Evaluation of physiological and biochemical aberration linked to effect of sodium dodecyl sulphate on barley seedlings

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Abstract

Sodium dodecyl sulphate (SDS) amount in domestic and industrial effluents is consistently increasing since SDS is available in almost all cleaning products. Therefore, it has become one of the potential destructive factors on habitats such as agricultural areas and aquatic environments. This study was carried out to evaluate the SDS-induced changes on barley (*Hordeum vulgare* L. cv. Olgun) seedlings. Barley seedlings were hydroponically cultivated for nine days at optimal conditions and, then, exposed to five different doses of SDS. After 72 h from SDS addition, root and coleoptile lengths were seriously reduced as compared to control. Also, SDS decreased soluble protein, chlorophyll *a*, chlorophyll *b* and carotenoid contents of the barley leaves. Furthermore, the SDS-induced reductive effects on protein profiles of barley leaves were also detected with electrophoresis. SDS caused serious decreases in activities of antioxidant enzymes, including superoxide dismutase, catalase, and ascorbate peroxidase and glutathione reductase except for peroxidase. Moreover, the SDS led to the emergence of oxidative damage symptoms that were manifested by the increased superoxide anion formation, hydrogen peroxide content and lipid peroxidation level of barley seedlings. Eventually, the results of the present study revealed that SDS cause to a severe reduction in the growth of barley seedlings.

Graphic abstract

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1 Introduction

Environmental pollution, defined as contamination of air, soil and water by various factors, has always been a serious threat for all organisms. The magnitude of this threat is increasing rapidly with domestic and industrial pollutants day by day [36]. Detergents, commonly used in the domestic and industrial process, are among these environmental pollutant factors [69]. The contamination from the detergent waste of the rivers, lakes and finally agricultural areas leads to irreversible damages at habitats of all organisms [48, 53]. To reduce this threat, The World Health Organization (WHO) has recommended 0.2 mg L^{-1} as the maximum detergent residue ratio in water resources [17]. However, it has been detected that the level of the detergent residue in some water resources is about fifty times greater than this critical ratio [13].

Detergents primarily comprise four main components: surfactants, foam regulators, adjuvants and additives [72]. Due to its better surfactant property in comparison to other surfactants, SDS is used in almost all detergents [5, 24]. It also exists in many products such as solid disinfectants, drug formulations, toothpastes, shampoos, liquid hand soaps, shower gels, shaving foam, floor cleaners, car wash soaps, lotions, laundry and dishwashing detergents (Atıcı 2016). In addition to these, SDS is also used in dentistry, skin softening, wool cleaning, metal processing as well as paint, paper and food industries [38, 42]. Because of its wide utilization and the waste disposal with water, SDS penetrates almost all habitats and inevitably pollutes those areas. Recently, some researchers have reported that the detergent wastes could cause allergic symptoms in organisms [8, 29, 39]. It has also been informed that detergent wastes could negatively affect physiological, biochemical metabolism of aquatic organisms [1, 44, 47, 56]. Additionally, it has also been detected that these wastes trigger the necrosis, chlorophyll degradation, growth retardation in plants [3, 16, 62]. Moreover, SDS, one of the raw material of detergents, leads to the misfolding and denaturation of proteins in cells since it can break down the peptide bonds of amino acids by loading with the negative charge to them [25, 55].

Unlike other organisms, plants are the sessile organisms, and therefore they are much more affected by various stress factors such as salt, cold, heat, drought and heavy metal. Those stress factors lead to troubles in the electron transport chain of organelles, and this situation results in overproduction of reactive oxygen species (ROS) in their cells.

When ROS are at low concentrations in the cytoplasm, they can play a role in cellular signalling events. However, excessive ROS seriously damage the membranes of cell and organelles. Therefore, ROS amounts in cells have to be stabilized by antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR) and peroxidase (POX) as well as non-enzymatic antioxidants containing ascorbate and glutathione.

