS accumulation **(Ozfidan-Konakci *et al.*, 2015).**

Combined use of CFE and silymarin increased proline, soluble sugars, AsA, α-TOC, and GSH levels in wheat plants stressed by cadmium **(Semida *et al.*, 2020).** Furthermore, analysis of CFE has identified various bioactive compounds, including flavonoids, phenolics, antioxidants, osmo-protective substances, essential nutrients, and vitamins **(Desoky *et al.*, 2021).** These diverse components play a crucial role in boosting non-enzymatic antioxidants such as α-TOC, AsA, GSH, and GB, which collectively enhance the plant's ability to tolerate stress.

Table 5 illustrates the combined effects of foliar CFE application under varying levels of salinity stress on: EL, MDA, $O_2^{\bullet-}$, and H_2O_2 in snap bean plants. The use of CFE mitigated effectively the detrimental effects of salinity stress, resulting in a significant increase in α-TOC, AsA, GSH, and GB across both growing seasons compared to the control. Also, our findings agree with that of **Arora *et al.* (2020), Desoky *et al.* (2020, 2021), Loudari *et al.* (2023), and Moradbeygi *et al*. (2020).**

2. Anatomical features

Anatomical analysis was performed in the second growing season (2023), with the results presented as follows.

2.1 Leaf anatomy

The data presented in Table 6 and Fig. 1 reveal that snap bean plants irrigated with tap water (control) and treated with foliar CFE showed notable improvements in all anatomical characteristics of snap bean leaf. The increases compared to the control were 32%, 29.8%, 17.4%, 32.5%, 22.3%, 20.8%, 51.5%, and 47.5% for blade thickness, palisade thickness, spongy tissue thickness, midvein length, midvein width, phloem thickness, xylem thickness, and vessel diameter, respectively. The results also suggest that foliar CFE application effectively mitigated the detrimental effects of salt stress (100 mM NaCl) on snap bean plants. In plants irrigated with 100 mM NaCl and not treated with CFE, reductions in leaf features were observed as follows: 25.7% for blade thickness, 27.9% for palisade thickness, 24.1% for spongy thickness, 1.87% for midvein length, 28% for midvein width, 22.9% for phloem thickness, 17.3% for xylem thickness, and 5.88% for vessel diameter. However, when plants were irrigated with 100 mM NaCl and sprayed with CFE, the reductions were much less, with decreases of 4.13%, 14.6%, 12.9%, 0.93%, 15.4%, 11.5%, 4.44%, and 1.36% for blade thickness, palisade thickness, spongy thickness, midvein width, phloem thickness, xylem thickness, and vessel diameter, respectively, compared to the control.

The data also reveal that salt stress (150 mM NaCl) caused significant reductions in several anatomical features of snap bean plants, including a 39.5% decrease in leaflet blade thickness, a 42.6% reduction in palisade thickness, a 46.3% decrease in spongy tissue thickness, a 30.8% reduction in midvein length, a 40.2% decrease in midvein width, a 36.9% reduction in phloem thickness, a 31.9% decrease in xylem thickness, and a 33.9% reduction in vessel diameter. However, when plants subjected to 150 mM NaCl stress were foliar sprayed with CFE, the negative effects of salinity were mitigated across all these anatomical parameters.

In this investigation, CFE improved the leaf anatomical traits of snap bean plants exposed to salt stress, suggesting that CFE aids in alleviating salinity harmful effects on leaf structure. So, promoted effective transport of assimilates and nutrients into plant cells, supporting various metabolic functions that contribute to healthy growth and satisfactory yields despite salinity stress. Foliar spraying with CFE also stimulated the development of protective leaf tissues, boosting ability to endure dehydration in the plant. In a similar study, **Rady *et al.* (2019)** reported that foliar applications of natural extracts and bio-stimulants, such as licorice (Glycyrrhiza glabra) root extract (LRE) which were rich in antioxidants, improved significantly the anatomical characteristics of salt-stressed snap bean plants. This treatment resulted in increases of blade thickness; palisade tissue thickness; spongy tissue thickness; midvein length and width; phloem and xylem thickness; and vessel diameter compared to the control.

