Acetone O-(2-naphthylsulfonyl)oxime Alleviates the Toxic Effects of Cadmium Stress in Maize Seedlings by Increasing the Phenolic Substance Content and Antioxidant System Activity

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Abstract

Cadmium (Cd) is a heavy metal that can have toxic effects on plants even in trace amounts in the soil. In this study, we aimed to elucidate the role of exogenous O-(2-naphthylsulfonyl)oxime (ANSO) in maize plants under Cd stress. For this purpose, the following experimental groups were formed: The control group will be kept in distilled water for 18 hours, the second group will be kept in ANSO (0.3 mM) for 6 hours and then in distilled water for 12 hours, the third group will be in distilled water for 6 hours and then in Cd solution (100 μM) for 12 hours, fourth group ANSO (0.3 mM) for 6 hours, followed by 12 hours of Cd solution (100 μM). When ANSO+Cd application is compared to Cd, the Cd content increased 7.8 times, while the ABA content decreased. RWC content, which was reduced by Cd stress, was not changed by ANSO pre-treatment. Chlorophyll content, which decreased with Cd treatment, increased with ANSO+Cd treatment. While the carotenoid content increased with Cd application, it increased much with ANSO+Cd application. The H$_2$O$_2$ content and lipid peroxidation increased in the plant with Cd stress and decreased with ANSO pre-treatment. With ANSO+Cd treatment, GPX activity decreased compared to Cd treatment, but CAT and APX values increased. ANSO pre-treatment did not significantly change SOD activity. Cd application increased proline content compared to control, but proline content decreased compared to Cd with ANSO pre-treatment. In ANSO+CD application, ascorbic acid, cinnamic acid and catechol values increased compared to the values in plants treated with Cd alone, but the trans-coumaric acid value decreased. As a result, it can be said that ANSO pre-application to maize seedlings under Cd stress provides the preservation of the ion balance of the cells by chelating Cd$^{+2}$ ions in the cell wall and vacuoles.

Introduction

Cadmium (Cd) is found naturally in the lithosphere; it is included in the ecosystem with events such as volcanic movements, erosion of rocks, and especially with mining activities in the zinc ores it is associated with. Cd is insoluble in water and resistant to abrasion; it is a heavy metal that is exported and imported because it is used in many fields such as solar panel and infrared optical window production, obtaining color pigments in paints, production of Ni-Cd batteries, production of speed control rods in nuclear fission (Bernhoft 2013; Genchi et al. 2020), production of phosphorus fertilizer (Asri et al. 2007). Unfortunately, this heavy metal, the industrial importance of which is undeniable, is biotoxic to all living organisms. Every year, an average of 25,000–30,000 tons of Cd is included in the ecosystem and thus the food chain through natural and anthropogenic ways (Yılmaz 2015). Epizootiological and epidemiological studies have revealed that Cd has carcinogenic effects in animals and humans (Huff et al. 2007). Major causes of cadmium toxicity in humans are contaminated water and food, as well as cigarette inhalation (Vlachou et al. 2022).

Cd slows root and shoot growth in plants, reduces the rate of photosynthesis, and causes changes in antioxidant enzyme activity (Ahmad et al. 2015; Khan et al. 2015). In the presence of Cd, ROS production increases, and oxidative stress develops (Hassoun & Stohs 1996). In a study with wheat, pea, and tomato, it was observed that Cd toxicity slowed seed germination (Baruah et al. 2019), while in a study conducted
with *Cicer arietinum* L., there was a decrease in protein yield due to nitrogen deficiency (Athar & Ahmad 2002). A study with the *Vicia faba* plant showed that Cd-induced oxidative stress triggered DNA damage in the plant (Lin et al. 2007) and caused a growth slowdown in the *Spinacia oleracea* plant (Younis et al. 2016). Cd is easily transported from the roots to the above-ground parts; disrupts the plant's stomatal activities, enzyme activities, and activates genes tasked with Cd tolerance, accumulation, and detoxification in the plant (Farinati et al. 2010). Another study showed accumulation of Cd in vegetative organs in maize plants exposed to Cd at different concentration levels and inhibited intake of copper, zinc, and manganese from soil (Akhtar et al. 2017). While herbal organisms fight against Cd with their defense systems, they either try to prevent the uptake of Cd from the soil, either try to develop tolerance to the Cd they are forced to take, or try to keep it away from their metabolic activities by accumulating Cd in various organs (El Rasafi et al. 2022).

