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Evaluating Ecological Nano-Calcium from Eggshells: Effects on Calcium Nutrition and Oxidative Stress in Lettuce Under Saline and Boron Toxicity

Ozge Sahin[1] · **Kiymet Deniz Yagcioglu**[2] · **Yusuf Kagan Kadioglu**[2] · **Aydin Gunes**[1]

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Abstract

Nano-fertilizers with higher efficacy compared to conventional fertilizers can provide advantage for plant cultivation in both productive and problematic soils. Therefore, this study aimed to determine the effect of nano-calcium (nano-Ca) on lettuce plants grown in saline-boron toxic soil. Nano-calcium fertilizer was prepared from eggshells. Functional and structural properties of nano-Ca was determined by scanning electron microscopy (SEM), x-ray diffraction (XRD) and fourier transform infrared spectroscopy (FTIR) before plant experiment. The treatments was; control, 40 mM NaCl and 20 mg kg^{-1} B (NaCl + B), and 40 mM NaCl and 20 mg kg^{-1} B with 4 mM Nano-Ca (NaCl + B + nano-Ca). The nano-Ca significantly increased the dry weight and calcium (Ca) concentration of lettuce plants under saline-B toxic conditions. Although there was a decrease in the concentrations of sodium (Na), chloride (Cl), and boron (B) with nano-Ca treatment, it was not statistically significant. Salinity and boron toxicity lead to increased lipid peroxidation. In the present study, the production of malondialdehyde (MDA) as a marker for lipid peroxidation, along with a significant decrease in hydrogen peroxide (H_2O_2) concentration, was observed with the application of nano-Ca. There was no significant alteration in superoxide dismutase activity (SOD) observed in lettuce grown under saline and boron toxic conditions. However, catalase activity (CAT) increased with nano-Ca application, while the activity of ascorbate peroxidase (APX) decreased. The study results suggest that nano-Ca serves a protective function for lettuce plants cultivated under saline and boron toxic conditions.

Keywords Nano-fertilizer · Leafy vegetable · Boron and salt stress · Oxidative stress · Calcium · Abiotic stress

Introduction

Salinity and B toxicity are two distinct yet related issues that can affect the growth and development of plants (Lu et al. 2023). The simultaneous occurrence of salinity and boron toxicity poses significant challenges to plant growth and crop productivity. These stressors can interact synergistically, amplifying their detrimental effects on plant physiology and ultimately leading to reduced yields and crop quality (Pandey et al. 2019). Since B transport in plants is mainly dependent on transpiration, in saline soils with high osmotic pressure, transpiration decreases, resulting in reduced B transport, and consequently, plants can be less affected by B toxicity (Mohammed et al. 2016). However, due to specific ion toxicity caused by salinity or osmotic stress, the biomass of plants decreases, leading to a relative increase in the amount of B in plant (Pandey et al. 2019; Karimi and Tavallali 2017). In order to meet the increasing food demand, ways to enhance productivity in saline and B toxic

Handling Author: Mikihisa Umehara.

✉ Ozge Sahin
osahin@ankara.edu.tr

Kiymet Deniz Yagcioglu
Kiymet.Deniz@eng.ankara.edu.tr

Yusuf Kagan Kadioglu
kadi@ankara.edu.tr

Aydin Gunes
agunes@agri.ankara.edu.tr

[1] Department of Soil Science and Plant Nutrition, Faculty of Agriculture, Ankara University, 06110 Ankara, Turkey

[2] Department of Geological Engineering, Faculty of Engineering, Ankara University, 06100 Ankara, Turkey

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soils should be explored. Salinity refers to the presence of high levels of salt in the soil or irrigation water. It can be caused by natural factors such as the proximity to coastal areas or human activities like excessive use of fertilizers or poor irrigation practices. High salt concentration in the soil creates an osmotic imbalance, drawing water out of the plant roots and reducing its ability to take up water (Khatri and Rathore 2022). This leads to water stress and subsequent wilting. Salinity disrupts the balance of essential ions, particularly Na and potassium (K), in plant cells. Excessive Na uptake can lead to toxicity, while reduced K levels hinder important physiological processes (Wakeel 2013). Salinity interferes with nutrient uptake by plants, especially for essential nutrients like nitrogen (N), phosphorus (P), and magnesium (Mg). This can result in nutrient deficiencies, affecting plant growth and yield. High salt levels can lead to the accumulation of toxic ions, such as Cl or Na, in plant tissues. These ions can disrupt cellular processes, damage cell membranes, and interfere with metabolic functions. Salinity can inhibit photosynthesis, the process by which plants convert sunlight into energy (Khatri and Rathore 2022). This results in reduced growth and diminished yield. Boron is an essential micronutrient for plants, but excessive levels can lead to toxicity. Boron plays a crucial role in cell wall formation and stability. However, excessive B levels can disrupt the structural integrity of the cell walls, leading to impaired growth and weakened plant tissues. High B concentrations can interfere with the uptake of other essential nutrients, such as Ca and Mg (Long and Peng 2023). This imbalance can disrupt various physiological processes and hinder plant growth. Boron toxicity can disrupt several metabolic processes, including carbohydrate metabolism and protein synthesis. This can negatively impact plant growth, development, and overall productivity. One characteristic symptom of B toxicity is the development of necrotic spots or browning on the edges and tips of leaves. This is often accompanied by stunted growth and reduced vigor. Boron toxicity can also interfere with flower and seed development, leading to abnormal reproductive structures and reduced seed set (Brdar-Jokanovic 2020).

