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Full Length Research Paper

Effects of reactive red 239 textile dye on total soluble protein content, peroxidase activity and lipid peroxidation of *Zea mays* L. cv. "Martha F1"

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This study examined the effects of Reactive red 239 on total soluble protein, peroxidase activity and malondialdehyde (MDA) content of corns. The corns were exposed to different concentrations of 4000, 5200, 6760, 8788 and 11424 mg/L Reactive Red 239. In comparison to the control group, there was an increase in the total soluble protein content of plant groups exposed to low-concentration dye application. On the contrary, total soluble protein content decreased as the concentration of the dye increased. In the analyzed plants, different effects were observed on peroxidase activity. When compared with the control group, a significant increase was identified in the plants which were treated with Reactive Red 239 excluding the group of 11424 mg/L application. Besides, the effects of Reactive Red 239 on MDA content were analyzed. MDA content of Reactive Red 239 treated plants was found to be lower in all application groups excluding 439 mg/L application group. Our findings indicated that Reactive Red 239 caused significant changes in total protein, peroxidase activity and MDA content.

Key words: Reactive red 239, total soluble protein, peroxidase activity, malondialdehyde (MDA).

INTRODUCTION

Plants are exposed to several environmental constrains generally attributed to abiotic (drought, extreme temperatures and salinity, etc) and biotic stresses induced by pathogens (fungi and viruses, etc). The ability of plants to modify their behavior appropriately in response to these environmental constrains is a major factor in their adaptation to these specific conditions. These responses inlude physiological as well as, biochemical and molecular changes (Jellouli et al., 2008).

Dye wastewater from textile and dyestuff industries is one of the most difficult industrial wastewaters to treat. The wastewaters from these industries is characterized by high alkalinity, biological oxidation demand, chemical oxidation demand, total dissolved solids with dye concentrations generally below 1 g/dm^3 (Kaushik and Malik, 2009). The synthetic origin and complex aromatic structures of dyes make them stable and difficult to be biodegraded (Fewson, 1998; Seshadri et al., 1994).

Reactive dyes, which are the only textile colorants are designed to bond covalently with cellulosic fibers (that is, cotton), are extensively used in the textile industry because of their wide variety of color shades, high wet fastness profiles, ease of application, brilliant colors, and minimal energy consumption (Aspland, 1997). Reactive Red 239 (RR 239) is a well known mono-azo dye and has been widely used in the dyeing process of textiles industry (Liu and Chiou, 2005).

In general, the chemical dye production wastewater effluent contanied relatively high levels of potentially toxic chemicals, indicating that the effects were probably due to a combination of pollutants (Sponza, 2006). Many biomarkers have been developed to determine the effects of environmental stresses. These biomarkers include the decrease of protein content and antioxidant enzyme

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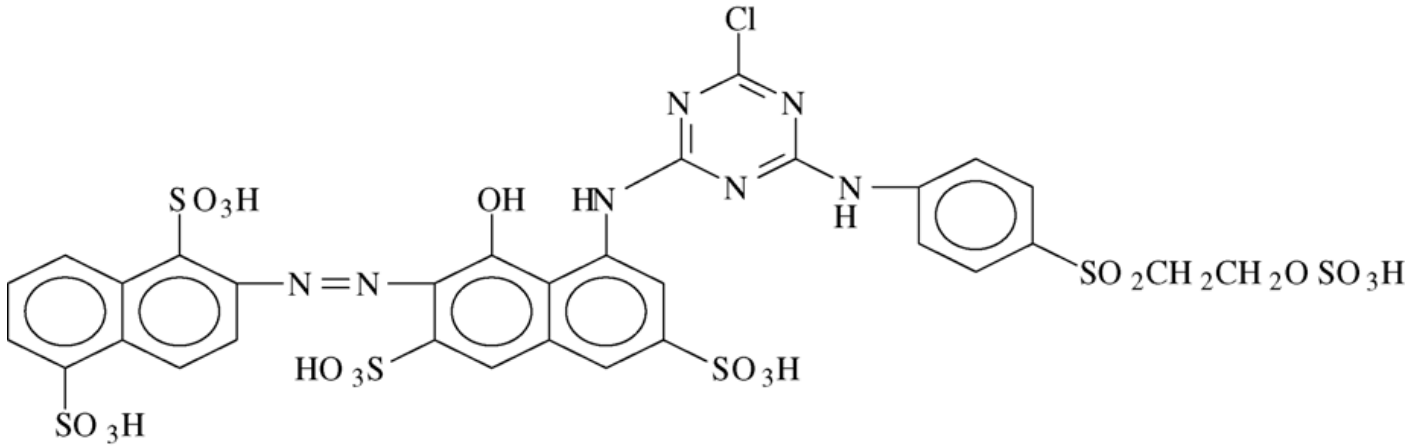