Many studies have been focused on the relationship between antioxidant enzyme activities and plant tolerance against the stressors such as salt, cold, hot and heavy metals [66, 67, 59–65, 59, 58]. Although SDS is a serious threat agent on plant growth, there are very limited studies about the toxic effects of SDS on plants. In these studies, it has been reported that SDS led to an important decrease in the growth rates and chlorophyll contents of duckweed and water fern [26, 27]. Another study has also revealed that the SDS caused the serious declines in biomass, germination percentage, CAT activity and protein content in wheat [19]. However, there is no study evaluating the effects of SDS-induced changes in protein profile, free radical and membrane damage of plants.

Over the recent years, the level and number of stress factors have been increasing in agricultural areas; therefore, limitations in yield and production of crops are rapidly growing. [12, 15]. Detergents, among these stress factors, are abundant in domestic and industrial wastewater and lead to the destructions in biological activities of organisms by disturbing oxygen transport in the water and soil [48]. Therefore, the detergent wastes are potential risk factor for plants. The toxic effects of detergents on living organisms arise from SDS which is the anionic raw material of almost all of the cleaning products [5]. In the present study, to contribute to the literature in these regards, it was evaluated the effects of SDS on antioxidant, biosynthesis and biochemical mechanisms of barley seedlings. Also, it was aimed to draw attention to the necessity of precautions to minimize the leakage of SDS into the natural environment from domestic and industrial wastewaters.

2 Materials and methods

2.1 Plant materials, growth conditions and treatments

Barley seeds (*Hordeum vulgare* L. cv. Olgun) were provided by East Anatolian Agricultural Research Institute. All chemicals used in the research were purchased from Sigma–Aldrich. The seeds were surface sterilized with 5% sodium hypochlorite (v/v) for 5 min, and then, they were washed five times deionized water. The seeds were planted in hydroponic systems containing Hoagland solution, aerated continuously with oxygen pumps [4]. The experiment was carried out in a greenhouse, under controlled conditions of humidity (70%), temperature (20–23 °C), photosynthetic active radiation ($60 \mu\text{m m}^{-2} \text{s}^{-1}$) and photoperiod (16-h light/8-h dark). In this experiment, the cultivation, application and harvest times for the barley seedlings were decided by many preliminary studies. In preliminary experiments, the nine-day seedlings were divided into five groups: only Hoagland (control); Hoagland plus SDS at four different concentrations (0.5, 1, 1.5, 2 mM). SDS concentrations were adjusted according to the volume of the Hoagland. After 72 h from SDS applications, it was detected that the root and shoot lengths of barley seedlings were reduced at the same rate with three SDS applications (1, 1.5, 2 mM) except for 0.5 mM. Following this experiment, to determine the phytotoxic effects of SDS amounts lower than 0.5 mM, a new experimental setup was designed in the same conditions.

In this new experiment, 0.1, 0.2, 0.3, 0.4 and 0.5 mM SDS were applied to the nine-day barley seedlings and 72 h after applications, the seedlings were harvested. Seven replicates were prepared from six plants for each application. The tissue replicates (0.2 g) were grinded in liquid nitrogen and stored in the deep freeze (– 80 °C) for analyses.

2.2 Determination of protein content and profile

Protein contents of the leaf samples were performed according to the method of Bradford [14]. The leaf tissues were homogenized in 0.1 M phosphate buffer (pH 6.75) after they were ground in liquid nitrogen. The homogenate was centrifuged at 15,000 rpm for 20 min and at 4 °C. Absorbance values of the supernatants were recorded at 595 nm. Results were expressed as mg g^{-1} FW and calculated through a calibration curve prepared with standard solutions of well concentration of bovine serum albumin.

Protein profile was detected with the method described by Laemmli [43]. The leaf samples were

homogenized with 125 mM Tris HCl (pH 6.8) containing 10% (v/v) glycerol, 2% sodium dodecyl sulphate (SDS), 0.1% bromophenol blue and 5% 2-mercaptoethanol. After the homogenates were centrifuged at 15,000 rpm for 15 min at 4 °C, they were kept in water at 90 °C for 5 min and cooled at room conditions. Protein bands were separated in 4–11% SDS–polyacrylamide gel (SDS–PAG) at 120 V. The polyacrylamide gel was stained by using Coomassie brilliant blue R-250. After the gel washing operations, protein bands were displayed.

