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Response of sorghum to effect of two azo dye bacteria

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ABSTRACT

A huge amount of azo-dye wastewater is generated annually by the textile industry. At present, the improper disposal of azo dyes to water bodies causes considerable concern as it can disrupt the ecosystem and, because of their toxicity and carcinogenicity, constitutes a possible environmental and health problem. Because of its cheap, environmentally safe, and sustainable properties, chemical, physical, and biological methods have been used to treat azo-dye wastewater, and biological technology has been recognized as a promising technique. Azo dyes are one of the most pollutant synthetic dyes to the environment. Azo dyes can be transmitted through a food chain and may be associated with problems of toxicity, carcinogenicity, and mutagenesis. In this study, contaminated soil with two Azo dyes, Reactive Blue (RB) and Reactive Red (RR) was used in sorghum planting (*Sorghum bicolor* L. Moench) using different dye concentrations (0, 0.05, 0.1, 0.2 g Kg^{-1} soil). Increased concentration of the dye caused significant alterations in the morphological characteristics of sorghum, such as steam length, leaves number and leaf area. There was a slight decrease in the plant content of total chlorophyll and carotenoids, while a significant variation in CAT and SOD enzyme activities was observed by increased dye concentration in the soil for both dyes compared to the control group. In addition, a significant increase in proline content was found at higher dye concentrations.

Keywords: Antioxidant, Azo dyes, Benzidine, Carotene, Enzymes, Proline.

INTRODUCTION

One of the biggest contributors to severe pollution problems worldwide is the textile industry and its dye-containing effluents. Specifically, the discharge of dye-containing effluent into the water body is unwanted due to their color and the excessive number of dyes dissolved in huge volumes of water, both in the dye bath as well as in the rinsing process. Without sufficient care, these dyes can stay in the environment for a long time. Having said that, even lesser concentrations of dyes may have sequentially environmental effects (Lata *et al.* 2007). The first step of azo dye degradation is decolorization, the mechanism by which the azo bond is broken down. Various bacteria and fungi have the potential to decolorize. However, few have been recognized by the textile industry. Their lack of adoption is primarily due to the technique's poor effectiveness, since the toxicity and salinity of azo-dye wastewater prevents the growth and operation of microorganisms. The potential of plants to induce azo-dye bio-decolorization has gained a great deal of attention recently. Bio-decolorization is expected to be promoted by plants. Some studies have shown that algae, such as *Oscillatoria*, *Chlorella pyrenoidosa*, and *Chlorella vulgaris*, have the ability to decolorize dye wastewater and have proposed that these algae be used to treat azo dyes polluted lakes and rivers. The azo-dye wastewater could successfully decolorize built wetlands that were previously used for sewage treatment. Researchers found that the rate of decolorization of built wetlands with plants was more than twice that of the unplanted bed. Azo dyes form the biggest production volume of dye chemistry at the present, and their relative significance may even increase in the future (Crespi *et al.* 2019; Fu *et al.* 2019; Benkhaya *et al.* 2020; Zhuang *et al.* 2020).

