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Protective Effects of Hesperidin and Salicylic Acid on *Lemna minor* L. Exposed to Evercion Yellow Textile Dye

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ABSTRACT
Hesperidin (HES) is a flavonone glycoside from the flavonoid family that is present in citrus species. It has potent anti-oxidant and anti-cancer properties. In times of stress, the phenolic chemical salicylic acid (SA), also known as a plant hormone, functions as a signal molecule, controlling the plant's reaction and maintaining its survival. For the removal of numerous harmful chemicals, phytoremediation, sometimes referred to as green reclamation, is an efficient, affordable, environmentally benign, and simple procedure. Duckweed (*Lemna minor* L.) is an important bioindicator species in phytoremediation study. Following the application of 75 ppm, 150 ppm, and 300 ppm reactive dye Evercion yellow 1X, the effects of 0.5 mM SA and 0.5 mM hesperidin on duckweed (*L. minor* L.) were examined in this study. The use of 0.5 mM SA against stress boosted the activities of peroxidase (POD), ascorbate peroxidase (APX), glutathione S-transferase (GST), glutathione reductase (GR), superoxide dismutase (SOD), and catalase (CAT). Additionally, total glutathione (GSH), total chlorophyll, and carotenoid content were altered by SA treatment. Similar to the SA application, the application of HES was effective in lowering stress. Lipid peroxidation content measured as malondialdehyde (MDA) content was found to be higher than the control groups. Results suggest that SA plays a positive role in *L. minor* against Evercion yellow 1X.

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Evercion Sarı Tekstil Boyasına Maruz Kalan *Lemna minor* L. Üzerinde Hesperidin ve Salisilik Asit'in Koruyucu Etkileri

ÖZET
Hesperidin (HES), turunçgil türlerinde bulunan flavonoid ailesinden bir flavonon glikozittir. Güçlü anti-oksidan ve anti-kanser özelliklere sahiptir. Stres zamanlarında, bitki hormonu olarak da bilinen fenolik kimyasal salisilik asit (SA) bir sinyal molekülü olarak işlev görür, bitkinin tepkisini kontrol eder ve hayatta kalmasını sağlar. Çok sayıda zararlı kimyasalın ortadan kaldırılması için, bazen yeşil ıslah olarak da adlandırılan fitoremediasyon, verimli, uygun maliyetli, çevreye zarar vermeyen ve basit bir prosedürdür. Su mercimeği (*Lemna minor* L.) fitoremediasyon çalışmalarında önemli bir biyoindikatör türdür. Bu çalışmada, 75 ppm, 150 ppm ve 300 ppm reaktif boya Evercion yellow 1X uygulamasının ardından, 0.5 mM SA ve 0.5 mM HES'in su mercimeği (*L. minor* L.) üzerindeki etkileri incelenmiştir. Strese karşı 0,5 mM SA kullanımı peroksidaz (POD), askorbat peroksidaz (APX), glutatyon S-transferaz (GST), glutatyon redüktaz (GR), süperoksit dismutaz (SOD) ve katalaz (CAT) aktivitelerini artırmıştır. Ayrıca, toplam gutatyon (GSH), toplam klorofil ve karotenoid içeriği SA uygulaması ile değişmiştir. Malondialdehit (MDA) içeriği olarak ölçülen lipid peroksidasyon düzeyi, kontrol gruplarına göre daha yüksek bulunmuştur.

Bitki Fizyolojisi

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INTRODUCTION

The textile industry is one of the world's main sources of pollution because it releases undesirable dye effluents (Yaseen & Scholz, 2019). Reactive dyestuffs are widely used in the textile industry because of their ease of use and stability. Both organic and inorganic pollutants can have a considerable negative impact on plant growth (Purwanti et al. 2018; Titah et al. 2018; Tangahu et al., 2019; Imron et al., 2019). Plants as bioremediation agents hold great promise for the breakdown of various hues as well as organic and inorganic contaminants (Ali, 2010; Singh & Singh, 2017).

A member of the Lemnaceae family, duckweed is a little and uncomplicated floating aquatic plant (Driever et al., 2005; Ceschin et al., 2018). *L. minor* is a useful plant for phytoremediation because it can quickly develop, adapt to a variety of aquatic circumstances, and collect pollutants (Ceschin et al., 2020; Can Terzi et al., 2021). Numerous physiological activities, such as gene transcription, signal transduction, redox signal pathways, and immunological response, require reactive oxygen species (ROS). However, excessive ROS generation results in damaging effects on DNA, proteins, and lipids that ultimately cause cell death (Li et al., 2017). Plants have developed defense mechanisms, both enzymatic and non-enzymatic, to scavenge ROS and lessen their harmful effects (Singh & Satheeshkumar, 2024).

Hesperidin is a flavanone that can be found in citrus and other plants naturally. It has been shown to have a variety of pharmacological actions, including antioxidant, anti-inflammatory, anti-hypercholesterolemic, and anticarcinogenic qualities (Tamilselvam et al., 2013; Janigashvili et al., 2024).

SA is a natural plant growth regulator that has a significant impact on a variety of physiological and metabolic processes that affect a plant's ability to grow and develop. Therefore, SA can be used to counteract elemental toxicities, heavy metal stressors, pathogen virulence, and salt stress (Wani et al., 2017).

The purpose of this study was to determine how SA and hesperidin protected *L. minor* from a reactive dye called Evercion Yellow 1X (EY). Antioxidant enzymes, pigment analysis, and lipid peroxidation tests were performed on samples collected on days 1, 4, and 7.

MATERIAL and METHOD

Hesperidin used in this study was originally synthesized in the chemistry laboratory at Inonu University. The hesperidin compound (Figure 1) was synthesized by organic orange fruits and identified by FT-IR spectroscopy.