2.3 Determination of pigment contents

The amount of chlorophyll *a*, chlorophyll *b* and carotenoids were determined with the method of Witham et al. [71]. Leaf tissues were homogenized in 80% acetone, and then the final volume of the homogenate was completed to 8 mL with 80% acetone. The homogenate was centrifuged at 3500 rpm for 7 min at 4 °C. The absorbance values of the supernatants were recorded at 450, 645 and 663 nm. The photosynthetic pigment amounts were calculated to be mg.g^{-1} FW according to the equivalents described by Witham.

2.4 Determination of antioxidant enzyme activities

Leaf samples (0.2 g) were grinded in liquid nitrogen and homogenized in 2 mL of 10 mM phosphate buffer (pH 7.0) containing 1 mmol L^{-1} ethylenediaminetetraacetic acid (EDTA) and 4% (w/v) polyvinylpyrrolidone. After the homogenate was centrifuged at 15,000 rpm for 15 min at 4 °C, the supernatant was used as the source for antioxidant enzymes.

SOD activity was determined by using the photochemical method described by Agarwal and Pandey [2]. This method is based on the determination of the photochemical reduction in formazan of nitro-blue tetrazolium (NBT) at 560 nm. One unit of SOD activity was accepted as the amount of enzyme causing photoreduction by 50% of NBT.

For determination of POX activity, it was used to the method described by Ye et al. [73]. According to this method, it was monitored the absorbance increase at 470 nm arisen from reacting of guaiacol, H_2O_2 and peroxidase. The amount of enzyme that increased 0.01 the absorbance value per minute is expressed as one unit of POX enzyme activity.

CAT activity was measured according to the method described by Gong et al. [30]. The method is based on the monitoring of the absorbance changes at 240 nm during H_2O_2 decomposition. According to the method, one-unit CAT activity is accepted as the amount of

enzyme required to decompose 1 $\mu\text{mol H}_2\text{O}_2$ per minute at 25 °C by using standard graphic.

APX activity was detected according to the method described by Nakano and Asada [52]. The changes of absorbance at 290 nm were determined. APX activity was calculated by using epsilon coefficient $2.8 \text{ mM}^{-1} \text{ cm}^{-1}$ for ascorbic acid.

GR activity measurement was performed according to the method of Foyer and Halliwell [28]. This method is based on the determination at 340 nm of NADPH oxidation during the conversion of oxide glutathione to glutathione by GR using NADPH as an electron donor. One unit of GR was expressed as the reduction in 1 mmol mL^{-1} oxidized glutathione (GSSG) per minute.

2.5 Determination of the hydrogen peroxide (H_2O_2) amount

The quantification of H_2O_2 amount was performed based on a modified version of the method described by Sergiev et al. [57]. Frozen leaf samples were homogenized in 0.1% TCA, and then the homogenate was centrifuged at 12,500 rpm for 15 min at 4 °C. The supernatant was mixed with the equal volume of 10 mM KH_2PO_4 (pH: 7.0) buffer and the double volume of KI solution. Absorbance values of the mixture and blind were recorded at 390 nm. H_2O_2 contents were calculated as $\mu\text{g.g}^{-1}$ FW based on the standard graph prepared by adding different concentrations of H_2O_2 solution within blind samples.

2.6 Determination of the superoxide (O_2^-) anion amount

The determination of the superoxide anion amount was accomplished by following the method of Elstner and Heupel [23]. Leaf samples were homogenized in 0.9 mL K_2HPO_4 buffer (pH 7.8). The homogenate was centrifuged at 12,500 rpm for 12 min at 4°C. The supernatants (350 μL) were incubated with 35 μL of 10 mM hydroxylamine hydrochloride and 315 μL of K_2HPO_4 for 60 min at 25 °C. The centrifugation process was repeated, and then, 17 mM aminobenzene sulphonic acid and 17 μM of 350 μL α -naphthylamine in equal volumes were added to the mixture. The final mixtures were incubated at room temperature for 60 min. The specific absorbance values of the mixtures were recorded at 530 nm. The results were expressed as $\mu\text{g.g}^{-1}\text{FW}$ and calculated through a calibration curve prepared with standard solutions of well concentration of NaNO_2 .