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Azo dyes are frequently used in a variety of applications in the food, pharmaceutical, paper, cosmetics, textile, leather industries, and others. They add up to be about half of all synthesized dyes (Benkhaya *et al.* 2020). They are the biggest group of dyes, which have a great deal of structural and color variety, used in the industry, making up 70% of the annual manufacturing (Tony *et al.* 2009). It can be defined as any class of artificial dyes that contains the azo group (-N=N-). When explaining a dye molecule, nucleophiles are known as auxochromes, while the aromatic groups are referred to as chromophore. Together, the dye molecule is frequently presented as a chromogen (Al-Rubaie & Mhessn 2012). Relying on the number of Azo groups, these dyes are arranged into mono Azo dyes having a single Azo group or poly Azo dyes carrying multiple Azo groups (Hunger 2007; Sandhya. 2010). Around half of the dyes applied in the textile industry include Azo dyes, and as a repercussion, toxicity issues have come to light due to the release of a few of these products into the environment (Pandey *et al.* 2007; Çinar & Demiröz 2010). The emergence of Azo dyes into the environment is a serious problem for life and is also a threat to the environment (Saquib & Muneer 2003). Azo dyes are used for dyeing cotton fabrics. Cotton is the most-commonly used fabric throughout all textiles (Olah *et al.* 1994). Azo dyes present good fiber-fixation factors as compared to other synthetic dyes showing up to 85% fixation. However, this clears up why so much dye is released into the environment, making up the other 10 to 15% of the amount used (Nam & Renganathan 2000; Oliveira *et al.* 2007). Numerous studies show that the release of Azo dyes into the environment is extremely alarming due to the toxic, mutagenic, and carcinogenic elements of these dyes and of their biotransformation products (Cisneros *et al.* 2002; Rehorek *et al.* 2002; Lin & Leu 2008). The setback of these dyes is that they are not straightforwardly degraded by aerobic bacteria, while with anaerobic or microaerobic reductive bacteria, they can produce toxic or mutagenic compounds such as aromatic amines (Chung & Cerniglia 1992; Wong & Yuen 1996). Mutagenic, carcinogenic, and toxic effects of the Azo dyes can result in consequence of direct action by the compound itself, or the formation of free radicals and aryl amine derivatives created while the reductive biotransformation of the Azo bond (Chung *et al.* 1992; Collier *et al.* 1993; Rajaguru *et al.* 1999). The environmental behavior of dyes drew attention and is prompted most importantly by the worry over their probable toxicity and carcinogenicity, intensified by the fact that many dyes previously were made up of known carcinogens like benzidine, which could possibly be reformed as an outcome of metabolism (Baughman & Perenich 1988; Clarke & Anliker 1980). Azo dyes are distributed throughout the body after absorption because they behave as xenobiotics. In the body, they either exert some kind of action themselves or are subjected to metabolism. Biotransformation may create less hazardous compounds. However, it may also create bioactive xenobiotics, i.e., compounds showing larger toxicity (Kleinow *et al.* 1987; Livingstone 1998). Industries can have an extremely negative impact on soil fertility and plant growth (Field *et al.* 2008). Plants interact with the surrounding soil, water, and air, extracting and releasing chemicals throughout their life cycle through photosynthesis and respiration. Plant systems of membranes and vascular transport adapted and evolved to manage water and nutrient uptake and transport but also allow many accidental compounds to traverse through the plant. The ability of some xenobiotics to enter plant tissues could cause a risk to plant consumers. Potentially with most attention at the moment are food crop interactions with arising contaminants, which showcase an unintentional type of phytoremediation, possibly providing an exposure pathway when edible plants interact with contaminated groundwater, soil, or irrigation water plants exhibit diverse suites of protective mechanisms against abiotic stresses. Environmental stresses typically lead to decreased growth rate, reduced reproduction and even death for particularly severe stress (Kleinow *et al.* 1987; Livingstone 1998; Field *et al.* 2008; Limmer & Burken 2016) So far, acknowledging and understanding the effects of widespread textile dyes on plant key physiological procedure is still very limited. Plants treated with industry sewage which contained dyes, had reduced concentrations of carbohydrates, proteins and chlorophyll (Field *et al.* 2008; Limmer & Burken 2016; Livingstone 1998). Kaushik *et al.* (2005), showed how the effluent of textiles caused a decline in plant shoot length, chlorophyll and carotenoid. To add on, intensive flooding of agricultural lands with polluted water from textile dyes concentrations in three different cultivars of wheat. Similar results of textile mill sewage containing have been reported for sorghum cultivars (Garg & Kaushik 2006: Arekhi & Jamshidi 2018).

MATERIALS AND METHODS

Sorghum bicolor L. Monech seeds were obtained from the local market and cultivated in soil which was polluted with different concentrations of two Azo dyes, Reactive Blue (RB) and Reactive Red (RR) which were brought from State Company for Textile Industries –Hilla Textile Factory.