Boron and Ca share similar characteristics, such as low phloem mobility, a structural role in the cell wall with low cytosolic concentration, and inducing growth alterations in case of deficiency. The amount and availability of either of these elements impacts on the distribution (Ramón et al. 1990) and the requirement of the other for optimal plant growth (Teasdale and Richards 1990; Bonilla et al. 2004; Etasami et al. 2021). It's important to note that while both B and Ca are essential nutrients, their application should be based on the specific needs of the plants being cultivated and the analysis of the soil to avoid deficiencies or excesses that can negatively impact plant growth. In addition, Ca plays crucial structural and signaling roles in plant development. Besides, it has a role in the cell wall structure, extension function, osmoregulation, extension with cell wall stiffness, and regulation of some enzymes, improves fruit density and decline the pathogen affect (Hamza et al. 2021; Mogazy et al. 2022).

Reactive oxygen species (ROS) production is one of the responses of plants to abiotic stresses such as salinity, drought and others, which plant developed antioxidant enzymes to overcome the excessive ROS production in salinity stressed-plant cells (You and Chan 2015). The effect of nano-fertilizer on increasing the amount of antioxidant enzymes activity which have been verified in many studies (Abdoli et al. 2020; Gaafar et al. 2020; Mushtaq et al. 2020; Rani et al. 2016). The researchers showed that nano-fertilizer have the properties of certain antioxidant enzymes and hence support the plant to cope with the oxidative stress conditions such as nano iron, zinc, calcium, silicon and others. Nano fertilizers possess distinct physico-chemical properties that confer advantages over traditional chemical fertilizers. With small particle dimensions (< 100 nm), nano fertilizers can effectively penetrate plant systems (Zahedi et al. 2020; Seleiman et al. 2021; Gil-Díaz et al. 2022). Due to their ultra-small size, nano fertilizers exhibit a high surface area (Babu et al. 2022; Hussain et al. 2022), which enhances their absorption and retention capacity compared to conventional bulky chemical fertilizers. The increased surface area of nano fertilizers enables them to contain higher nutrient loads and release these nutrients gradually in accordance with crop demands, without causing any negative consequences (Babu et al. 2022; Gil-Díaz et al. 2022). In contrast, common synthetic fertilizers often suffer from low plant uptake efficiency, leading to nutrient wastage through leaching, volatilization, and gaseous emissions, contributing to environmental degradation (Dimkpa et al. 2015a, b; Benlamlih et al. 2021; Yadav et al. 2023). The slow and gradual nutrient release characteristic of nano fertilizers mitigates these negative environmental outcomes. As a result, nano fertilizers show promise in sustainable agriculture practices by improving nutrient utilization efficiency and minimizing environmental impacts.

In saline and B toxic conditions, Ca uptake by plants can be influenced by various factors, including the presence of other ions in the soil solution. One such factor is Na, which can have an antagonistic effect on Ca uptake. When plants are exposed to high levels of Na in the soil, it can disrupt the balance of ions around the plant roots. Sodium ions can compete with Ca ions for uptake by plant roots. This competition can lead to a decreased uptake of Ca by plants (Acosto-Motos et al. 2017; Zhao et al. 2021). Additionally, previous studies have reported the effectiveness of Ca applications in preventing boron toxicity (González-Fontes et al. 2014). Studies have explored obtaining nano-Ca from eggshells (Habte et al. 2019; Jalu et al. 2021). Nevertheless, there is a lack of research regarding the

influence of nano-Ca on plants cultivated in soils characterized by salinity and B toxicity. The objective of this study is to investigate the impact of nano-Ca derived from eggshell waste as an alternative calcium source on plant growth, and the uptake of Ca, B, Na and Cl. Additionally, the study aims to examine oxidative stress mechanisms in the context of saline and boron toxic conditions.