2.2 Stem anatomy

The results shown in Table 7 and Fig. 2 indicate that snap bean plants irrigated with tap water (control) and treated with foliar CFE demonstrated considerable enhancements in all stem anatomical characteristics. The increases in these features were 23.7% for epidermis thickness, 20.9% for cortex thickness, 35.5% for phloem thickness, 25.3% for xylem thickness, 7.14% for stem diameter, 25.2% for vessel diameter, and 20.8% for the number of vascular bundles compared to the control,.

The results show that foliar application of CFE effectively alleviated negative impacts of salt stress (100 mM NaCl). In plants irrigated with 100 mM NaCl without CFE treatment, the reductions in stem anatomical features compared to the control (tap water + DW) were as follows: a 20.1% decrease in epidermis thickness, 37.5% in cortex thickness,

20.7% in phloem thickness, 14.3% in xylem thickness, 6.98% in stem diameter, 26.7% in vessel diameter, and 10.5% in the number of vascular bundles. However, plants exposed to the same saline conditions but treated with CFE, the reductions were less severe, with decreases of 6.22% in cortex thickness, 16.3% in phloem thickness, 4.78% in xylem thickness, 1.99% in stem diameter, 4.46% in vessel diameter, and 5.26% in the number of vascular bundles. Notably, the epidermis thickness in the CFE-treated plants exhibit non significant difference compared to the control.

The data clearly demonstrate that salt 150 mM NaCl treatment substantially impaired stem anatomical features. Salt stress caused reductions of 44% in epidermis thickness, 48.3% in cortex thickness, 36.4% in phloem thickness, 30.5% in xylem thickness, 91.5% in stem diameter, 32.6% in vessel diameter, and 26.3% in the number of vascular bundles compared to the control. Nevertheless, foliar treatment with CFE under identical salt stress conditions significantly reduced these harmful effects, resulting in enhanced values for all the assessed parameters. In this investigation, foliar CFE notably improved the stem anatomical features in salt-stressed snap bean plants, indicating that CFE helps mitigate salt stress detrimental effects on stem structure. These anatomical enhancements facilitated more efficient assimilates and nutrients movement into the cells, which used in various metabolic activities. This, in turn, positively influenced plant growth and contributed satisfactory yield under salt stress conditions. Furthermore, CFE treatment facilitated formation of protective tissues in the leaves and boosting the plant's capacity to resist dehydration. In this concern, **Taha (2016)** reported that moringa leaf extract foliar spray (MLE) enhanced the anatomical structure of sunflower stems under moderate soil salinity. Also, Combined application of MLE (seed soaking + foliar spray) proved particularly effective, resulting in significant improvements in stem anatomy, such as increased stem diameter, greater number of xylem vessels, as well as their thickness and diameter, in addition to enhanced pith diameter and the number of pith layers. This underscores the potential of MLE in promoting structural development and strengthening plant resilience under saline stress.

Table 3. Effect of salinity stress, foliar CFE treatment, and their interactions on EL, MDA, O_2^-, and H_2O_2 concentrations of snap bean plants during the 2022 and 2023 growing seasons

