



Ectoine Reduces Nickel Stress and Boosts Antioxidant Defences in Wheat

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Abstract

This study aimed to determine whether ectoine (ET), a bacterial compatible solute, can mitigate the adverse effects of nickel (Ni) toxicity in wheat plants. Treatments included 50 and 75 ppm Ni, with ET applied at 5 mM and 10 mM concentrations. Results showed that Ni stress significantly increased nickel accumulation in plant tissues (from 348 ppb in the control to 17.203 ppb under 50 ppm Ni), while reducing chlorophyll content from 1.38 to 1.01. However, application of 10 mM ET effectively ameliorated these effects by reducing nickel levels to 6.610 ppb (a 61.56% improvement) and increasing chlorophyll content from 1.01 to 1.22 (a 20.79% improvement). Nickel exposure also elevated oxidative stress markers, such as hydrogen peroxide and malondialdehyde (MDA), and increased the activities of antioxidant enzymes. Co-application of ET reduced these oxidative stress indicators and moderated enzyme activity, with 10 mM ET demonstrating the greatest protective effect. In the docking studies of ET with antioxidant enzymes, it was observed that ET exhibits a binding affinity ranging from −6.3 to −4.6 kcal/mol. It was determined that a significant portion of ET interactions with antioxidant enzymes occurred with glutamine and lysine residues of these enzymes. These findings suggest that the role of ET as a compatible solute contributes to its ability to stabilize cellular structures, decrease reactive oxygen species (ROS) accumulation, and enhance antioxidant defences, indicating its potential as an effective agent for alleviating heavy metal stress in plants.

Keywords Ectoine · Heavy metal stress · *Triticum aestivum* · Cellular protection · Prediction analysis

Introduction

Nickel (Ni) is a naturally occurring metal in the Earth's crust and is increasingly released into the environment through industrial activities such as mining, metallurgy, and the production of batteries, pigments, and stainless steel (Iyaka

2011; Janas et al. 2018). While Ni is valued for its physical and chemical properties, it also plays an essential biological role in certain microorganisms and plants (Polacco et al. 2013; Boer et al. 2014; Brazzolotto et al. 2016). In plants, Ni functions primarily as a micronutrient cofactor for urease, the enzyme responsible for catalysing urea breakdown (Polacco et al. 2013). Ni deficiency can lead to urea accumulation, disruption of nitrogen metabolism, and reduced plant growth and yield (Brown et al. 1990; Gerendas and Sattelmacher 1997). Despite its essential role in trace amounts, Ni can become toxic at elevated concentrations (Tveito et al. 1989; Brown et al. 1990; Gad et al. 2007; Dudek-Adamska et al. 2021). It enters soils through both natural processes (e.g., volcanic activity, windblown dust) and anthropogenic inputs such as mining, fossil fuel combustion, and fertilizer or manure application (Kumar and Subrahmanyam. 2021). Once in the soil, Ni can accumulate in plants and subsequently enter the food chain, posing health risks to insects, animals, and humans (Wang et al. 2020; Heidari et al. 2020). High Ni exposure in humans (whether from consuming

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Ni-containing foods, occupational contact in Ni-processing industries, or living near industrial sources) has been linked to diseases affecting the kidney, lungs, cardiovascular system, and nervous system, as well as dermal problems and an increased cancer risk (Tveito et al. 1989; Dudek-Adamska et al. 2021). The toxic effects are primarily attributed to the overproduction of ROS such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($\bullet OH$) (Gajewska and Skłodowska 2007; Wu et al. 2012).

In plants, Ni uptake occurs mainly through the root system via active transport or passive diffusion (Yusuf et al. 2011). Following absorption, Ni is transported to aerial tissues via the xylem, with its mobility controlled by Ni-binding proteins and metal-ligand complexes including organic acids, histidine, and nicotianamine (Chen et al. 2009; Ahmad and Ashraf 2011; Yusuf et al. 2011). Elevated Ni levels have been demonstrated to disrupt cellular processes, including impaired cell division, stunted growth, reduced uptake of Fe and other essential micronutrients, inhibit germination, and interfere with photosynthetic efficiency (Matraszek and Hawrylak-Nowak 2010; Lešková and Araya 2019; Jahan et al. 2020). Symptoms such as chlorosis and necrosis in leaves of plants directly contribute to quality and yield losses (Sun, 1998; Matraszek and Hawrylak-Nowak 2010).

Cereals form a fundamental pillar of global food security, and the agricultural implications of their productivity are therefore substantial. Wheat, rice, and maize together occupy over half a billion hectares worldwide (FAOSTAT, 2012). Despite the population growth from 2012 to 2020, the wheat cultivation area, which stood at 217 million hectares in 2012, saw a marginal increase to 219 million hectares based on data from 2020 (Peña-Bautista et al. 2017; FAO 2018; FAOSTAT 2020). Addressing yield losses from heavy metal stress, including Ni toxicity, is therefore critical. Field and greenhouse studies have demonstrated that Ni contamination can markedly reduce crop productivity. For example, a greenhouse study in Canada reported a 25% yield reduction in plants grown in Ni-contaminated soils (Kukier and Chaney 2004). In Egypt, wheat and rice cultivated in Ni-rich soils accumulated proportionally more Ni, correlating with impaired growth (Badawy et al. 2023). Similar observations in wastewater-irrigated fields have confirmed elevated Ni accumulation in wheat tissues and associated yield declines (Gajewska et al. 2006; Singh et al. 2012b; Nawaz et al. 2021).

Since humankind relies on Ni in various industries, and it is also essential for the metabolism of certain organisms, its importance is evident. However, at high concentrations, Ni can be toxic to many organisms, including humans, and can lead to significant economic losses by inhibiting plant growth. ET, a microbially derived compound, has been shown to exert protective effects on a wide range of

macromolecules, including proteins, nucleic acids, and membrane lipids, and its protective properties have been demonstrated under various environmental and physiological stress conditions, such as high salinity, extreme temperatures, dehydration, and oxidative stress. Building on this background, the present study aimed to investigate whether ET could mitigate the toxic effects of nickel, with a particular focus on wheat, an essential crop for human nutrition. To achieve this, the study investigated various parameters of wheat plants grown in a hydroponic system, including antioxidant enzyme levels, H_2O_2 and MDA content under different Ni concentrations. Furthermore, docking studies were conducted to investigate the possible interaction of ET with antioxidant enzymes in order to clarify its role in ROS regulation and antioxidant defence.

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Material and Method

Plant Material and Growth Conditions

The seeds of *Triticum aestivum* cv. Yıldırım were subjected to surface sterilization using a 3% solution of sodium hypochlorite for 5 min, followed by five rinses with sterilized distilled water. After sterilization, the seeds were placed in net pots filled with expanded clay pellets (hydrotons) and allowed to germinate at 26 ± 2 °C for 4 days. To determine

appropriate concentrations for Ni stress, a preliminary trial was conducted using different concentrations of NiCl_2 (10, 20, 30, 40, 50, and 75 ppm). While wheat seedlings exhibited visible symptoms of stress at 30 and 40 ppm, significant growth inhibition was observed at 50 and 75 ppm. A stock solution of NiCl_2 was prepared and added in appropriate volumes to 2 L containers to achieve the desired final concentrations. Throughout the experiment, the Hoagland solution supplemented with Ni and ectoine was completely renewed every two days to prevent depletion of nutrients and supplements. Therefore, these two concentrations were selected for use in the subsequent experiments. Following the determination of effective Ni concentrations, the concentration of ectoine ($\geq 95.0\%$ purity by HPLC; powder form; product code 81619, Sigma-Aldrich, USA) was determined. The required amount was then weighed and dissolved directly in the Hoagland nutrient solution prior to application. Different ET concentrations (0.5, 1, 5, 10, and 15 mM) were tested, revealing that 0.5 and 1 mM had little to no alleviating effect, while 15 mM did not enhance the response beyond that achieved with 10 mM. Consequently, the 0.5, 1, and 15 mM concentrations of ET were excluded from the experimental groups; namely, only 5- and 10-mM ET were used in the subsequent experiments. For the new experiment, germination was carried out as described above. Following germination, plants were treated with Ni, ET, and their combination in half-strength Hoagland nutrient solution within a hydroponic system under controlled growth chamber conditions. Four net pots were used per treatment group. The photoperiod was set to 16 h light/8 h dark with a light intensity of $200 \mu\text{mol m}^{-2} \text{s}^{-1}$. A constant temperature of $26 \pm 2^\circ\text{C}$ was maintained throughout both the light and dark phases, and relative humidity was kept at 60–70%. The Hoagland nutrient solution contained the following components (mg/L): K_2SO_4 , 146.5; $\text{CaCl}_2 \cdot 2 \text{H}_2\text{O}$, 73.5; $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$, 51.5; KNO_3 , 18.05; $\text{NaH}_2\text{PO}_4 \cdot 2 \text{H}_2\text{O}$, 2.51; H_3BO_3 , 0.47; $\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$, 1.66; $\text{MnCl}_2 \cdot 4 \text{H}_2\text{O}$, 0.19; $\text{ZnSO}_4 \cdot 7 \text{H}_2\text{O}$, 0.04; $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$, 0.015; and H_2MoO_4 , 0.11, adjusted to pH 6.0 (Orhan et al. 2022, 2023).