2.7 Determination of lipid peroxidation level

Lipid peroxidation levels were determined according to the method described by Heath and Packer [34]. Leaf tissue (0.2 g) was homogenized in 1.2 mL of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 12,500 rpm for 15 min. 1 mL of 0.5% thiobarbituric acid (TBA) in 0.1% TCA solution was mixed with 500 μL of the supernatant. The mixtures were incubated in boiling water for 40 min and then cooled in an ice cap. They were centrifuged again at 12,000 rpm for 5 min. The absorbance values of samples were recorded at 532 nm and 600 nm. Malondialdehyde (MDA) amounts were calculated by using the extinction coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$.

2.8 Statistical analysis

The data are the mean of six repetitions for each application. The data were analysed by using one-way ANOVA and compared with Duncan's multiple comparison at $p < 0.05$ significance level with SPSS 20. Standard errors were indicated in all the tables.

3 Results

3.1 Root and coleoptile lengths

When looking to Figs. 1 and 2, it is clear that SDS induced morphological changes on the barley seedlings. The root length in the 0.1 mM SDS application was 0.2% higher than the control, but this increase was not statistically significant ($p > 0.05$). On the other hand, other SDS applications (0.2, 0.3, 0.4 and 0.5 mM) caused a decline by 5, 8, 14 and 19% in the root length, respectively ($p < 0.05$). The SDS-induced symptoms in coleoptiles lengths were similar to those of root lengths. Indeed, when compared to control, 0.1, 0.2, 0.3, 0.4 and 0.5 mM SDS seriously reduced by 5, 11, 14, 18 and 19% the coleoptiles lengths, respectively ($p < 0.05$).

3.2 Soluble protein contents

As given in Table 1, when compared to control, soluble protein contents in SDS groups exhibited a downward trend. The protein amounts in 0.1, 0.2, 0.3, 0.4 and 0.5 mM SDS applications were determined to be less approximately 4, 7, 7, 8 and 8% than in control, respectively ($p < 0.05$). When looking to Fig. 3, it is clearly seen that the SDS applications induced significant changes as manifested by the different intensity of protein bands in relation to the control. In SDS dose-dependent manner, while the densities of the bands in the area (50–60 kDa) corresponding to RuBisCO were

Fig. 1 Effects of SDS application on root and coleoptile lengths of barley seedlings. Different letters in same groups indicate statistically significant differences ($p < 0.05$)

Fig. 2 Digital photographs of barley seedlings after 72 h from SDS applications

Table 1 Effects of SDS applications on the contents of protein and photosynthetic pigments of the barley leaves

Treatments	Protein (mg g ⁻¹ FW)	Chlorophyll <i>a</i> (mg g ⁻¹ FW)	chlorophyll <i>b</i> (mg g ⁻¹ FW)	Carotenoid (mg g ⁻¹ FW)
Control	15.52 ± 0.01a*	6.08 ± 0.03a	2.47 ± 0.02a	5.23 ± 0.04a
0.1 SDS	14.96 ± 0.03b	4.94 ± 0.02d	1.95 ± 0.08d	3.76 ± 0.03bc
0.2 SDS	14.5 ± 0.01c	4.91 ± 0.04d	2.09 ± 0.03c	3.81 ± 0.05b
0.3 SDS	14.4 ± 0.01d	5.45 ± 0.04b	2.21 ± 0.05b	3.86 ± 0.11b
0.4 SDS	14.33 ± 0.03e	5.14 ± 0.06c	2.12 ± 0.03c	3.62 ± 0.05bc
0.5 SDS	14.32 ± 0.02e	4.49 ± 0.05e	1.93 ± 0.02d	3.54 ± 0.13c

*Different letters in the same column indicate statistically significant differences ($p < 0.05$)

seriously reduced, the intensities of the low molecular weight (15–25 kDa) bands containing the stress proteins were significantly increased.