Parameter / Treatments		EL (%)		MDA (µmol g^{-1})		$O_2^{\bullet-}$ (µmol g^{-1} FW)		H_2O_2 (µmol g^{-1} FW)	
Effect of salinity		**First season**	**Second season**	**First season**	**Second season**	**First season**	**Second season**	**First season**	**Second season**
Control		6.67±^{c}	6.59±^{c}	0.87±^{c}	0.84±^{c}	0.45±^{c}	0.43±^{c}	1.42±^{c}	1.37±^{c}
100 Mm NaCl		10.4±^{b}	9.37±^{b}	1.78±^{b}	1.71±^{b}	0.67±^{b}	0.62±^{b}	1.96±^{b}	1.89±^{b}
150 Mm NaCl		13.5±^{a}	12.5±^{a}	2.44±^{a}	2.35±^{a}	0.89±^{a}	0.82±^{a}	2.46±^{a}	2.37±^{a}
Effect of foliar spray									
DW		11.2±^{a}	10.5±^{a}	1.90±^{a}	1.84±^{a}	0.73±^{a}	0.69±^{a}	2.09±^{a}	2.02±^{a}
CFE		9.21±^{b}	8.49±^{b}	1.48±^{b}	1.42±^{b}	0.60±^{b}	0.56±^{b}	1.80±^{b}	1.73±^{b}
Effect of interaction									
Salinity	**Foliar spraying**								
Control	**DW**	7.18±^{e}	7.10±^{e}	0.94±^{d}	0.91±^{d}	0.49±^{e}	0.47±^{e}	1.55±^{e}	1.50±^{e}
	CFE	6.16±^{f}	6.08±^{f}	0.80±^{e}	0.77±^{e}	0.41±^{f}	0.39±^{f}	1.29±^{f}	1.24±^{f}
100 Mm NaCl	**DW**	11.6±^{c}	10.6±^{c}	2.01±^{b}	1.94±^{b}	0.75±^{c}	0.70±^{c}	2.15±^{c}	2.08±^{c}
	CFE	9.18±^{d}	8.15±^{d}	1.54±^{c}	1.47±^{c}	0.58±^{d}	0.53±^{d}	1.77±^{d}	1.70±^{d}
150 Mm NaCl	**DW**	14.7±^{a}	13.7±^{a}	2.76±^{a}	2.67±^{a}	0.95±^{a}	0.88±^{a}	2.58±^{a}	2.49±^{a}
	CFE	12.3±^{b}	11.2±^{b}	2.11±^{b}	2.02±^{b}	0.82±^{b}	0.75±^{b}	2.33±^{b}	2.24±^{b}
ANOVA	**Df**	***P-value***							
S	**2**	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
F	**1**	0.0000	0.0000	0.0000	0.0000	0.0000	0.0003	0.0000	0.0000
S.F	**2**	0.0001	0.0001	0.0002	0.0002	0.0030	0.0030	0.0115	0.0115

EL= electrolyte leakage, MDA= malondialdehyde), O2•−= superoxide, and H2O2= hydrogen peroxide

Table 4. Influence of salinity stress, foliar CFE application, and their interactions on the activities of POX, CAT, APX, SOD, and GR in snap bean plants during the 2022 and 2023 growing seasons.

Parameter / Treatments		POX (A470 µmol mg^{-1} protein)		CAT (A240 µmol mg^{-1} protein)		APX (µmol mg^{-1} protein)		SOD (A560 µmol mg^{-1} protein)		GR (A340 µmol mg^{-1} protein)	
Effect of salinity		First season	Second season	First season	Second season	First season	Second season	First season	Second season	1st season	2nd season
Control		0.88±^{c}	0.92±^{c}	61.6±^{c}	62.7±^{c}	51.1±^{c}	52.2±^{c}	3.40±^{c}	3.49±^{c}	30.9±^{c}	31.8±^{c}
100 Mm NaCl		1.38±^{b}	1.47±^{b}	67.3±^{b}	68.3±^{b}	56.7±^{b}	58.1±^{b}	5.40±^{b}	5.63±^{b}	43.4±^{b}	44.5±^{b}
150 Mm NaCl		1.65±^{a}	1.77±^{a}	70.7±^{a}	71.8±^{a}	61.2±^{a}	62.7±^{a}	7.89±^{a}	8.25±^{a}	50.3±^{a}	51.6±^{a}
Effect of foliar spray											
DW		1.22±^{b}	1.31±^{b}	65.4±^{b}	66.5±^{b}	55.3±^{b}	56.6±^{b}	4.94±^{b}	5.16±^{b}	40.0±^{b}	41.1±^{b}
CFE		1.38±^{a}	1.47±^{a}	67.6±^{a}	68.7±^{a}	57.4±^{a}	58.7±^{a}	6.19±^{a}	6.41±^{a}	43.1±^{a}	44.2±^{a}
Effect of interaction											
Salinity	Foliar feeding										
Control	DW	0.82±^{f}	0.86±^{f}	60.7±^{f}	61.7±^{f}	50.4±^{f}	51.5±^{f}	3.24±^{f}	3.33±^{f}	30.3±^{f}	31.2±^{f}
	CFE	0.93±^{e}	0.97±^{e}	62.6±^{e}	63.6±^{e}	51.8±^{e}	52.9±^{e}	3.56±^{e}	3.65±^{e}	31.5±^{e}	32.4±^{e}
100 Mm NaCl	DW	1.28±^{d}	1.37±^{d}	65.8±^{d}	66.9±^{d}	55.8±^{d}	57.1±^{d}	4.37±^{d}	4.60±^{d}	41.6±^{d}	42.7±^{d}
	CFE	1.48±^{c}	1.57±^{c}	68.7±^{c}	69.8±^{c}	57.6±^{c}	59.1±^{c}	6.42±^{c}	6.65±^{c}	45.2±^{c}	46.3±^{c}
150 Mm NaCl	DW	1.56±^{b}	1.68±^{b}	69.7±^{b}	70.8±^{b}	59.7±^{b}	61.2±^{b}	7.21±^{b}	7.57±^{b}	48.1±^{b}	49.4±^{b}
	CFE	1.74±^{a}	1.86±^{a}	71.6±^{a}	72.7±^{a}	62.7±^{a}	64.2±^{a}	8.58±^{a}	8.94±^{a}	52.5±^{a}	53.8±^{a}
ANOVA	Df	*P-value*									
S	2	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
F	1	0.0001	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
S.F	2	0.1016	0.1016	0.0379	0.0379	0.0013	0.0013	0.0001	0.0001	0.0016	0.0016