Treatments

After determining the concentrations of the heavy metal and the alleviating agent, the experiments were arranged into seven groups: a control group (0Ni0ET, no Ni and no ET), 50 ppm Ni (50Ni0ET), 50 ppm Ni with 5 mM ET (50Ni5ET), 50 ppm Ni with 10 mM ET (50Ni10ET), 75 ppm Ni (75Ni0ET), 75 ppm Ni with 5 mM ET (75Ni5ET), and 75 ppm Ni with 10 mM ET (75Ni10ET). These groups were designed to assess the effects of different Ni concentrations and ET combinations. To ensure a uniform distribution of nutrients in the Hoagland medium and optimal

oxygen availability for plant roots, the hydroponic systems were consistently aerated using an air pump throughout the experiments. Regular water addition was implemented to prevent water loss caused by evapotranspiration. Each treatment was replicated three times for statistical reliability. On the 10th day following the germination period, the root and shoot length, as well as the total fresh weight of the plants, were measured. The collected plant samples were subsequently analysed to determine antioxidant enzyme activities, MDA and H_2O_2 levels.

Measurement and Analysis

Ni Content Determination

To determine Ni content, plant samples were washed with tap water, rinsed with distilled water, and dried at 40°C for three days. After drying, the samples were carefully weighed and a 500 mg portion of each was transferred to a polytetrafluoroethylene container for acid digestion with HNO_3 and H_2O_2 using a microwave system (Milestone Start D). Ni content of each sample was measured by Inductively coupled plasma mass spectrometry (ICP-MS) (Thermo Scientific X Series II) after two-stage microwave digestion and dilution, following a previously described method (Orhan and Ceyran 2024).

Estimation of Chlorophyll Content

Photosynthetic pigment content in plant tissues was determined using a modified version of a previously described method (Knudson et al. 1977). A 200 mg leaf sample was immersed in 20 mL of 95% ethanol for pigment extraction, and the absorbance of the extracts was measured at 470, 649, and 665 nm using a spectrophotometer. Chlorophyll a, chlorophyll b, and total chlorophyll concentrations were calculated based on equations provided previously (Lichtenthaler and Wellburn 1983).

Estimation of H_2O_2 Content

During the assessment of H_2O_2 quantification, minor modifications were introduced to a previously established methodology (Velikova et al. 2000). Freshly harvested plant tissue (200 mg) from each experimental group was mixed with 2 mL of 0.1% trichloroacetic acid (TCA) to achieve efficient homogenization. Following sample homogenization, the mixture was centrifuged at 11,000 rpm for 20 min at 4°C . After centrifugation, 200 μL of supernatant from each sample was mixed with 200 μL of potassium phosphate buffer (10 mM, pH 7.20). Following this, 500 μL

of 1 M potassium iodide was added to the mixture, and the absorbance at 390 nm was recorded at a temperature of 4 °C. To establish the calibration curve, standard H₂O₂ solutions were prepared using 0.1% TCA at eight distinct concentrations, spanning from 0.0125 to 5 nM. The H₂O₂ content of each sample was then determined by referencing the calibration curve.

Estimation of Lipid Peroxidation (MDA)

The MDA content in plant tissues was determined following a previously described method, with some modifications (Razinger et al. 2008). In summary, 300 mg of plant tissue from each experimental group was meticulously homogenized in a solution comprising 3 mL of 0.01% butylated hydroxytoluene, 15% TCA, 0.25 M hydrochloric acid, and 0.37% thiobarbituric acid (TBA). Following homogenization, samples were incubated at 90 °C for 30 min. Afterwards, the samples were immediately cooled on ice, then centrifuged at 11,000 rpm for 15 min at 4 °C. Subsequently, the supernatant was mixed 1:1 (v/v) with 0.3 mM TCA. Following this, samples were centrifuged at 12,000 rpm for 10 min, and MDA content was determined by measuring absorbance at 532 and 600 nm wavelengths. The latter (600 nm) was subtracted from the former to rectify for non-specific turbidity. MDA equivalents per gram fresh weight (FW) were then computed, per the previously outlined methodology (Landi 2017).

Extraction of Enzymes

Each sample, comprising 200 mg of fresh plant tissue, underwent thorough homogenization in a cold mortar using 2 mL of 100 mM cold potassium phosphate buffer (pH 7.0). The buffer was supplemented with 1% polyvinyl pyrrolidone and 0.1 mM ethylenediaminetetraacetic acid (EDTA). After centrifugation for 15 min at 14,000 rpm at 4 °C, the supernatant from each sample was collected and employed for the determination of the relevant enzymes.

Superoxide Dismutase (EC1.15.1.11)

The activity of the superoxide dismutase (SOD) enzyme was assessed by monitoring the inhibition of the photochemical reduction of nitro blue tetrazolium (NBT) (Beauchamp and Fridovich 1971). A volume of 20 µL of supernatant from each group was mixed with 1500 µL reaction mixture. Aliquots (20 µL) of supernatant from each treatment group were mixed with 1500 µL of the reaction mixture. The reaction mixture consisted of 300 µL of 750 µM NBT, 300 µL of 130 mM methionine, 300 µL of 20 µM riboflavin, 300 µL of 100 µM EDTA-Na₂, and 300 µL of H₂O₂ in 50

mM potassium phosphate buffer (pH 7.8). The alterations in absorbance were recorded at 560 nm. An enzyme unit was defined as a 50% inhibition in the reaction involving the mentioned chemicals. SOD activity was expressed as units per gram fresh weight (U/g FW), where one unit corresponds to 50% inhibition of NBT reduction.

Catalase (EC1.11.1.6) Activity

Catalase (CAT) activity of the plant samples was assessed by partially adapting a prior method (Aebi 1984). In summary, the reaction mixture contained 20 µL enzyme extract, 100 µL of 150 mM potassium phosphate buffer (pH 7.0), 150 µL of 30 mM H₂O₂, and 30 µL distilled water, resulting in final concentrations of 50 mM phosphate buffer and 15 mM H₂O₂ in a total volume of 300 µL. The mixture was incubated at 25±2 °C for 30 min. Changes in absorbance at 240 nm were monitored using a spectrophotometer. CAT activity was expressed as micromoles of H₂O₂ decomposed per minute per milligram protein (µmol/min/mg protein).

Guaiacol Peroxidase (EC1.11.1.7) Activity

The guaiacol peroxidase (GPOX) activity in plant samples was assessed by partially adapting a prior method (Baycu et al. 2006). In brief, the reaction mixture, with a total volume of 300 µL, contained 215 µL of 100 mM sodium potassium phosphate buffer (pH 5.8), 45 µL of 100 mM guaiacol, 15 µL of 100 mM hydrogen peroxide (H₂O₂), and 25 µL of enzyme extract. Enzyme activity was expressed as the change in absorbance at 470 nm per gram FW per minute (ΔA470/g FW/min).

Ascorbate Peroxidase (EC 1.11. 1.11) Activity

The assessment of ascorbate peroxidase (APX) activity in plant samples was conducted through a partial modification of a previously established method (Nakano and Asada 1981). In brief, the reaction mixture, with a total volume of 300 µL in 50 mM phosphate buffer (pH 7.0), contained 50 µL of enzyme extract, 50 µL of 3 mM ascorbate, 50 µL of 0.6 mM H₂O₂, and 50 µL of 0.6 mM EDTA. The enzyme activity was assessed by monitoring the absorbance at 290 nm. The APX activity was expressed as micromoles of ascorbate oxidized per minute per millilitre of enzyme extract (µmol/min/mL).

Preparation of Complexes and Molecular Docking

The receptor molecules of ascorbate peroxidase (1OAG), catalase (1DGB), glutathione peroxidase (4A78), and superoxide dismutase (6DQY) were downloaded from

RSCB Protein Data Bank (RCSB 2024). ET was selected as a ligand and downloaded from PubChem (PubChem CID: 126041) in SDF format (PubChem 2024). The ligand and receptor in SDF format was converted to PDB format using the PyMOL program (Schrodinger 2021). The receptor molecules were prepared in the Autodock Vina program (Trott & Olson, 2010) by removing water molecules, adding polar hydrogens, and adding Kollman charges (Bozari 2024). The ET molecule was also prepared using the same program. The best five binding sites of each receptor molecule were identified using the Discovery Studio Visualizer program (Biovia 2023). The grid dimensions were selected as indicated for the five best binding sites. The best binding poses of the ligand to the receptors were saved as output files in PDB format and were designed using PyMOL. Then Biovia Discovery Studio was used to visualize the results in both 2D and 3D.

Statistical Analysis

Statistical analysis involved the execution of a one-way ANOVA test using SPSS for Microsoft Windows (SPSS v.22.0). The outcomes were presented as the means of three experiments $\pm$ standard deviation (SD). Significance of differences between means was determined using the Duncan's multiple range test at a confidence level of 0.05.