3.3 Photosynthetic pigment contents

Pigment analysis results showed that the control group had the highest pigment contents (Table 1). When compared to the control, all of the SDS applications caused the dramatic reductions in the chlorophyll *a* (by 19, 19, 10, 15 and 26%), chlorophyll *b* (by 21, 16, 11, 14 and 22%) and carotenoid contents (by 18, 26, 14, 19 and 33%) ($p < 0.05$).

3.4 Antioxidant enzyme activities

The SOD activity has exhibited an increase in 0.1 and 0.2 mM SDS applications as compared to control. However, the increase in 0.2 SDS was statistically meaningless ($p > 0.05$). On the other hand, the values of SOD activity in 0.3, 0.4 and 0.5 mM SDS were notably lower than that of control (Table 2) ($p < 0.05$). After the SDS applications, POX activities were markedly increased relative to the control ($p < 0.05$). The highest increase in about 93% was detected in response to 0.5 mM SDS. CAT activities were markedly lower than that of control. When compared to control, the

Fig. 3 Effects of SDS application on protein profiles of barley leaves

reductions in the CAT activities were recorded by 47, 69, 65, 76 and 68%, respectively (Table 2), ($p < 0.05$). When looking to Table 2, APX activity was also exhibited the significant decline trends in SDS-treated plants relative to control ($p < 0.05$). However, the decline ratios in APX activity as compared to control were almost the same among all of the SDS application. As compared to the control, GR activity was also reduced about 10% in 0.1 SDS, 12% in 0.2 SDS, 16% in 0.3 SDS, 28% in 0.4 SDS and 24% in 0.5 SDS ($p < 0.05$).

3.5 H_2O_2 content and superoxide anion ($O_2^{\cdot-}$) production

As can be seen in Table 3, the lowest values of superoxide anion and H_2O_2 were recorded in the control. When compared to the control, SDS applications led to critical increases in superoxide anion content. Also, in a dose-dependent manner, SDS applications seriously increased

Table 3 Effects of SDS applications on the contents of superoxide anion, hydrogen peroxide and MDA of the barley leaves

Treatments	Superoxide anion ($\mu\text{g g}^{-1}\text{FW}$)	Hydrogen peroxide ($\mu\text{g g}^{-1}\text{FW}$)	MDA ($\text{nmol g}^{-1}\text{FW}$)
Control	$24.27 \pm 0.77c^*$	$22.52 \pm 0.28f$	$4.84 \pm 0.05e$
0.1 SDS	$28.55 \pm 0.41b$	$23.83 \pm 0.18e$	$5.12 \pm 0.04e$
0.2 SDS	$32.85 \pm 0.61a$	$26.91 \pm 0.07d$	$5.99 \pm 0.23d$
0.3 SDS	$32.32 \pm 0.51a$	$28.1 \pm 0.12c$	$6.85 \pm 0.08c$
0.4 SDS	$32.9 \pm 0.37a$	$43.77 \pm 0.69b$	$12.25 \pm 0.49b$
0.5 SDS	$31.33 \pm 0.28a$	$51.15 \pm 0.28a$	$15.05 \pm 0.16a$

*Different letters in the same column indicate statistically significant differences ($p < 0.05$)

the H_2O_2 content. The highest value of H_2O_2 content was measured in 0.5 mM SDS (an approximate increase of 127%) relative to the control ($p < 0.05$).

3.6 Lipid peroxidation levels

MDA is accepted as one of the important indicators of lipid peroxidation. In the present experiment, all of the SDS applications except for 0.1 mM SDS have critically increased the lipid peroxidation level. When compared to the control, the MDA contents in 0.2, 0.3, 0.4 and 0.5 mM SDS applications increased by approximately 24, 42, 153 and 210%, respectively (Table 3) ($p < 0.05$).

