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*Original Scientific Paper*

# Physiological response of *Moringa oleifera* exposed to bisphenol A

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**ABSTRACT:**
The physiological and morphological effects of different concentrations of bisphenol A (BPA) on *Moringa oleifera* seedlings were determined in this study. Significant chlorosis and abscission were observed in leaves exposed to 50 mg/L of BPA. Photosynthetic pigment levels were affected differently by varying doses of BPA. Although the total carbohydrate content of seedling parts was increased by BPA, protein content was lowered by it, except in the case of roots at 1.5 mg/L of BPA. However, it was determined that the content of non-protein sulphhydryl groups of seedling parts did not change significantly. The total phenolic content of root tissues showed an insignificant change; however, it was found that phenolic content increased in the stems and leaves following application of BPA. The content of hydrogen peroxide ($H_2O_2$) in seedling tissues increased with increasing concentrations of BPA. Statistical analysis indicated that $H_2O_2$ content was significantly correlated with malondialdehyde content. These results clearly show that the application of BPA causes oxidative stress in seedling tissues.

**Keywords:**
bisphenol A, *Moringa oleifera*, growth, physiological effect

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## INTRODUCTION

Man-made chemicals can cause serious environmental pollution. Bisphenol A [BPA; 2,2-bis(4-hydroxyphenyl) propane, CAS No. 80-05-7] is unquestionably one such chemical. As a chemical produced in high volume worldwide, BPA is mainly used in polycarbonate plastics and epoxy resins. However, it is also used in polyester, polysulphone, polyacrylate resins and flame retardants. Polycarbonates are widely used in food-contacting materials, such as baby-feeding bottles, tableware and microwave safe containers. Epoxy resins are used as a protective coating for some canned food and beverages and as a coating on metal lids for glass jars and bottles (WHO/FAO 2009). Although BPA does not occur naturally, it has been widely distributed in the environment due to its high production and large consumption volumes (Tsai 2006), allowing all organisms to come into contact with the chemical. Even though most BPA is removed from wastewater, detection in environmental samples has still been reported (Musolff *et al*. 2010; Xu *et al*. 2014).

*Moringa oleifera* Lam. (drumstick tree, horseradish tree, ben oil tree) is a fast-growing evergreen or deciduous tree with tripinnate leaves and fragile branches, and it usually grows to a height of 10-12 m.The horseradish tree is a species native to the Himalayan foothills in Southern Asia, its range stretching from northeastern Pakistan to the northern West Bengal state in India and northeastern Bangladesh (Troup 1921; Nasir & Ali 1972; Ramachandran *et al*. 1980; Parrotta 2001). The vegetative and generative parts of this valuable tree are used in many products, such as foodstuffs, cosmetics, newsprint and textiles (Roloff *et al*. 2009). Furthermore, it is characterized by considerable medicinal properties of almost all of its parts, including the root, leaf, flowers, seed, fruit and bark, which have been utilised in both indigenous and modern medicine, with proven effectiveness of several active constituents reported in recent studies (Anwar *et al*. 2007; Farooq *et al*. 2012).

An endocrine-disrupting chemical, BPA is an important industrial raw material. The widespread use of BPA, its release into the environment and its lack of a life cycle in nature have increased the risk its becoming a toxic substance worldwide and have made it a significant environmental pollutant. The present investigation was carried out in order to assess the physiological and morphological effects of BPA applied in different concentrations on *M. oleifera* seedlings.

## MATERIALS AND METHODS

***Plant exposure.*** Seeds of *Moringa oleifera* (Moringaceae) were used for the experiment. The seeds were sterilised with a 5% NaOCl solution for 15 minutes and washed with distilled water three times. This was followed by germination in perlite at 24±1°C. After germination, 30-day old *M. oleifera* seedlings were transferred to 2l plastic vessels (three seedlings per vessel) containing an aerated nutrient solution (Table 1).