Table 5. Influence of salinity stress, foliar application of CFE, and their interactions on the levels of α-TOC, AsA, GSH, and GB in snap bean plants during the 2022 and 2023 growing seasons

Parameter / Treatments		***α*-TOC (µmol g^{-1} DW)**		**AsA (µmol g^{-1} FW)**		**GSH (µmol g^{-1} FW)**		**Glycine betaine (µg g^{-1} DW)**	
Effect of salinity		First season	Second season	First season	Second season	First season	Second season	First season	Second season
Control		2.04±^{c}	2.06±^{c}	1.26±^{c}	1.28±^{c}	0.92±^{c}	0.94 ±^{c}	41.6±^{c}	42.7±^{c}
100 Mm NaCl		2.70±^{b}	2.75±^{b}	1.56±^{b}	1.61±^{b}	1.47±^{b}	1.52±^{b}	51.2±^{b}	52.2±^{b}
150 Mm NaCl		3.33±^{a}	3.40±^{a}	1.83±^{a}	1.90±^{a}	1.87±^{a}	1.94 ±^{a}	56.7±^{a}	57.8±^{a}
Effect of foliar spray									
DW		2.49±^{b}	2.54±^{b}	1.49±^{b}	1.54 ±^{b}	1.34±^{b}	1.38±^{b}	48.4±^{b}	49.4±^{b}
CFE		2.89±^{a}	2.94±^{a}	1.61±^{a}	1.65±^{a}	1.50±^{a}	1.55 ±^{a}	51.3±^{a}	52.4 ±^{a}
Effect of interaction									
Salinity	**Foliar feeding**								
Control	**DW**	1.93±^{f}	1.95±^{f}	1.21±^{f}	1.23±^{f}	0.87±^{f}	0.89±^{f}	40.5±^{f}	41.5±^{f}
	CFE	2.15±^{e}	2.17±^{e}	1.31±^{e}	1.33 ±^{e}	0.97±^{e}	0.99±^{e}	42.8±^{e}	43.8±^{e}
100 Mm NaCl	**DW**	2.46±^{d}	2.51±^{d}	1.52±^{d}	1.57±^{d}	1.37±^{d}	1.42±^{d}	49.7±^{d}	50.7±^{d}
	CFE	2.94±^{c}	2.99±^{c}	1.60±^{c}	1.65±^{c}	1.56±^{c}	1.61±^{c}	52.7±^{c}	53.7 ±^{c}
150 Mm NaCl	**DW**	3.08±^{b}	3.15±^{b}	1.74±^{b}	1.81±^{b}	1.77±^{b}	1.84±^{b}	54.9±^{b}	56.0±^{b}
	CFE	3.59±^{a}	3.66±^{a}	1.91±^{a}	1.98±^{a}	1.96±^{a}	2.03±^{a}	58.6±^{a}	59.6±^{a}
ANOVA	**Df**	***P-value***							
S	**2**	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
F	**1**	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
S.F	**2**	0.0008	0.0008	0.0276	0.0276	0.0000	0.0000	0.0040	0.0040

α-TOC = alpha tocopherol, **AsA** = ascorbic acid, GSH = glutathione, and GB = glycine betaine.