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Pots of 25 cm (in diameter) × 30 cm (in height) were filled with 6 kg of soil polluted with different concentrations of two Azo dyes (Reactive Blue & Reactive Red) separately (0.05, 0.1and 0.2 g kg^{-1} soil) and another group with zero concentration as a control for comparison. Each treatment had three replications. Six seeds of *S. bicolor* were cultivated in each pot. The pots were irrigated with tap water throughout the study period. In the present study, the height of the stems of the plants was measured using a tape measure, starting from the surface of the soil to the base of the final leaf according to Pendleton & Seif (1961). In addition, the number of leaves on plants was counted. The leaf area was measured according to Abd Ahmed & Abood (2016).

Leaf Area (cm^2) = Maximum Leaf Width (cm) × Length of Leaf (cm) × 0.75

The chlorophyll and carotenoid content of the plants were measured according to Arnon (1949) and Mackinney (1941). The chlorophyll content was estimated by extracting fresh leaves using 80% acetone followed by centrifuging at 8000 rpm for 20 min and then measuring the color intensity of the extract at 645 and 663 nm for the carotenoid content according to Lichtenthaler & Wellburn. (1983) under wavelength 470 nm. Preparation of plant extracts to estimate activities of enzymes was according to Luhova *et al.* (2003). Plant material was homogenized gently with a morter and pestle on ice groats at a ratio of 1:2 (w/v) with 0.1 M potassium phosphate buffer, PH 7.2-7.4, and also 0.3g poly vinyl pyrocallol (PVP). The homogenate was centrifuged at 8,000 g for 20 min at 4 °C, and the obtained supernatant was used for enzyme assays. Super oxide dismutase (SOD) was measured according to Marklund & Marklund (1974), while estimating catalase (CAT) activity was carried out according to Hadwan & kadhum Ali (2018). The method of Bates *et al.* (1973) was used to estimate the plant content of proline. So that, 0.1 g was taken from the powdered dry plant sample, then homogenized with 5 mL of 3% aqueous sulfosalicylic acid, thereafter the homogenate was centrifuged at 14,000 g for 2 min, followed by adding 2 mL ninhydrin and glacial acetic acid, and also 4 mL toluene. Absorbance is at a wavelength of 520 nm of the toluene phase to estimate the proline concentration in plant tissue. The study data were analyzed using complete randomized design (CRD) followed by descriptive statistics (mean and standard deviation), while least significant difference (LSD) ≤ 0.05 were used as a significant level.

RESULTS AND DISCUSSION

Morphological characteristics

The results showed a reduction in both stem length (Fig. 1) and number of leaves (Fig. 2), leaf area (Fig. 3) in the plants treated with the two Azo dyes (RB and RR) compared to control group. The reason for these adverse result on morphological indicators may be attributed to Azo dyes inhibition on the ATPase activity of the plant, which could finally inhibit the photosynthetic oxygen evolution and plant growth (Zhou & Xiang 2013). In addition, the toxicity of organic pollutants may affect plant cell ultrastructure, biosynthesis, membrane stability, and DNA. When organic pollutants are absorbed into plant cells, they can cause disorder of the cell ultrastructure (Zhang *et al.* 2017). The essential function of a cell is controlled by these ultra-structures. Plant cells will become abnormal or even die if the cell's ultrastructures are damaged or destroyed (Gunning & Steer 1975; Agarwal 2006). This is in agreement with the results of a study conducted to find out the effect of methyl orange on flower plants, Marigold (yellow and orange), *Celosia Argentea*, finding that there was a reduction in the morphological characteristics of plants exposed to the dye compared to control group. Statistical analysis showed that LSD was 0.046 in steam length, 0.001 in leaf number and 0.0001 in leaf area, exhibiting significant difference in comparison with control group ($p < 0.05$).