Distilled water was used to prepare the nutrient solution. The seedlings were grown in a climate chamber (Snijders Scientific, Netherlands) (light/dark regime of 16/8 h, light level of ~120 µE. $m^{-2}$. $s^{-2}$, temperature of 24±1°C). They were supplied with 0, 1.5, 17.2 and 50 mg/L of BPA following a seven-day acclimatisation period. The solutions applied were changed every two days, and the test solutions were refreshed. The *M. oleifera* seedlings were harvested after four days for observation of toxicity symptoms such as detachment of some leaves and chlorosis. The seedling roots were washed with distilled water three times. The roots, stems and leaves were separated for determination of fresh weight and physiological analyses.

***Physiological analyses.*** Fresh seedling leaves were homogenised in 80% acetone to determine the content of photosynthetic pigments. After the supernatants were separated, the samples were read in a spectrophotometer (CINTRA 202, Australia) at 662, 645 and 450 nm. Chlorophyll-a (Chl-a), chlorophyll-b (Chl-b) and carotenoid levels were calculated according to the methods of Lichtenthaler & Wellburn (1985). The protein content of seedling parts was determined spectrophotometrically using the Lowry method (Lowry *et al.* 1951). The content was estimated using the calibration curve of BSA (bovine serum albumin). The total soluble carbohydrate content of seedling parts was determined by the anthrone method (Plummer 1998). Non-protein sulphhydryl content was determined according to Ellman's method (Ellman 1959). Reduced glutathione (GSH) was used as a standard. Carbohydrate content was calculated using the standard glucose curve. The total phenolic content of seedling parts was determined using Folin-Ciocalteu reagent according to Ratkevicius *et al.* (2003). Gallic acid was used as a standard. The level of lipid peroxidation was determined by detecting the amount of malondialdehyde (MDA) using the method proposed by Zhou (2001). The $H_2O_2$ content of seedling tissues was determined according to Sergiev *et al.* (1997).

**Table 1**. Nutrient composition used in the study (Ozturk *et al.* 2003).

| | | | | | |
|---|---|---|---|---|---|
| $K_2SO_4$ | $Ca(NO_3)_2$ | $KH_2PO_4$ | $MgSO_4$ | KCl | Fe-EDTA |
| 0.88 mM | 2.0 mM | 0.25 mM | 1.0 mM | 0.1 mM | 100 µM |
| $H_3BO_3$ | $MnSO_4$ | $ZnSO_4$ | $CuSO_4$ | $(NH_4)6Mo_7O_{24}$ | |
| 1.0 µM | 0.5 µM | 1.0 µM | 0.2 µM | 0.02 µM | |

The tolerance indices (TI) of seedling parts were calculated as follows:

$$TI = \frac{\textit{Fresh weight of seedling part treated with BPA}}{\textit{Fresh weight of seedling part not treated with BPA}} \times 100$$

***Data analysis.*** All analyses were carried out with three replicates. The least significant difference (LSD) test was used to compare the parameters on SPSS 11.0. The Pearson correlation coefficient was used to determine the relationship between the data obtained.

## RESULTS AND DISCUSSION

According to our observations, there were no toxicity symptoms at 1.5 mg/L of BPA (Fig. 1). On the other hand, it was determined that other doses of BPA caused some toxicity symptoms in *M. oleifera* seedlings. Local chlorosis was observed at 17.2 mg/L of BPA, especially in older leaves of the seedlings. The chemical also caused partial abscission of the leaves. At 17.2 mg/L, there was a significant decrease in clear primary and secondary root growth, as well as abnormalities such as fractures and browning in the secondary roots tips. Significant chlorosis and abscission were observed in the leaves at 50 mg/L of BPA. Roots exposed to the highest concentration showed toxicity symptoms such as softening and browning. These toxicity symptoms may have been due to negative effects of the high BPA concentration on many physiological and biochemical processes in the seedlings.