Hesperidin synthesis from the orange fruits

Although hesperidin is an insoluble flavonoid in water, its solubility varies depending on pH. First, the dried oranges were ground and powdered. 12.5 g of ground orange powder was dispersed in 125 mL distilled water for 1 hour and mixed with a magnetic stirrer. Since Hesperidin was insoluble in water, the water-soluble species were removed by filtration. $CaCO_3$ was used to adjust the pH of the environment. 25 g of $CaCO_3$ was dissolved in 250 ml of distilled water, the $Ca(OH)_2$ solution obtained by removing the insoluble part was used to adjust the pH of the environment. $Ca(OH)_2$ solution was added dropwise onto the orange mixture and the pH of the medium was adjusted to 10, and it was mixed for 24 hours to solubilize hesperidin. After solubilization, the mixture was filtered with filter paper, the insoluble part was removed and the pH of the solution was neutralized with HCl. Hesperidin, which dissolves in basic pH but does not dissolve in neutral pH, was obtained by precipitation. Efficiency 85% (Figure 2).

In FTIR spectra (Figure 3), hydroxyl tension peaks at 3400 cm^{-1}, C-H tension peaks of the aromatic ring at 2800 cm^{-1} circumference, and aromatic C = C tension peaks at 1600 cm^{-1}.

Experimental Design

L. minor mature plants in good condition were obtained from the Turkish seed firm Erciyes in Kayseri. EY, a reactive dyestuff, was selected for this study. Plants were acclimated to Hoagland medium in the greenhouse (temperature: 23 ± 2°C; light/dark cycle: 16/8 h; light intensity: 10,000 lux) for one week before dye treatment (Hoagland & Arnon, 1950). Healthy fronds (30-40 g) from plants were detached and placed in 250 ml glass beakers

with 1/30-diluted Hoagland culture solution with one of the following treatments; Hoagland medium, 75 ppm EY, 150 ppm EY, 300 ppm EY, 0.5 mM SA, 0.5 mM SA + 75 ppm EY, 0.5 mM SA + 150 ppm EY, 0.5 mM SA + 300 ppm EY, 0.5 mM HES, 0.5 mM HES + 75 ppm EY, 0.5 mM HES + 150 ppm EY and 0.5 mM HES + 300 ppm EY. Each experiment was replicated three times. The fronds were picked on days one, four, and seven.

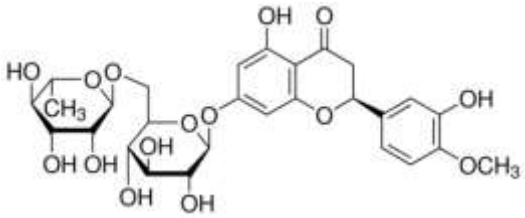

Figure 1. Hesperidin molecule
Şekil 1. Hesperidin molekülü

Figure 2. Hesperidin
Şekil 2. Hesperidin

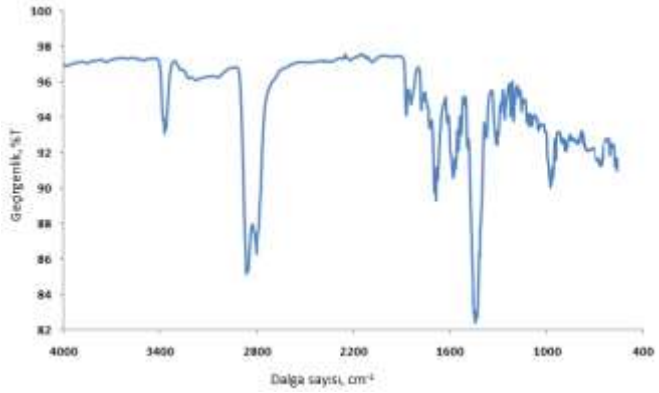

Figure 3. FT-IR spectrum of hesperidin
Şekil 3. Hesperidinin FT-IR spektrumu

Enzyme extraction and protein content

According to Huang et al. (2013), enzyme extractions were evaluated. A 5 ml solution of 0.1 M potassium phosphate buffer (pH 7.8) was used to homogenize 0.5 g of *L. minor*. At 4°C and 10000 g, homogenates were centrifuged for 15 minutes. Using bovine serum albumin as the reference protein, the protein content was assessed using a Bradford protein assay (Bradford, 1976).

POD activity

POD activity was calculated in accordance with Peters et al. (1989) and Mac Adam et al. (1992). A solution was prepared by vortexing 3 ml of 0.1 M potassium phosphate buffer (pH 6.0), 0.04 ml of 0.03 M H_2O_2, and 0.05 ml of 0.2 M guaiacol. To 0.9 mL of this solution, 0.1 mL of extract was added. The increase of absorbance at 436 nm was recorded within 1 min. The extinction coefficient of guaicol is 26.6 mM^{-1} cm^{-1}.

APX activity

The measurement of APX activity was followed Nakano and Asada's (1981) guidelines. The reaction mixture was prepared by adding 550 µl of phosphate buffer (pH 7.6), 100 µl of 10 mM EDTA, 12 mM H_2O_2, 250 µl of extract, and 100 µl of 0.25 mM AsA. Enzyme activity was determined as the absorbance change obtained in 1 minute at a wavelength of 290 nm. APX activity was calculated with an extinction coefficient of 2.8 $mM^{-1}cm^{-1}$.

GST activity

The GST activity was determined using the Habig et al. (1974) technique. The reaction mixture included 400 µl of 0.1 M potassium phosphate buffer, 400 µl of reduced glutathione, 100 µl of sample, and 150 µl of CDNB. The absorbance change over 1 minute at a wavelength of 344 nm was used to determine enzyme activity. CDNB has an extinction coefficient of 9.6 mM^{-1} cm^{-1}.

GR activity

According to Carlberg & Mannervik (1985), an assessment of GR activity was carried out. A solution containing 2

mM EDTA in 0.2 M potassium phosphate buffer (pH 7), 10 mM Tris-HCl (pH 7), 2 mM NADPH, and 20 mM GSSG in 10 ml distilled water was prepared. The reaction mixture was prepared by combining 500 µl of 0.2 M potassium phosphate buffer, 50 µl of 2 mM NADPH, 50 µl of 20 mM GSSG, 250 µl of distilled water, and 150 µl of the sample. The enzyme activity was measured as the change in absorbance per minute at 340 nm and calculated using the NADPH extinction coefficient of 6.2 mM^{-1} cm^{-1}.