Material and Methods

Preparation and Characterization of Nano-Ca

The collected eggshells underwent a thorough washing process in deionized water, following which they were dried at 120 °C for 2 h and subsequently ground to a particle size of 100 µm using a sieve. For the nano-Ca preparation, 12.5 g of coarse eggshell powder was treated with 250 mL of 1 M HCl. Simultaneously, 250 mL of 1 M NaOH was added drop by drop. This process resulted in the formation of a gel-like substance, which was left to age overnight at room temperature. The aged gel was then filtered using Whatman No. 42 filter paper and washed with distilled water to remove any impurities. Subsequently, the filtered material was dried at 60 °C for 24 h in an oven and later subjected to calcination at 900 °C for 1 h, following the method described by Habte et al. 2019. The properties of the nano-Ca are presented in Table 1.

Figure 1 displays scanning electron microscopy (SEM) images, while Fig. 2 shows SEM coupled with energy-dispersive X-ray spectroscopy (SEM–EDX) results. The SEM micrographs were acquired using a Zeiss Evo40 microscope, operating at an accelerating voltage of 200 kV. The particle size and size distribution were determined from these micrographs.

Fourier transform infrared spectroscopy (FTIR) was employed to analyze the functional groups associated with PEB (Presumed Experimental Biomaterial) in the range of 700–4000 cm^{-1}, following the methodology reported by Sahin et al. 2017. The FTIR analysis was conducted using a Shimadzu Infinity FTIR-Spectrometer. The FTIR spectra were recorded with 20 scans per sample, where 1–2 nano grams of nano-Ca sample were used for pelletization along with 100 mg of KBr. The recorded FTIR spectra, as shown in Fig. 3, revealed the characteristic infrared absorption bands corresponding to the identified functional groups and phases.

Chemical Composition of the Nano-Ca

The total concentrations of Ca, Na, and Cl in the nano-Ca samples were measured using X-ray fluorescence (XRF) spectroscopy, following the methodology described

Table 1 The properties of the experimental soil and nano-Ca

Properties/methods	Amount/ Quantification	
	Soil	Nano-Ca
Texture	Clay loam	–
EC	0.42	–
pH	7.90	–
	g kg^{-1}	
$CaCO_3$	57.8	–
Organic Matter	7.46	–
N Kjeldahl	2.30	–
P $NaHCO_3$ available	0.08	–
K NH_4OAc ext	470	–
Ca NH_4OAc ext	2570	–
Mg NH_4OAc ext	1.86	–
Na NH_4OAc ext	65.0	–
Total P	0.95	1.92
Total K	14.1	2.18
Total Ca	37.6	486
Total Mg	14.6	7.11
Total S	0.95	1.39
Total Na	2.30	0.27
Total Cl	0.02	0.41
Total Si	217	2.15
	mg kg^{-1}	
Total Fe	10,170	432
Total Zn	68.0	0.6
Total Cu	33.3	9.4
Total Mn	829	6.6
Total Mo	15.2	6.7
B $CaCl_2$ ext	1.25	–