Terrestrial plants can take BPA from the soil via their roots and transport it to the above-ground parts (Nakajima *et al.* 2004). This suggests the potential of BPA to affect growth. In the present study, BPA had a different effect on the growth of *M. oleifera* seedlings depending on the concentrations applied. The fresh weights of roots increased by 117.3% ($p < 0.05$) and 17.7% ($p > 0.05$) at 1.5 and 17.2 mg/L of BPA, respectively, but decreased by 24.9% ($p < 0.05$) at 50 mg/L (Fig. 2A). The fresh weight of stems increased insignificantly at 1.5 mg/L of BPA ($p > 0.05$). In contrast, stem weights at 17.2 and 50 mg/L of

**Fig. 1.** Growth of *M. oleifera* seedlings after BPA applications.

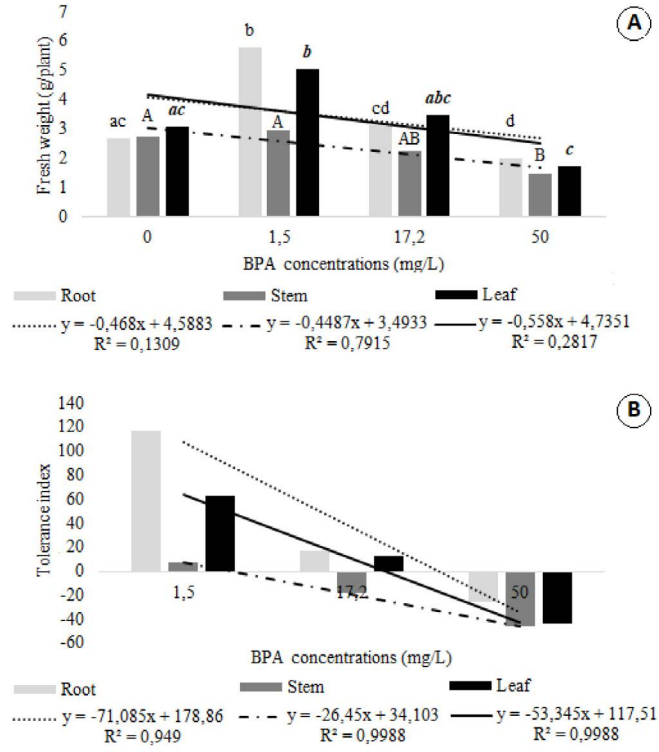


**Fig. 2.** Effects of BPA on growth of *M. oleifera* seedlings and their statistical evaluation. The control had only a 10% nutrient solution. Bars indicate the mean of three replicates. Means with different letters are significantly different from one another according to the LSD test ($p < 0.05$).

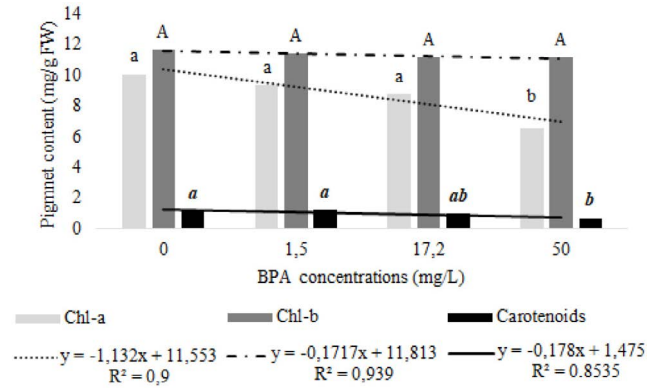


**Fig. 3.** Effects of BPA on content of photosynthetic pigments in *M. oleifera* seedlings and their statistical evaluation. The control had only a 10% nutrient solution. Bars indicate the mean of three replicates. Means with different letters are significantly different from one another according to the LSD test ($p < 0.05$).