4 Discussion

In the present study, it was aimed to evaluate as the physiological and biochemical the effects of SDS on the barley seedlings. As far as we know, only a few works have explored the effects of SDS on plants. Also, this is the first study which reveals the SDS-induced changes on barley seedlings. Therefore, it is expected that this study will provide important contributions to the literature.

Table 2 Effects of SDS applications on the activities of superoxide dismutase (SOD), peroxidase (POX) and catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) of the barley leaves

Treatments	SOD ($\text{U mg}^{-1}\text{ protein}$)	POX ($\text{U mg}^{-1}\text{ protein}$)	CAT ($\text{U mg}^{-1}\text{ protein}$)	APX ($\text{U mg}^{-1}\text{ protein}$)	GR ($\text{U mg}^{-1}\text{ protein}$)
Control	$5.23 \pm 0.09bc^*$	$1580 \pm 32.2f$	$39.06 \pm 2.62a$	$5.23 \pm 0.04a$	$6.5 \pm 0.17a$
0.1 SDS	$5.7 \pm 0.23a$	$1838 \pm 32.8e$	$20.61 \pm 0.53b$	$3.76 \pm 0.03bc$	$5.86 \pm 0.22b$
0.2 SDS	$5.37 \pm 0.05ab$	$2071 \pm 26.7d$	$11.92 \pm 0.38c$	$3.81 \pm 0.05b$	$5.74 \pm 0.04b$
0.3 SDS	$4.95 \pm 0.05c$	$2231 \pm 35.5c$	$13.75 \pm 0.38c$	$3.86 \pm 0.11b$	$5.49 \pm 0.17b$
0.4 SDS	$4.39 \pm 0.03d$	$2480 \pm 42.1b$	$9.3 \pm 1.2c$	$3.62 \pm 0.05bc$	$4.71 \pm 0.06c$
0.5 SDS	$3.7 \pm 0.09e$	$3054 \pm 59.7a$	$12.7 \pm 2.42c$	$3.54 \pm 0.13c$	$4.92 \pm 0.11c$

*Different letters in the same column indicate statistically significant differences ($p < 0.05$)

4.1 Effects of SDS applications on root and coleoptile growth

The results of the present study clearly showed that SDS applications lead to negative effects in normal growth of barley, inducing a decrease in root and coleoptile lengths. It was also determined that the levels of reduction in growth exhibited a positive correlation with the increase in SDS dosage. As compatible with our results, Chang et al. [19] reported that SDS reduced the growth of wheat. It is possible that the SDS-induced growth retardation is due to destructions on the synthesis reactions. After SDS applications, the declines in protein content of barley seedlings study support this situation. It is well known that sodium, a raw component of SDS, is toxic for plants [60]. Therefore, initially, the toxic effects of SDS might be attributed to its sodium content. However, Tuna et al. [70] reported that the sodium-induced the growth retardations in different barley varieties occur at the very higher concentration than sodium content of SDS used in this study. In the present case, it cannot be expressed that the SDS-induced retardations in the barley growth arose from sodium cation. On the other hand, when sulphate, another component of SDS, is at a high amount in the cells, it leads to disruption of the intracellular colloidal structure. In such a case, excessive sulphate in plant tissues will retard the plant growth by disrupting the processes of synthesis reactions and the cell division [18]. In this study, the results of protein content have already shown that biosynthesis reactions strongly affected by SDS.

4.2 Effects of SDS applications on soluble protein content and protein profiles

Proteins have many functions from catalytic activity to defence in organisms. Recently, diverse studies have revealed the effects of SDS on proteins. Rajagopal et al. [55] reported that SDS increased the denaturation of bacterial proteins. Also, it was determined in vitro that SDS breaks down the peptide bonds in amino acids and therefore denatures the proteins [25]. Our results clearly pointed out that SDS led to the critical decline in soluble protein content, corroborating previous result recorded for wheat grown in the presence of SDS [19].