–:not determined

ext: extractable

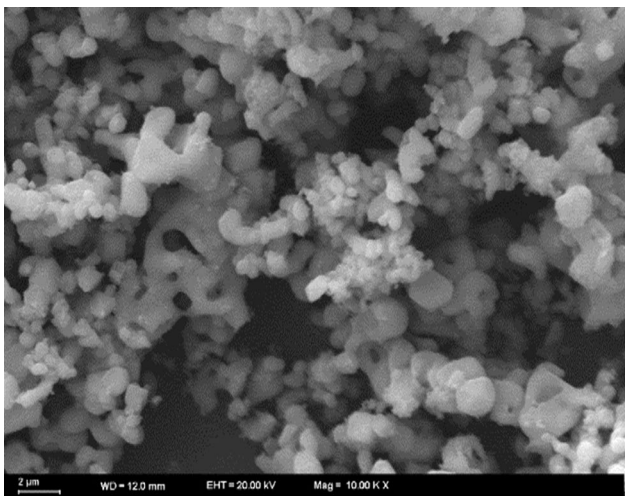


Fig. 1 Scanning electron microscopy (SEM) image of nano-Ca

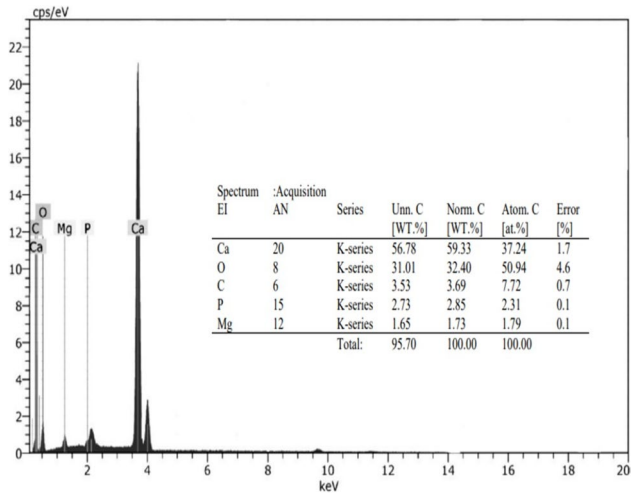


Fig. 2 Scanning electron microscopy -EDX (Sem–EDX) images of nano-Ca

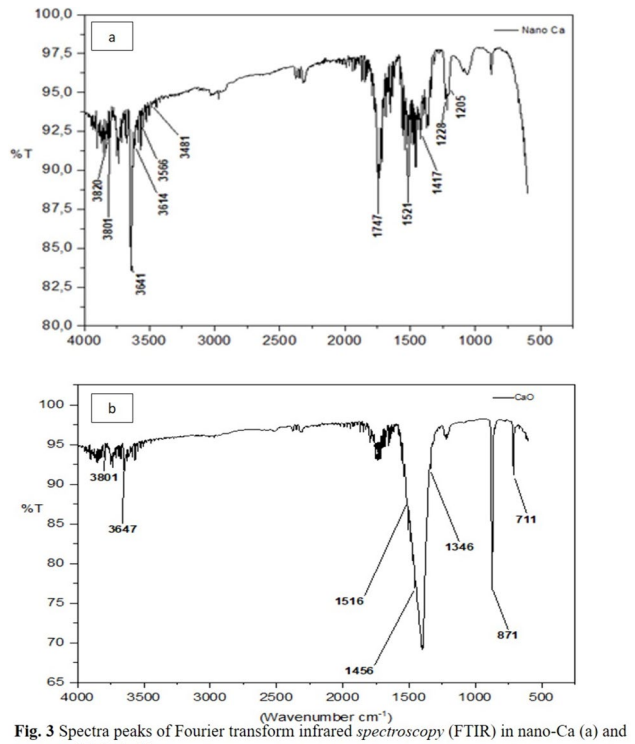


Fig. 3 Spectra peaks of Fourier transform infrared *spectroscopy* (FTIR) in nano-Ca (a) and commercial CaO (b)

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by Gunes et al. (2009). This non-destructive analytical technique allows for the determination of elemental composition without sample preparation. On the other hand, the B concentration in the nano-Ca samples was determined using inductively coupled plasma optical emission spectrometry (ICP-OES). Prior to analysis, acid digestion of the samples was performed using a mixture of nitric acid (HNO_3) and perchloric acid ($HClO_4$) in a 4:1 ratio. This acid digestion process helps in solubilizing the boron from the nano-Ca samples. The ICP-OES instrument used for this analysis was the Perkin Elmer 1200 V model, following the procedure outlined by Sahin et al. (2017).

Growth Conditions and Treatments

Lettuce is salt-sensitive vegetable (Shannon and Grieve 1999). Salt stress reduces lettuce germination, leaf water content, photosynthesis rate, chlorophyll content, root and shoot growth, and increased Na and Cl ion concentration and lipid peroxidation (Xu and Mou 2015). Curly lettuce (*Lactuva sativa* L. Cv. Sementel) plant was selected as test plant and cultivated in a naturally illuminated greenhouse at the Faculty of Agriculture, Ankara University, from March 9, 2021, to April 27, 2021. During this period, the daytime temperature ranges from 20–24 °C, and the nighttime temperature is between 10–15 °C, with a daytime light intensity in the range of 200-400W m^{-2} and the average humidity levels were recorded as approximately 65 ± 5%. Each lettuce seedlings were sown individually in 2 L PVC containers (without drainage hole, 20 cm depth, 22 cm top diameter, and 15 cm bottom diameter) filled with soil. Throughout the experiment, a daily weight measurement was conducted on randomly selected pots and manually water was added to maintain the soil at 70% of the field capacity.