BPA decreased by 17.8% ($p > 0.05$) and 45.8% ($p < 0.05$), respectively, when compared to the control. Similarly, the fresh weights of leaves increased at concentrations of 1.5 and 17.2 mg/L of BPA, while at 50 mg/L of BPA they decreased by 43.6% ($p < 0.05$). According to calculations of the the tolerance index, high BPA application clearly had a negative effect on the growth of seedlings (Fig. 2B). In agreement with our findings, high BPA concentrations were found to inhibit growth in tomatoes (*Solanum lycopersicum* L.), durum wheat (*Triticum durum* Desf.), broad beans (*Vicia faba* L.) and lettuce (*Lactuca sativa* L.) (Ferrara *et al.* 2006). There are also studies showing that low BPA doses promote the growth of soybean [*Glycine max* (L.) Merr.], rice (*Oryza sativa* L.) and carrot (*Daucus carota* L.) seedlings (Terouchi *et al.* 2004; Qiu *et al.* 2013; Ali *et al.* 2016), as seen in our study.

Photosynthesis is the most important process for plants. The application of BPA, like many stress factors, may cause changes in photosynthesis and content of photosynthetic pigments (Jiao *et al.* 2015, 2017). Rapala *et al.* (2017) reported decreasing content of chlorophyll and carotenoids in *Arabidopsis thaliana* at high concentrations of BPA (25 and 50 mg/L). That said, low doses of BPA (up to 5 mg/L) had no negative effects on photosynthetic pigment content. The content of photosynthetic pigments in *M. oleifera* leaves and their statistical evaluations are shown in Fig. 3. The content of Chl-a at 1.5, 17.2 and 50 mg/L BPA was found to be decreased by 6.82% ($p > 0.05$), 13.24% ($p > 0.05$) and 35.15% ($p < 0.05$), respectively, when compared to the control. Similarly, Chl-b content decreased by up to 4.20% ($p > 0.05$). Carotenoid content insignificantly increased by 2.48% at 1.5 mg/L of BPA ($p > 0.05$). In contrast, the content decreased by up to 41.88% ($p < 0.05$) at 50 mg/L of BPA. Qiu *et al.* (2013) suggested that the reduction of Chl with BPA application may be due to the peroxidation of chloroplast membrane lipids. According to correlation analysis, there was a negative relationship between the content of MDA and photosynthetic pigments ($r = -0.837$ at $p < 0.01$ for Chl-a, $r = -0.156$ at $p > 0.05$ for Chl-b and $r = -0.809$ at $p < 0.01$ for carotenoids). In addition, negative correlations were discerned between the content of $H_2O_2$