GSH content

The amount of GSH was calculated in accordance with Akerboom & Sies (1981). 2.378 mg of DTNB and 0.248 mg of NADPH were prepared in 1 ml of 125 mM sodium diphosphate buffer (pH 7.5) containing 6.3 mM EDTA. 700 µl of NADPH and 100 µl of DTNB were incubated in a water bath at 30 °C, then 100 µL of distilled water, 5 ml of GR, and 100 µl of the extract were added. The reaction was calculated at 412 nm.

SOD activity

The McCord Fridovich (1969) approach was used to assess SOD activity. A solution A was prepared by mixing 100 mL of a 50 mM K_2HPO_4 buffer at pH 7.8 with a solution containing 24.8 mg of cytochrome c and 10 mL of distilled water with 0.76 mg of xanthine. Solution B was prepared separately by dissolving 0.2 U/ml of xanthine oxidase. A reaction mixture was prepared by combining 900 µl of solution A, 50 µl of solution B, and 100 µl of the sample. At 550 nm, enzyme activity was determined.

CAT activity

The Luck (1965) technique was used to evaluate CAT activity. A 1/15 M pH 7 potassium phosphate buffer has been prepared, and 160 µl of H_2O_2 was added to 100 ml of this buffer. Then 200 µl of sample was added to 800 µl of phosphate buffer to which H_2O_2 was added, and absorbance changes over 1 minute were determined by reading at 240 nm. The molar extinction coefficient for H_2O_2 is 0.0396 cm^2 μmol^{-1}.

Pigments analysis

De Kok and Graham's (1980) approach was utilized to determine pigment content. To begin, a 1 g leaf sample was homogenized in 50 ml of acetone and stored at +4°C for 24 hours. After adding 1/5 water to the homogenate, it was homogenized again in a shaking incubator for 15 minutes and kept at +4 °C for 24 hours. At the end of the period, the samples were centrifuged at 5000 g for 10 minutes. The absorbance values of the centrifuged samples were read in a spectrophotometer at 662, 645, 470 nm according to Lichtenthaler and Welburn (1983).

Lipid peroxidation assay

Heath and Packer (1968) used the thiobarbituric acid reactive chemicals approach to measure MDA levels. A 0.5 g leaf sample was homogenized with 5 mL of 0.1% trichloroacetic acid (TCA), and the homogenate was centrifuged at 10,000 g for 10 minutes. From the centrifuged sample, 2 mL of the supernatant was taken and 2 mL of 0.5% thiobarbituric acid (TBA) was added. The mixture was incubated in a water bath at 95°C for 30 minutes. The absorbance was measured using both 532 and 600 nm wavelengths. MDA content was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1}.

Statistical Analysis

The results were reported as the mean with standard error (SE). The entire data set from all studies was statistically evaluated using SPSS version 25.0. In the study, the conformity of the data to normal distribution was checked with the Shapiro-Wilk Test, and the homogeneity of the variances was checked with the LEVENE test. Since the data provided normal distribution, the ANOVA test, one of the parametric test methods, was used in the analysis of multi-group variables and the Duncan multiple comparison test was used for pairwise comparisons between groups (Duncan, 1955).

RESULTS and DISCUSSION

Antioxidant Enzyme Activities

The synthetic dyes used in the textile industry pollute a significant amount of water. Textile dyes do not adhere tightly to the fabric and are released as effluent into the aquatic environment (Rania et al., 2022).

In nature, plants are continually exposed to abiotic and biotic stresses. Furthermore, plants have both enzymatic and non-enzymatic antioxidant defense systems that help to minimize ROS production and cellular damage.

Lemnaceae are among the world's tiniest and quickest growing angiosperms, producing massive amounts of biomass. Lemna species have a high economic value and diverse applications in biotechnology and ecology (Alp et

al., 2023). Their morphological and physiological characteristics enable them to perform valuable bioassays in restricted environments in a short amount of time, making them ideal laboratory organisms. Lemna species are used as bioindicators for ecotoxicological studies, both in vivo and in vitro (Basiglini et al., 2018; Beker Akbulut et al., 2020; Beker Akbulut & Özhan Turhan, 2021). HES is one of the most common flavonoids discovered in citrus species. It is an active pharmacological compound having antihypertensive, anti-inflammatory, and anticancer properties (Iranshahi et al., 2015; Tabeshpour et al., 2020; Arikan et al., 2022; Ji et al., 2024).

POD is a common enzyme found in animals, plants, microbes, and cultured cells. It catalyzes the oxidation of phenols and amines with hydrogen peroxide, removing their toxicity. POD activity was shown to be higher in the EY-applied groups than in the control group. It rose with time in those treated with 0.5 mM SA and dye. The 0.5 mM HES and dye groups demonstrated an increase in POD activity over time. In the 0.5 mM SA +300 ppm dye groups, the maximal POD activity was 15.42 U/mg protein. The groups treated with 0.5 mM HES +150 ppm dye showed the highest POD activity (16.33 U/mg protein). The changes in POD activity were found to be statistically significant, $F(11,24)= 2.85$, $p=.015$. (Figure 4).