The experimental setup included three different treatments, each with four replicates. The treatments were as follows: (1) Control: (2) NaCl + B [40 mM NaCl + 20 mg kg^{-1} B]: (3) NaCl + B + nano-Ca [40 mM NaCl + 20 mg kg^{-1} B + 4 mM nano-Ca]. Prior to planting the lettuce seedlings, the soil in each pot was enriched with the following basal fertilizers: 50 mg P kg^{-1} soil in the form of KH_2PO_4, 100 mg N kg^{-1} soil as $(NH_4)_2SO_4$, 50 mg K kg^{-1} soil as K_2SO_4, and 25 mg Mg kg^{-1} soil as $MgSO_4.7H_2O$.

The total N (Walinga et al. 1989) and total concentrations of Ca of the soil were determined by X-ray flouresence (PEDXRF) as described by Gunes et al. 2009. Other soil analyses were carried out according to Page et al. 1982. The extractable concentration of B of soil were determined according to Bingham 1982 which the properties of the soil is presented in Table 1.

Sampling and Harvest Procedure

Fresh samples of fully matured leaves were collected randomly for analysis. The remaining leaves were stored at −20 °C in aluminum foil for enzyme extraction and assay, performed on April 27, 2021. At the end of the experiment, the plants were harvested. The plant leaves were carefully

washed with tap water, followed by two washes with deionized water to remove any surface contaminants. Subsequently, the shoots were dried in a thermo-ventilated oven at 60 °C until a constant mass was achieved. The dried plant material was then ground through a 200 µm sieve to facilitate the determination of Ca, Na, Cl, and B concentrations.

For the analysis of Ca, Na, and Cl concentrations in the plants, polarized energy dispersive X-ray fluorescence (PEDXRF) was employed, following the detailed methodology outlined by Gunes et al. 2009 and Sahin et al. 2014. This non-destructive analytical technique allows for the measurement of elemental concentrations in the samples without altering their integrity.

To determine the total B concentrations of the plants, a dry ashing process was carried out. Initially, 250 mg subsamples of the plant material were subjected to dry ashing in a muffle furnace, maintained at 550 °C for 8 h. The resulting ash was dissolved in 10N nitric acid (HNO_3), and the B concentration was analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES) with a Perkin Elmer 1200 V instrument. The methods employed for B analysis were based on the works of Horwitz 1980 and Kalra 1998.

Enzyme Extraction and Assay

All enzyme extraction procedures were conducted under controlled temperature conditions (0–4 °C) to maintain sample integrity. Samples were taken from the youngest matured leaves, and 0.5 g of fresh shoot tissue were homogenized in 5 mL of 100 mM potassium phosphate buffer (pH 7.5) containing 1 mM EDTA-Na_2 and 0.5 mM ascorbate using a Heidolph, Diax 900 homogenizer.

The homogenized samples were then subjected to centrifugation at 10,000 g for 5 min to separate the soluble enzyme extracts from the cellular debris. Enzyme activities were measured at 25 °C using a Shimadzu UV/VIS1201 spectrophotometer. The specific activities of the enzymes were determined using the following methods:

Superoxide dismutase activity was assayed using the nitroblue tetrazolium (NBT) method as described by Gong et al. 2002. The absorbance was measured at 560 nm, and one unit of SOD activity was defined as the amount of enzyme that produced 50% inhibition of NBT reduction under the given assay conditions.

Ascorbate peroxidase activity was determined by monitoring the decrease in ascorbate concentration and measuring the change in absorbance at 290 nm for 1 min, following the procedure established by Nakano and Asada (1981). The activity was calculated using the extinction coefficient (2.8 mM^{-1} cm^{-1}) for ascorbate.

Catalase activity was assessed by observing the decrease in absorbance at 240 nm for 1 min after the decomposition of H_2O_2, following the method by Cakmak et al. (1993). The activity of H_2O_2 was calculated using the extinction coefficient (40 mM^{-1} cm^{-1}).

Determination of Lipid Peroxidation (MDA) and Hydrogen Peroxidase (H_2O_2)

To evaluate membrane damage in the shoot samples, lipid peroxidation (MDA) content was quantified. The MDA-TBA (Thiobarbituric acid) complex was measured as an indicator of lipid peroxidation, following the method described by Hodges et al. (1999). The amount of MDA-TBA complex was determined using the extinction coefficient of 155 mM^{-1} cm^{-1} with a Shimadzu UV/VIS1201 spectrophotometer. Furthermore, the H_2O_2 content in the shoots was determined calorimetrically, following the procedure outlined by Mukherjee and Choudhuri (1983).

Statistical Analysis

The experimental design followed a completely randomized design with four replications. Each replicate consisted of one plant per pot. The data obtained from the experiment subjected to analysis of variance (ANOVA) to assess the variation among the treatments by MINITAB17. To determine significant differences among the treatment means, Duncan's multiple range test was applied at a significance level of $P < 0.05$.

