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The Effect of Bisphenol A on Root Development and Chlorophyll a:b Ratio in *Lens culinaris*

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Abstract

Bisphenol A is an industrial chemical that has become widely prevalent in aquatic and terrestrial habitats. Although extensive research has been directed to its harmful effect on mammalian cells, there are fewer studies on the effects of bisphenol A on plants. The aim of the current study was to investigate the effect of bisphenol A on root elongation and chlorophyll a:b ratio in lentils (*Lens culinaris*). The results showed that bisphenol A at concentrations of 10, 50 and 100 mg dm^{-3} inhibited germination and root development in *Lens culinaris*. Furthermore, exposure to bisphenol A at concentrations of 50 and 100 mg dm^{-3} significantly increased the chlorophyll a:b ratio, meaning that the amount of chlorophyll b relative to chlorophyll a was decreased. This effect was only observed in seedlings treated with bisphenol A both during germination and growth, and not those treated only during the germination period, which indicates that the seeds were able to recover from the toxicity of bisphenol A before sprouting. Due to its effects on root development and chlorophyll content, bisphenol A could be a significant hazard to plant life.

Keywords: Bisphenol A; *Lens Culinaris*; Chlorphyll a:b ratio; Germination; Root development.

1. Introduction

Bisphenol A is an important industrial chemical that is widely used as a monomer in the production of epoxy resins and other common plastics [1, 2].

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Often abbreviated to BPA, it is involved in the manufacture of a variety of products, including adhesives, thermal paper, dental sealants, and plastic food containers [2]. Given its widespread use and annual global production of approximately 6.8 million tons [3], it is unsurprising that BPA has evolved to become a significant environmental contaminant that leaches into water and soil [4, 5, 6]. Much research has gone into the endocrine disrupting effects of BPA on animals and humans since it exhibits estrogenic properties and binds to estrogen receptors [7]. These properties have been shown to disrupt the reproductive systems of various species and ultimately deteriorate the health and fecundity of animals exposed to the chemical [8]. Our more distant relatives, plants, have unfortunately not been deemed worthy of such attention in this regard. Indeed, it is only more recently that any extensive research has gone into the effects of BPA on the primary producers of our ecosystems. Given the stark differences between the endocrine systems of animals and plants, it is not the estrogenic properties of BPA that interest us in this present study, but instead the cytotoxic effects that BPA can have on plant tissue, which must be studied at several growth stages. Bisphenol A is reported to increase the levels of reactive oxygen species (ROS) in both plant and animal tissues [9, 6]. Plants maintain a delicate balance of oxidants and reductants, and any chemicals that disrupt this balance may exert toxicity. Plants are usually most vulnerable to toxicity during the germination phase [10] as the seed draws in large amounts of water via imbibition, exposing the vulnerable plant embryo to whatever solutes may have been dissolved in the water [11]. Bisphenol a is one such solute. Indeed, Dogan and his colleagues [10] have found that BPA inhibits seed germination in chickpeas and also adversely affects root development. The same study found that BPA significantly increased the levels of hydrogen peroxide, a reactive oxygen species. Photosynthesis, one of the most important metabolic reactions of life, is largely dependent on the pigment chlorophyll for the absorption of light. Qiu and his colleagues [12] found that BPA decreases the total content of chlorophyll in soybean seedlings. However, they did not investigate the effects of BPA on chlorophyll a and chlorophyll b individually. In fact, an extensive review of the literature found no studies that investigate the relative amounts of chlorophyll a and b (chlorophyll a:b ratio) in relation to BPA, as per the author's knowledge. Chlorophyll a is the main photosynthetic pigment in the majority of plants [11], but chlorophyll b absorbs a different range of wavelengths of light. As a means of acclimation, plants increase the amount of chlorophyll b relative to chlorophyll a in low light conditions in order to expand the range of wavelengths that can be absorbed and make more efficient use of available light [13]. Indeed, in shade-adapted chloroplasts, there is a higher ratio of photosystem II to photosystem I as photosystem II contains a higher proportion chlorophyll b [14]. This reveals the significance of chlorophyll a:b ratio: plants can control their chlorophyll a:b ratio in order to acclimate to different light conditions. Should BPA be found to affect this ratio, then it very well may affect the shade tolerance of plants. This present study therefore aims to investigate the effects of bisphenol A on root elongation and chlorophyll a:b ratio using lentils (*Lens culinaris)* as a model organism.