Maize (*Zea mays* L.) ranks third after wheat and rice in terms of human nutrition. (Chaudhary et al. 2014). Today, the grain of maize is used in nutrition both directly and indirectly as starch and glucose; in addition, herbaceous parts are heavily preferred in the feed industry. When the maize plant was exposed to cadmium stress, Rubisco and phosphoenolpyruvate carboxylase activities were inhibited and biomass reduction was observed (Wang et al. 2009). Soils where maize, one of the most important crops used in human and animal nutrition around globe, is produced are at risk of Cd stress (Chumbley and Unwin 1982; Dharma-Wardana 2018). It has been noted that in maize plants exposed to Cd stress, the cell wall composition changes, reduces growth by disrupting the chloroplast structure, disrupts the membrane structure, and adversely affects the metabolism of polyamines (Anjum et al. 2015; Vatehová et al. 2016; Seifikalhor et al. 2020). In addition, the positive effect of the increase in the expression levels of genes that control the synthesis of metallothioneins (MTs) and phytochelatins (PCs) in the maize plant under the stress of heavy metals such as copper, Cd and lead on growth and development has enabled maize to be evaluated as a hyperaccumulator plant. However, this assessment should be approached with caution, as the detailed molecular mechanisms that would allow it to be classified as a hyperaccumulator plant are not sufficiently known (Gao et al., 2022; Sharma et al., 2023). Overexpression of ZmPCS1, a phytochelatin synthesis gene, in maize plants under Cd stress has been reported to significantly increase the phytoremediation ability of maize (Jin et al. 2022).

Oximes, formulated with R1R2C = NOH, are chemical compounds whose chemical functions have not been fully elucidated, as well as performing very important functions in the secondary metabolism of known life forms on globe (Sørensen et al., 2017). After it has been determined that naturally synthesized oximes have high biological activity, chemically synthesized oximes are used for a number of different purposes, especially in the agricultural sector (Mahadevan, 1973). The literature review has shown that; Various sulfonate derivative oximes have antimicrobial, anticancer, antiviral, antihypertension, enzyme inhibition, antioxidant, heavy metal chelator, and antitubercular effects (Korkmaz and Bursal, 2022; Asif et al. 2021; Popli et al., 2021; Taslimi et al., 2021; Yetişsin and Kardeş, 2021; Mishra et al., 2019; Venugopala et al., 2019; Gabr et al., 2015; Ke et al., 2013). While these studies note that sulfonate derivatives have many uses from medicine to aesthetics, they point to the horizon of practical solutions to the potential problems that humanity may face in the future.
Acetone O-(2-naphthylsulfonyl)oxime (ANSO), a sulfonate derivative, is a variety of ketoxime. ANSO does not work as well as acetone O-(4-chlorophenylsulfonyl)oxime (AO) as an electrophilic agent (Korkmaz 2021\textsuperscript{a}). However, in the electrophilic amination of arylcadmium iodides with ketoximes, the naphthyl group in ANSO produces a high steric effect in the oxidative reaction (Korkmaz 2021\textsuperscript{b}).

It has been shown that the adverse effects of copper and Cd stress can be alleviated with acetone O-(4-chlorophenylsulfonyl)oxime (Yetişişin and Kardeş, 2022; Demiralay, 2022). We think that ANSO, a synthetic sulfonate derivative oxime, may have effects on the phytoremediation of Cd in Cd-contaminated environments and attenuating the adverse effects of Cd stress by affecting different metabolic pathways in plants under Cd stress. With this hypothesis, we investigated the effect of ANSO pretreatment on biochemical processes and effects on Cd accumulation in tissues in Cd-stressed maize seedlings.