Results and Discussion

Characterization of Nano-Ca

Figure 1 displays the scanning electron microscope image of nano-Ca, revealing the morphology and size of spherical nano-Ca produced through the thermal decomposition of waste eggshell at 900 °C (Alobaidi et al. 2022). Additionally, certain CaO particles undergo sintering during the calcination process, as evident in the SEM images. The vibrations between particles during calcination cause them to split, resulting in smaller particle sizes (Nuryantini et al. 2019). Furthermore, Mirghiasi et al. (2014) reported that the SEM images of nanoparticles also depicted a spherical morphology for CaO nanoparticles after calcination, which aligns with the findings of our study.

As shown in Fig. 2, C, O, Ca, P and Mg peaks were identified in the spectra of the nano-Ca particles. The results of EDX test showed that the elements contained in nano-Ca

were C 3.69%, O 32.40%; Ca 59.33%, P 2.85%, and Mg 1.73%. The elements O and C are the highest chemical elements in number. This indicates that the outcomes from both calcination and precipitation processes collectively yield calcium oxide (CaO), aligning with findings reported by Prayitno et al. (2019). Similar result was determined by Habte et al. (2019) as an 87% nano-Ca. Khan et al. (2018) described that nano-Ca product was mainly consists of dumb bell like microstructures. These structures possess bulgy terminal ends like as our results. The particle sizes of the resultant powders appeared slightly smaller than the initial eggshell powder particle sizes. The morphologies of the synthesized powders became more rounded following heat treatment, and showed spheroidal shapes. After heat treatment, an agglomeration phenomenon was produced by high-temperature sintering (Ho et al. 2013). The eggshell constitutes 11% of the egg's total weight, composed of $CaCO_3$ (94%), calcium phosphate (1%), organic matter (4%), and magnesium carbonate (1%) as reported by Rivera et al. (1999) and Ho et al. (2013). While occasionally utilized as fertilizer due to their rich Ca and N content, unfortunately, most eggshells are discarded as waste. Additionally, chicken eggshells contain trace elements like Na, Mg and Sr, which are also present in human bone.

The peaks are identified at specific 2-theta values whose detail is given in Fig. 3. The FTIR spectrum of the $CaCO_3$ sample is obtained in the form of a graph that relates the wave number to its transmittance, located at the absorption peak of 4000–500 cm^{-1} (Diningsih and Rohmawati 2022). The FTIR spectrum of the nano-Ca sample is generated by the absorption of electromagnetic radiation, which has absorption peaks at 3820 cm^{-1}, 3801 cm^{-1}, 3614 cm^{-1}, 3566 cm^{-1}, 3481 cm^{-1}, 1747 cm^{-1}, 1521 cm^{-1}, 1417 cm^{-1}, 1228 cm^{-1} and 1205 cm^{-1}. According to Huang et al. (2020) the distinct peak of nearly 3433 cm^{-1} is attributed to the O–H stretching vibration of residual water and 1799 cm^{-1} corresponds to the C=O bonds of carbonate. Comparable findings were previously documented by Habte et al. (2019), where an absorbance at 3639 cm^{-1} was attributed to the O–H bond arising from water molecules on the nanoparticle surface, and a prominent band at 512 cm^{-1} was identified as the vibration of the Ca–O bond. According to Mirghiasi et al. (2014), FTIR spectrum of the CaO for powder which was shown at the strong band at 3647 cm^{-1} corresponds to the O–H bonds from the remaining $Ca(OH)_2$ or from water molecules on the external surface of the samples during handling to record the spectra. The peak around 1400–1500 cm^{-1}, as well as a weak band at 873 cm^{-1} indicates the C-O bond related to carbonation of CaO nanoparticles. There is a tiny dip in the spectra at 2359 cm^{-1} due to the presence of atmospheric CO_2. The broad transmission band at approximately 3428 cm^{-1} can attributed to OH stretching vibration from residual waster. The weak band at 1747 cm^{-1} corresponds to C=O bonds from carbonate. 1521 cm^{-1} peak showed that the C=N stretching vibrations, two well-defined infrared bands at 1228 and 1205 cm^{-1} are characteristic of the OH and, C–O stretching and bending modes of $CaCO_3$, respectively (Witoon 2011).