2. Materials and Methods

2.1. Preparation of Treatments

Analytical grade solid bisphenol A was obtained from Sigma-Aldrich Inc. (USA). Aqueous solutions of BPA were prepared with the following concentrations: 10 mg dm^{-3}, 50 mg dm^{-3}, and 100 mg dm^{-3}.

2.2. Root Length Test

Four-hundred lentil seeds were counted. The seeds were immersed in a 1.5% sodium hypochlorite solution for 5 minutes for surface sterilization, and were then washed 4 times with tap water. They were then split into 4 groups of 100 seeds each, 3 groups for the three BPA treatments, and one for the control. Each group of seeds was evenly distributed onto two layers of paper towel, and 20 cm^3 of each treatment solution was applied. After 3 days of exposure in a dark room, the seeds were removed for root length measurements.

Outlines of each of the roots were drawn onto sheets of paper. The sheets of paper were scanned and imaging software was used to measure the length of each root relative to an accurate scale. Seeds that had excessively convoluted roots were difficult to draw and measure accurately and were therefore excluded.

2.3. Planting the Seeds

Eighty germinated seeds were randomly selected from each of the above 100-seed groups. Each set of 80 seeds was then split in two groups of forty.

The resulting eight groups were sowed in separate containers of artificial soil (Polo Peatmoss ®). Four groups were irrigated with the same BPA treatment that they were exposed to during germination, and the other 4 were irrigated with water. The containers were watered with 200 cm^3 of the treatment solution on the first day, and then 50 cm^3 daily for a total of 7 days. During growth, the containers were exposed to continuous lighting from incandescent lightbulbs.

The result was a total of 8 separate sets of 40 seedlings each. Four of the sets were watered with either 10 mg dm^{-3}, 50 mg dm^{-3}, 100 mg dm^{-3} BPA, or the control treatment (water), during both the germination and growth stages. The other 4 sets were exposed to the treatments only during germination and not during growth. This enabled us to assess the recovery of the plants after BPA exposure during the germination phase.

2.4. Chlorophyll Extraction

The leaves were removed from each of the sets of lentil plants. Five 0.3 g samples of leaf tissue were randomly selected from each set. Each sample was homogenized with 90% acetone. The homogenate was centrifuged at 14000 RPM for 5 minutes.

A nano-drop spectrophotometer was used to find the absorption indices of the supernatant at 647nm and 664nm.

2.5. Chlorophyll a and b Concentration Calculations

The equations of Jeffrey and Humphrey [15] for chlorophyll a and b content of higher plants using a 90% acetone solvent were used to compute relative concentrations of chlorophyll a and b:

Chlorophyll a $= 11.93\ E_{664} - 1.93\ E_{647}$

Chlorophyll b $= 20.36\ E_{647} - 5.50\ E_{664}$

Where E_x is the absorbance at wavelength *x* nm.

Chlorophyll a:b ratio was computed by dividing the values of chlorophyll a by those of chlorophyll b.

2.6. Statistical Analysis

Data for both the root length and chlorophyll a:b ratio was analyzed statistically by a one-way analysis of variance (ANOVA). A follow-up comparison between the groups was carried out using Tukey's honest significant difference test ($\alpha = 0.05$). The Student's t-test was then used to compare the groups treated with BPA solely during germination with those that were treated with BPA during both germination and growth. $P<0.05$ was considered to be statistically significant. Minitab version 14 (Minitab Inc., State College, PA, USA) was used for all statistical analysis.

3. Results

3.1. Root Length

The effects of bisphenol A on the root length of Lens culinaris seeds are represented in Figure 1, which shows that as BPA concentration increased, the average root length values decreased. A one-way ANOVA showed highly significant differences between all groups ($P<0.0005$). A follow up analysis between the groups using Tukey's test showed that all BPA-treated groups had significantly lower mean root lengths than the control ($P<0.05$). The 100 mg dm^{-3} group also had significantly lower root length than the 10 mg dm^{-3} group ($P<0.05$), but differences between the 10 mg dm^{-3} group and 50 mg dm^{-3} group, as well as between the 50 mg dm^{-3} and 100 mg dm^{-3} groups were not statistically significant ($P>0.05$).