**Materials And Methods**

**Preparation of samples before measurements**

In the study conducted using ADA 523 maize variants from the Sakarya Maize Research Institute, maize seeds were regularly watered and grown in the plant growth room of the Mus Alparslan University Central Laboratory under conditions of 22°C day/18°C night temperature, 65 ± 5% relative humidity and 400 µMol m\(^{-2}\)s\(^{-2}\) light density. After a period of 25 ± 3 days, the samples were collected by cutting 1 cm above the soil surface of the seedlings suitable for use. As a result of the literature review, it was decided that a Cd concentration of 100 µM would be appropriate (Han et al, 2016; El Rasafi et al, 2022). In order to determine the ANSO concentration suitable for the current study, the H\(_2\)O\(_2\) and MDA contents of the seedlings were investigated after ANSO pre-application at 0.01, 0.1, 0.3, 0.6, 0.9, 1.2, 1.5, and 1.8 mM concentrations on maize seedlings under 100 µM Cd stress. Findings obtained from these parameters showed that 0.3 mM ANSO concentration was appropriate. Created to be Control (18 hours pure water), ANSO (6 hours 0.3 mM ANSO followed by 18 hours pure water), Cd (6 hours pure water followed by 12 hours 100 µM CdCl\(_2\) solution), ANSO + Cd (6 hours 0.3 mM ANSO followed by 12 hours 100 µM CdCl\(_2\) solution) samples obtained by experimental assemblies are preserved at -20 ° C for evaluation in different parameters.

**Measurement of cadmium in maize leaves**

The maize seedlings were digested with hydrogen peroxide (35%, Merck, Germany) and nitric acid (65%, Merck, Germany). The Cd content was analyzed by ICP-MS (Agilent 7900, Agilent, USA) after microwave-assisted acid digestion. First 0.1 g of each sample was digested using 1 mL of 35% (v/v) H\(_2\)O\(_2\) and 8 mL of 65% (v/v) HNO\(_3\) in PTFE vessels and then the vessels were put into a microwave system (Anton Paar, Microwave Reaction System). The following conditions were used for the microwave system: up to 185°C for 20 min, then constant temperature for 15 min, and finally cooling to 60°C for 21 min. Each sample was diluted by adding 50 mL of distilled water. This prepared solution was used to determine the amount
of Cd by an ICP-MS device that included a quartz torch with a quartz injector tube, a concentric nebulizer, and a cyclonic spray chamber.

**Measurement of ABA content in maize leaves**

100 mg of fresh leaf sample was lyophilized for 3 hours. Lyophilized samples were extracted in MilliQ (Water/tissue ratio ratio 50:1, v/w) at 4 °C for 16 hours. Quantitative ABA analyzes were performed with the Phytodetek ABA ELISA kit. (±) cis-trans ABA (Sigma, St. Louis) was used as standard. Results were expressed as pmol per gram fresh weight.

**Measurement of relative water content (RWC) in maize leaves**

RWC was determined using the following formula reported by Barr and Weatherley, (1962): RWC (%) = [(FW - DW)/(TW - DW)] x 100,

where FW refers to fresh weight, DW to dry weight, and TW to Turgid weight.

**Measurement of Photosynthetic pigment content in maize leaves**

Photosynthetic pigment determination was made according to the technique developed by Arnon (1949). The homogenate obtained from 0.25 g maize leaves homogenized in 5 mL 80% acetone was centrifuged at 5000 rpm for 5 minutes; the absorbance of the obtained supernatant was measured at 450, 645, 663 nm (Nicolet evolution 100, Thermo Scientific, USA). According to Lichtenthaler (1987) chlorophyll and carotenoid values were found by replacing the values in the equation used.

**Measurement of malondialdehyde (MDA) content in maize leaves**

The measurement of lipid peroxidation was made by determining MDA content using the Heath & Packer (1968) method. The homogenate extracted in 0.1% trichloroacetic acid (TCA) was centrifuged at 15,000 g for 5 minutes. 0.5% thiobarbituric acid (TBA) prepared in 4 mL of 20% TCA was added to 1 ml of the resulting homogenate and the absorbance of the formed supernatant was read at 532 nm. The MDA concentration value was found by substituting the obtained measurement results in the formula A = E.c.l ( =155 mM⁻¹ cm⁻¹). Non-specific values at 600 nm are excluded.