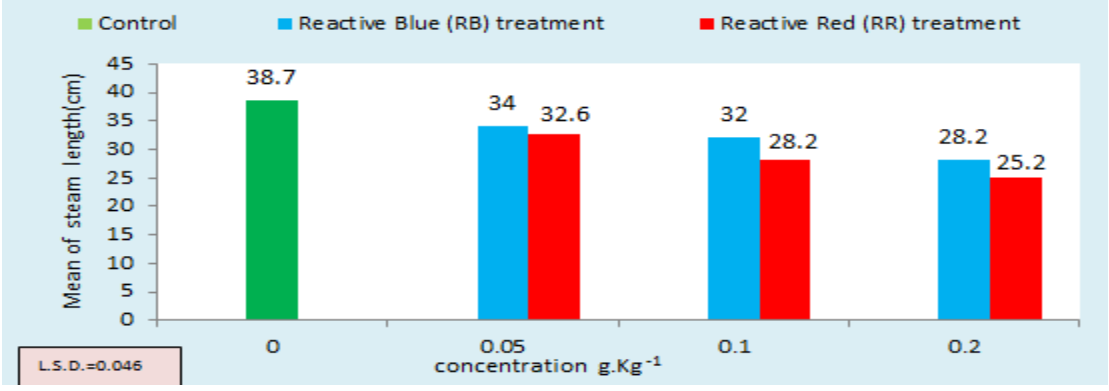

Fig. 1. The variation in mean steam length in plants exposed to different concentrations of the two Azo dyes compared to the control group.

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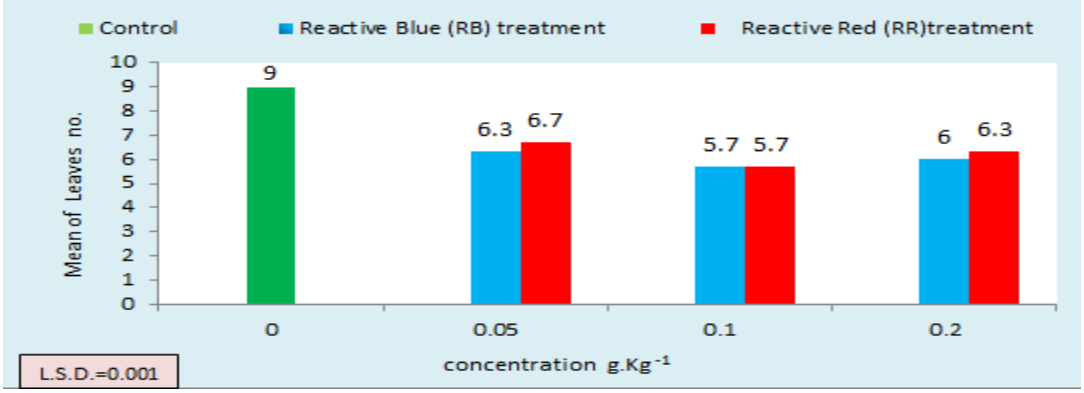

Fig. 2. The variation in the average number of leaves in plants exposed to different concentrations of the two Azo dyes compared to the control group.

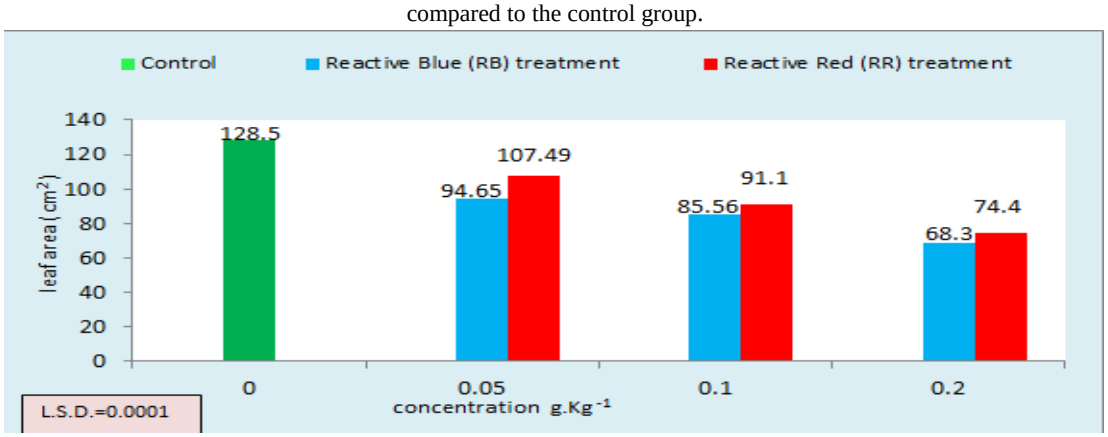

Fig. 3. The variation in leaf area in plants exposed to different concentrations of the two Azo dyes compared to the control group.