Table 6. Effect of salt stress, foliar application of CFE, and their combined effects on the count and measurements of specific anatomical features in cross-sections of the fourth upper compound leaf's leaflet blade on the main stem of snap bean plants during the second growing season (2023).

Parameter / Treatments		Blade thickness (µ)	Palisade thickness (µ)	Spongy thickness (µ)	Midvein Lenth (µ)	Midvein width (µ)	Phloem thickness (µ)	Xylem thickness (µ)	Diameter of vessel average (µ)
Effect of salinity									
Control		237.4±4a	86.4±2a	97.9±5a	1245±8a	1130±9a	190.1±5a	237.4±6a	31.3±2a
100 Mm NaCl		172.0±3b	56.5±2b	71.9±4b	975±6b	800±8b	140.1±3b	140.1±5b	21.3±1b
150 Mm NaCl		164.9±2c	46.8±3c	67.1±3c	895±5c	751 ±6c	119.3±4c	118.8±5c	17.7±1c
Effect of foliar spray									
DW		247.5±6b	82.6±2b	109.04±b	1340±12b	1206±8b	203.6±5b	197.3±5b	28.4±2b
CFE		326.8±4a	107.2±3a	128.0±2a	1775±15a	1475±9a	246.0±3a	299.0±4a	41.9±3a
Effect of interaction									
Salinity	Foliar spray								
Control	DW	210.8±3b	72.4±2b	95.4±4b	1070±13b	1040±12b	170.2±4b	157.6±6b	22.1±.5b
	CFE	264±5a	100.4±5a	100.4±5a	1430±11a	1220±16a	210.1±3a	317.2±8a	25.8±.6a
100 Mm NaCl	DW	156.6±6d	52.2±3d	72.4±4d	1050±14d	749±14d	131.3±4d	130.3±2d	20.8±.9d
	CFE	202.1±5c	61.8±4c	83.12±3c	1060±9c	880±7c	150.6±3c	150.6±6c	21.8±.9c
150 Mm NaCl	DW	127.6±9f	41.56±2f	51.2±2f	740 ±8f	622±8f	107.4±5f	107.4±3f	14.6±.6f
	CFE	187.5±5e	51.2±3e	71.4±3e	879±8e	850±9e	129.6±2e	129.6±4e	20.4±.4e

Table 7. Effect of salinity stress, foliar application of CFE, and their interactions on the counts and measurements of specific anatomical features in transverse sections of the fourth internode of the main stem in snap bean plants during the second growing season (2023).

Parameter / Treatments		Epidermis thickness (µ)	Cortex thickness (µ)	Phloem thickness (µ)	Xylem thickness (µ)	Stem diameter (µ)	Vessel diameter (µ)	No. of vascular bundle (µ)
Effect of salinity								
Control		57.5±2a	172.5±5a	68.5±3a	266.7±9a	5544±16a	178.1±6a	21.0±.5a
100 Mm NaCl		47.0±3b	130.7±4b	57.2±2b	198.7±8b	5281±1b	141.8±5b	16.5±.4b
150 Mm NaCl		33.4±.9c	92.5±3c	44.6±2c	166.1±5c	482±9c	115.4±3c	15.5±.8b
Effect of foliar spray								
DW		61.7±2b	179.2±5b	72.3±6b	280.3±8b	5459±9b	193.3±6b	24±.4b
CFE		76.3±4a	216.6±9a	98±3a	351.3±9a	5849±11a	242.1±9a	29±.6a
Effect of interaction								
salinity	Foliar spray							
Control	DW	52.3±3b	167.3±6b	63.8±.8b	219.7±3b	5472±13b	163.6±4c	19±.3b
	CFE	62.7±5a	177.8±4a	83.6±.9a	313.8±7a	5726±18a	200.0±5a	23±.6a
100 Mm NaCl	DW	41.8±3c	104.6±3d	50.6±.3d	188.3±6d	5090±16d	120.0±6d	17±.6d
	CFE	52.3±2b	156.9±2c	53.4±.8c	209.2±7c	5363±12c	156.3±3c	18±.8c
150 Mm NaCl	DW	29.3±.5e	86.5±3f	40.6±.4f	152.6±5f	465±11f	110.3±3f	14±.4f
	CFE	37.6±.6d	98.6±5e	48.6±.6e	179.6±6e	499±12e	120.6±2e	15±.5e