**Measurement of hydrogen peroxide (H₂O₂) content in maize leaves**

In the method developed by Velikova et al. (2000) homogenate samples of leaves (0.25g), primarily homogenized in a solution created with 0.1g of activated coal and 5mL 0.1% TCA, are centrifuged for 15 mins at 15000 g + 4C. Later, the supernatant (1000 µl) was put into a glass cuvette and mixed with 1 M KI (1500 µl) and phosphate buffer (10mM, pH 7.0). The solution absorbance was measured at 390 nm.
Measurement of antioxidant enzyme activities in maize leaves

Measuring the GPX (Guaiacol peroxidase) activity

According to the method of Urbanek et al. (1991) guaiacol peroxidase (EC 1.11.1.7) activity; it was determined by measuring 2 mL samples formed with 100 ml of potassium phosphate buffer (PH 7.0), 0.1 mM EDTA, 5 mM guaiacol, 15 mM \( \text{H}_2\text{O}_2 \) and 50 µL enzyme extract at 470 nm for 1 min. Enzyme activity was then calculated using \( \text{ext. coeff} = 26.6 \text{mM}^{-1}\text{cm}^{-1} \) extraction coefficient.

Measuring the CAT (Catalase) activity

Enzyme activity (CAT: EC 1.11.1.6) was found by measuring a 1 mL reaction mixture consisting of 50 mM potassium phosphate buffer (PH 7,0), 30 mM \( \text{H}_2\text{O}_2 \) and 20µl enzyme extract at 240 nm for 5 min (Aebi 1983). It was then calculated using \( \text{ext. coeff} = 39.4 \text{µM}^{-1}\text{cm}^{-1} \) extraction coefficient.

Measuring the SOD (Superoxide dismutase) activity

While determining the SOD (SOD: EC 1.15.1.1) value, first of all, according to the method developed by Beauchamp and Fridovich (1971) potassium buffer (50 mM, pH 7.8), EDTA (0.1mM), methionine (13mM), nitro blue tetrazolium (75 µM) and 50 µl extract were mixed and 2 µM riboflavin was added into 1 ml sample obtained from the mixture. This mixture was kept in white light of 375 \( \mu\text{mol m}^{-2}\text{s}^{-1} \) intensity for 10 minutes and then absorbance values were read at 560nm.

Measuring the APX (Ascorbate peroxidase) activity

The enzyme activity (APX: EC 1.11.1.11) found by the method used according to Nakano and Asada (1981) was calculated by reading the absorbance at 290 nm of 1mL samples prepared with 50 mM potassium phosphate buffer (pH 7.0), 250 µM ASC (ascorbate), 5 mM \( \text{H}_2\text{O}_2 \) and 20µl enzyme extract.

Measuring the protein content

To measure protein content, bovine serum albumin standards were prepared and protein complex was measured at 595 nm with Coomassie Brilliant Blue G250 stain according to the method reported by Bradford (1976). Protein concentration was calculated in milligrams and used to express enzyme activity.

Measurement of proline content in maize leaves

Homogenate filtered formed by homogenization of 0.2 g dried sample in 10 mL 3% sulpho salicylic acid. 1 mL was taken from the supernatant obtained by centrifugation of the filtrate formed as a result of filtration at 22°C at 5000 rpm for 5 minutes. 1 mL of acetic acid and 1 mL of ninhydrin (prepared by using acetic acid and ortho-phosphoric acid) were added to the supernatant and put into tubes to be kept in a water bath at 100°C for 1 hour. Then, 3 mL of toluene was added to the samples taken into the ice bath and mixed with vortex. Finally, the samples were centrifuged for 5 minutes at 4000 rpm, and the upper
phase was taken and read at 520 nm at the spectrophotometer (Bates et al. 1973). Results were evaluated in µg per gram fresh weight (FW).