Results

The Presence of Ni in Root and Stem Samples

Ni content in shoots under control conditions (0Ni0ET) was 348 ppb. Treatment with 50 ppm Ni (50Ni0ET) increased shoot Ni content by 49.4-fold (17203 ppb). Co-application of 5 mM ET with 50 ppm Ni (50Ni5ET) reduced shoot Ni content to approximately 2.46-fold (6982 ppb), corresponding to a 59.4% improvement compared to 50 ppm Ni alone (50Ni0ET). Increasing ET to 10 mM (50Ni10ET) further lowered Ni content to about 2.60-fold (6610 ppb), achieving a 61.56% improvement (Fig. 1). Compared to the control group (0Ni0ET), shoot Ni content increased to 43.3-fold (19411 ppb) in the 75 ppm Ni application (75Ni0ET). Co-application of 5 mM ET with 75 ppm Ni (75Ni5ET) decreased shoot Ni content to 9938 ppb, corresponding to a 48.8% improvement compared to 75 ppm Ni alone (75Ni0ET). Increasing ET to 10 mM (75Ni10ET) further decreased shoot Ni content to 8405 ppb, achieving a 56.7% improvement (Fig. 1). Ni content in roots under control conditions (0Ni0ET) was 379 ppb. Compared to control (0Ni0ET), treatment with 50 ppm Ni (50Ni0ET) increased root Ni content by 130.7-fold (49537 ppb). Co-application of 5 mM ET with 50 ppm Ni (50Ni5ET) reduced root Ni content to 46,995 ppb, corresponding to a 5.13% decrease compared to 50 ppm Ni alone (50Ni0ET). Increasing ET

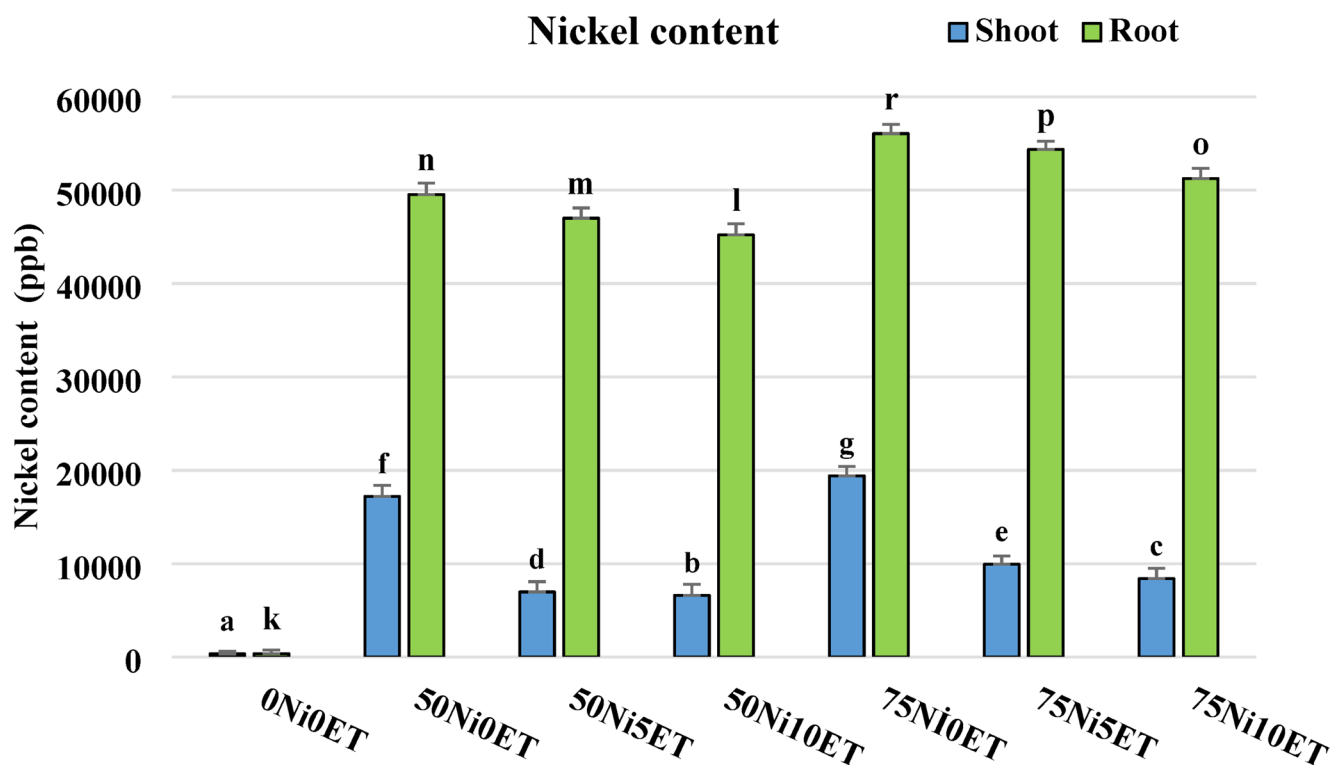


Fig. 1 Nickel (Ni) accumulation in *T. aestivum* under various treatments (nickel, ectoine (ET) and their combinations). Means within each column followed by the same letter are not significantly different at the $p < 0.05$ level as determined by Duncan's multiple range test

to 10 mM (50Ni10ET) further lowered Ni content to about 1.10-fold (45196 ppb), achieving an 8.76% improvement (Fig. 1). Compared to the control group (0Ni0ET), root Ni content increased to 147.9-fold (56051 ppb) in the 75 ppm Ni application (75Ni0ET). Co-application of 5 mM ET with 75 ppm Ni (75Ni5ET) decreased root Ni content to 1.03-fold (54350 ppb), corresponding to a 3.04% reduction compared to 75 ppm Ni alone (75Ni0ET). Increasing ET to 10 mM (75Ni10ET) further decreased root Ni content to 1.09-fold (51244 ppb), achieving an 8.58% reduction (Fig. 1).

Chlorophyll (Chl) a and b Levels in Response to Ni and ET Applications

Under the 50Ni0ET treatment, Chl-a content decreased by 26.81% compared to the 0Ni0ET treatment, from 1.38 to 1.01 mg/g FW (Fig. 2). Chl-a content increased to 1.12 mg/g FW under the 50Ni5ET treatment, representing a 10.89% improvement relative to 50Ni0ET. Chl-a content was 1.22 mg/g FW under the 50Ni10ET treatment, representing a 20.79% improvement relative to 50Ni0ET. Under the 75Ni0ET treatment, Chl-a content decreased by 33.34% compared to the 0Ni0ET treatment, from 1.38 to 0.92 mg/g

FW (Fig. 2). Chl-a content increased to 0.97 mg/g FW under the 75Ni5ET treatment, representing a 5.43% improvement relative to 75Ni0ET. Chl-a content was 1.02 mg/g FW under the 75Ni10ET treatment, representing a 10.87% improvement relative to 75Ni0ET. Under the 50Ni0ET treatment, Chl-b content decreased by 18.03% compared to the 0Ni0ET treatment, from 0.61 to 0.50 mg/g FW (Fig. 2). Chl-b content increased to 0.55 mg/g FW under the 50Ni5ET treatment, representing a 10.0% improvement relative to 50Ni0ET. Chl-b content was 0.58 mg/g FW under the 50Ni10ET treatment, representing a 16.0% improvement relative to 50Ni0ET. Under the 75Ni0ET treatment, Chl-b content decreased by 34.43% compared to the 0Ni0ET treatment, from 0.61 to 0.40 mg/g FW (Fig. 2). Chl-b content increased to 0.45 mg/g FW under the 75Ni5ET treatment, representing a 12.50% improvement relative to 75Ni0ET. Chl-a content was 0.51 mg/g FW under the 75Ni10ET treatment, representing a 27.50% improvement relative to 75Ni0ET.

H₂O₂ Content in Response to Ni and ET Applications

Under the 50Ni0ET, H₂O₂ content increased from 192.56 to 254.41 μ mol/g FW, representing a 32.11% rise compared