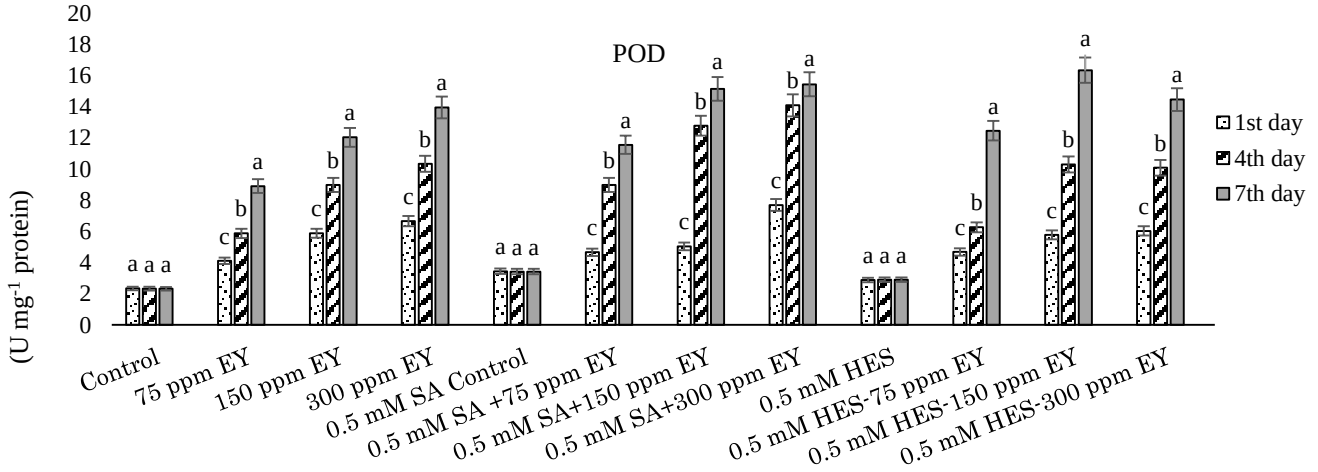

Figure 4. POD activity in L. minor treated with EY, SA and HES. Vertical columns represent the standard error of the average of three replications. Data followed by different letters are significantly different from each other according to Duncan's test, F(11,24) = 2.85, p=.015

Şekil 4. EY, SA ve HES uygulanan L. minor'da POD aktivitesi. Dikey sütunlar üç tekrarın ortalamasının standart hatasını temsil eder. Farklı harflerle takip edilen veriler Duncan testine göre birbirinden anlamlı derecede farklıdır, F(11,24)= 2.85, p=.015

Ascorbate peroxidase enzymes serve a critical function in regulating ROS in cells, particularly H_2O_2. Following 75, 150, and 300 ppm EY treatment, APX activity increased with the number of days. The 0.5 mM SA and dye administered groups increased as the number of days increased. On the seventh day, the maximum APX activity (15.26 U/mg protein) was identified in the groups treated with 0.5 mM HES (Figure 5).

GSTs are multifunctional proteins that are involved in a variety of intracellular events such as stress metabolism, primary and secondary metabolism, herbicide detoxification, plant defense against heavy metals, ozone destruction, and xenobiotics. GST activity increased with increasing dye concentrations and days. The changes in GST activity were found to be statistically significant, $F(11,24)= 3.57$, $p<.004$. Only in the dye-applied groups did the maximum GST activity result from 300 ppm EY application. GST activation was found to be greater in the 0.5 mM SA-treated groups than in the dye-only groups. GST activity was shown to be decreased in the 0.5 mM HES treated groups compared to the 0.5 mM SA applied groups (Figure 6).

GR reduces glutathione by using NADPH as a substrate. On the fourth and seventh days, dye-only groups had lower GR activity than the control group. (GR activity decreased on the fourth and seventh days in the groups treated with 0.5 mM SA). The lowest GR activity in the 0.5 mM HES treated groups was seen on the seventh day in the 300 ppm EY applied groups. This is due to the fact that there are notable variations between the administered dose groups and the control, $F(11,24)= 1.36$, $p<.254$ (Figure 7).

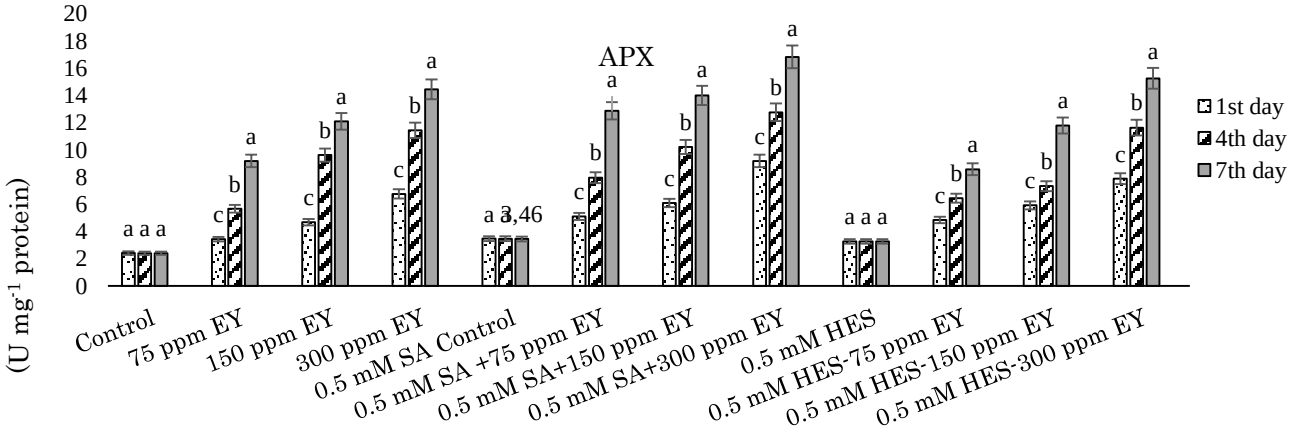

Figure 5. APX acitivity in L. minor treated with EY, SA and HES. Vertical columns represent the standard error of the average of three replications. Data followed by different letters are significantly different from each other according to Duncan's test, F(11,24)= 3.88, p=.003

Şekil 5. EY, SA ve HES uygulanan L. minor'da APX aktivitesi. Dikey sütunlar üç tekrarın ortalamasının standart hatasını temsil eder. Farklı harflerle takip edilen veriler Duncan testine göre birbirinden anlamlı derecede farklıdır, F(11,24)= 3.88, p=.003