**Measurement of phenolic compounds in maize leaves**

To determine the amount of phenolic contents by HPLC, the last concentrations of ascorbic acid, trans-coumaric acid, catechol, and cinnamic acid standards were weighed to obtain solutions with a concentration of 10 mg mL\(^{-1}\). Then, 1% acetonitrile and acetic acid were added (at a rate of 1/9, respectively) to the standards, and methanol was added at the same rates to prepare stock standards. The stock standards were used for the calibration curve after they were diluted as 10, 25, 50, 75, and 100 µg mL\(^{-1}\) (Tapan, 2016). The concentration of maize leaf extracts was diluted as 20 mg mL\(^{-1}\) by using the solutions that were used in the standard. The extracts were loaded to an HPLC by filtering with a 0.45 µm membrane filter. HPLC analysis was performed by using the Agilent Technologies 1260 Infinity II HPLC (Agilent, USA). The HPLC configuration was as follows: a G7130A column furnace (28°C), a 1260 Quat Pump VL pump with a flow rate of 1.0 mL minutes\(^{-1}\), a 1260 DAD WR detector (at 272, 280, and 310 nm), and a 1260 Vial sampler (20 µL injected). ACE 5 C18 (250 x 4.6 mm) was used as the analytical column for the analysis.

**Statistical analysis**

Experiments were designed as randomized blocks with at least 3 repetitions. The numerical data were analyzed using SPSS (v.17, SPSS Inc., USA). Duncan's multiple range test was used to establish statistical significance. In all the analyses, statistical significance was shown by \( P < 0.05 \).

**Results And Discussion**

**Cd content**

Phytochelatins are compounds used in heavy metal detoxification synthesized from glutathione. Chelating compounds can provide the feature of use in phytoremediation to plants that do not have hyperaccumulatory properties in their nature (Souza et al. 2013). In another study, it was noted that exogenous glutathione administration increased Cd accumulation (Huang et al, 2019). The Cd values in maize leaves are shown in Fig. 2A. Cd accumulation in samples exposed to ANSO + Cd treatment was 7.8-fold higher than the content in samples exposed to Cd alone. The reason for the increase in Cd accumulation by ANSO treatment may be that the synthesis of phytochelatins is promoted in the presence of ANSO. In a study using EDTA, a synthetic phytochelatin, absorption of Pb, Zn and Cd from soil increased 48 times in the plant *Sinapis alba*, 4,6 times in *Raphanus sativus oleifomis* and 3,3 times in *Amaranthus spp.* (Kos et al. 2003). In addition, in another study, it was revealed that the organic chelators secreted by *Solanum nigrum* L. grown in Cd-contaminated soils increase the amount of Cd absorbed from the soil (Bao et al. 2011).

**ABA content**
ABA, a multifunctional phytohormone with vital roles in the plant life cycle, alleviates the adverse effects of Cd stress by increasing the activity of antioxidant enzymes (Finkelstein, 2013; Han et al., 2016). In the measurements, it was observed that there was no statistically significant difference in ABA content between ANSO and control applications. However, it was determined that the effect of Cd stress increased significantly compared to the control. When compared to the Cd application, a significant decrease was recorded in the ABA content in the ANSO + Cd application. In parallel with the current study; It is stated that in *Glycine max* plant under 40 mM Cd stress, ABA content starts to decrease over time after reaching the highest level in the first 24 hours (Perez Chaca et al., 2014).

### RWC content

RWC values from maize leaves are shown in Fig. 3A. Measurements show that ANSO application alone does not increase RWC values compared to control. However, Cd has reduced RWC content by creating a stress environment in the plant. A previous study revealed that RWC values in potato (*Solanum tuberosum* L.) plants exposed to Cd stress showed a marked decline (Li et al. 2019). In a study of moth beans (*Vigna aconitifolia* L.), Cd stress caused RWC reductions of around 67% in leaves and 56% in roots (Vijendra et al. 2016). It was observed that ANSO pre-application to maize plants under Cd stress did not make a statistically significant difference in the RWC value.