Biochemical characteristics

Pigments of photosynthesis

The content of photosynthetic pigments in plants is considered as one of the agents sensitive to stress conditions (Kholghi *et al.* 2014; Banimahd Keivani 2018). The results indicate a reduction in the total chlorophyll and carotenoids content with an increase in Azo dyes concentration. Therefore, it can be deduced that both RB and RR inhibited the biosynthesis of photosynthetic pigments or favored their breakdown. Moreover, declining photosynthesis capacity under dye stress conditions may be a protective response to limit ROS production in chloroplasts (Vafaei *et al.* 2013) as shown in Fig. 4. By comparing with the control, the higher values of chlorophyll were 0.22 and 0.21 in 0.05 g kg^{-1} RB and RR respectively. These values decrease with the increased Azo dye concentration, reaching 0.17 with RB and 0.15 with RR in 0.2 g kg^{-1}. A similar trend was previously found by Mustafa & Hayder (2020) reporting a reduction of chlorophyll by *Phragmites australis*, Typha *domingensis*, *Ceratopyllum demersum*, *Potamegatone Perfoliatus*, and *Hydrilla varcillata* when exposed to three different concentration (0.01, 0.02, 0.03 ppm) of Reactive Blue, Reactive Yellow, and the mixture of them. Their statistical analysis showed that LSD was 0.937 > 0.05 exhibiting that there is no significant difference with control group.

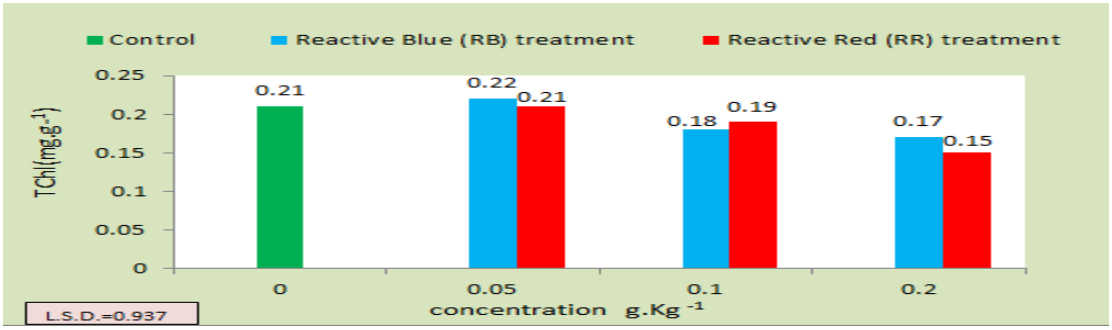

Fig. 4. The variation in total chlorophyll (TChl) content in plants exposed to different concentrations of the two Azo dyes compared to the control group.

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The results of the present study (Fig. 5), showed a decrease in the carotenoid contents in the stressed plants due to exposure to different concentrations of Azo dyes compared to the control group, where the highest content of carotenoids was 3.29 and 3.79 for RB and RR respectively within the concentration of 0.05 g kg^{-1} soil. The lowest value at the highest concentration, 2.17, belonged to RB and 3.13 to RR, which may be due to destruction of carotenoid under severe stress (Munné-Bosch & Alegre 2000).