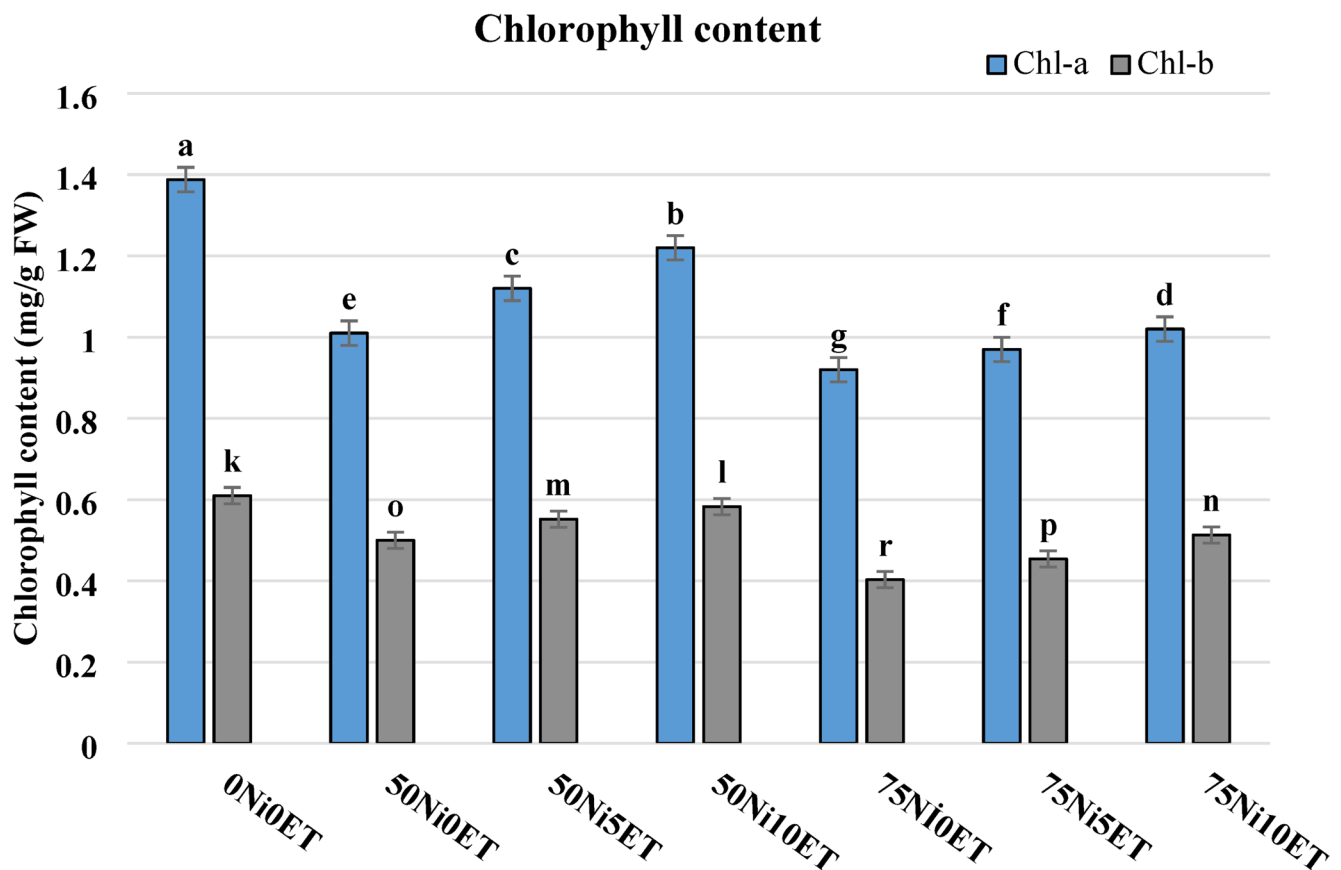


Fig. 2 Chlorophyll content in *T. aestivum* in response to nickel (Ni) and ectoine (ET) treatments. Means within each column followed by the same letter are not significantly different at the $p < 0.05$ level as determined by Duncan's multiple range test

Fig. 3 H_2O_2 levels in *T. aestivum* in response to nickel (Ni) and ectoine (ET) treatments. Means within each column followed by the same letter are not significantly different at the $p < 0.05$ level as determined by Duncan's multiple range test

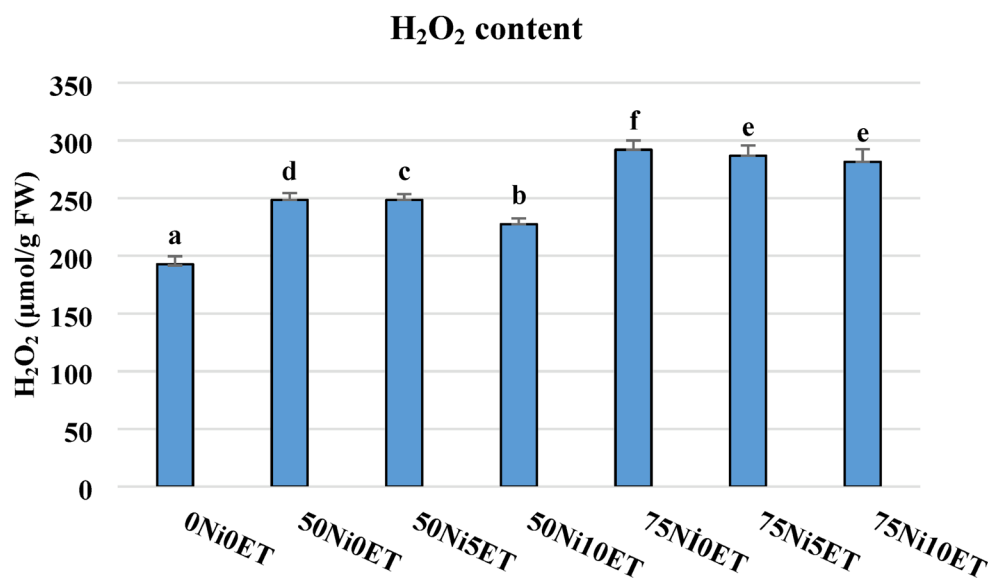
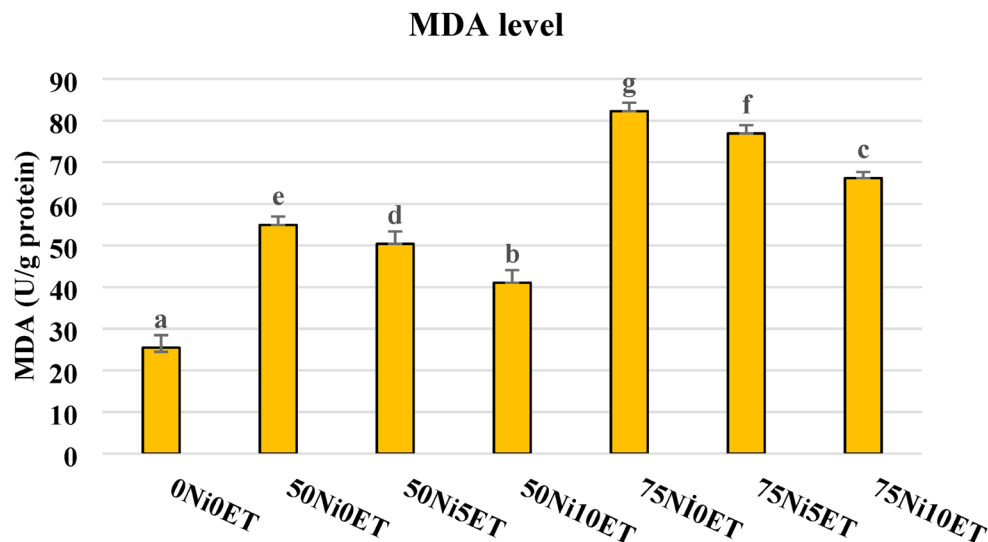


Fig. 4 MDA levels in *T. aestivum* in response to nickel (Ni) and ectoine (ET) treatments. Means within each column followed by the same letter are not significantly different at the $p < 0.05$ level as determined by Duncan's multiple range test



to the 0Ni0ET. Under the 50Ni5ET, H_2O_2 content decreased to 248.38 $\mu\text{mol/g FW}$, representing a 2.37% improvement compared to the 50Ni0ET. Under the 50Ni10ET, H_2O_2 content decreased to 227.32 $\mu\text{mol/g FW}$, representing a 0.64% improvement compared to the 50Ni0ET (Fig. 3). Under the 75Ni0ET, H_2O_2 content increased from 192.56 to 291.96 $\mu\text{mol/g FW}$, representing a 51.62% rise compared to the 0Ni0ET. Under the 75Ni5ET, H_2O_2 content decreased to 286.64 $\mu\text{mol/g FW}$, representing a 1.82% improvement compared to the 75Ni0ET. Under the 75Ni10ET, H_2O_2 content decreased to 281.44 $\mu\text{mol/g FW}$, representing a 3.61% improvement compared to the 75Ni0ET (Fig. 3).

MDA Level in Response to Ni and ET Applications

Under the 50Ni0ET, MDA content increased from 25.45 to 54.93 U/g protein, representing a 115.83% rise compared to

the 0Ni0ET. Under the 50Ni5ET, MDA content decreased to 50.97 U/g protein, representing an 8.26% improvement compared to the 50Ni0ET. Under the 50Ni10ET, MDA content decreased to 41.08 U/g protein, representing a 25.21% improvement compared to the 50Ni0ET (Fig. 4). Under the 75Ni0ET, MDA content increased from 25.45 to 82.28 U/g protein, representing a 223.31% rise compared to the 0Ni0ET. Under the 75Ni5ET, MDA content decreased to 76.90 U/g protein, representing a 6.53% improvement compared to the 75Ni0ET. Under the 75Ni10ET, MDA content decreased to 66.18 U/g protein, representing a 19.56% improvement compared to the 75Ni0ET (Fig. 4).