Figure 1. Molecular structure of RR 239 (Liu and Chiou, 2005).

activity and the increase of MDA. Being an important organic constituent protein plays significant role in cellular metabolism and as constituents of cell membrane. It regulates the process of interaction between the intra and extracellular media (Kharat et al., 2009). It is known that soluble protein content is an important indicator of physiological status of plants (Doganlar et al., 2010). Stressfull environments induce the generation of reactive oxygen species (ROS) such as superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-) etc. in plants thereby creating a state of oxidative stress in them (Asada, 1994; Gille and Singler, 1995; Prasad et al., 1999; Panda et al., 2003a, b). This increased ROS level in plants caused oxidative damage to biomolecules such as lipids, proteins and nucleic acids, thus, altering the redox homeostasis (Smirnoff, 1993; Hayat et al., 2010). Plants respond to a rise in ROS that the defence system is unable to remove with increased enzymatic or non-enzymatic antioxidant processes (Alscher et al., 2002).

Peroxidases (POD), (E.C. 1.11.1.7) are heme containing enzymes able to oxidize a wide range of organic and inorganic compounds, using H_2O_2 as a co-substrate (Halliwell, 2006). A large group of POD enzymes are considered a part of the general protective mechanisms in plants (Wang and Zhoua, 2006). Many types of environmental stresses both biotic and abiotic produce characteristic changes in physiological and metabolic processes of higher plants. Thus, POD activity usually increases in plant tissues under various stress conditions such as the influence of toxic elements (Mocquot et al., 1996; Weeckx and Clijsters, 1996; Miteva and Peycheva, 1999; Miteva et al., 2005), mechanical injuries (Espelie et al., 1986) or attack by parasitic organisms (Shimoni et al.,1991). In the present study, we investigated the changes in total soluble protein, peroxidase activity and lipid peroxidation in *Zea mays* cv. ‘‘Martha F1’’ caused by RR 239 was applied in different concentrations.

MATERIALS AND METHODS

Preparation of plant samples

In this study, as textile dye RR 239 (Figure 1) and as the plant *Zea mays* L. cv. “Martha F1” were selected. Perlite was used as the plant growth media. The studies were carried out in climate room. The temperature of the climate room was adjusted to 23 ± 2°C and humidity was adjusted as 60%. Application groups were prepared in 5 different concentrations (4000, 5200, 6760, 8788 ve 11424 mg/L). The trials were performed with three replications. The plants were irrigated every three days during the plant growth period. In the first three irrigations, the plants were treated with a test solution containing dye and Hoagland solution was used in the subsequent irrigations. The composition of Hoagland culture solution was prepared according to Hoagland and Arnon (1938). Collected samples were frozen in liquid nitrogen and were kept at -80°C.

Identification of total soluble protein

Total soluble protein amount was identified by Bradford (1976) method.

Identification of peroxidase activity

The method was performed according to Mac Adam et al. (1992). The change in enzyme activity was measured with spectrophotometer for 1 min at 436 nm (Mac Adam et al., 1992).

Malondialdehyde (MDA) analysis

The method was performed according to Heath and Packer (1968). The measurements made at 600 nm were dedected from the measurements at 532 nm and with 155 mM^{-1} cm^{-1} extinction coefficient and MDA amount was also calculated (Heath and Packer, 1968).

Statistical analysis

Statistical analyses were performed using SPSS 15.0 for Windows. Duncan’s (1955) Multiple Range Test was employed to determine the statistical significance of differences among the means