Dry Weight, Boron, Sodium, Chloride and Calcium Concentrations of Lettuce

The created salinity and B toxicity caused a slight decrease in the dry weight of lettuce plants (Table 2). However, the application of nano-Ca significantly increased the dry weight of lettuce plants compared to the control and salinity (NaCl + B) treatments. Applying nano-Ca resulted in a notable rise in the plants' Ca concentration compared to both the control and the NaCl + B treatment. The NaCl + B treatments caused an increase in the concentrations of Na, Cl, and B in the plants compared to the control. While the application of nano-Ca led to a reduction in these increases to some extent, the changes were not statistically significant. It is known that plant development is affected by salinity and B toxicity. This effect is due to excessive amounts of salt increasing osmotic pressure in the soil, causing plants to receive insufficient water, and specific toxic effects of Na, Cl, and B ions (Hailu and Mehari 2021). Under saline and B toxic conditions, it is known that the Na, Cl, and B ions have an antagonistic effect on the Ca influx in plants

Table 2 Effect of nano-Ca on dry weight, calcium (Ca), sodium (Na), chlorine (Cl), and boron (B) concentrations of lettuce plant grown in saline-B toxic soil

Treatments	Dry Weight g $plant^{-1}$	Ca g kg^{-1}	Na g kg^{-1}	Cl g kg^{-1}	B mg kg^{-1}
Control	6.98 ± 0.14 b	9.10 ± 0.56 b	6.55 ± 0.82 b	24.27 ± 0.69 b	63.45 ± 8.38 b
NaCl + B	6.44 ± 0.14 b	10.01 ± 0.23 b	15.15 ± 1.10 a	32.31 ± 1.17 a	153.9 ± 8.62 a
NaCl + B + nano-Ca	7.33 ± 0.25 a	11.29 ± 0.22 a	13.88 ± 0.66 a	31.95 ± 0.65 a	137.5 ± 15.3 a
F	45.71*	10.38**	28.05**	27.20**	18.89**
LSD	0.59	1.18	2.60	2.78	36.0

The values are means of four replicates ± standard errors. Different letters in each column represent significant difference at $p < 0.05$ level based on Duncan's multiple range test. ns: non-significant, *$p < 0.05$, **$p < 0.01$

(Bastías et al. 2010; Javadi et al. 2016). In our study, the application of nano-Ca improved plant growth and increased Ca concentrations. There are not many studies investigating the effects of nano-Ca under saline and B toxic conditions. However, a few previous studies support our findings. Hamza et al. (2021) reported that the highest yield and quality were obtained with the $Ca(NO_3)_2$ and 100% nano $Ca(NO_3)_2$ treatments. Furthermore, Tantawy et al. (2014) observed a positive impact on tomato fruit yield with a lower concentration of nano-calcium carbonate. Similarly, positive effects of nano-Ca were reported in apple by Ranjbar et al. (2018, 2020), and in tomatoes by Sajyan et al. (2018) and Haghighi and Naghavi (2019). In addition to all these, salt stress is an osmotic stress similar to drought. In their previous studies, Ayyaz et al. (2022; 2024) have determined that Ca nanoparticles are effective in alleviating drought stress in *Brassica napus*.

Malondialdehyde and Hydrogen Peroxide Concentrations, and Catalase, Superoxide Dismutase and Ascorbate Peroxidase Activity of Lettuce

The assessment of MDA and H_2O_2 concentrations in the shoot samples offers valuable insights into the extent of oxidative stress and potential damage to cellular membranes. These analyses serve as important indicators of the plant's response to stress conditions and shed light on its oxidative defense mechanisms. Table 3 presents the data on MDA and H_2O_2 levels, alongside the activity of essential antioxidant enzymes like CAT, SOD, and APX. Malondialdehyde is the decomposition product of fatty acids of membranes and its increase is the result of greater accumulation under high antioxidant stress (Ebrahimi et al. 2016; Mahmoud et al. 2021). It is well known that reactive oxygen species (ROS) induced lipid peroxidation of membranes is a reflection of oxidative stress condition damage at the cellular level (Eraslan et al. 2007).