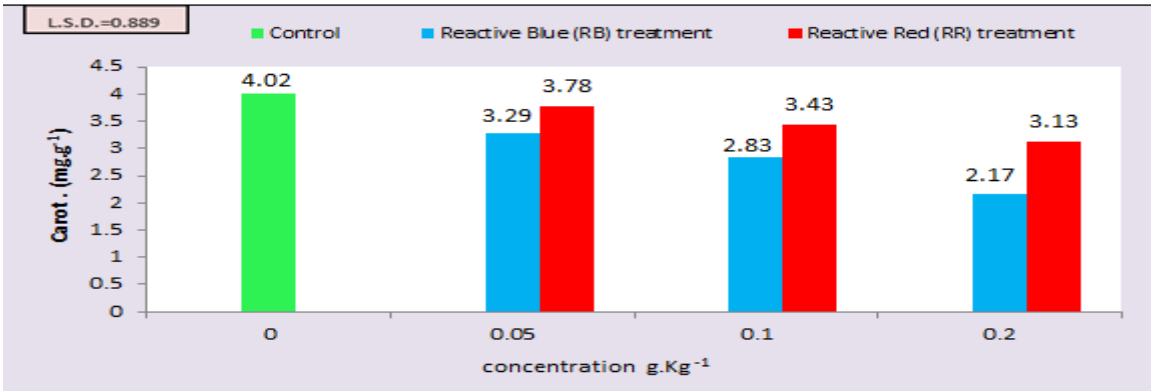

Fig. 5. The variation in carotenoid contents in plants exposed to different concentrations of the two Azo dyes compared to the control group.

These results are in line with those of Raghda'a Ali Al-Khafajy *et al.* (2020), who reported a decrease in the level of carotenoids in leaves of chrysanthemum (*Calendula officinalis* L.) exposed to salt stress. The statical analysis showed that LSD was 0.889 (> 0.05) exhibiting no significant difference with control group.

Super oxide dismutase (SOD) activity

The result (Fig. 6) revealed that there is a slight raise in SOD activity by elevating the Azo dyes concentrations compared to the control, ranging from 11.6 -14.74 U/g.fw and 11.57-13.55 U/g.fw for RB and RR respectively. The reason for this raise may be the exposure of the plant to environmental stress leading to generate reactive oxygen species (ROS) as O_2 and H_2O_2. etc. Plants protect cells and sub-cellular systems from the cytotoxic effects of these active oxygen radicals by anti-oxidant enzymes like catalase and superoxide dismutase (Fu *et al.* 2019). Patade *et al.* (2017) showed that activities of SOD increased under abiotic stresses. The statistical analysis showed that LSD was 0.049 (< 0.05) exhibiting significant difference in comparison with control group.

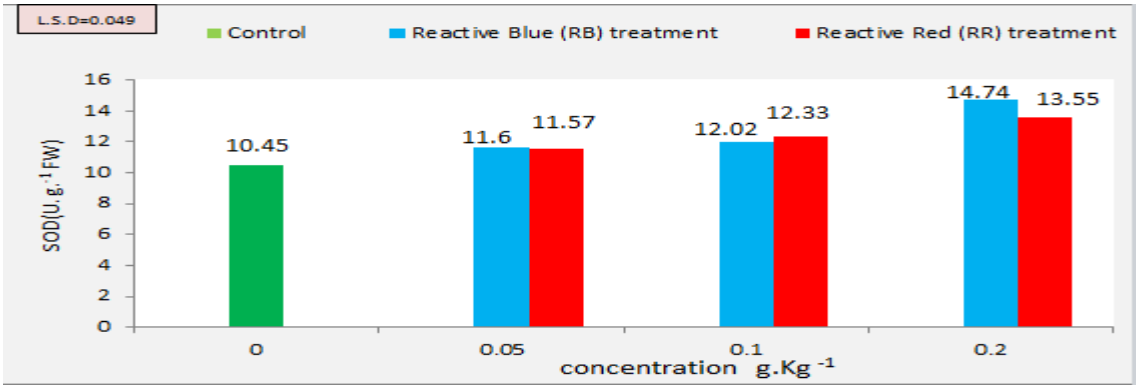

Fig. 6. The variation in super oxide dismutase (SOD) activity in plants exposed to different concentrations of the two Azo dyes compared to control group.