The possible cause of these declines could be the disruptions in the cellular metabolic events during transcription, translation and post-translation. Previous studies also showed that SDS-induced decreases in plant protein contents are associated with the disruption of peptide bonds after translation [19, 22]. Along with protein content, the protein profile images obtained from electrophoresis confirmed the negative effects of SDS on protein biosynthesis. When looking to Fig. 3, it can be clearly seen that SDS

caused a decrease in densities of protein bands among 50–60 kDa. It is well known that these band ranges are the location of ribulose 1–5 bisphosphate carboxylase oxygenase (RuBisCO) that is the key enzyme in photosynthesis [40]. Therefore, the decline in this protein bands may be an indicator of the SDS-induced retardation of the photosynthesis process. On the other hand, the intensities of protein bands under the 25 kDa significantly increased with SDS applications (Fig. 3). It is well known that a lot of stress proteins are located among these protein bands and, the increase of these proteins is one of the plant defence strategies against stress conditions [45].

4.3 The effects of SDS applications on photosynthetic pigment contents

For the ecological balance as well as plant development, the continuation of photosynthesis is essential but this metabolic process is adversely affected by stress factors such as drought, salt and heavy metal [20, 63]. The deteriorations of chlorophyll *a* and chlorophyll *b* are accepted as an indicator of the reduced photosynthetic efficiency [33]. The results of the present study shown that the SDS-induced symptoms on photosynthesis manifested as the critical declines in chlorophyll *a* and *b* contents. These results are in accordance with a previous work, which showed that SDS declined the chlorophyll contents in duckweed [27]. In this way, the detrimental effect of SDS on pigments may be associated with the SDS-induced disruption in the chlorophyll–protein complex and/or separation of magnesium from chlorophyll centre [62]. In the present study, the content of carotenoids, considered to perform as the accessory pigment for photosynthesis, was markedly decreased by SDS applications. The carotenoids have also antioxidant properties due to a large number of double bonds in their linear molecular structure, and therefore, they can directly scavenge the reactive oxygen particles [54]. In that case, the decrease in carotenoid content might have been led a reduction in the total antioxidant defence capacity against SDS-induced oxidative damage of the barley seedlings.

4.4 Effect of SDS applications on the antioxidant defence system

In plant cells, the level of ROS is controlled by the antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD), glutathione reductase (GR) and by the antioxidant substances such as ascorbate and glutathione. However, the power of this defence system may not be enough for their elimination due to the excessive formation of the ROS

under stress conditions. In this case, the ROS lead to the oxidative damages on all cell components [32, 50].

SOD forms the first line of the enzymatic defence mechanism against oxidative damage and has a role in reducing of superoxide anion to hydrogen peroxide [32, 50]. In the present study, the SOD activities were generally diminished by the SDS applications except for 0.1 and 0.2 mM SDS. Meanwhile, the SOD and superoxide anion data indicate that although the highest SOD activity was measured in 0.1 mM SDS application, this increase in SOD activity was insufficient for detoxification of superoxide anions. On the other hand, in other SDS groups, SOD activities were very low than control. When considered that SDS can damage peptide bond among amino acids, the reductions in SOD activities can be attributed to degradation of the active molecular structure of the enzyme by SDS [25].

The exposure of barley to SDS led to different effects in activities of antioxidant enzymes such as POD, CAT, APX and GR, which are responsible for detoxification of H_2O_2 [31]. SDS applications caused the activation of POX. This activation might arise from stimulation of the defence mechanism against the SDS-induced oxidative stress. However, it is clear that this activation alone could not prevent the SDS-induced increases in H_2O_2 amounts.

Unlike POX, the activity of CAT, responsible for the prevention of the hydroxyl radical formation by catalysing the conversion of H_2O_2 into water and oxygen [6], was seriously reduced by SDS applications. This result has corroborated the result of a previous study which recorded a decline of CAT activity in wheat in the presence of SDS [19]. After the exposure of plants to SDS, the inactivation of CAT might result from the separation from the enzyme molecules of the hem groups which is essential for CAT activation [41, 68].

