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PHYTOTOXIC EFFECTS OF BISPHENOL A ON GROWTH INDICANTS AND CHLOROPHYLL CONTENT OF *PISUM SATIVUM* L.

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Abstract

In the environment, Bisphenol A (BPA) is an emerging universal pollutant and it disrupts endocrine system and is substantially toxic for living beings and significantly harmful to the plants as well. Toxicologic effects of BPA on plants is not fairly documented. In the present study, effects of BPA on growth and chlorophyll content were studied in *Pisum sativum* L. (pea) seedlings. After treatment of pea seeds with varying concentrations of BPA (2, 5, 10, 15, 20, and 25 mg/l) for 3 hrs at room temperature (21°C), they were sown in pots and growth *viz.*, height of plants, fresh and dry weights of stems and leaves and chlorophyll content were recorded after 15 and 30 days from sowing. The current study confirmed that BPA can affect the growth and chlorophyll content in a dose related manner. BPA at lower concentration (2 mg/l) increased the growth in pea seedlings whereas BPA at higher concentration exhibited a significant inhibitory effect on the growth of pea seedlings. Consequently, the results of this study may be applied in future researches of environmental hazards linked to soil due to BPA as well as managing of BPA in relation to soil.

Introduction

Bisphenol A is a phenolic compound used for more than 50 years to manufacture phenol and epoxy resins, polycarbonates, polyesters and is present in refillable water and baby bottles and lacquer and plastic coatings in food cans (Goodson *et al.* 2002). BPA gets into water bodies by municipal and industrial wastes (Gatidou *et al.* 2007, Fu and Kawamura 2010). Globally, 3700000 tons of BPA is produced every year (Mihaich *et al.* 2009). Since, BPA leaches from plastic materials and bottles, human beings come into its contact by eating food and drinks stored in these bottles, cans and packs (Huang *et al.* 2012) and hence it jeopardize the healthiness of humans (Le *et al*. 2008, Wagner and Oehlmann 2009, Cooper *et al*. 2011, Tabaa *et al*. 2022). Humans risk themselves by consuming fish obtained from BPA polluted marine waters (Mita *et al*. 2011). Canada is the first in the world to proclaim that BPA is a toxic material (Canada-Gazette 2010).

Farm lands generally gets supplemented by actuated sewage mud having BPA (Gatidou *et al.* 2007, BPA pollution in soils have gravely endangered the safety of the environment (Hunt *et al.* 2009). Plants could get affected by BPA polluted soils (Pop *et al.* 2021, Yamamoto *et al.* 2001, Zaborowska *et al.* 2021). Few studies are available on the impact of BPA on land plants (Terouchi *et al.* 2004, Ferrara *et al.* 2006) in relation to the impact of BPA on animals (Flint *et al.* 2012, Tabaa *et al.* 2022). There is limited documented work available on the toxicologic effect of BPA on plants which take in and accumulate it from BPA polluted agricultural soils (Ferrara *et al.* 2006). Comprehensive studies have proved that BPA disrupts endocrine system (Staples *et al*. 1998, Clarke and Smith 2011). However, plants can make BPA-glycosides by breaking down BPA (Noureddin *et al.* 2004), clastogenic and phytotoxic effects on plants of BPA were

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ascertained (Ferrara *et al*. 2006). In kiwifruit pollens, BPA influenced its germination, produced stress reaction and changed its steroid hormone creation (Speranza *et al*. 2011). BPA induced mitotic as well as chromosomal abnormalities in cells of root tips of onion (Jadhav *et al.* 2012). A dose of 0.01 to 1 mg/l BPA concentration promotes soybean growth and in carrot, it stimulates differentiation of shoot and 0.1 mg/l BPA induces highest simulating effect (Terouchi *et al.* 2004). Conversely, BPA at 10 and 50 mg/l concentrations exhibits substantial toxicity on tomato, broad bean, lettuce and durum wheat (Ferrara *et al*. 2006).

Wang *et al*. 2015a, b, Ali *et al.* 2016, 2017, Siddiqui *et al.* 2021 stated about BPA toxicity in plants. But, these research work dealt about hydoponic treatments of BPA. They did not examine the effects of BPA treatment in the fields. There is considerable difference between the impacts of chemicals in soils than in solutions. Hence, it is essential to evaluluate in practical settings precisely in soil related to toxicity. Thus the present work was undertaken to investigate phtotoxic impacts of BPA on the chlorophyll content and growth on pea plant which is a vital commercial crop.