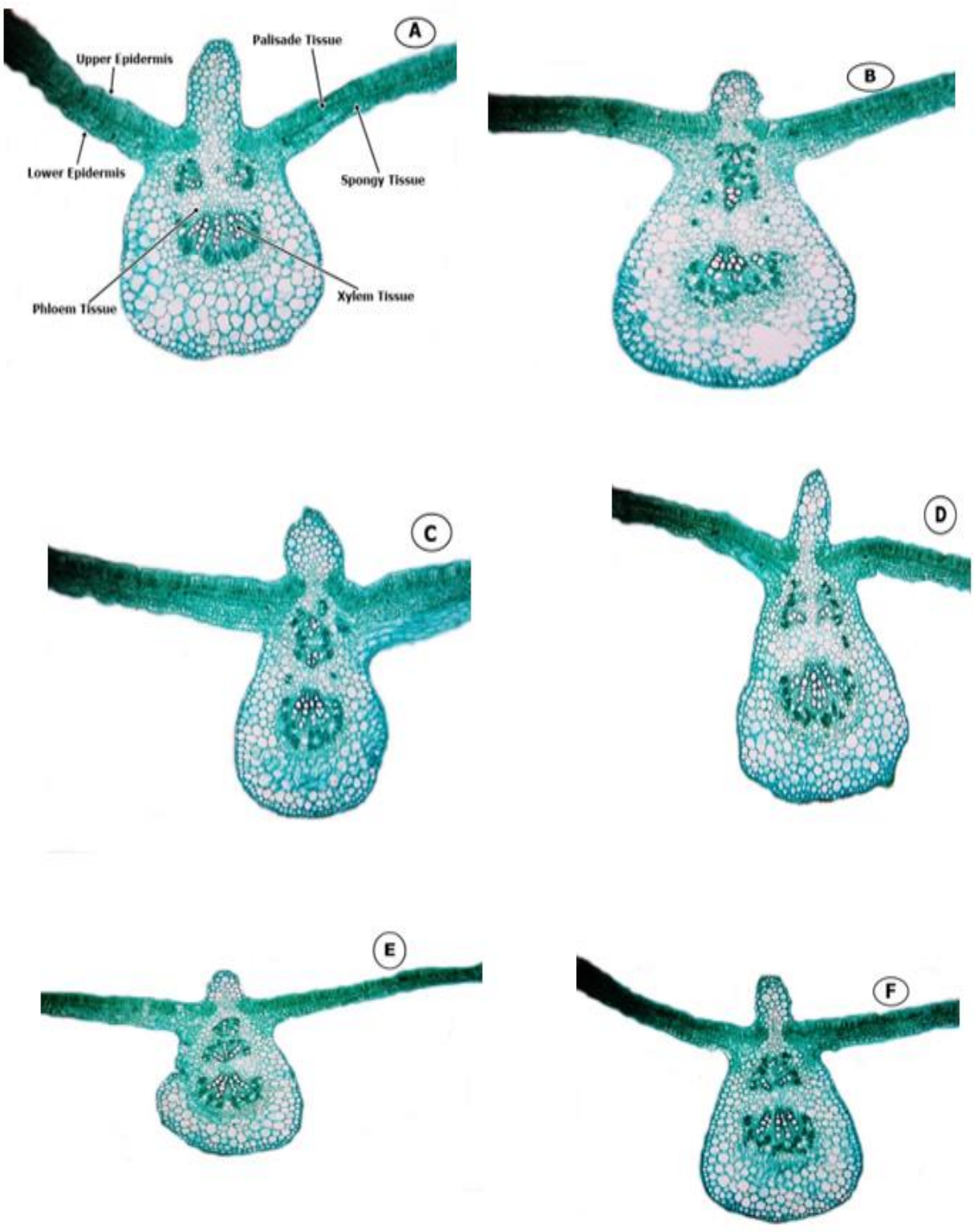

Fig. 1: Cross-section of the leaflet blade of snap bean plants exposed to the combined treatments of foliar application with CFE and salinity stress during the second growing season (2023).