Fig. 5 SOD contents in *T. aestivum* in response to nickel (Ni) and ectoine (ET) treatments. Means within each column followed by the same letter are not significantly different at the $p < 0.05$ level as determined by Duncan's multiple range test

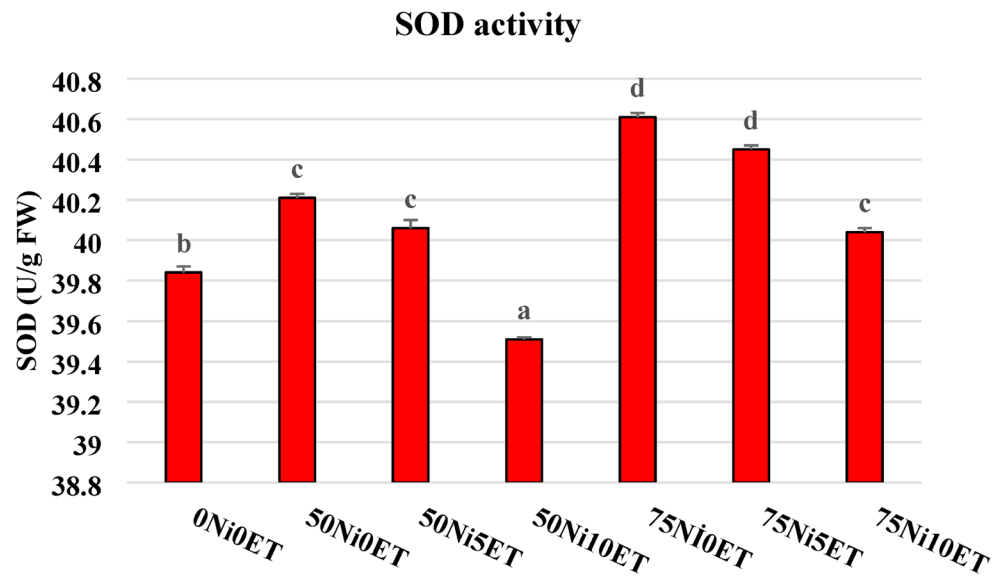
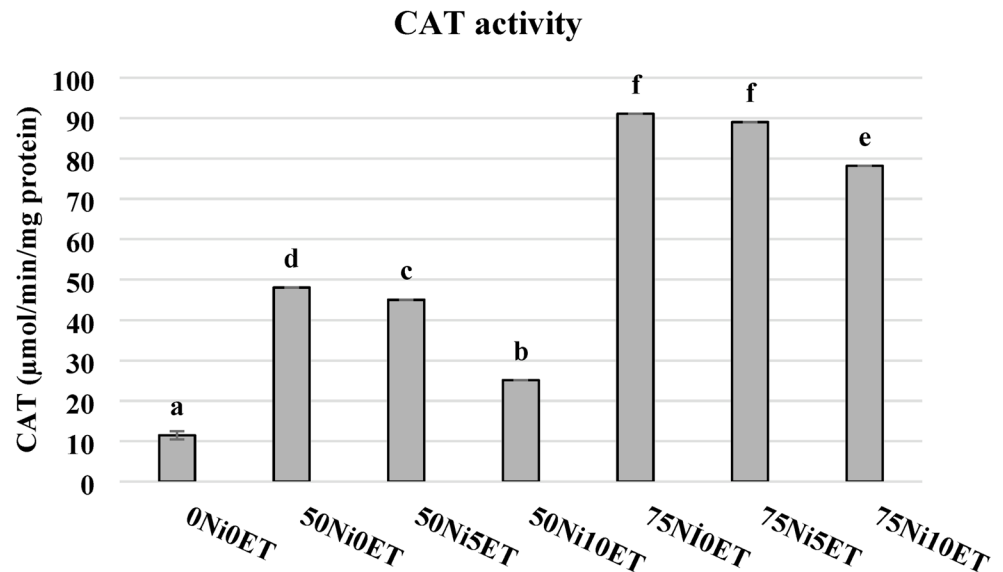


Fig. 6 CAT contents in *T. aestivum* in response to nickel (Ni) and ectoine (ET) treatments. Means within each column followed by the same letter are not significantly different at the $p < 0.05$ level as determined by Duncan's multiple range test



Antioxidant Enzyme in Response to Ni and ET Applications

With respect to the control group (0Ni0ET), SOD activity under the 50Ni0ET exhibited a modest rise of 0.93%, increasing from 39.84 to 40.21 U/g protein. Under the 50Ni5ET application, SOD activity was 40.06 U/g protein, representing an improvement of 0.37% compared to 50Ni0ET. Under the 50Ni10ET application, SOD activity was 39.51 U/g protein, representing an improvement of 1.74% compared to 50Ni0ET (Fig. 5). With respect to the control group (0Ni0ET), SOD activity under the 75Ni0ET exhibited a modest rise of 1.93%, increasing from 39.84 to 40.61 U/g protein. Under the 75Ni5ET application, SOD activity was 40.45 U/g protein, representing an improvement of 0.39% compared to 75Ni0ET. Under the 75Ni5ET

application, SOD activity was 40.04 U/g protein, representing an improvement of 1.41% compared to 75Ni0ET (Fig. 5).

Ni application led a significant increase in CAT enzyme activity (Fig. 6). Under the 50Ni0ET treatment, CAT activity increased 4.19-fold compared to the 0Ni0ET treatment, rising from 11.48 to 48.02 μmol/min/mg protein. CAT activity decreased to 45.03 μmol/min/mg protein under the 50Ni5ET treatment, representing a 6.23% improvement relative to 50Ni0ET. CAT activity was 25.12 μmol/min/mg protein under the 50Ni10ET treatment, representing a 47.69% improvement relative to 50Ni0ET. Under the 75Ni0ET treatment, CAT activity increased 7.93-fold compared to the 0Ni0ET treatment, rising from 11.48 to 91.08 μmol/min/mg protein. CAT activity decreased to 89.01 μmol/min/mg protein under the 75Ni5ET treatment,

Fig. 7 GPOX contents in *T. aestivum* in response to nickel (Ni) and ectoine (ET) treatments. Means within each column followed by the same letter are not significantly different at the $p < 0.05$ level as determined by Duncan's multiple range test

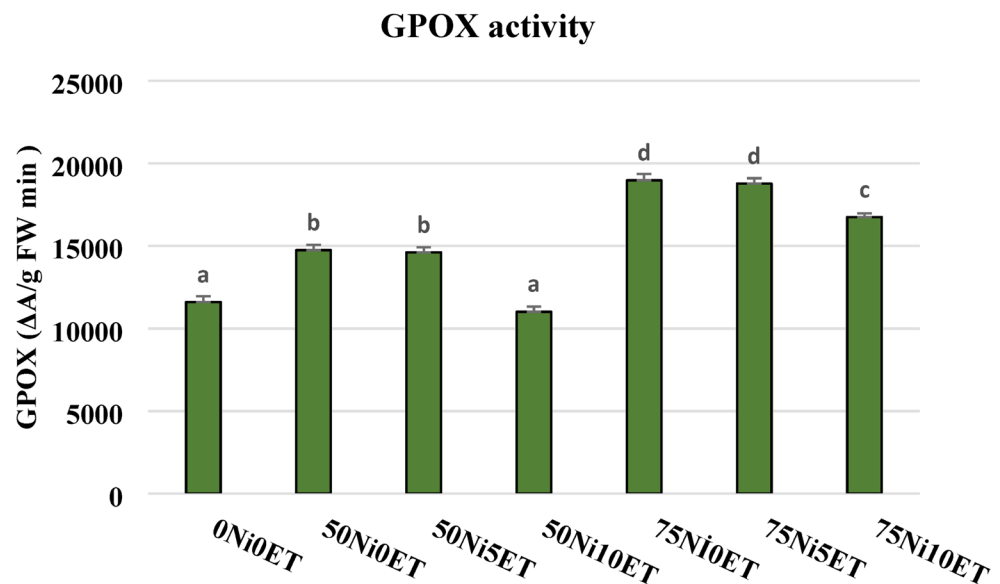
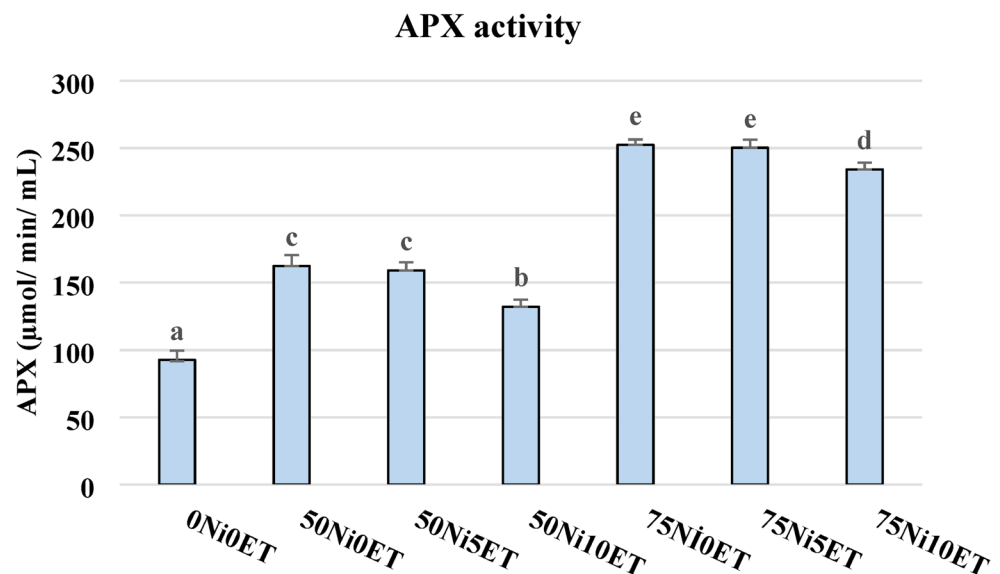


Fig. 8 APX contents in *T. aestivum* in response to nickel (Ni) and ectoine (ET) treatments. Means within each column followed by the same letter are not significantly different at the $p < 0.05$ level as determined by Duncan's multiple range test



representing a 2.27% improvement relative to 75Ni0ET. CAT activity was 78.17 $\mu\text{mol}/\text{min}/\text{mg}$ protein under the 75Ni10ET treatment, representing a 14.17% improvement relative to 75Ni0ET (Fig. 6).