Catalase (CAT) activity

The current result showed an increase in the activity of CAT (Fig. 7). So that, it was at a lower concentration of 0.05 g kg^{-1} for both Azo dyes compared to the control group and in the case of RB (9.5) it was higher than that of RR (6.43). The enzyme activity decreased by elevating in the concentration of the two dyes. This decline may be caused by inhibiting the construction of the enzyme, or an alteration in the structural enzyme units, in the presence of these two organic pollutants, the Reactive blue RB and the Reactive red RR (Movafeghi *et al.* 2013). These results are consistent with those of Carias *et al.* (2008), who detected decreased CAT activity at a high concentration of 700 mg L^{-1}, while it was low at 130 mg L^{-1} due to acid orange 7 (an Azo dye) in *Phragmites*

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australis by 14 days. The statistical analysis showed that LSD was 0.0001 (< 0.05) exhibiting significant difference with the control group.

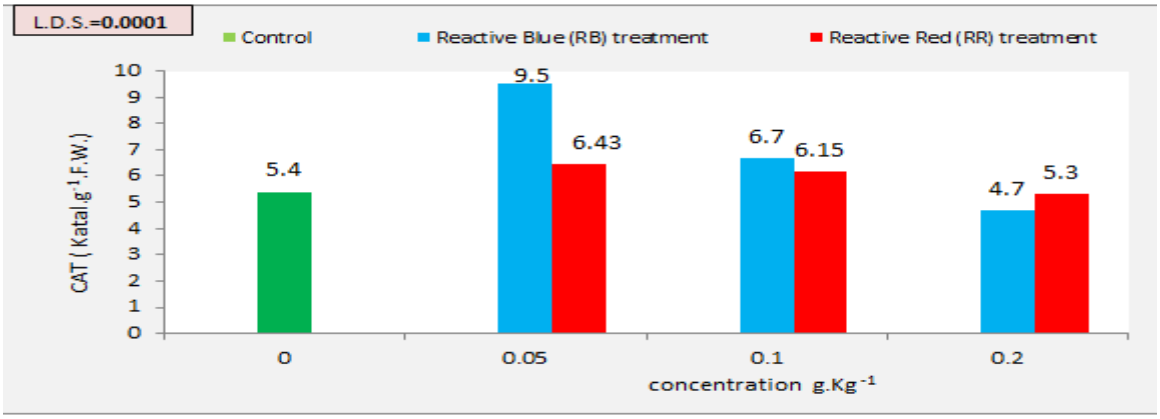

Fig. 7. The variation in catalase (CAT) activity in plants exposed to different concentrations of the two Azo dyes compared to control group.

Proline

The results (Fig. 8) showed that the proline content of leaves was high in plants grown in Azo dyes-contaminated soil and upraised by the elevated concentration of Azo dyes. Proline is an amino acid present in higher plants, and is the first indicator which increases under different abiotic stresses (Saibi *et al.* 2015). It is also known to act indirectly by scavenging ROS due to enhancing the plant antioxidant defense system (Rejeb *et al.* 2014). This is consistent with the study of Al-Zurfi *et al.* (2020) who reported that when *Lemna minor* is treated with 0.01, 0.04 and 0.07 µg L^{-1} of Congo Red for 21 days, proline content raised by elevating the dye concentration. Their statistical analysis showed that LSD was 0.001 (< 0.05) displaying significantly difference with the control group.