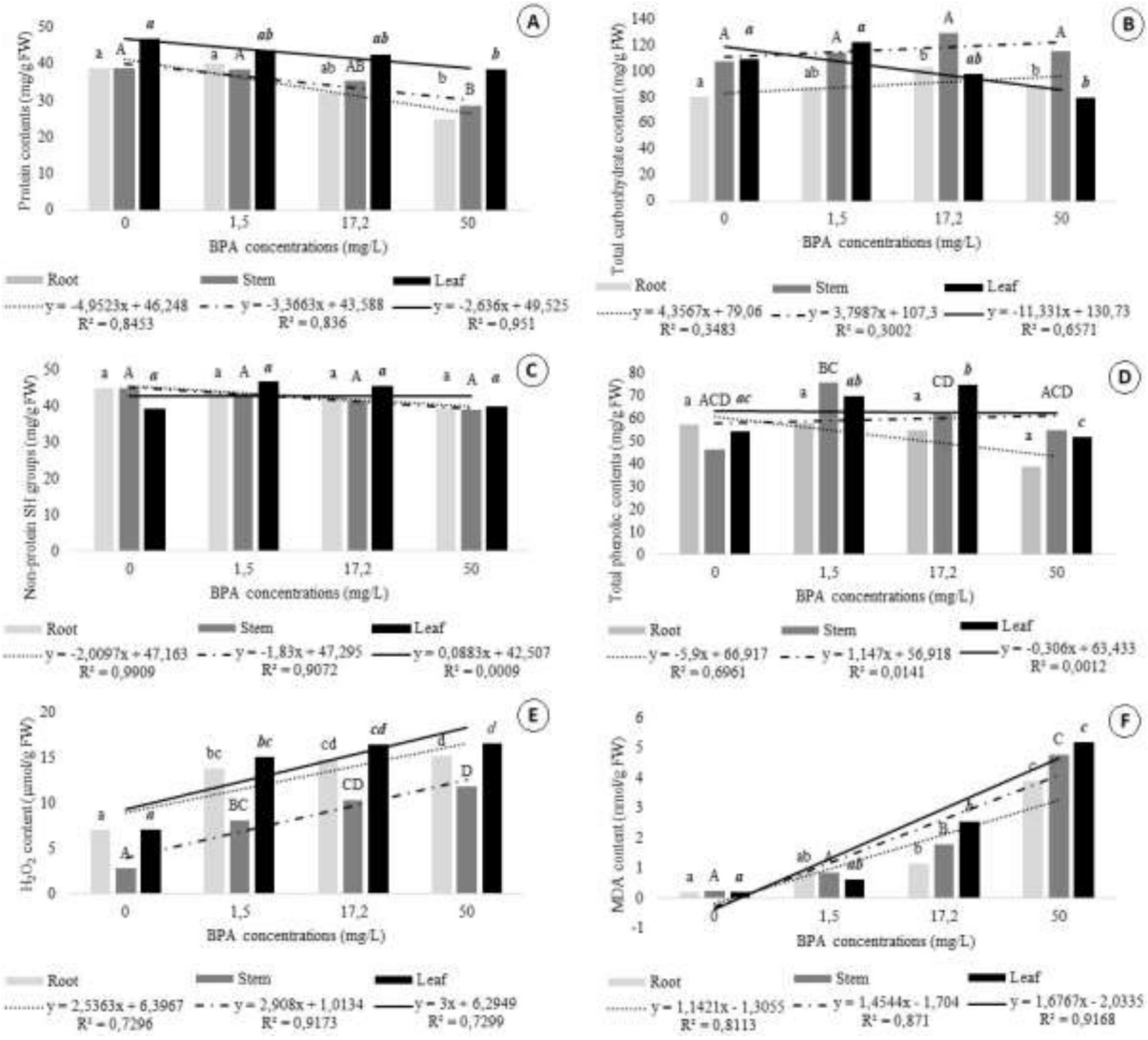


**Fig. 4.** Effects of BPA on protein content (A), total carbonhydrate content (B), content of non-protein sulphhydryl groups (C), total phenolic content (D), content of hydrogen peroxide (E) and that of MDA (F) in *M. oleifera* seedlings and their statistical evaluation. The control had only a 10% nutrient solution. Bars indicate the mean of three replicates. Means with different letters are significantly different from one another according to the LSD test ($p < 0.05$).

and photosynthetic pigments ($r = -0.611$ at $p < 0.05$ for Chl-a, $r = -0.108$ at $p > 0.05$ for Chl-b and $r = -0.541$ at $p < 0.05$ for carotenoids), which suggests that oxidative stress induced by BPA accelerates the peroxidation of chloroplast membrane lipids and leads to their decrease.

The protein content of the *M. oleifera* seedling parts and their statistical evaluations are presented in Fig. 4A. Protein content was decreased by the tested BPA doses, except in the case of 1.5 mg/L of BPA in the roots. The maximum decreases in root, stem and leaf tissues were calculated at 50 mg/L of BPA as 35.83, 26.30 and 18.02% ($p < 0.05$), respectively. Regression analysis showed that there was a negative relationship between protein content and $H_2O_2$ content ($r = -0.530$ at $p > 0.05$ for roots, $r = -0,604$ at $p < 0.05$ for stems and $r = -0.624$ at $p < 0.05$ for leaves). Previous studies have shown that reactive oxygen species (ROS) are responsible for the destruction of proteins (Halliwell 1987; Dogan *et al*. 2010, 2012; Rapala *et al*. 2017). It follows that ROS produced due to BPA in *M. oleifera* tissues caused oxidative stress resulting in the reduction of protein content.