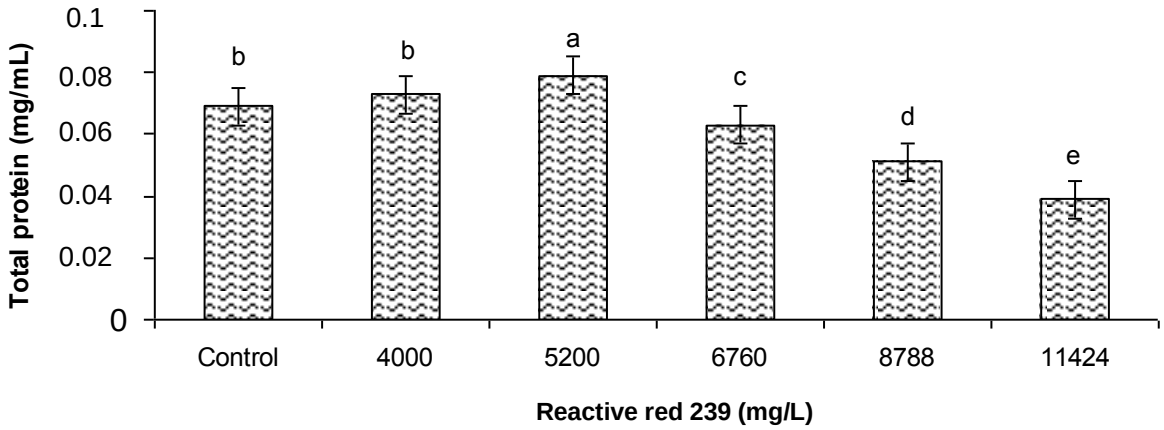

Figure 2. Changes in total soluble protein contents in *Z. mays* L. cv. "Martha F1" leaves exposed to RR 239. Vertical bars represent standart error of average of three replications. Data followed by different letters are significantly different from each other (P<0.05) according to Duncan's test.

(Duncan, 1955).

RESULTS

Compared to control dye treatment caused a decrease of the total soluble protein content at 6760 to 11424 mg/L dye concentrations. On the other hand, total soluble protein content increased at 4000 and 5200 mg/L. The highest total soluble protein was found to be 0.079 mg/mL in 5200 mg/L application group, while the lowest total soluble protein content was found to be 0.039 mg/mL in 11424 mg/L application group (Figure 2). In comparison to the control group, there was a decrease in the peroxidase activity of plant groups exposed to low-concentration dye application. On the contrary, peroxidase activity increased together with the increase in concentration. The highest peroxidase activity was detected as 6.08 U/mg protein in the 11424 mg/L application group and the lowest peroxidase activity was determined as 1.28 U/mg protein in the 5200 mg/L application group. This difference was statistically found significant ($P < 0.05$) (Figure 3).

In comparison to control leaves, exposing leaves to 4000 and 5200 mg/L concentrated dye applications caused the MDA content to decrease while high-concentration dye applications increased MDA content. Maxiumum decrease in MDA content was seen as 0.51 µmol MDA/g FW in the 5200 mg/L application group. The highest MDA content was detected as 5.94 µmol MDA/g FW in the 11424 mg/L application group. This difference was statistically found significant ($P < 0.05$) (Figure 4).

DISCUSSION

Though industrialization and urbanization in recent years brought economical boom to the world but at the same time, it is the major cause of environmental pollution (Chowdhury et al., 2011). The environmental pollutants are spread through different channels, many of which finally enter into the food chain of livestock and man (Rajaganapathy et al., 2011). Synthetic dyes and heavy metals are widely used in the industries like textiles, leather, paper, plastic, electroplating, cement, metal processing, wood preservatives, paints, pigments and steel fabricating industries (Ponnusami et al., 2008). These industries discharge large quantities of toxic wastes and the untreated effluents from these industries cause soil and water pollution (Dar et al., 2011; Sundaram et al., 2011).

In recent past, because of rapidly growing industrialization pollution due to dyes has been amplified to the great extent. Worldwide, thousands of the dye stuffs are being released in the environment in the form of effluents during synthesis and dyeing processes. Azo dyes are one of the major constituents of this pollution (Jadhav et al., 2011). Azo dyes accounts for the majority of all textile dyestuffs produced and have been the most commonly used synthetic dyes in the textile, food, paper making, printing, leather and cosmetic industries (Chang et al., 2001). In addition to their visual effect and their adverse impact in terms of chemical oxygen demand (COD), many synthetic azo dyes show their toxic, carcinogenic and genotoxic effects (Sharma et al., 2009). Being xenobiotic substance, their degradation in nature is very difficult (Rosu et al., 2008).