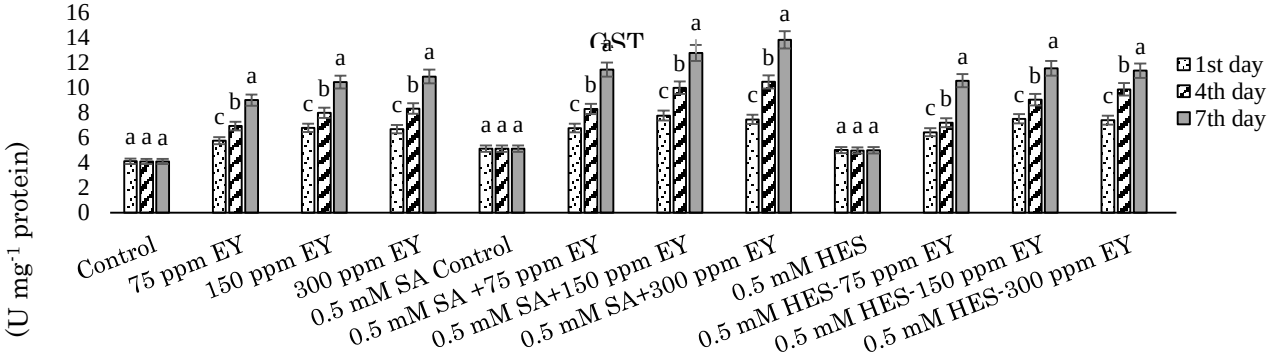

Figure 6. GST acitivity in L. minor treated with EY, SA and HES. Vertical columns represent the standard error of the average of three replications. Data followed by different letters are significantly different from each other according to Duncan's test, F(11,24)= 3.57, p<.004

Şekil 6. EY, SA ve HES uygulanan L. minor'da GST aktivitesi. Dikey sütunlar üç tekrarın ortalamasının standart hatasını temsil eder. Farklı harflerle takip edilen veriler Duncan testine göre birbirinden anlamlı derecede farklıdır,F(11,24)= 3.57, p<.004.

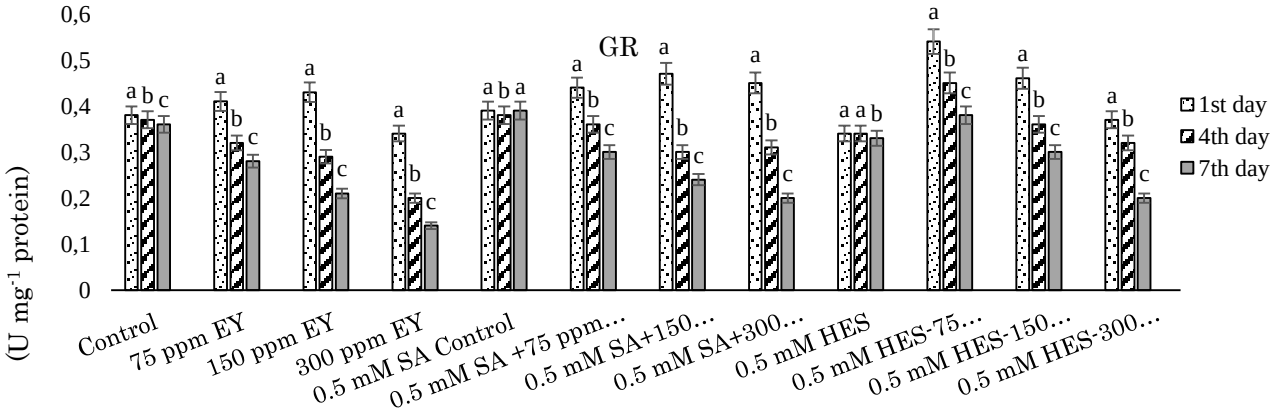

Figure 7. GR acitivity in L. minor treated with EY, SA and HES. Vertical columns represent the standard error of the average of three replications. Data followed by different letters are significantly different from each other according to Duncan's test, F(11,24)= 1.36, p<.254

Şekil 7. EY, SA ve HES uygulanan L. minor'da GR aktivitesi. Dikey sütunlar üç tekrarın ortalamasının standart hatasını temsil eder. Farklı harflerle takip edilen veriler Duncan testine göre birbirinden anlamlı derecede farklıdır, F(11,24)= 1.36, p<.254

GSH is a key molecule in sulfur metabolism and plant response to oxidative stress. GSH levels increased in response to increasing dye concentrations and days. The dye-applied groups had the highest GSH concentration. The 0.5 mM SA treated groups had higher GSH content than the dye-only groups. On the seventh day, the groups that applied 0.5 mM HES-300 ppm EY had the highest GSH content (8.36 U/mg protein). This is because significant differences exist between the applied dose groups and the control, $F(211,24)= 6.36$, $p<.000$ (Figure 8).