The concentrations of MDA and H_2O_2 significantly increased with the NaCl + B treatment, indicating that the elevated H_2O_2 is associated with membrane damage, as reflected by the increased MDA levels. Conversely, when nano-Ca was applied under stress conditions, a significant reduction in H_2O_2 levels was observed, indicating a decrease in membrane damage. One of the crucial defense mechanisms against toxic oxygen radicals in plants under stress is the activity of antioxidant enzymes. In this study, CAT activity increased significantly, while APX activity decreased by nano-Ca treatment. Catalase is known to convert H_2O_2 to H_2O, thereby reducing its toxicity (Javadisaber et al. 2023). The increased CAT activity with nano-Ca application corresponded to the reduced H_2O_2 levels and, consequently, reduced membrane damage. The decrease in APX can be explained by the rising CAT activity, leading to a decrease in ROS. The increased levels of SOD due to salinity and B toxicity did not show any significant change with nano-Ca application. Under conditions of salinity and B toxicity stress, plant cells undergo an accumulation of ROS, leading to ionic, osmotic, and mineral element stress. These ROS, including superoxide anion radicals ($O_2^{\bullet -}$), singlet oxygen ($^1O_2$), H_2O_2, and hydroxyl radicals ($OH^{\bullet}$), are commonly produced within plant cells, particularly in chloroplasts, and they play a crucial role in regulating cellular enzyme activity (Singh et al. 2023). One of the essential enzymes involved in the response to ROS is SOD, which catalyzes the conversion of H_2O_2 to H_2O, utilizing ascorbate. Moreover, SOD converts $O_2^{\bullet -}$ radicals into H_2O_2 and O_2. Other enzymes like CAT and APX are responsible for breaking down H_2O_2 into H_2O and O_2, leading to a decrease in the levels of MDA, an indicator of oxidative damage (Nasrallah et al. 2022). Calcium also plays a defensive role in mitigating the effects of salt and B stress by regulating ROS levels, which in turn enhances the activity of SOD and CAT (Ahmad et al. 2018). Notably, nano-sized Ca has been found to significantly affect the activity of SOD, CAT, and APX. It's important to note that the specific response of plants to B and salt stress can vary depending on factors such as the plant species, cultivar, and the severity and duration of the oxidative stress. Additionally, nano-sized particles have been found to alleviate salt stress by increasing K uptake and reducing Na ion toxicity (Nasrallah

Table 3 Effect of nano-Ca on lipid peroxidation (Malondialdehyde: MDA) and hydrogen peroxide (H_2O_2) concentrations, and superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) enzyme activity of lettuce plant grown in saline-B toxic soil

Treatments	MDA nmol g^{-1} FW	H_2O_2 mmol kg^{-1} FW	CAT mmol g^{-1} FW min^{-1}	SOD Unit	APX mmol g^{-1} FW min^{-1}
Control	5.84 ± 0.81 b	16.80 ± 0.98 c	0.05 ± 0.01 c	33.52 ± 1.08 b	2.63 ± 0.64 b
NaCl + B	10.16 ± 0.43a	85.54 ± 1.56 a	0.14 ± 0.02 b	59.56 ± 7.02 a	4.63 ± 0.38 a
NaCl + B + nano-Ca	5.93 ± 0.54 b	54.83 ± 1.54 b	0.45 ± 0.02 a	58.16 ± 1.99 a	1.37 ± 0.20 b
F	16.0**	616**	166**	27.63**	13.83**
LSD	1.97	4.44	0.052	11.84	1.42

The values are means of four replicates ± standard errors. Different letters in each column represent significant difference at $p < 0.05$ level based on Duncan's multiple range test. **$p < 0.01$

et al. 2022). Furthermore, nano fertilizers have shown an increase in enzymatic activity, including APX, GPX, SOD, and CAT, making the plants more tolerant to salt stress conditions (P'erez-Labrada et al. 2019).

Conclusion

Nano-calcium, which can be synthesized from readily available raw eggshells, serves as a promising alternative calcium fertilizer. The findings of this study demonstrate that nano-Ca significantly enhances the growth of lettuce plants. Moreover, plants possess specific antioxidant mechanisms to combat the stress induced by salt and B toxicity. The results also indicate that lettuce plants under salt and B toxicity stress experience significant alterations in the levels of antioxidative compounds such as SOD, CAT, APX, and MDA and H_2O_2. Applying Ca to plants can boost the activity of antioxidant enzymes, thereby enhancing the plant's defense mechanisms against oxidative damage to the cellular membrane. Calcium, as an essential element, aids in safeguarding the membrane against the adverse effects of MDA and H_2O_2 under conditions of B and salt toxicity. Consequently, nano-Ca emerges as an effective agricultural practice for mitigating the detrimental impacts of salinity and B toxicity on crops.

Author Contribution Ozge Sahin: Data curation; Formal analysis; Funding acquisition; Investigation; original draft; Writing. Kıymet Deniz Yagcioglu; Software; characterization of nano-Ca by SEM and FTIR. Yusuf Kagan Kadioglu: Characterization of nano-Ca by SEM and FTIR Validation; Visualization; Roles/Writing–original draft; Writing. Aydin Gunes: Conceptualization; Data curation; Investigation; Methodology; Roles/Writing – original draft; Writing – review & editing.

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Declarations

Conflict of Interest The authors declare that they have no competing interests.

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