Several methods are available for the removal of such pollution. However, implementation of physicochemical methods for the removal of dyes and their effluents have inherent drawbacks such as they require more energy and chemicals so economically unfeasible, being unable to completely remove the recalcitrant azo dyes and/or their organic metabolites, accumulation of toxic byproducts, generating a significant amount of sludge that may cause secondary pollution problems and

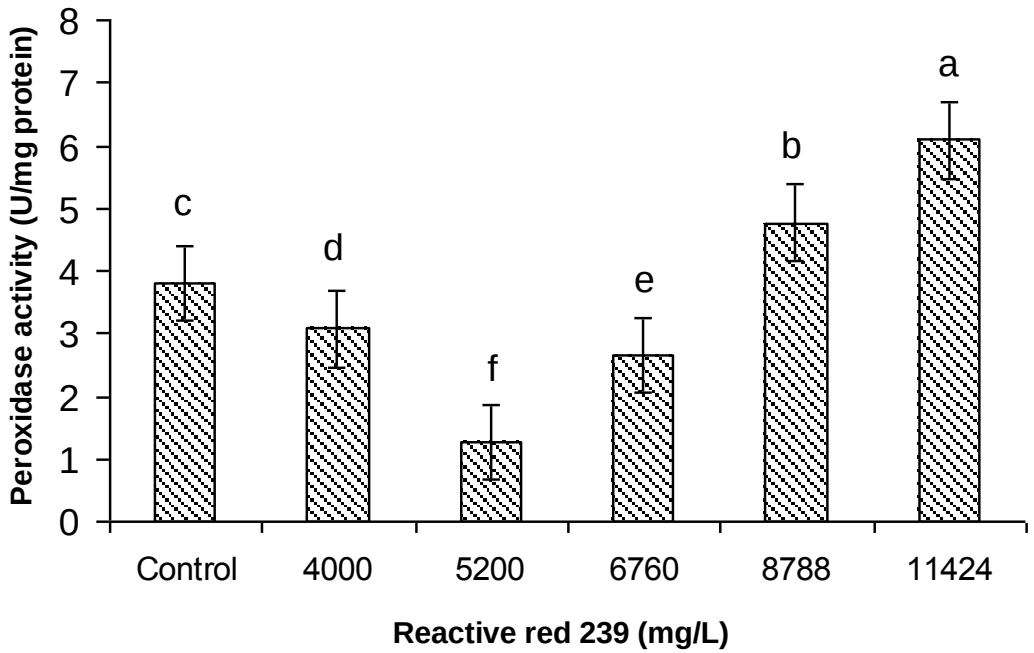

Figure 3. Changes in peroxidase activity in *Z. mays* L. cv. "Martha F1" leaves exposed to RR 239. Vertical bars represent standart error of average of three replications. Data followed by different letters are significantly different from each other (P<0.05) according to Duncan's test.

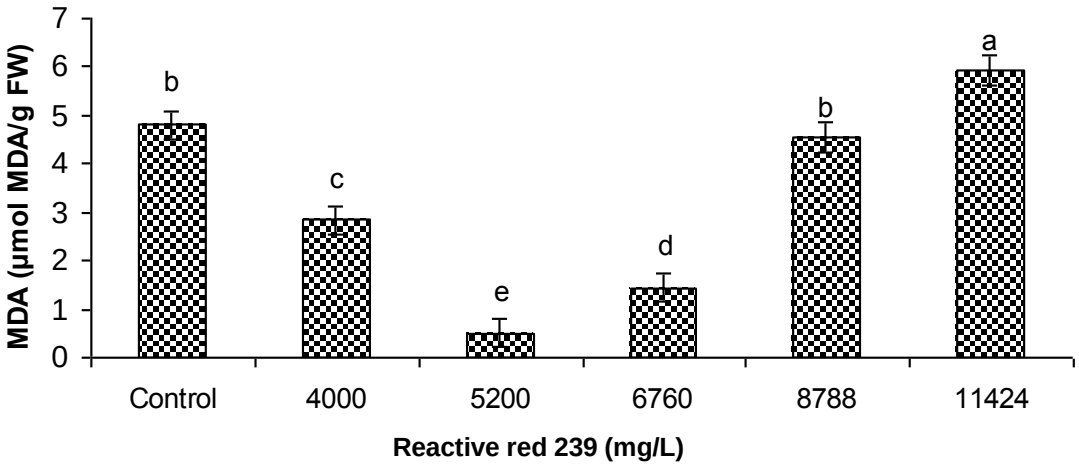

Figure 4. Changes in MDA levels in *Z. mays* L. cv. "Martha F1" leaves exposed to RR 239. Vertical bars represent standart error of average of three replications. Data followed by different letters are significantly different from each other (P<0.05) according to Duncan's test.