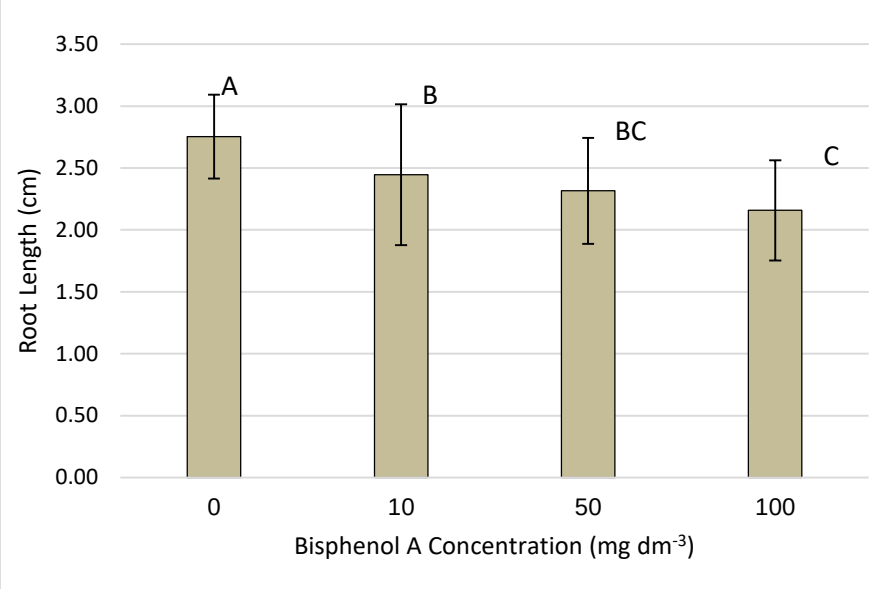

Figure 1: The Effect of Different BPA Concentrations on Mean Root Length in Lens culinaris Seeds.

Error bars represent standard deviation. Means with different letters are significantly different according to Tukey's test ($P<0.05$).

3.2. Chlorophyll a:b Ratio

The mean values of chlorophyll a:b ratio for the groups that were treated with BPA during germination only are represented in Figure 2. Clearly, the mean ratios were all relatively close to each other, and this was confirmed by the ANOVA test which showed no significant differences between the groups ($P>0.05$).

Figure 3 illustrates the average chlorophyll a:b ratios for the seeds treated during both germination and growth. Contrary to before, differences between the groups were highly significant according to the ANOVA test ($P<0.0005$). Tukey's test revealed that the 100 mg dm^{-3} group had a significantly higher chlorophyll a:b ratio than all other groups ($P<0.05$). Similarly, the chlorophyll a:b ratio in the seeds exposed to the 50 mg dm^{-3} treatment was significantly higher than that of that of the seeds treated with lower concentrations ($P<0.05$). However, the difference in chlorophyll a:b ratio between the 10 mg dm^{-3} and the control group was not significant ($P>0.05$).

As a follow-up analysis, the student's t-test was used to compare every group in Figure 2 with the matching group in Figure 3 in order to determine the effect of BPA treatment during the seedling growth stage.

The differences between the two control groups and between the two 10 mg dm^{-3} groups were not statistically significant ($P>0.05$). However, the chlorophyll a:b ratios of the 50 mg dm^{-3} and 100 mg dm^{-3} groups treated with BPA during both germination and growth were significantly higher than the corresponding groups treated during germination alone ($P<0.0005$).