It was determined that the total carbohydrate content of seedling parts was increased by the application of BPA (Fig. 4B). While the carbohydrate content of roots was significantly increased at 17.2 and 50 mg/L of BPA ($p < 0.05$), these increases were insignificant in the case of stems ($p > 0.05$). The total carbohydrate content of leaves increased insignificantly at 1.5 mg/L as well ($p > 0.05$). In contrast, it decreased by up to 26.97% at 50 mg/L ($p < 0.05$). There was a positive correlation between $H_2O_2$

content and carbohydrate content for root and stem tissues ($r = 0.397$ at $p > 0.05$ for roots and $r = 0.364$ at $p > 0.05$ for stems). The findings clearly indicate that carbohydrates may play an important role in the response to BPA toxicity. However, a negative relationship between $H_2O_2$ and carbohydrate content in the leaves ($r = -0.402$ at $p > 0.05$) suggests that other mechanisms may play a role in that response.

Most of the non-protein sulphhydryl (SH) groups in plants form glutathione (GSH) (Grill *et al*. 1979). Under stress, GSH, a thiol-containing tripeptide, can scavenge ROS and reduce ROS-induced injury in plants (Gill & Tuteja 2010). The levels of non-protein sulphhydryl groupsare shown in Fig. 4C. It can be seen that the levels in roots and stems underwent insignificant reductions ($p > 0.05$). In contrast, the content non-protein sulphhydryl groups in leaves increased at 1.5 and 17.2 mg/L of BPA ($p > 0.05$) and reached the control level at 50 mg/L ($p > 0.05$). Regression analysis showed that there was a negative correlation between the content of non-protein SH groups and that of $H_2O_2$ ($r = -0.170$ at $p > 0.05$ for roots, $r = -0.516$ at $p < 0.05$ for stems and $r = -0.165$ at $p > 0.05$ for leaves). This may be due to oxidative stress. It can be concluded that ROS are scavenged by antioxidant mechanisms other than non-protein sulphhydryl groups.

Phenolic compounds play an important role in the response to many stress factors (Dogan & Gultekin 2017; Dogan & Demirors Saygideger 2018). The total phenolic content of root tissues showed no significant change with the application of BPA ($p > 0.05$) (Fig. 4D). Regression analysis showed that there was an insignificant and negative correlation between $H_2O_2$ and total phenolic content in the roots ($r = -0.266$ at $p > 0.054$). The content in stems at 1.5, 17.2 and 50 mg/L BPA increased by 63.67% ($p < 0.05$), 33.20% ($p > 0.05$) and 18.40% ($p > 0.05$), respectively, when compared to the control. The content in leaf tissues increased at up to 17.2 mg/L of BPA ($p < 0.05$), but decreased at 50 mg/L ($p > 0.05$). In addition, positive correlations were discerned between $H_2O_2$ and total phenolic content ($r = 0.290$ at $p > 0.05$ for stems and $r = 0.431$ at $p > 0.05$ for leaves). The BPA-induced increase in the compounds represented another defensive mechanism against oxidative stress in stems and leaves. Decrease in the amount of phenolic compounds may have been caused by peroxidases and phenol oxidases (Thypyapong *et al*. 1995). These enzymes have been found to exhibit activity increases in many stressful situations (Kwak *et al*. 1996; Ruiz *et al*. 1998). Increase in their activity may also be a cause of the decrease in the amount of phenolic compounds under BPA stress.