Materials and Methods

Purchase of pea seeds (*Pisum sativum* L. cv. Arkil; 2n = 14) were done from a shop located in the Kingdom of Saudi Arabia. Bisphenol A (2,2-(4,4 dihydroxydiphenyl) propane) of Sigma–Aldrich Merck (Germany) is bought from Bayouni Trading Co. Ltd., Jeddah, Saudi Arabia. CAS number of BPA is 80-05-07, water solubility at 25 ºC is 123–300 mg/l, melting point is 153-157 ºC, molecular weight is 228.29 and $C_{16}H_{18}O_2$ is its formula. Seeds of pea were sterilized for 15 min. in a solution of 0.1% $HgCl_2$ and were washed 2-3 times in distilled water. A set of 10 seeds for each concentration were soaked for 3 hrs in BPA solutions at room temperture (21^0C) of different concentrations (2, 5, 10, 15, 20 and 25 mg/l) and were seeded in pots (size 20 cm x 25 cm). Ten seeds were soaked in distilled water and seeded in a pot for control. The complete experimentation was done thrice in similar settings. After 15 and 30 days of sowing seeds of pea, growth indicants for example height of plants, fresh and dry weights of stems, fresh and dry weights of leaves were recorded. By using a scale, height of plant were measured. For each plant, after 12 hrs of drying in an oven at 80ºC, dry weight of stems and leaves were noted.

Extraction of chlorophyll was done after the leaves of pea seedlings were soaked in 80% acetone and it was centrifuged for 10 min at 5300g. Reading for absorbance of supernatant was taken respectively at 645 and 663 nm. As per equation, chlorophyll content was assessed: $20.2A_{645} + 8.02A_{663}$ (Lichtenthaler and Lester Packer 1987.). By using Graph Pad software (San Diego, CA, USA), statistical analysis (ANOVA with Dunnett's multiple-comparison test) was completed having significance at $p \leq 0.05$. Data were exhibited as mean ± standard error (SE).

Results and Discussion

Results after sowing for 15th and 30th day, of the fresh and dry weights in BPA treated pea seedlings for 3 hrs at different concentrations are shown in Tables 1 and 2 and Figs 1, 2 and 3.

On 15th day, the height of plant (Fig 1 and 2) and dry weight of leaves of 2 mg/l BPA treated pea seedling were enhanced by 13.44% ($p < 0.05$) and 55.27% ($p < 0.001$) and fresh weight of leaves and stems and dry weight of stems was decreased by 12.63% ($p < 0.001$), 5.75% ($p < 0.001$) and 7.66% ($p < 0.05$) respectively, as compared with control. After treatment of pea seedlings with 5 mg/l BPA, there was an increase in dry weight of leaves (6.03%) and decrease in plant height (11.70%), fresh weight of leaves (25.27%, $p < 0.001$), fresh weight (16.52%, $p < 0.01$) and dry weight of stems (16.66%, $p < 0.001$) as compared with control. In case of 10 mg/l BPA treated pea seedlings, there was a decrease in plant height (30.38%, $p <0.001$), fresh weight

(8.84%, $p < 0.001$) and dry weight of leaves (10.55%, $p < 0.01$), fresh weight (20.91%, $p < 0.001$) and dry weight of stems (33.33%, $p < 0.001$).

In 15 mg/l, there was decrease in plant height (18.03%, $p < 0.01$), fresh weight (31.59%, $p < 0.001$) and dry weight of leaves (22.11%, $p < 0.001$), fresh weight (13.35%, $p < 0.001$) and dry weight of stems (38.33% $p < 0.001$). In 20 mg/l, there was decrease in plant height (43.32%, $p < 0.001$), fresh weight (47.39%, $p < 0.001$) and dry weight of leaves (38.19%, $p < 0.001$), fresh weight (43.10%, $p < 0.001$) and dry weight of stems (50.33%, $p < 0.001$). In case of 25 mg/l, there was decrease in plant height (55.50%, $p < 0.001$), fresh weight (68.40%, $p < 0.001$) and dry weight of leaves (61.30%, $p < 0.001$), fresh weight (56.43%, $p < 0.001$) and dry weight of stems (56.66%, $p < 0.001$) (Table 1 and Figs 1 and 2.)

Table 1. Effect of BPA on fresh and dry weights of pea seedlings on the 15th day after pea seeds were sown.

	Concentration of BPA in mg/l						
	0.0	2	5	10	15	20	25
FWL (g)	6.33 ± 0.06 (100%)	5.53 ± 0.02** (-12.63%)	4.73 ± 0.17** (-25.27%)	5.77 ± 0.34** (-8.84%)	4.33 ± 0.01** (-31.59%)	3.33 ± 0.03** (-47.39%)	2.00 ± 0.01** (-68.40%)
DWL (g)	1.99 ± 0.01 (100%)	3.09 ± 0.03** (55.27%)	2.11 ± 0.15 (6.03%)	1.78 ± 0.26* (-10.55%)	1.55 ± 0.01** (-22.11%)	1.23 ± 0.04** (-38.19%)	0.77 ± 0.03** (-61.30%)
FWS (g)	10.95±0.66 (100%)	11.58 ± 0.23** (-5.75%)	9.77 ± 0.21* (-16.52 %)	8.66 ± 0.06** (-20.91%)	9.66 ± 0.02** (-13.35%)	6.23 ± 0.01** (-43.10%)	4.77 ± 0.05** (-56.43%)
DWS (g)	3.00 ± 0.03 (100%)	2.77 ± 0.02$^{\#}$ (-7.66%)	2.50 ± 0.31** (-16.66%)	2.00 ± 0.24** (-33.33%)	1.85 ± 0.03** (-38.33%)	1.49 ± 0.01** (-50.33%)	1.30 ± 0.03** (-56.66%)