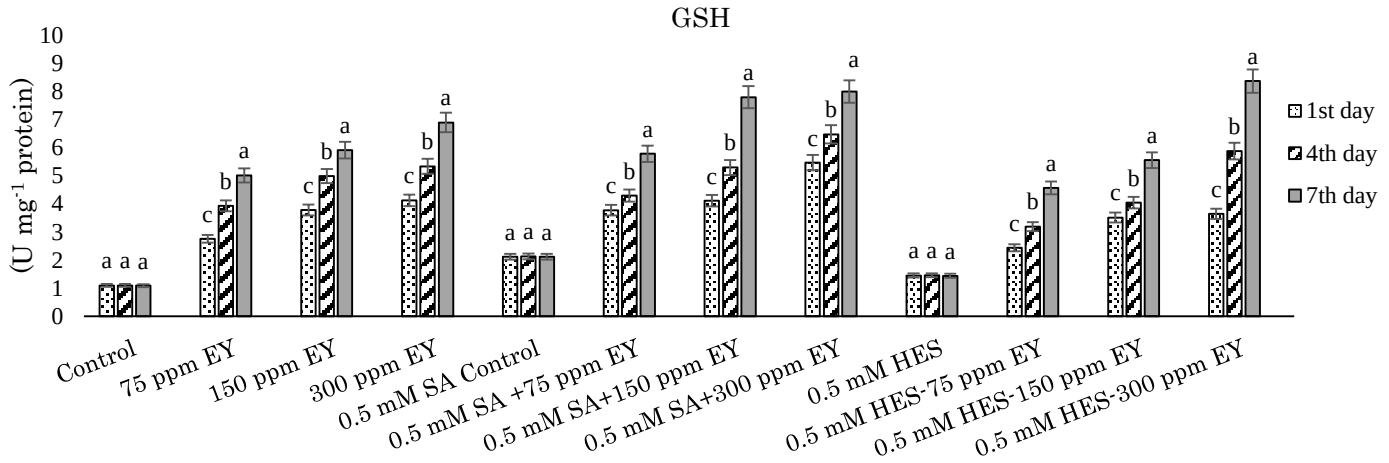

Figure 8. GSH content in L. minor treated with EY, SA and HES. Vertical columns represent the standard error of the average of three replications. Data followed by different letters are significantly different from each other, F(11,24)= 6.36, p<.000

Şekil 8. EY, SA ve HES uygulanan L. minor'da GSH içeriği. Dikey sütunlar üç tekrarın ortalamasının standart hatasını temsil eder. Farklı harflerle takip edilen veriler Duncan testine göre birbirinden anlamlı derecede farklıdır, F(11,24)= 6.36, p<.000.

SOD defends plants against abiotic stress-induced ROS. SOD activity increased in dye-treated groups compared to the control. The changes in SOD activity was found to be statistically significant, $F(11,24)= 3.10$, $p<.010$. The group that applied 300 ppm EY on the seventh day showed the highest SOD activity. Increased concentrations in the SA and HES applied groups resulted in an increase in SOD activity (Figure 9).

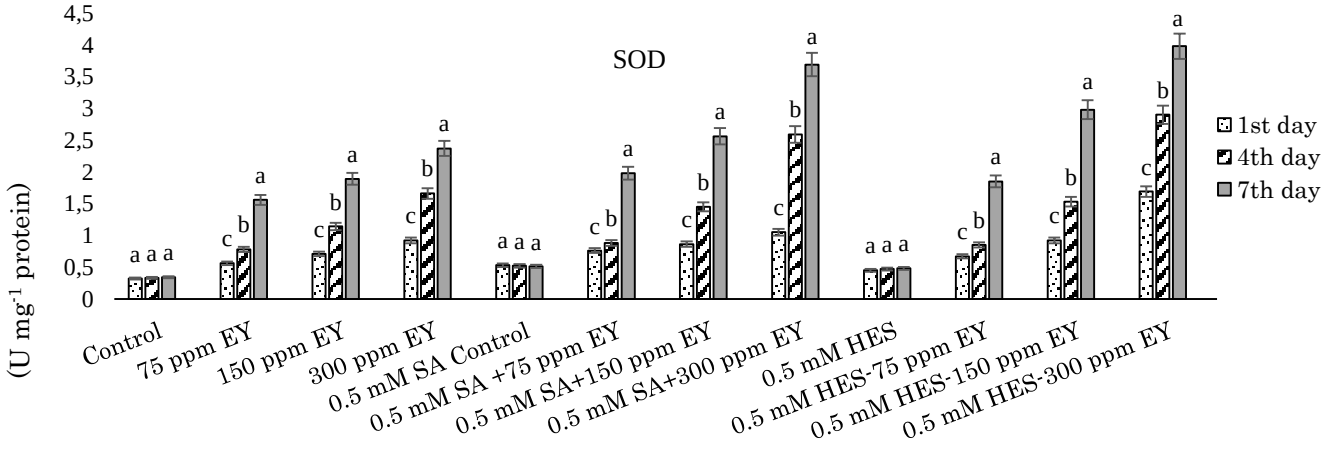

Figure 9. SOD acitivity in L. minor treated with EY, SA and HES. Vertical columns represent the standard error of the average of three replications. Data followed by different letters are significantly different from each other according to Duncan's test, F(11,24)= 3.10, p<.010

Şekil 9. EY, SA ve HES uygulanan L. minor'da SOD aktivitesi. Dikey sütunlar üç tekrarın ortalamasının standart hatasını temsil eder. Farklı harflerle takip edilen veriler Duncan testine göre birbirinden anlamlı derecede farklıdır F(11,24)= 3.10, p<.010

CAT is a tetrameric heme-containing enzyme, removes H_2O_2 from plants under stress to prevent oxidative damage CAT activity increased on the fourth day solely in the dye-applied groups, but declined on the seventh day. The

groups that applied 300 ppm EY on the seventh day showed the highest CAT activity. On the fourth day, the groups who received both SA and 300 ppm dye showed the highest CAT activity. CAT activity reduced on the seventh day in HES-applied groups. This is because significant differences exist between the applied dose groups and the control, $F(11,24)= 1.94$, $p<.084$ (Figure 10).