involving complicated procedures (Robinson et al., 2001; Forgacs et al., 2004). Due to this reason, biological methods have gotten significant attention and these provide advantages over the conventional treatments (Verma and Madamwar, 2003). In many cases, metabolites generated after azo dye degradation have been found to be more toxic. Chen (2002) observed that biotoxicity to cells was possibly attributed by intermediate metabolites of Reactive Black B. Metabolites obtained after azo dyes degradation were found to be more toxic than parent dye compounds in the presence of araclor-induced rat liver microsome preparations (Mansour et al., 2007). Toxicity of azo dyes has been assessed by phytotoxicity and microbial toxicity, genotoxicity, mutagenicity assays widely (Brown and Dietrich, 1983; Jadhav et al., 2010a; Parshetti et al., 2010). However, toxicity studies with respect to generation of oxidative stress in plants are yet to get much significant attention. Under certain environmental conditions, plants may experience oxidative stress due to increased levels of

ROS and decreased activities of antioxidant enzymes (Smirnoff, 2005). From previous studies, it can be seen that the environmental pollutants can induce the oxidative stress which can be accompanied with lipid peroxidation and/or protein oxidation or even DNA damage in plant as well as mammalian cells (Schutzendubel and Polle, 2002; Achary et al., 2008; Jeng et al., 2010). Lipid peroxidation chain reaction and protein oxidation can be generated by the formation of ROS and these two parameters are stronger indicators of induction of oxidative stress than the altered antioxidant enzyme levels (Zaman et al., 1995).

The studies on the reactions of industrial wastes on the plants did not mainly focus on the evaluation of the reactions to textile wastes. Significant changes were determined in the total soluble protein level, the peroxidase activities, and MDA contents of plants exposed to different stress conditions (for example, extreme temperatures, salinity and herbicide) (Doganlar et al., 2010; Wang and Zhoua, 2006; Gulen and Eris, 2004). These intrinsic changes seen in *Zea mays* exposed to RR 239, are in same line with our results. MDA is a cytotoxic product of lipid peroxidation and an indicator of free radical production and consequent tissue damage (Ohkawa et al., 1979). Thus, cell membrane stability has widely been utilized to study the effects of stress on plants (Zhang et al., 2007). MDA levels in the leaves exposed to the high concentration of RR 239 were higher than those of the control and only the low concentrations of RR 239 decreased the MDA levels in leaves (Figure 4). There was a decrease in peroxidase activity together with the decrease in MDA contents. Peroxidase activity increased in line with the significant increase of MDA contents for 8788 and 11424 mg/L concentrated application groups. High concentrations dye application severely reduced leaf total soluble protein contents in leaves but however, the low concentrations of dye application increased the total soluble protein content in leaves of *Zea mays.* There was an increase in the total soluble protein level of leaves belonging to plants with a decreased MDA content, and decreased peroxidase activity (Figures 2 to 4). These results illustrate that there is a relationship between the total soluble protein level, peroxidase activity, and MDA content in corn of RR 239 application.

ROS are chemically-reactive molecules containing oxygen which includes superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH^-). These are produced as by-products of the partial reduction metabolism of oxygen and plays crucial role in cell signaling and development in plants, including plant defense response, cell death and oxidative stress, yet ROS can cause protein, lipid, and DNA damage (Smirnoff, 2005).

The total soluble protein level, peroxidase activity, and MDA content are parameters commonly used to determine stress in plants. In this study, the total soluble protein level increased and peroxidase activity and MDA content decreased in plant groups exposed to low-concentration dye applications in comparison to the control group and plant groups exposed to high-concentration dye applications (Figures 2 to 4). Based on these results, Reactive Red 239 did not cause stress in plants at low concentrations; however, it displays toxic effects at high concentrations.

Conclusion

Turkish government has declared that the azo dyes are banned dyes since tye 1st of March, 1995 due to the relevant aromatic amines. However, due to their efficiency of dyeing and cost, they have been in use in textile dyeing processes in Turkey and some countries (Isik and Sponza, 2003). These results indicated that there was a relationship between total soluble protein, peroxidase activity and MDA content in *Zea mays*. Thus, the used Reactive Red 239 is considered as an important stress factor. Considering the negative effects of Reactive Red 239 on plant growth in increasing concentrations, it becomes evident that the organizations in textile industry should show due diligence to avoiding direct disposal of dye wastes to environment with no treatment. This study indicates that further studies should be carried out in testing the effect of wastewaters containing dye charged to the nature.

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