(**A**) CFE Irrigation with tap water + foliar spray with distilled water (**B**) Irrigation with tap water + foliar spray with (**B**) Irrigation with 100 Mm NaCl + foliar spray with distilled water (**D**) Irrigation with 100 Mm NaCl + foliar spray with CFE (**E**) Irrigation with 150 Mm NaCl + foliar spray with distilled water (**F**) Irrigation with 150 Mm NaCl + foliar spray with CFE.

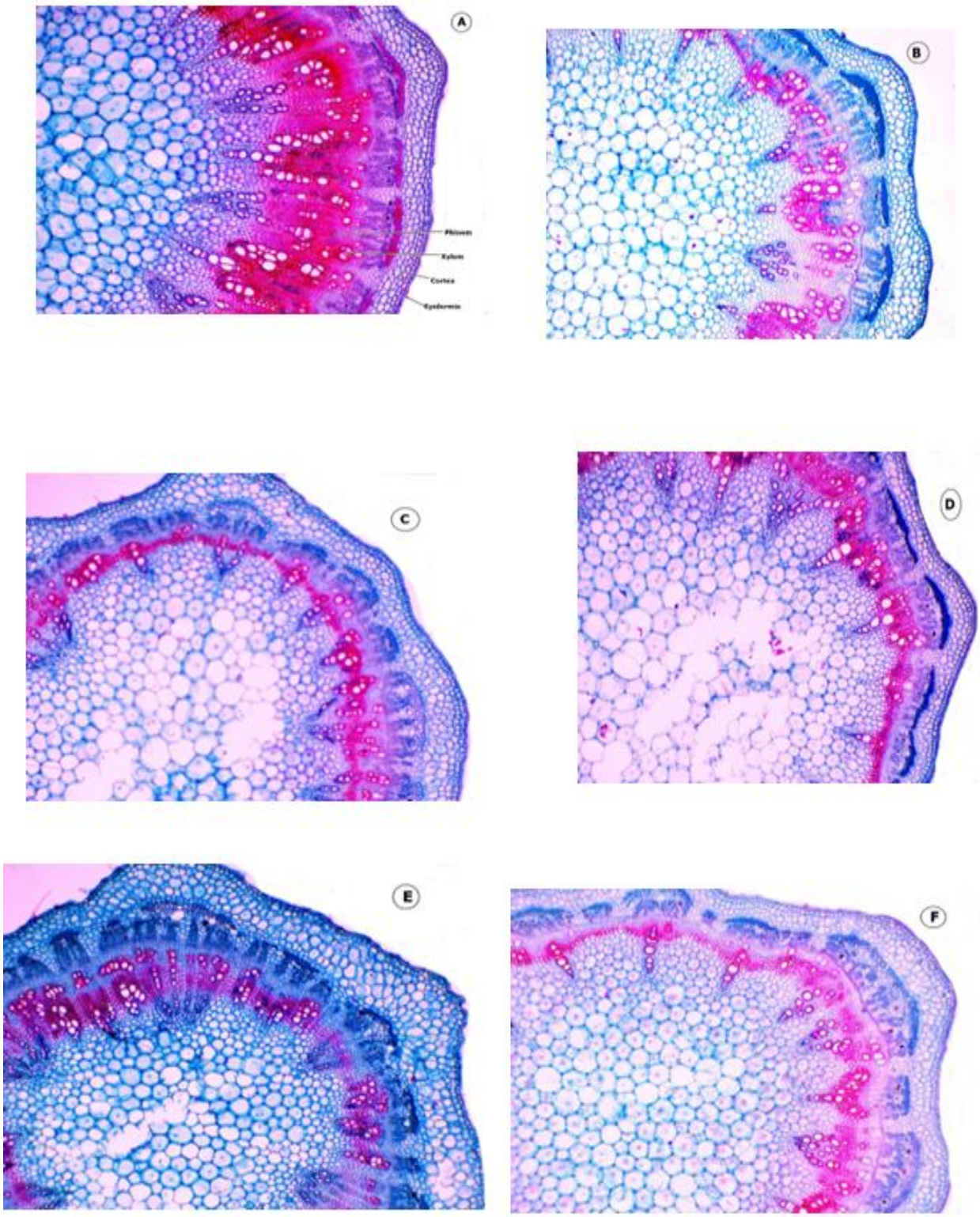

Fig. 2: Cross-sectional view of the main stem of snap bean plants exposed to the combined treatments of foliar application of CFE and salinity stress during the second growing season (2023).

(A) Irrigation with tap water + foliar spray with distilled water (B) Irrigation with tap water + foliar spray with CFE (C) Irrigation with 100 Mm NaCl + foliar spray with distilled water (D) Irrigation with 100 Mm NaCl + foliar spray with CFE (E) Irrigation with 150 Mm NaCl + foliar spray with distilled water (F) Irrigation with 150 Mm NaCl + foliar spray with CF.

Clove fruit extract effectively alleviated the salt stress harmful effects on snap bean plants due to enhance physiological and biochemical processes, so, improvs the feasibility to cultivate snap beans in conditions with high salinity.4o mini

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الملخص العربي

الاستجابات الفسيولوجية والتشريحية لنبات الفاصوليا للرش الورقي بمستخلص ثمرة القرنفل تحت إجهاد الملوحة