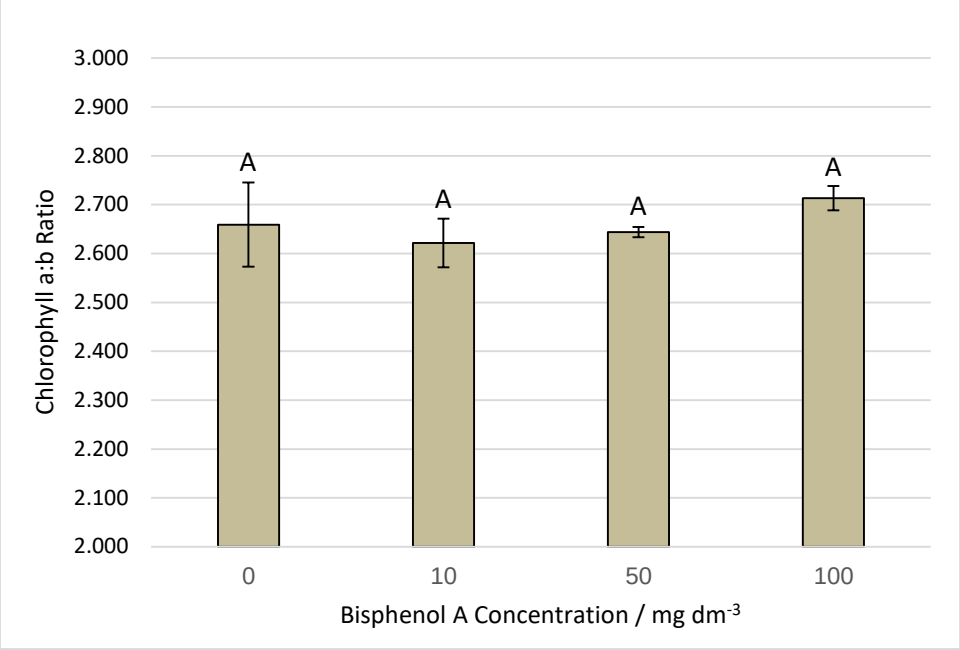

Figure 2: The Effect of Different BPA Concentrations on Mean Chlorophyll a:b Ratio in Samples Treated with BPA Solely During Germination

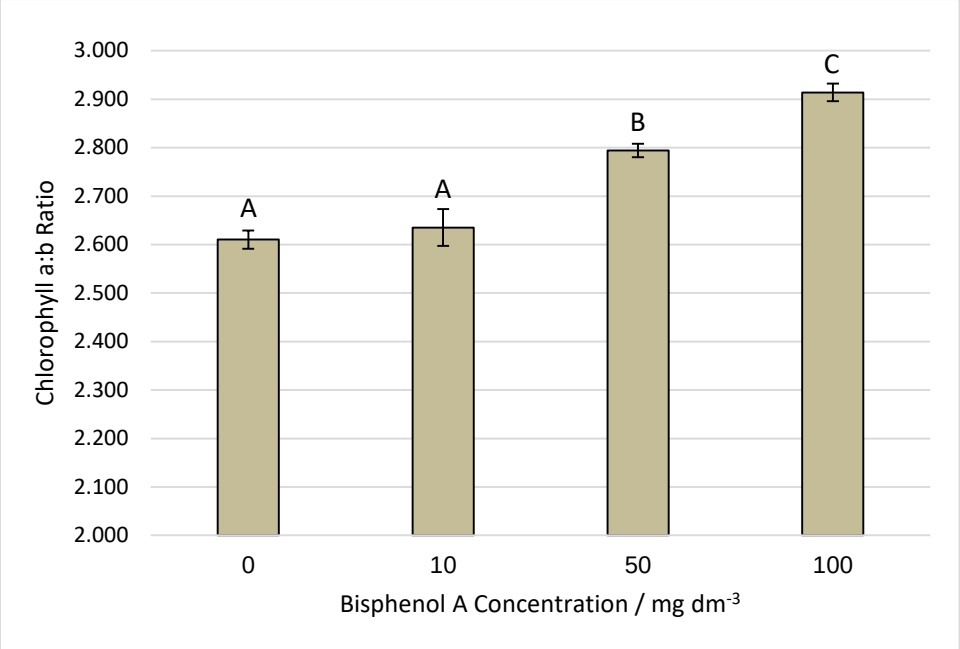

Figure 3: The Effect of Different BPA Concentrations on Mean Chlorophyll a:b Ratio in Samples Treated with BPA During Both Germination and Growth

In both figures, error bars represent standard deviation. Means with different letters are significantly different according to Tukey's test ($P<0.05$).

4. Discussion

The results of this study show that aqueous solutions of BPA at concentrations of 10, 50 and 100 mg dm^{-3} inhibit root development in *Lens culinaris*. This is in agreement with the results of Dogan and his colleagues [10], who found that BPA was inhibitory to germination and root development in chickpea seeds.

Furthermore, doses of 50 mg dm^{-3} and 100 mg dm^{-3} BPA were found to significantly increase chlorophyll a:b ratio in *Lens culinaris*, meaning that the amount of chlorophyll b relative to chlorophyll a was decreased. This effect was only observed in seedlings treated with BPA both during germination and growth, and not those treated only during germination, which indicates that the seeds were able to recover from the toxicity of BPA before sprouting.