APX and GR have significant roles in reducing the H_2O_2 into the water at the ascorbate–glutathione cycle which is active in mitochondria, chloroplasts, cytosol and peroxisomes [49]. Besides H_2O_2 elimination, they are also responsible for the reduction in glutathione and ascorbate which are essential for the continuity of the cycle [50]. Therefore, the inactivation of the APX and GR causes to the limitation of the elimination of the H_2O_2 in stressed plants [49, 50]. The data of the present work showed that SDS caused serious reductions in both APX and GR activities. This means that SDS can interrupt the bio-regulation of the ascorbate–glutathione cycle.

4.5 Effect of SDS applications on the reactive oxygen formation

ROS are formed during the oxidation–reduction reaction of the electron transport events in cell organelles such as chloroplasts and mitochondria. They can function as

signal molecules in the simulation of defence mechanisms against stress damages [11]. However, the formation of ROS may excessively increase due to the destructive effects of the stressors on the metabolic processes, including photosynthesis and respiration. In this case, the excessive ROS damages to all compartments of the cells [75]. The superoxide radical, the first radical product of the oxidation–reduction reactions, is a precursor molecule of other ROS leading to the irreversible degradation of macromolecules such as lipid, protein and carbohydrate and DNA [46, 49]. In the present research, the amount of this radical showed an increase in depending on the SDS doses. Due to the disruption of the membrane lipids and proteins by SDS, this increase in superoxide formation may be attributed to the excessive electron leaks from the electron transport chains on cell membranes [37].

H_2O_2 is thought to be less toxic than the superoxide radical for cells since it has no free electrons. Meanwhile, when H_2O_2 is at low concentration, it functions as a signal molecule which stimulates the defence systems for stress tolerance [7, 10, 31]. Therefore, intracellular H_2O_2 content has to be always kept low by antioxidant enzymes. If the capacities of these enzymes are insufficient for the elimination of excessive H_2O_2 , it transforms to hydroxyl radical which is the most dangerous radical for cell membranes [74]. In this case, the excessive H_2O_2 inevitably will lead protein denaturation, DNA breakage and lipid peroxidation [31, 51]. The present study results showed the H_2O_2 contents increased with the rise of SDS concentration. It is strongly possible that these increases in H_2O_2 amounts are a result of SDS-induced decreases in CAT, APX and GR activities.

4.6 Effect of SDS applications on the cell membrane

The changes of the MDA amount are accepted as an indicator of the level of the lipid peroxidation which arises from reaction with ROS of the unsaturated bonds in phospholipids, cholesterol and fatty acids in the cell membrane. In the presence of ROS, lipid peroxidation continues in form of chaining on cell membranes and leads to the degradation of membrane integrity [9, 21, 35]. As far as we know, there are no studies reporting the effects on the plant cell membranes of SDS. The results of this study clearly showed that SDS caused to damage in the cell membranes as depending on the dose of the SDS. It is strongly possible that this damage in the membrane of barley seedlings was a result of the marked increase in ROS triggered by SDS.

In addition to this, another reason for the high membrane damage may be the fragmentation of peptide bonds of integral and peripheral proteins in the cell membrane by SDS. Some experiment evidence support the idea that

SDS disrupted the peptide bonds of the integral proteins in the cell membranes cultivated in vitro [25, 43]. The SDS-induced disruptions on the protein and membrane complex may also lead to the deterioration on membranes of the organelle such as chloroplast and mitochondria. In this case, the redox reactions in these organelle membranes are interrupted, and thus, ROS formation is excessively increased. When considering the compatible increase in the ROS and MDA results of the present study, it can be asserted that the high lipid peroxidation stemmed from the disruption of not only lipids but also proteins in the membranes by SDS.

5 Conclusion

Overall, the results of the present work revealed that SDS led to a clear negative effect on the physiological performance of barley seedlings. SDS reduced the biosynthesis of protein and photosynthetic pigments. In addition, SDS made difficult the control of ROS levels by decreasing the efficiency of antioxidant defence systems, and therefore, it caused serious damage in the cell membranes. As an inevitable result of these, it dramatically reduced the growth of barley seedlings. It is hoped that this study will draw attention to the necessity of the precautions against the random disposal into agricultural areas of wastewater containing SDS.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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