### Pigment contents

The chlorophyll and carotenoid content in the leaves of the maize plants is shown in Fig. 3B. The amount of chlorophyll in leaves is key for photosynthesis. Because the decrease in the amount of chlorophyll reduces the production of organic matter. A study has revealed that the parsley (*Petroselinum hortense* L.) plant slows down chlorophyll biosynthesis when exposed to Cd stress (Ulusu et al. 2017). In the present study, it was observed that the amount of chlorophyll in the plant samples treated with ANSO alone did not differ significantly when compared with the control group. However, the amount of chlorophyll in the Cd-treated samples decreased. A significant increase was observed in the ANSO + Cd application compared to the Cd application. Cd stress significantly disrupts photosynthesis by having negative effects on structures such as the synthesis of photosynthetic pigments, the activity of various enzymes, the deterioration of CO₂ fixation, and the internal concentration of Zn, Mn, and Fe ions in plants (Nikolic et al., 2014; Saleh et al., 2020). The decrease in chlorophyll content under stress suggests that the structure of the molecule has deteriorated as a result of the displacement of the Cd⁺² ions in the environment and the Mg⁺² ions, which have a central role in the architecture of the chlorophyll molecule. In addition, the amount of chlorophyll in plant samples treated with ANSO + Cd was higher than the amount of chlorophyll in plant samples treated with Cd. It can be thought that ANSO supplementation has a protective effect on chlorophyll. Carotenoids, which can provide protection to photosynthetic pigments, are important antioxidants. However, their response to Cd stress varies from plant to plant (Parmar et al. 2013). In this study, when ANSO + Cd application is compared with Cd application, it can be said that ANSO significantly increases the carotenoid content and strengthens the antioxidant system to protect against the adverse effects of stress.
**Hydrogen Peroxide (H\textsubscript{2}O\textsubscript{2}) contents**

The production of H\textsubscript{2}O\textsubscript{2} by Cd stress in maize plant leaves is shown in Fig. 4A. The production of reactive oxygen species such as H\textsubscript{2}O\textsubscript{2} creates oxidative stress in plants and causes organelle damage (Cho & Seo 2005). It has been shown that cadmium-induced oxidative stress can cause DNA damage in the plant *Vicia faba* (Lin et al. 2007). In this study, it was observed that the H\textsubscript{2}O\textsubscript{2} content of ANSO-treated maize leaves was lower compared to C. But the level of H\textsubscript{2}O\textsubscript{2} in Cd-treated plant leaves has increased significantly over other applications. In plants exposed to ANSO + Cd treatment, H\textsubscript{2}O\textsubscript{2} production decreased compared to plants exposed to Cd alone. This shows that oxidative stress can be alleviated with ANSO.

**Malondialdehyde (MDA) contents**

MDA content measurements in maize plant leaves are shown in Fig. 4B. Cd-triggered ROS production indirectly generates an MDA increase, causing lipid peroxidation that disrupts the permeability of plasma membranes (Skórzyńska-Polit et al. 2010). In this study, while ANSO treatment did not cause a significant increase in MDA content compared to control group, it was observed that MDA content increased significantly in plant leaves treated with Cd. However, it was determined that MDA content decreased significantly in the ANSO + Cd application compared to the Cd application. The ANSO pre-treatment appears to reduce MDA production. A study on *Pistia stratiotes* L. also observed an MDA increase in the plant with Cd stressor (Li et al. 2013).

**Antioxidant enzyme activity measurements**

The antioxidant system helps maintain plant cell redox balance by converting ROS to less toxic products and indirectly preserves membrane structure and functions (Huang et al., 2019; Song et al., 2019). Although antioxidant enzymes such as SOD, CAT, GPX, and APX are important resistance points in the fight against free radicals, their activities in plants tend to increase or decrease according to the type and density of heavy metals, and the exposure time of the plant to heavy metals (Ayhan et al. 2005).

**SOD activity measurements**

The SOD value obtained from the ANSO + Cd treated group did not differ from the SOD value obtained from the Cd-treated group. However, the SOD value in the ANSO-treated group is higher than the values in the two experimental groups mentioned above. However, the highest SOD value was found in group C (Fig. 5A). It is stated that SOD activity is decreased in Thlaspi and mung bean seedlings exposed to Cd stress (Pongrac et al. 2009; Leng et al. 2021). SOD uses Cu\textsuperscript{+2} and Zn\textsuperscript{+2} ions as cofactors. Since Cd heavy metal is also a +2 valent metal, it raises the idea that the SOD enzyme causes an imbalance in the electrical charge state and causes a significant decrease in its activity compared to C in Cd and ANSO + Cd applications.