Although the differences in the data points may seem small, one must not forget that they are ratios and not absolute values, and can therefore represent significant amounts of chlorophyll a and b. The remarkably low standard deviation values in most of the chlorophyll a:b ratio data, specifically the values in Figure 3, serve to illustrate the biological stability of chlorophyll a:b ratio. Indeed, Dale and Causton [13] have found it to be unaffected by several environmental factors, going so far as to consider it a reliable bioassay for light conditions.

An interesting comparison can be made between these results and those of Qiu and his colleagues [12], who

found that BPA decreased the activity of photosystem II in the chloroplasts of soybeans. Given that photosystem II contains most of a plant's chlorophyll b content [14], it would not be unreasonable to assume that our results may be related to the effect of BPA on photosystem II.

The effects of BPA on *Lens culinaris* can all be explained in relation to the aforementioned tendency of BPA to induce reactive oxygen species generation [9, 6]. Regarding the effects of BPA on root elongation, Sun and his colleagues [16] found that high-dose BPA (17.2 and 50 mg dm^{-3}) caused the inhibition of glutamine synthetase/glutamate synthetase cycles and the promotion of glutamate dehydrogenase pathways, which, according to Zhang and his colleagues [6] leads to the excessive production of amino acids and proteins which inhibits germination and root development.

To explain the effects of BPA on chlorophyll content, it is important to note that photosynthesis is a reaction that requires precise redox homeostasis. The reactions of photosynthesis, especially those that occur at photosystem II, produce ROS. These same ROS can destroy photosynthetic apparatus and disrupt the aerobic environment required for the electron transport chain to operate. As a result, plants have developed a variety of repair and antioxidant systems that regulate the concentrations of ROS and reverse their damage to photosynthetic apparatus [17].

These antioxidant systems have evolved to work precisely at the levels of ROS produced naturally by metabolic reactions like photosynthesis, and not at the elevated levels brought about by the introduction of BPA. In the case of photosynthesis, this means that the repair mechanisms that reverse the damage inflicted by ROS may not be able to keep up with the artificially inflated ROS levels, leading to the net destruction of photosynthetic apparatus. According to Rüdiger [18], chlorophyll a is formed from the reduction of chlorophyll b. This reductive pathway may also be affected by the elevated levels of ROS or by the antioxidant systems that are activated by BPA [6], which could affect chlorophyll a:b ratio. More research should be carried out to investigate the effect of BPA or ROS on this pathway.

Furthermore, the antioxidant systems that BPA activated in the study of Zhang and his colleagues [6] could offer a possible explanation for why the seeds that were treated only during germination did not exhibit any difference in chlorophyll a:b ratio as seedlings. As seeds, the samples had no leaves in which chlorophyll could be expressed. The withdrawal of BPA probably gave the seeds ample time to recover, since the antioxidant systems would be able to clear out any excess ROS before chlorophyll production accelerated. Again, further analysis is necessary to verify this.

The results of this investigation show that the environmental contaminant, BPA, is indeed hazardous to plant life. An inhibitor of germination and early root development, it could affect the survival of seedlings and ultimately reduce the reproductive efficiency of plant species. Indeed, the seed is the link between one plant generation and the next, and is therefore of great importance.

Our findings regarding chlorophyll a:b ratio offer new insights into the effect that BPA may have on shade tolerance in plants. Although further investigations must be conducted in this regard, the increases in

chlorophyll a:b ratio due to BPA exposure indicate that BPA can reduce the proportion of chlorophyll b in a plant's chloroplasts, which eventually reduces the range of wavelengths that a plant can absorb. This would theoretically reduce the efficiency of light-harvesting mechanisms in the chloroplasts and hence reduce shade tolerance [13]. Given that plants are the main producers in most terrestrial ecosystems, any effects on photosynthetic efficiency can reverberate through the ecosystem and reduce the amount of energy available for terrestrial species.

The major limitation of the current study was the fact that ROS levels were not evaluated at the different growth stages. More research could be directed into this to further explain our results. Moreover, the specific effects of BPA on the biosynthesis of chlorophyll a and b should be investigated further.

5. Conclusion

In conclusion, we have found that BPA inhibits root development and increases chlorophyll a:b ratio in *Lens culinaris.* Taking the results of this investigation into account, as well as those of similar studies in the literature, it becomes clear that BPA contamination may be a serious problem facing our ecosystems.

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