FWL- Fresh weight of leaves; DWL- Dry weight of leaves; FWS- Fresh weight of stems; DWS- Dry weight of stems. Data within parenthesis indicates fresh and dry weight of leaves and stems.

******p < 0.001 compared to control; *p < 0.01 compared to control; #p < 0.05 compared to control. Data are mean of three replicates ± SEM; 0.0 = Control group.

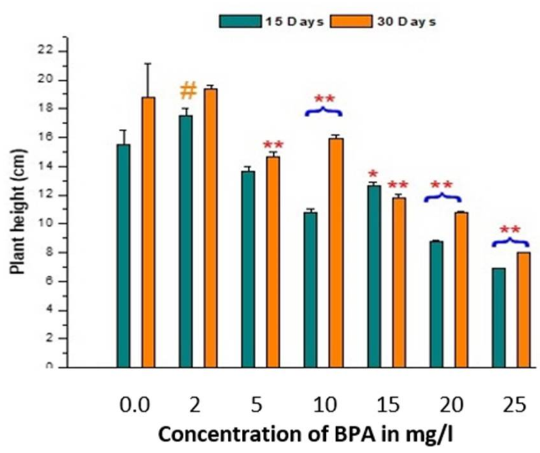

Fig. 1. Outcomes of different concentrations of BPA on plant height for 15 and 30 days after pea seeds were sown. **p < 0.001 compared to control; *p < 0.01 compared to control; #p < 0.05 compared to control. Data are mean of three replicates ± SEM; 0.0 = Control group.

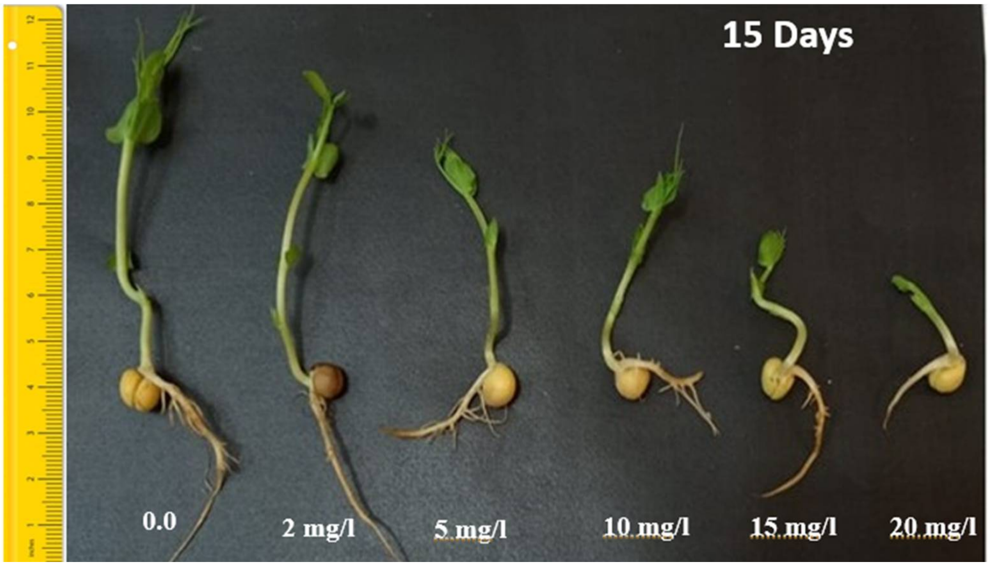

Fig. 2. Image representing the outcomes of BPA on the growth of pea seedlings on the 15th day after pea seeds were sown.

After sowing on 30th day, there was an increase in plant height (2.98%) (Figs 1 and 3), fresh weight of leaves (20.23%) and decrease in dry weight of leaves (9.90%), fresh weight of stems (13.95%), dry weight of stems (60.58%) in 2 mg/l and in case of 5 to 25 mg/l BPA treated pea seedlings; plant height, fresh and dry weight of leaves and fresh and dry weight of stems were decreased by (21.89, 91.25, 23.42, 10.87 and 4.61%) at 5 mg/l; (15.39, 14.49, 39.93, 21.68 and 27.67%) at 10 mg/l; (37.29, 27.54, 40.54,19.20 and 46.54%) at 15 mg/l; (42.62, 42.03, 53.45, 26.70 and 57.86 %) at 20 mg/l and (57.48, 45.03, 69.96, 60.84 and 74.63%) at 25 mg/l respectively when compared to control as shown in (Table 2.)