**CAT activity measurements**
CAT activity in the ANSO-treated plant group was detected at the lowest value compared to other groups (Fig. 5B). The CAT value in the Cd-treated group was higher than the value in the C and ANSO groups, but lower than the ANSO + Cd-treated group. The highest CAT value was found in the ANSO + Cd treated group. In a study with *Oryza sativa* L., it was shown that the increase in CAT activity in plant roots exposed to Cd reaches even higher values with selenite supplementation, which reduces Cd accumulation (Wan et al. 2019).

**GPX activity measurements**

GPX is involved in the detoxification of H$_2$O$_2$ from the cell wall (Morales & Barceló 1997). GPX activity was detected at the lowest value in samples from plants treated with ANSO (Fig. 5C). The GPX value produced by Cd treatment in the plant is the highest compared to the values obtained from other groups. The GPX value in the ANSO + Cd-treated group was lower than the Cd-treated group, but higher than the ANSO and C groups.

**APX activity measurements**

The group with the highest APX activity was the ANSO + CD treated group. (Fig. 5D). There was no significant difference in the values obtained from the C and ANSO groups. However, the APX value obtained from the Cd-treated samples is higher than the values in the C and ANSO groups, but lower than the values obtained from the ANSO + Cd group. It is known that there is an increase in APX activity in plants exposed to Cd stress (Rahman et al. 2016). APX enzyme, which is dependent on ascorbic acid to perform its antioxidant function, appears to increase significantly in APX activity in parallel with the increase in ascorbate content in ANSO + Cd application compared to Cd application.

The severity of oxidative damage by Fenton reactions increases in parallel with the increase in the Cd$^{+2}$ content of the ROS formed as a result of Haber-Weiss reactions in maize plants under cadmium stress (Smirnoff, 1993). The increase in H$_2$O$_2$ concentration, which is a rapidly spreading and effective ROS with the effect of Cd Stress, activates tolerance mechanisms to alleviate the negative effects of stress through many mechanisms from the hormonal signal transmission to the structure of the cell wall, from the expression of genes to physiological regulations (Swanson and Gilroy, 2010). In ANSO + Cd application, antioxidant system enzymes (Fig. 5) were activated and non-enzymatic antioxidants ascorbic acid, catechol, cinnamic acid, proline, and carotenoid contents (Fig. 7, 6, and 3B) increased compared to Cd application. This situation resulted in a significant decrease in the H$_2$O$_2$ level (Fig. 4A). Parallel to this decrease, the ABA level decreased (Fig. 2B). A significant increase in total chlorophyll content occurred concurrently with the improvement in membrane damage (Fig. 4B). Although the Cd level increased 7.8-times, the fact that the negative effects of stress have been alleviated to this extent may be a sign that the ANSO molecule may be a highly effective synthetic oxime.

**Proline contents**
The accumulation of proline in the leaves of the maize plant is shown in Fig. 6. The proline, one of the osmoprotectants, is involved in the scavenging of ROS in plants (Büyük et al. 2012). In the current study, we see increased proline accumulation in ANSO-treated samples of the plant compared to the C group. However, when it comes to Cd treatment, the plant increased its proline accumulation even more than in control group and ANSO-treated group. ANSO + Cd samples revealed significant decreases in proline values compared to Cd-treated samples. This situation supports the findings that the plant tries to cope with Cd stress by increasing proline accumulation. Similar findings are found in other studies. One of the responses of Cajanus cajan, Vigna mungo and Triticum aestivum plants when exposed to Cd stress is to accumulate proline in their roots, shoots and cotyledons (Saradhi 1991).

Measurement of phenolic compounds

Although it varies according to the phenolic compound, the type and the dose of Cu, Pb and Cd heavy metals, a study on Zea mays showed that heavy metal treatments increased the total phenolic compound values in the plant (Kısa et al. 2016). It has been shown that some phenolics such as cinnamic acid and coumaric acid produced by Cd and Zn stress in Candelia obovata are involved in the scavenging of free radicals (Chen et al. 2020).

Ascorbic acid contents

The highest ascorbic acid value was measured in plant samples treated with ANSO. The ascorbic acid value in the plants exposed to ANSO + Cd treatment was lower compared to the C and ANSO groups. The lowest value was measured in plant samples treated with Cd (Fig. 7A). In a study it was suggested that ascorbic acid, which functions as a cofactor in the scavenging of ROS products and in the active center of APX, can be considered as an indicator of the antioxidant property of the plant (Mazid et al. 2011). According to the results obtained in this study, it can be thought that heavy metal is suppressive on ascorbic acid content, but this stress can be alleviated in the presence of ANSO.