Under the 50Ni0ET treatment, GPOX activity increased 1.27-fold compared to the 0Ni0ET treatment, rising from 11,596 to 14,735 $\Delta\text{A}470/\text{g}$ FW/min (Fig. 7). GPOX activity decreased to 14,605 $\Delta\text{A}470/\text{g}$ FW/min under the 50Ni5ET treatment, representing a 0.88% improvement relative to 50Ni0ET. GPOX activity was 11,021 $\Delta\text{A}470/\text{g}$ FW/min under the 50Ni10ET treatment, representing a 25.21% improvement relative to 50Ni0ET. Under the 75Ni0ET treatment, GPOX activity increased 1.64-fold compared to the 0Ni0ET treatment, rising from 11,596 to 18,969 $\Delta\text{A}470/\text{g}$ FW/min (Fig. 7). GPOX activity decreased to 18,769 $\Delta\text{A}470/\text{g}$ FW/min under the 75Ni5ET treatment,

representing a 1.05% improvement relative to 75Ni0ET. GPOX activity was 16,746 $\Delta\text{A}470/\text{g}$ FW/min under the 75Ni10ET treatment, representing a 11.72% improvement relative to 75Ni0ET.

Under the 50Ni0ET treatment, APX activity increased 1.75-fold compared to the 0Ni0ET treatment, rising from 97.67 to 162.32 $\mu\text{mol}/\text{min}/\text{mL}$ (Fig. 8). APX activity decreased to 159.01 $\mu\text{mol}/\text{min}/\text{mL}$ under the 50Ni5ET treatment, representing a 2.03% improvement relative to 50Ni0ET. APX activity was 132.3 $\mu\text{mol}/\text{min}/\text{mL}$ under the 50Ni10ET treatment, representing a 18.49% improvement relative to 50Ni0ET. Under the 75Ni0ET treatment, APX activity increased 2.72-fold compared to the 0Ni0ET treatment, rising from 97.67 to 252.32 $\mu\text{mol}/\text{min}/\text{mL}$ (Fig. 8). APX activity decreased to 250.21 $\mu\text{mol}/\text{min}/\text{mL}$ under the 75Ni5ET treatment, representing a 0.84% improvement

relative to 50Ni0ET. APX activity was 234.13 $\mu\text{mol}/\text{min}/\text{mL}$ under the 75Ni10ET treatment, representing a 7.15% improvement relative to 75Ni0ET (Fig. 8).

Docking Predictions of ET and Antioxidant Enzymes

As indicated in Table 1 the best binding positions of ET to the ascorbate peroxidase, catalase, guaiacol peroxidase, and superoxide dismutase were determined as the initial positions of each region. Accordingly, the binding energy of the molecule to the ascorbate peroxidase enzyme was calculated as -5.1 kcal/mol at S3 site, -6.3 kcal/mol to catalase at S2 and S3 site, -4.6 kcal/mol to guaiacol peroxidase at S2 and S3 site, and -5.2 kcal/mol to superoxide dismutase at S1 and S5 site. The binding energies are indicated in Table 1, and the molecule-ligand interactions are shown in Fig. 9.

Discussion

Halophilic microorganisms exhibit an extraordinary ability to thrive in environments characterized by low water activity, typically associated with high salt concentrations (Brown 1976; Oren 2006). ET is one of the compounds synthesized *de novo* in response to salt stress and is the most extensively studied metabolite among the halophiles. This compound has been shown to effectively stabilize the structures of macromolecules such as DNA, proteins, and lipid bilayers (Yu and Nagaoka 2004; Hahn et al. 2015a; Zaccari et al. 2016; Czech et al. 2018; Keller et al. 2021). The stabilizing effects of ET result from a mechanism known as preferential exclusion (Yu et al. 2007). This process involves the exclusion of ET from the hydration layer surrounding the surface of macromolecules, leading to changes in the solvent structure around these macromolecules. The remarkable properties of ET have propelled it into the spotlight of numerous scientific investigations and have facilitated its application across diverse fields including cosmetics, medicine and biotechnology. For instance, ET stabilizes enzymes and protects cells under stress conditions. In biodiesel production, ET enhances the catalytic efficiency of lipase enzymes, leading to increased biodiesel yields (Wang and Zhang 2010). Moreover, ET stabilizes lactoperoxidase enzymes, commonly used in the food industry, and improves the growth and glucose utilization of *Zymomonas mobilis*, a widely employed ethanol producer. It also protects these cells from ethanol toxicity during fermentation (Zhang et al. 2008). The cell-stabilizing properties of ET extend beyond these applications; it also serves as a cryoprotectant for human cells (e.g., oocytes, red blood cells, and natural killer cells) and microorganisms, such as lactic acid bacteria (Assal et al. 2014; Pasley et al. 2017; Choi et

Table 1 The binding affinity of ectoine (ET) to nine different positions in five different sites with Ascorbate Peroxidase, Catalase, Guaiacol Peroxidase, and Superoxide Dismutase

Mode	S1 position Affinity (kcal/mol)				S2 position Affinity (kcal/mol)				S3 position Affinity (kcal/mol)				S4 position Affinity (kcal/mol)				S5 position Affinity (kcal/mol)			
	APX	CAT	GPOX	SOD	APX	CAT	GPOX	SOD	APX	CAT	GPOX	SOD	APX	CAT	GPOX	SOD	APX	CAT	GPOX	SOD
1	-4.8	-5.9	-4.5	-5.2	-4.6	-6.3	-4.6	-4.8	-5.1	-6.3	-4.6	-4.9	-4.6	-5.8	-4.5	-4.7	-4.8	-6.3	-4.0	-5.2
2	-4.6	-5.8	-4.4	-5.1	-4.4	-6.3	-4.3	-4.7	-4.4	-6.3	-4.5	-4.7	-4.3	-5.8	-4.4	-4.7	-4.5	-6.3	-4.0	-5.1
3	-4.4	-5.8	-4.4	-4.9	-4.2	-6.2	-4.1	-4.5	-4.3	-6.2	-4.5	-4.5	-4.1	-5.7	-4.3	-4.6	-4.5	-6.2	-3.9	-5.1
4	-4.3	-5.7	-4.3	-4.8	-4.2	-6.2	-4.1	-4.5	-4.3	-6.0	-4.4	-4.4	-4.1	-5.7	-4.3	-4.6	-4.3	-6.2	-3.8	-4.8
5	-4.2	-5.7	-4.3	-4.8	-4.2	-6.0	-4.0	-4.5	-4.3	-6.0	-4.4	-4.3	-3.9	-5.6	-4.2	-4.4	-4.3	-6.2	-3.8	-4.6
6	-4.2	-5.7	-4.2	-4.7	-4.2	-6.0	-4.0	-4.4	-4.3	-6.0	-4.3	-4.3	-3.9	-5.6	-4.1	-4.2	-4.3	-6.0	-3.8	-4.6
7	-4.2	-5.6	-4.2	-4.7	-4.2	-6.0	-3.9	-4.4	-4.2	-5.9	-4.3	-4.3	-3.9	-5.6	-4.0	-4.2	-4.1	-6.0	-3.8	-4.4
8	-4.1	-5.4	-4.2	-4.6	-4.1	-6.0	-3.8	-4.4	-4.2	-5.9	-4.2	-4.2	-3.9	-5.5	-3.9	-4.2	-4.1	-6.0	-3.8	-4.4
9	-4.1	-5.4	-4.1	-4.6	-4.1	-6.0	-3.7	-4.4	-4.2	-5.9	-4.2	-4.2	-3.9	-5.5	-3.9	-4.2	-4.0	-5.9	-3.7	-4.3