Oxidative stress is very serious because it promotes cell death and causes a wide range of disorders as a pathophysiological condition (Melchiorri *et al*. 1996). As reported in the vast majority of studies, ROS production appears as a common consequence of exposure to BPA (Bindhumol *et al*. 2003; Chitra *et al*. 2003; Dogan *et al*. 2010, 2012). It was therefore concluded that the levels of $H_2O_2$ in parts of *M. oleifera* seedlings confirm that ROS were involved in the oxidative stress caused by BPA. The hydrogen peroxide content of seedling tissues increased with increasing BPA concentrations (Fig. 4D). The maximum increase in content of $H_2O_2$ was found in root, stem and leaf tissues exposed to 50 mg/L of BPA, in which its values were 2.16, 4.12 and 2.35 times higher than in their respective controls ($p < 0.05$). Similar to the situation with $H_2O_2$ levels, those of lipid peroxidation increased with increasing BPA concentrations (Fig. 4E). Lipid peroxidation refers to the oxidative degradation of lipids, and MDA is one of the preferred biomarkers of this (Dogan *et al*. 2010). The maximum increases in MDA content were determined in roots, stems and leaves exposed to 50 mg/L of BPA, in which MDA levels were respectively 18.37, 18.76 and 21.54 times higher than in their respective controls ($p < 0.05$) (Fig. 4F). Regression analysis showed that there was a significant and positive correlation between MDA content and that of $H_2O_2$ ($r = 0.576$ at $p < 0.05$ for roots, $r = 0.772$ at $p < 0.01$ for stems and $r = 0.644$ at $p < 0.05$ for leaves). Thus, the application of BPA was clearly shown to cause lipid peroxidation by inducing oxidative stress.

## CONCLUSION

The aim of this study was to determine the effects of different concentrations of BPA on *M. oleifera* seedlings. Dose-dependent changes were found in the physiological parameters examined. While a low concentration of BPA had positive effects on growth of the seedlings, a high concentration was found to be toxic. In other words, BPA was found to have a biphasic effect. The tolerance indexes confirmed this situation as well. On the other hand, the correlation was found to be positive between content of $H_2O_2$ and that of MDA in all tissues examined. The findings clearly indicate that BPA has a potential to induce oxidative stress. Although there are a number of studies dealing with the effects of BPA on humans, animals, aquatic life and aquatic organisms, there has been a limited amount of research into its effect on terrestrial plants. Moreover, no study has investigated the effects of BPA on *M. oleifera*. In this context, our findings may prove to be a stimulus for similar studies.

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**REZIME**

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## Fiziološki odgovor vrste *Moringa oleifera* izložene bisfenolu A

Gulcan Cinar i Muhittin Dogan

U ovoj studiji su utvrđeni fiziološki i morfološki efekti različitih koncentracija bisfenola A (BPA) na klijance *Moringa oleifera*. Primećena je značajna hloroza i apscisija listova izloženih 50 mg / L BPA. Na sadržaje fotosintetskih pigmenata različito su uticale različite doze BPA. Iako je ukupni sadržaj ugljenih hidrata u klijancima povećan zbog BPA, sadržaj proteina je smanjen, izuzev u korenu na 1,5 mg/L BPA. Međutim, utvrđeno je da se sadržaj proteinskih sulfhidrilnih grupa u delovima klijanaca nije značajno promenio. Ukupni fenolni sadržaj korenskog tkiva pokazao je beznačajnu promenu; međutim, utvrđeno je da se sadržaj povećao u stabljici i listovima nakon primene BPA. Sadržaj vodonik peroksida ($H_2O_2$) u tkivima klijanaca je porastao sa povećanjem koncentracije BPA. Prema statističkim analizama, sadržaj $H_2O_2$ je značajno korelisan sa sadržajem malondialdehida. Ovi rezultati jasno pokazuju da primena BPA uzrokuje oksidativni stres u tkivima klijanaca.

**Ključne reči**: bisphenol A, *Moringa oleifera*, rast, fiziološki efekat