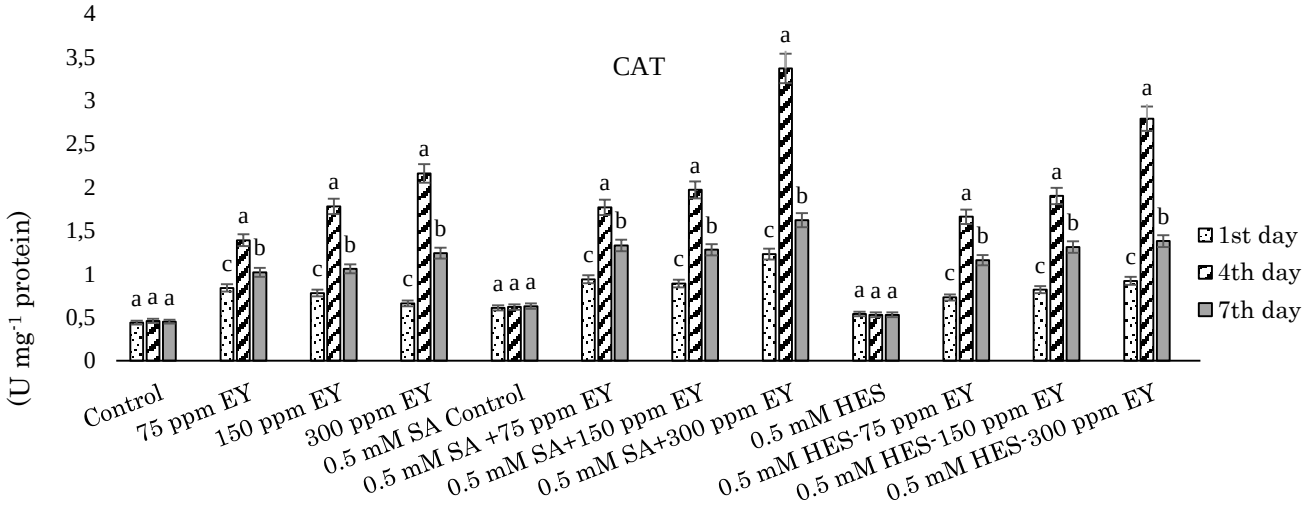

Figure 10. CAT acitivity in L. minor treated with EY, SA and HES. Vertical columns represent the standard error of the average of three replications. Data followed by different letters are significantly different from each other according to Duncan's test, F(11,24)= 1.94, p<.084

Şekil 10. EY, SA ve HES uygulanan L. minor'da CAT aktivitesi. Dikey sütunlar üç tekrarın ortalamasının standart hatasını temsil eder. Farklı harflerle takip edilen veriler Duncan testine göre birbirinden anlamlı derecede farklıdır, F(11,24)= 1.94, p<.084

Photosynthetic Pigment Analysis

Stress conditions negatively affect photosynthetic activity in plants. In comparison to the control group, the overall chlorophyll concentration in the 75 ppm, 150 ppm, and 300 ppm dye treated groups decreased as time passed. The total chlorophyll content increased on the first day compared to the control, but then decreased on consecutive days in the groups treated with 0.5 mM SA and 75, 150, and 300 ppm dyes. On day 7, total chlorophyll content increased compared to day 4 in groups treated with 0.5 mM HES and 75, 150, or 300 ppm dye. This is due to the fact that there are notable variations between the administered dose groups and the control, $F(11,24)= 11.61$, $p<.000$ (Figure 11).

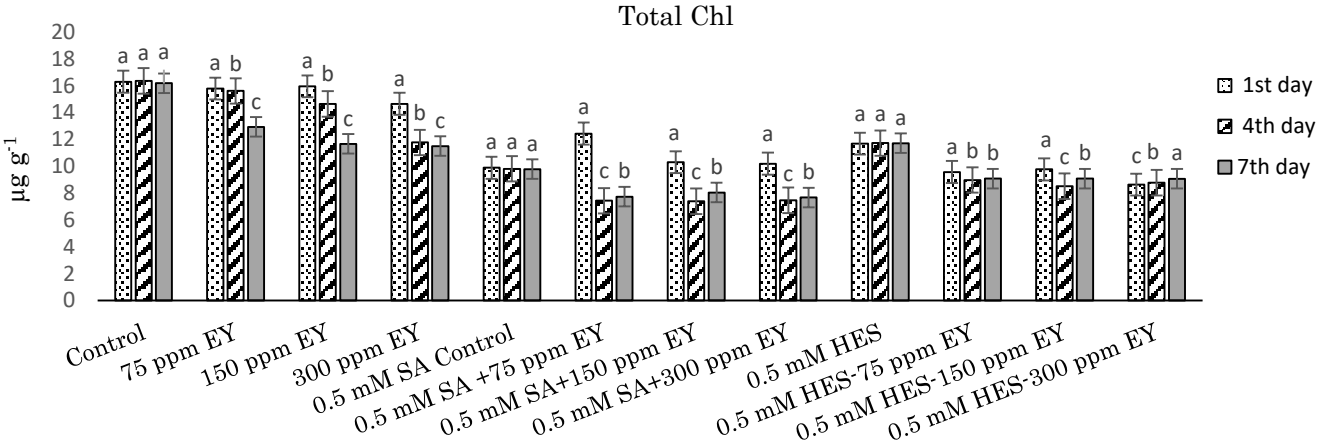

Figure 11. Changes in total chlorophyll content in L. minor treated with EY, SA and HES.Vertical columns represent the standard error of the average of three replications. Data followed by different letters are significantly different from each other according to Duncan's test, F(11,24)= 11.61, p<.000

Şekil 11. EY, SA ve HES uygulanan L. minor'da total klorofil içeriğindeki değişiklikler. Dikey sütunlar üç tekrarın ortalamasının standart hatasını temsil eder. Farklı harflerle takip edilen veriler Duncan testine göre birbirinden anlamlı derecede farklıdır, F(11,24)= 11.61, p<.000

The dye-treated groups' carotenoid concentration reduced by 75 ppm, 150 ppm, and 300 ppm compared to the control. On the first day, groups receiving 0.5 mM SA and 75 ppm dye had the greatest carotenoid levels (4.07 µg g-1). On the seventh day, those treated with 0.5 mM SA and 75 ppm dye had the lowest carotenoid levels (1.78 µg/g). The carotenoid content of the HES treated groups reduced in comparison to the control, with the exception of the HES and 75 ppm dye applied groups on the first day. The changes in carotenoid content were found to be statistically significant, $F(11,24)= 2.24$, $p<.048$ (Figure 12).