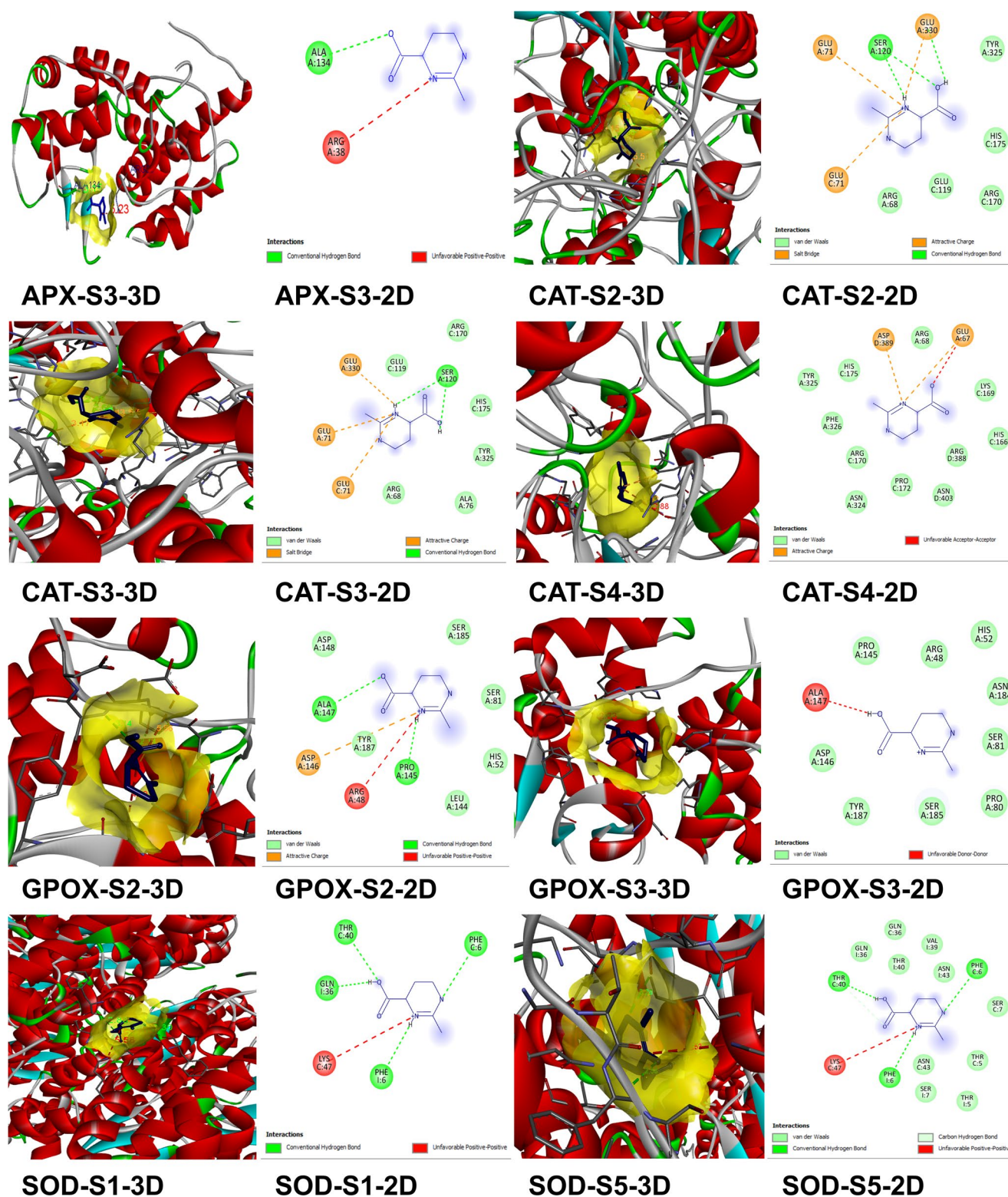


Fig. 9 The 3D and 2D interaction of the best binding sites of ectoine (in blue) to the pocket of Ascorbate Peroxidase (APX), Catalase (CAT), Guaiacol Peroxidase (GPOX), and Superoxide Dismutase (SOD) (in yellow) (color figure online)

al. 2018; Orhan et al. 2024). In medical applications, ET has demonstrated promising cell-protective and anti-inflammatory effects. Studies on human epithelial cells and animal models have shown its potential in alleviating intestinal disorders (Abdel-Aziz et al. 2015; Castro-Ochoa et al. 2019). Furthermore, ET has been found to be more effective than saline in relieving symptoms in patients with acute respiratory infections and acute bronchitis (Tran et al. 2019). It also protects eye health by addressing dry eye disorders and maintaining tear fluid balance (Dwivedi et al. 2014). Additionally, ET has shown potential in reducing the side effects of radiotherapy or chemoradiation (Rieckmann et al. 2019). As mentioned earlier, ET has the potential to protect cells and cellular macromolecules from a wide range of stress factors. Therefore, in this study, we investigated whether ET can protect plants against nickel stress.

Our investigation revealed that exposure to 50 ppm and 75 ppm Ni resulted in significant reductions in Chl-a pigment content, with decreases of 27.23% and 33.34%, respectively, compared to the control. Similarly, Chl-b pigment content declined by 18.03% and 34.43% at these nickel concentrations. In fact, many researchers indicated that Ni toxicity reduces the chlorophyll content in different plants. For example, in a study on a variety of wheat (*Triticum aestivum*), increasing nickel concentrations (25 and 50 μM) resulted in a concentration-dependent reduction in total chlorophyll content (chl a and b) (Uruç Parlak 2016). Similarly, research on *Triticum aestivum* revealed that elevated nickel concentrations (50, 100, and 150 ppm) also led to a concentration-dependent decrease in total chlorophyll content (Saleh et al. 2020). This trend has been observed in other plant species as well, including *Brassica oleracea*, *Vigna mungo*, and *Pistia stratiotes* (Pandey and Sharma 2002; Singh and Pandey 2011; Singh et al. 2012a).

On the other hand, ET, the compound investigated for its potential to reduce Ni toxicity, increased chl-a content at both 50 ppm and 75 ppm Ni concentrations. Among the two ET concentrations tested (5 and 10 mM), the higher concentration (10 mM) was more effective in mitigating the negative impact, with amelioration rates of 20.79% for 50 ppm nickel and 5.43% for 75 ppm nickel. Similarly, chl-b content also increased with the 10 mM ET treatment, showing increases of 10.0% for 50 ppm nickel and 12.50% for 75 ppm nickel. The mechanism through which ET enhances chlorophyll content likely arises from its ability to improve plants' photosynthetic efficiency, thereby facilitating a more effective conversion of light energy into chemical energy, as demonstrated with another osmoprotectant compound, glycine betaine (Mäkelä et al. 2000).

The H_2O_2 level in the control was 192.56 $\mu\text{mol/g FW}$. With the application of 50 ppm nickel, the H_2O_2 level increased to 254.41. The addition of 5 mM ET with 50 ppm

nickel resulted in a level of 252.36, while the application of 10 mM ET with 50 ppm nickel was more effective, reducing the level to 227.32. For the 75 ppm nickel treatment, the H_2O_2 level reached 291.96 $\mu\text{mol/g FW}$. The application of 5 mM ET with 75 ppm nickel resulted in a level of 286.64 $\mu\text{mol/g FW}$, and 10 mM ET was again more effective, decreasing the level to 281.44 $\mu\text{mol/g FW}$. Thus, 10 mM ET demonstrates greater effectiveness than 5 mM ET at both nickel concentrations, particularly at 50 ppm. Numerous studies on various plants, including *Triticum aestivum*, *Zea mays*, and *Solanum lycopersicum*, have shown that H_2O_2 levels increase under nickel stress, supporting the findings of the current study (Jahan et al. 2020; Vaculík et al. 2021; Sabir et al. 2022). The reduction of nickel toxicity through the co-administration of ET suggests that ET may be effective by directly neutralizing reactive oxygen species (ROS), as its ROS-scavenging properties have been reported (Brands et al. 2019), or by enhancing the activity of antioxidant enzymes (Ozfidan-Konakci et al. 2022).

During stress conditions (like heavy metal exposure, drought, or salinity), ROS levels increase and attack the methylene groups (CH_2) in the polyunsaturated fatty acids of the lipid bilayer, creating a lipid radical ($\text{L}^\bullet$). This $\text{L}^\bullet$ then reacts with molecular oxygen (O_2) to form a lipid peroxyl radical ($\text{LOO}^\bullet$). The $\text{LOO}^\bullet$ can react with another lipid molecule, producing a lipid hydroperoxide (LOOH) and generating a new lipid radical, thereby perpetuating the chain reaction. The lipid hydroperoxides (LOOH) formed during this process can break down into reactive aldehydes, such as MDA, which is measured as a biomarker to determine the stress level in cells, including plants (Ayala et al. 2014).

Our study showed that, consistent with the changes in H_2O_2 levels, MDA levels increased with both Ni treatments. Compared to the control group 50 and 75 ppm Ni elevated MDA levels by 115.82% and 223.25%, respectively. This aligns with previous studies reporting that Ni application raises MDA levels in various plants (Sirhindi et al. 2016; Xalxo et al. 2017; Jahan et al. 2020; Vaculík et al. 2021). However, the co-application of Ni with ET, particularly at 10 mM, reduced the elevated MDA levels, improving the condition by 25.21 for 50 ppm Ni toxicity and 19.57% for 75 ppm Ni toxicity. The decrease in MDA levels with ET application under Ni stress is likely due to the role of ET as an osmoprotectant, which stabilizes cellular membranes and shields them from oxidative damage. ET likely mitigates lipid peroxidation by reducing the generation of ROS and enhancing the plant's antioxidant defence mechanisms, thereby lowering MDA levels. This aligns with previous findings that ET helps maintain the stability of lipid monolayer and bilayer membranes (Harishchandra et al. 2010), in addition to its ability to scavenge hydroxyl radicals (Brands et al. 2019). Another scenario is that ET might have

improved plant growth by inhibiting Ni uptake, similar to how the application of glycine betaine has been shown to promote growth of *Zea mays* under salt stress by decreasing Na uptake (Zhu et al. 2022).