Catechol contents

The lowest catechol content was found in the ANSO group. Remarkably, the catechol value is close to the ANSO group in the plants treated with Cd. However, the catechol value detected in the ANSO + Cd group is higher than the ANSO and Cd groups and significantly lower than the C group (Fig. 7B).

Cinnamic acid contents

It was determined that the cinnamic acid value in the ANSO + Cd group was highest compared to other groups. In addition, the cinnamic acid value detected in the plants treated with Cd was relatively high compared to the results obtained from the ANSO and C groups (Fig. 7C).

Trans-P-coumaric acid contents

It was observed that the trans-P-coumaric acid value was lower in the ANSO group compared to the trans-P-coumaric acid value obtained from the control group. The highest value of trans-P-coumaric acid was
measured in the group treated with Cd. The trans-P-coumaric acid value measured by ANSO + Cd treatment was higher than the value in the ANSO group but lower than in the C group (Fig. 7D).

The increase in the content of phenolic compounds, which have antioxidant properties due to their electron donating abilities, can serve to protect plants more actively against the adverse effects of stress by reinforcing their defense systems (Michalak, 2006). In a study conducted by Abbas et al. (2022), it was stated that the total phenolic content of pea plants (*Pisum sativum* L.) under Cd stress increased significantly, and a significant improvement occurred with jasmonic acid treatment. In the current study, the content of total phenolics increased with ANSO pre-application in maize seedlings under Cd stress, and this increase may have contributed to the strengthening of the antioxidant system and contributed to the plants forming a more efficient defense line against the adverse effects of stress.

**Conclusions**

In the present study, we aimed to elucidate the effect of ANSO pre-application on maize plants exposed to Cd stress on alleviating the adverse effects of stress. In conclusion, although exogenous ANSO increased the Cd content 7.8-fold, ANSO pre-application had a positive effect on the enzymatic and non-enzymatic components of the antioxidant system and ABA content. Thus, it was seen that it alleviated the adverse effects of stress. It was understood that this effect contributed significantly to the improvement of the membrane system in parallel with the decrease in H$_2$O$_2$ content. Simultaneously, a significant increase in photosynthetic pigment contents occurred. These results, which are evaluated as a whole, can only be achieved by chelating the Cd$^{+2}$ ions of the ANSO molecule, accumulating them in the cell wall and packing them in vacuoles. This situation shows that ANSO makes significant contributions to the continuation of the functions maintained by ions such as Cu$^{+2}$, Zn$^{+2}$, Mn$^{+2}$, Se$^{+2}$, and Ca$^{+2}$, which are very important functions in the cell. In addition, the 7.8-fold increased Cd content of ANSO molecule indicates the heavy metal chelating potential of the molecule and its importance for phytoremediation studies.

**Declarations**

**Acknowledgments**

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**Declaration of Competing Interest**

The authors declare no conflicts of interest.

**Author contribution statement**
FY designed and conceived the study. FY and EA carried out the experiments, analyzed the data, written, read, and approved the manuscript.

References


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Figures
Figure 1

Structure of acetone O-(2-naphthyl sulphonyl)oxime (ANSO).
**Figure 2**

Change in Cd (A) and ABA (B) values with ANSO supplementation in maize plants exposed to cadmium stress.
Figure 3

Change in RWC (A) and pigment contents (B) values with ANSO supplementation in maize plants exposed to cadmium stress.
Figure 4

Change in H$_2$O$_2$ (A) and MDA (B) contents values with ANSO supplementation in maize plants exposed to cadmium stress.
Figure 5

SOD (A), CAT (B), CPX(C), and APX (D) activity values with ANSO supplementation in maize plants exposed to Cd stress.
Figure 6

Proline content values with ANSO supplementation in maize plants exposed to Cd stress.
Figure 7

Ascorbic acid (A), catechol (B), cinnamic acid (C), and trans-P-coumaric acid (D) contents with ANSO supplementation in maize plants exposed to Cd stress.