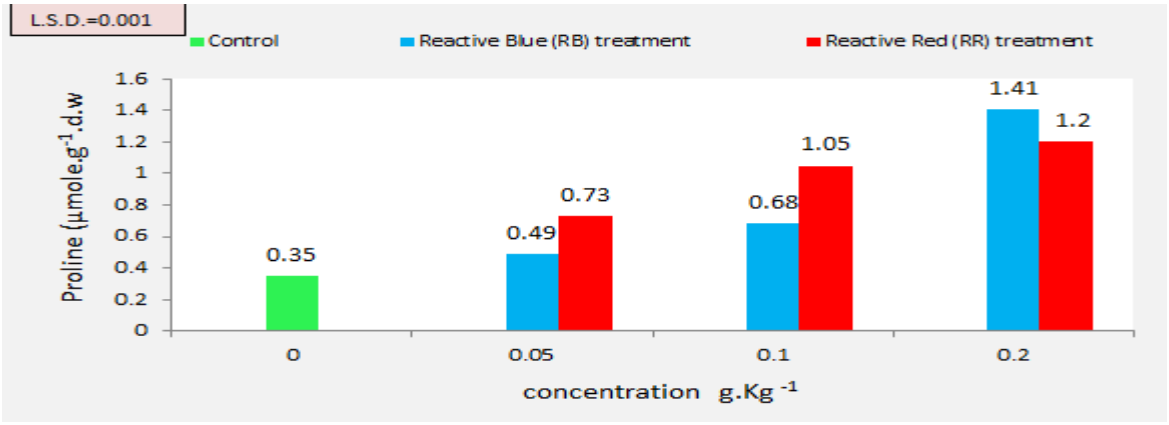

Fig. 8. The variation in proline content in plants exposed to different concentrations of the two Azo dyes compared to the control group.

CONCLUSION

This study concluded that Reactive Blue and Reactive Red Azo dyes could affect the morphological and physiological characteristics of *Sorghum bicolor* L. Muench. The analyses revealed a significant reduction in its leaves number, leaf area, and steam length coinciding with the elevation of the two dyes concentration. On the other hand, there was no significant difference in photosynthetic pigments of treatments in comparison with control. Exposure to Azo dyes leads to a significant raise in SOD activity and a significant alteration in CAT activity, such that it raised in low concentration, while then decreased by elevated Azo dye concentrations in soil. Proline values in the treatments also are shown a significant increase in comparison with the control group.

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پاسخ سورگوم (ذرت خوشه‌ای) به اثر دو باکتری رنگ آزو

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چکیده

سالانه حجم عظیمی از فاضلاب رنگ آزو، توسط صنعت نساجی تولید می‌شود. در حال حاضر، دفع نامناسب رنگ‌های آزو به منابع آبی موجب بروز نگرانی قابل توجهی شده است زیرا می‌تواند باعث اختلال در اکوسیستم شده و به دلیل سمیت و خاصیت سرطان‌زایی خود، موجب بروز مشکلات زیست محیطی و سلامتی شود. روش‌های شیمیایی، فیزیکی و زیست شناختی به دلیل ارزان بودن، ایمنی زیست محیطی و ویژگی‌های پایدار، برای تصفیه فاضلاب رنگ آزو استفاده شده‌اند و فناوری زیستی به‌عنوان روشی نویدبخش و ارزشمند شناخته‌شده است. رنگ‌های آزو، یکی از آلاینده‌ترین رنگ‌های صناعی در محیط‌زیست هستند. آنها از طریق زنجیره غذایی قابل‌انتقال هستند و با مسائلی مانند سمیت، سرطان‌زایی و موتاژنز (جهش‌زایی) ارتباط دارند. در این مطالعه، خاک‌آلوده با دو رنگ آزو، راکتیو آبی (RB) و راکتیو قرمز (RR) در کشت سورگوم (*Sorghum bicolor* L. Moench) با استفاده از غلظت‌های رنگ مختلف (۰، ۰,۰۵، ۰,۱، ۰,۲ کیلوگرم خاک) استفاده شد. افزایش غلظت رنگ، منجر به تغییرات معنی‌داری در ویژگی‌های ریخت شناختی ذرت خوشه‌ای مانند طول ساقه، تعداد برگ و سطح برگ شد. کاهش اندکی در محتوای کلروفیل کل و کارتنویید های گیاه وجود داشت، درحالی‌که تغییرات معنی‌دار در فعالیت‌های آنزیم CAT و SOD با افزایش غلظت رنگ در خاک برای هر دورنگ در مقایسه با گروه شاهد مشاهده شد. همچنین، افزایش معنی‌دار در محتوای پرولین در غلظت‌های رنگ بالاتر مشاهده شد.

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