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تم إجراء تجربتين زراعيتين في أصص داخل الصوب الزراعية بكلية الزراعة، جامعة الزقازيق، مصر؛ خلال موسمي النمو 2022 و2023، بهدف الحد من تأثير إجهاد الملوحة على نبات *الفاصوليا* باستخدام مستخلص ثمرة القرنفل. شملت معاملات الملوحة ثلاث مستويات هي الكنترول (غير معرض للإجهاد)، و100ملى مول كلوريد صوديوم، و 150ملى مول كلوريد صوديوم، بينما تضمنت معاملات مستخلص ثمار القرنفل معاملة الكنترول (الرش بالماء المقطر)، ومعاملة الرش بمستخلص ثمار القرنفل (10 جم من ثمار القرنفل المجففة لكل 1 لتر من الماء المقطر). تم تنفيذ التجربة باستعمال تصميم القطاعات المنشقة بنظام القطاعات العشوائية الكاملة في ثلاث مكررات؛ حيث تم تخصيص القطاعات الرئيسية لمستويات الملوحة، بينما خُصصت القطاعات الفرعية لمعاملات مستخلص ثمرة القرنفل. تم زراعة بذور الفاصوليا في 15 أكتوبر 2022 و17 أكتوبر 2023 في أصص بلاستيكية تحتوي على 8 كجم من التربة الطينية المجففة هوائيًا . أدى إجهاد الملوحة إلى زيادة كبيرة في مؤشرات الإجهاد التأكسدي (MDA، EL، O_2^-، H_2O_2) مع تعزيز مضادات الأكسدة غير الإنزيمي (α-TOC، وGSH، والجليسين بيتين ،)وزيادة نشاط الإنزيمات المضادة للأكسدة (CAT، و POD، وAPX، وSOD، و GR). كما تسببت المستويات العالية من الملوحة في تدهور تشريحي شديد. وقد أدى الرش بمستخلص ثمار القرنفل إلى التخفيف من آثار إجهاد الملوحة من خلال تقليل الضرر التأكسدي، وتحسين استجابات مضادات الأكسدة، وتحسين التركيب التشريحي للنبات . أخيراً فإنه قد تبين فعالية استخدام مستخلص ثمار القرنفل في الحد من تأثير إجهاد الملوحة على نبات الفاصوليا، والذي نتج عنه تحسن الصفات الفسيولوجية والتشريحية. ويُعد هذا المنشط الحيوي استراتيجية واعدة لتحسين زراعة الفاصوليا في الظروف المالحة.

الكلمات المفتاحية: الفاصوليا، إجهاد الملوحة، مستخلص ثمرة القرنفل، منشط حيوي