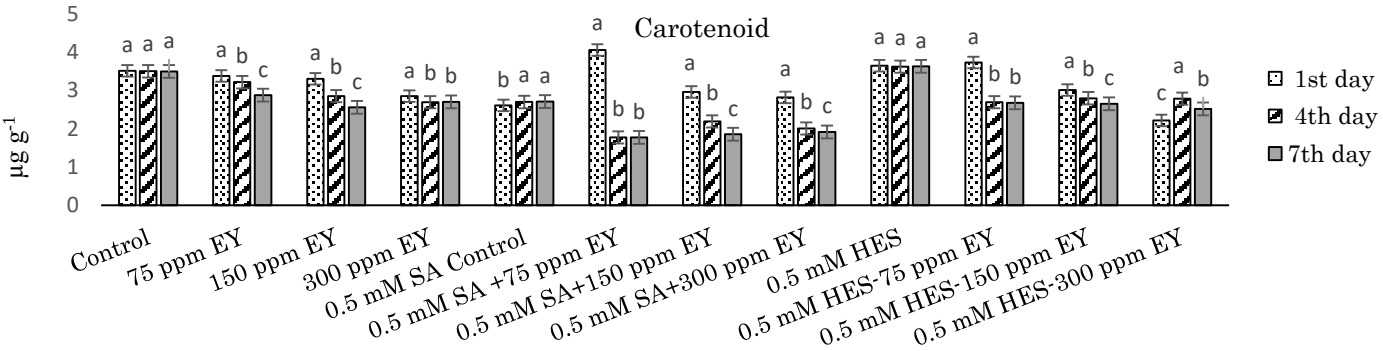

Figure 12. Changes in Carotenoid content in L. minor treated with EY, SA and HES. Vertical columns represent the standard error of the average of three replications. Data followed by different letters are significantly different from each other according to Duncan's test, F(11,24)= 2.24, p<.048

Şekil 12. EY, SA ve HES uygulanan L. minor'da karotenoid miktarındaki değişiklikler. Dikey sütunlar üç tekrarın ortalamasının standart hatasını temsil eder. Farklı harflerle takip edilen veriler Duncan testine göre birbirinden anlamlı derecede farklıdır, F(11,24)= 2.24, p<.048

Lipid Peroxidation Analysis

Increased Lipid peroxidation content in membranes weakens their barrier function and increases permeability to organic substances and ions. This causes sulfhydryl group destruction and enzyme inactivation. On the fourth day, groups that received 300 ppm EY had the highest MDA activity. On the seventh day, MDA levels in the dye-applied groups decreased. MDA concentrations peaked on day four in those treated with 0.5 mM SA and 300 ppm EY. MDA levels in the groups treated with 0.5 mM HES and dye increased on day four but decreased on day seven. This is due to the fact that there are notable variations between the administered dose groups and the control, $F(11,24)= 2.60$, $p<.025$ (Figure 13).

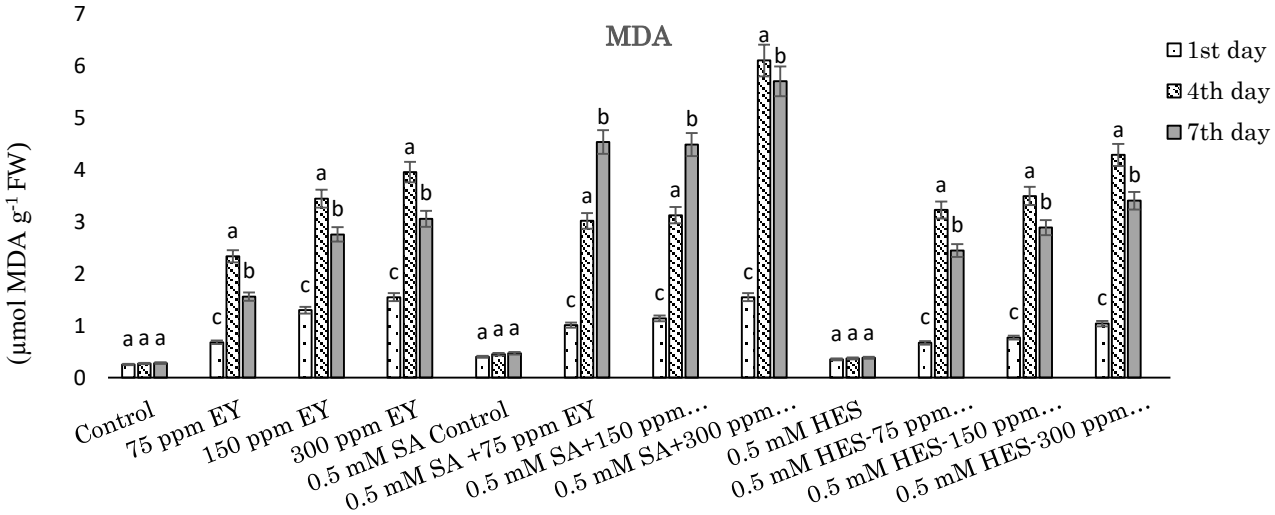

Figure 13. Changes in MDA content in L. minor treated with EY, SA and HES. Vertical columns represent standard error of the average of three replications. Data followed by different letters are significantly different from each other according to Duncan's test F(11,24)= 2.60, p<.025

Şekil 13. EY, SA ve HES uygulanan L. minor'da MDA miktarındaki değişiklikler. Dikey sütunlar üç tekrarın ortalamasının standart hatasını temsil eder. Farklı harflerle takip edilen veriler Duncan testine göre birbirinden anlamlı derecede farklıdır F(11,24)= 2.60, p<.025

CONCLUSION

In conclusion, when employed in agriculture, water containing reactive dyes can end up in human bodies. Reactive dyes provide a significant risk to human health and may be a primary contributor to cancerous disorders. A very promising species for natural-environment technologies is *L. minor*. Oxidative stress was brought on by Everzol yellow dye (EY) in *L. minor*. Antioxidant activity and pigmentation were significantly decreased. POD, APX, GST, GSH, and SOD were all elevated upon application of SA and hesperidin. Day 7 saw a decline in GR and CAT activity in the hesperidin and SA groups. Day 4 saw an increase in MDA content. The results of this investigation indicate that the combination of hesperidin and exogenous SA helped *L. minor* recover from dye-induced stress.

Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflict of Interests and Ethical Statement

The authors of the articles declare that they have no conflict of interest.

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