Studies on Ni toxicity in plants have applied various concentrations of Ni to different plant species (*Oryza sativa*, *Zea mays*, *Phaseolus vulgaris*), reporting that the Ni is transported to the roots, stems, and leaves (Mihailovic and Drazic 2011; Aziz et al. 2015; Vaculik et al. 2021). In this study, applying 50 ppm of Ni resulted in root levels being 130.2 times higher and stem levels 29.3 times higher compared to the control. At 75 ppm, the increase was 147.8-fold in the roots and 32.8-fold in the stems. Comparing 5 mM ET to 10 mM ET reveals that the higher concentration is more effective in reducing nickel accumulation in both shoots and roots under both Ni stress conditions (50 and 75 ppm Ni). While 5 mM ET provides some mitigation of Ni toxicity, 10 mM ET demonstrates a greater capacity to alleviate nickel uptake, suggesting that increasing the ET concentration enhances its protective effects against nickel stress. It was also observed that the nickel translocated to the plant stems decreased at both ET concentrations applied (5 and 10 mM). The mechanism by which ET reduces nickel content in plants (both roots and shoots) may be similar to the action of the compatible solute proline, which has been shown to enhance antioxidant enzyme activities in *Cicer arietinum* under cadmium toxicity (Hayat et al. 2013). Additionally, a study on the algal cell *Chlorella vulgaris* found that proline application reduced lipid peroxidation caused by heavy metal stress, including nickel (Mehta and Gaur 1999). Recent findings revealed that ET stimulates antioxidant enzymes, enhances proteins linked to fruit disease resistance, and activates the biosynthetic pathways of phenylpropanoids and flavonoids (Xu et al. 2025). Another study also demonstrated that ET enhances antioxidant defence, reduces inflammation, promotes collagen synthesis, activates erythroid 2-related factor 2 (Nrf2), and protects against UVB-induced damage in human skin fibroblast (HSF) cells and a 3D skin model (Wang et al. 2025). Previous research across various plants has demonstrated that nickel exposure increases levels of H₂O₂, MDA, and nickel accumulation (Sirhindi et al. 2016; Xalxo et al. 2017; Kotapati et al. 2017; Jahan et al. 2020; Vaculik et al. 2021). The results of the current study show that co-application of ET and Ni significantly reduces Ni uptake by plant and limits the transport of nickel from the roots to the shoots. The subsequent reduction in these parameters, along with a decrease in Ni content in roots and shoots following the co-application of ET and Ni, supports the hypothesis that ET mitigates nickel-induced oxidative damage by reducing nickel accumulation in plants. The effect of ET in reducing nickel uptake may be attributed to its potential to stabilize the cell membrane.

Stabilization of the cell membrane would consequently reduce nickel uptake into the cells, thereby limiting its transport when uptake is decreased. The reduction in H₂O₂ and MDA levels observed with the co-application of ET and Ni also supports the idea that ET stabilizes the cell membrane. This decrease in oxidative stress markers indicates that ET effectively mitigates nickel-induced damage, thereby helping to protect the integrity of the cell membrane. This aligns with previous findings that ET helps maintain membrane stability, including lipid monolayer and bilayer membranes (Harishchandra et al. 2010).

Our results show that SOD, CAT, APX and GPOX antioxidant enzymes levels increased with both Ni applications. The increase in the levels of these enzymes has risen as the Ni concentration increased from 50 to 75 ppm. These results coincide with the findings of previous studies conducted with various plants under Ni stress (Mihailovic and Drazic 2011; Sirhindi et al. 2016; Zhang et al. 2020; Kumar et al. 2022). Ni exposure significantly increased the activities of CAT, GPOX, APX, and SOD enzymes in wheat plants, with higher enzyme levels observed under 75 ppm Ni compared to 50 ppm. The addition of ET (5 mM or 10 mM) moderated these increases, with 10 mM ET showing a more substantial reduction in all enzyme activities, particularly for CAT and APX. Considering the changes in these enzymes, GPOX is the enzyme least affected under Ni stress. The slight increase in GPOX activity under Ni stress and its relatively minor reduction with ET could be due to its structure. As known, Ni has a high affinity for amino acids like histidine, cysteine, and methionine. If the structure of GPOX contains fewer of these residues in critical regions (such as its active site), Ni ions might have less impact on GPOX than on enzymes with more vulnerable configurations, like CAT or APX (Kozłowski et al. 1999; Kowalik-Jankowska et al. 2007).

The enzyme least affected by Ni stress after GPOX was SOD. The fact that SOD, like GPOX, is less impacted by Ni stress suggests that SOD is relatively resilient in the presence of Ni, potentially due to the presence of amino acids in its structure that interact less with nickel (Kozłowski et al. 1999; Kowalik-Jankowska et al. 2007; Szunyog et al. 2019). At 50 and 75 ppm Ni concentrations, CAT and APX enzyme activities increased significantly compared to the control, suggesting that H₂O₂ levels exceeded what APX could manage alone. This indicates that CAT and APX work effectively together to regulate H₂O₂ levels. Although CAT is noted for its swift turnover rate, it has a lower affinity for H₂O₂ than peroxiredoxins and APX, making both enzymes essential for maintaining plant health under stress (Mittler and Zilinskas 1991; König et al. 2002).

It was observed that among the 5 and 10 mM ET concentrations used to reduce Ni toxicity, 10 mM was more effective

than 5 mM in restoring all antioxidant enzyme levels. These two ET concentrations showed the least improvement in SOD enzyme activity, while significant improvements were seen in the other three enzymes. The minimal effect of Ni toxicity on the SOD enzyme and the limited improvement observed with ET at both concentrations suggest that this enzyme is less affected by nickel toxicity, and hence, ET has a restricted impact on enhancing this enzyme. Both of the ET concentrations applied led to increased activity of the other three enzymes (POD, APX, and CAT), indicating an enhanced ability to resist nickel-induced damage. This increase may be due to the stabilizing effects of ET, as it forms strong interactions with surrounding water molecules, creating a stable hydration shell that indirectly stabilizes enzyme structures (Smiatek et al. 2012; Hahn et al. 2015b; Zaccai et al. 2016). Through the mechanism of preferential exclusion, ET is excluded from the enzymes' hydration layers, modifying the solvent structure around them. This process could help preserve enzyme conformation and reduce their direct interactions with Ni. ET might also regulate the gene expression of antioxidant enzymes, similar to the effects observed with glycine betaine, which has been shown to upregulate the expression of 165 genes, including those associated with heavy metal tolerance, in transgenic rice (*Oryza sativa*) (Kathuria et al. 2009). In addition, ET is well known for its free radical-scavenging effect (Brands et al. 2019), which could reduce the need for antioxidant enzymes, making a decrease in enzyme levels an expected outcome.

Based on the docking results obtained in this study, the binding strength, from strongest to weakest, is as follows: CAT>SOD>APX>GPOX. These results demonstrate that CAT is the most effectively protected, with binding affinities ranging from -6.3 to -5.4 kcal/mol, followed by SOD, whose binding affinities range from -5.2 to -4.8 kcal/mol. APX shows moderate protection, with affinities between -5.1 kcal/mol and -4.1 kcal/mol, while GPOX is the least protected, exhibiting binding affinities from -4.6 kcal/mol to -3.7 kcal/mol. Building on these findings, a study utilizing experimental methods and molecular docking analysis revealed that ET and its derivatives (homoectoine, hydroxyectoine, 2-methyl-6-phosphonomethyl-1,4,5,6-tetrahydro-pyrimidine-4-carboxylic acid, and 2-methyl-6-(3-phosphono-propyl)-1,4,5,6-tetrahydro-pyrimidine-4-carboxylic acid) interact with the tyrosinase enzyme, with the strongest interaction observed for 2-methyl-6-phosphonomethyl-1,4,5,6-tetrahydro-pyrimidine-4-carboxylic acid (Behazin and Ebrahimi 2018). More recently, a study explored the potential of four compatible solutes (trehalose, inositol, glucosylglycerol, and sucrose) to protect the RNase enzyme. Using Molecular Mechanics Poisson-Boltzmann Surface Area analysis, it was reported

that glucosylglycerol and sucrose provided the best protection based on their binding energies (Ilyas et al. 2024). By demonstrating binding affinity for specific amino acids within antioxidant enzymes, ET may enhance these enzymes' activity, thereby alleviating stress in the plant.

Conclusion

The study highlights the protective effects of ET against nickel-induced stress in wheat plants, with 10 mM ET proving more effective than 5 mM. ET application significantly alleviated chlorophyll degradation, limited nickel accumulation, and reduced oxidative stress markers (H_2O_2 and MDA) in response to nickel toxicity. Additionally, ET moderated the activities of key antioxidant enzymes (SOD, CAT, APX, and GPOX), with the greatest reduction observed in CAT activity, indicating its stabilizing influence on antioxidant defence systems. Docking analysis further confirmed these findings, indicating a binding affinity trend of CAT>SOD>APX>GPOX, with CAT displaying the strongest interaction with ET (-6.3 to -5.4 kcal/mol) and GPOX the weakest (-4.6 to -3.7 kcal/mol). The observed protective effects likely stem from the ability of ET to reduce reactive oxygen species and stabilize enzyme structures, supporting its potential utility in enhancing plant resilience under heavy metal stress conditions. This research highlights the potential of ET as an eco-friendly solution to enhance plant health and productivity in metal-polluted environments. However, the role of ET in effectively mitigating Ni toxicity remains unclear and warrants further investigation.

Author Contributions Furkan Orhan: conceptualization, methodology, investigation, writing—original draft, and supervision, Kadriye Uruç Parlak: Investigation, Data collection, and Formal analysis, Sedat Bozari: investigation, validation, formal analysis, writing—review and editing, and software analysis, Derya Efe: methodology, investigation, writing. All authors commented on the previous versions of the manuscript, and all authors read and approved the final version of the manuscript.

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Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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