Table 2. Effect of BPA on fresh and dry weights of pea seedlings on the 30th day after pea seeds were sown.

	Concentration of BPA in mg/l						
	0.0	2	5	10	15	20	25
FWL (g)	7.66 ± 0.01 (100%)	9.21 ± 0.21 (20.23%)	8.33 ± 0.23 (-91.25%)	6.55 ± 0.04 (-14.49%)	5.55 ± 0.04** (-27.54%)	4.44 ± 0.77** (-42.03%)	4.21 ± 0.55** (-45.03%)
DWL (g)	3.33 ± 0.01 (100%)	3.00 ± 0.01 (-9.90%)	2.55 ± 0.16** (-23.42%)	2.00 ± 0.01** (-39.93%)	1.98 ± 0.01** (-40.54%)	1.55 ± 0.21** (-53.45%)	1.00 ± 0.11** (-69.96%)
FWS (g)	13.33 ± 0.16 (100%)	11.4 7 ± 0.15** (-13.95%)	11.88 ± 0.02** (-10.87%)	10.77 ± 0.25** (-21.68%)	9.77 ± 0.17** (-19.20%)	6.88 ± 0.18** (-26.70%)	5.22 ± 0.18* (-60.84%)
DWS (g)	4.77 ± 0.03 (100%)	4.55 ± 0.21 (-60.58%)	3.45 ± 0.02** (-4.61%)	2.55 ± 0.06** (-27.67%)	3.85 ± 0.06** (-46.54%)	2.01 ± 0.01** (-57.86%)	1.21 ± 0.11** (-74.63 %)

FWL- Fresh weight of leaves; DWL- Dry weight of leaves; FWS- Fresh weight of stems; DWS - Dry weight of stems. Data within parenthesis indicates fresh and dry weight of leaves and stems.

**$p < 0.001$ compared to control; *$p < 0.01$ compared to control. Data are mean of three replicates ± SEM; 0.0 = Control group.

Fig. 3. Image representing the outcomes of BPA on the growth of pea seedlings on the 30th day after pea seeds were sown.

Fig. 4 illustrates the outcome of chlorophyll content in pea seedlings treated with 2 to 25 mg/l BPA for 15 and 30 days after sowing. In case of 15 days BPA treatment, chlorophyll content was found to decrease in 2 mg/l (13.46%), 5 mg/l (19.82%), 10 mg/l (21.70%, $p < 0.05$), 15 mg/l (32.50%, $p < 0.01$), 20 mg/l (43.86%, $p < 0.01$) and 25 mg/l (49.54%, $p < 0.01$) respectively in comparison to control. Similar pattern were reported after 30 days BPA treatment which were (14.38%) in 2 mg/l, (13.01%) in 5 mg/l, (29.31%, $p < 0.01$) in 10 mg/l, (37.46%, $p < 0.01$) in 15 mg/l, (47.53%, $p < 0.01$) in 20 mg/l and (58.08%, $p < 0.01$) in 25 mg/l respectively in comparison to control.

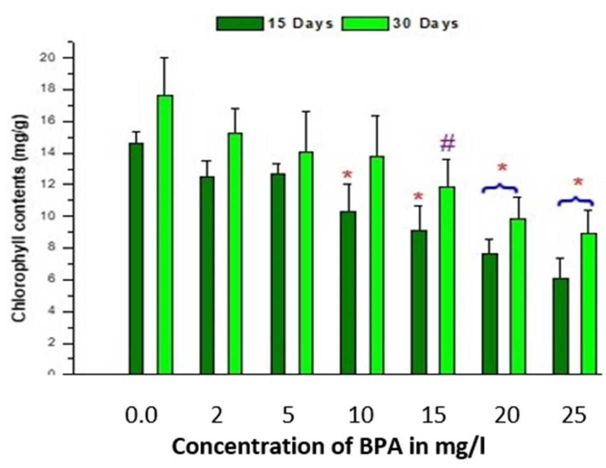

Fig. 4. Outcomes of different concentrations of BPA on chlorophyll contents for 15 and 30 days after pea seeds were sown. $**p < 0.001$ compared to control; $* p < 0.01$ compared to control; $\#p < 0.05$ compared to control. Data are mean of three replicates ± SEM; 0.0 = Control group.

As per visual observation, plant growth is the utmost evident phytotoxicity test factor. As per the observed data, treatment with BPA affects growth indicants of pea plant. *Pisum sativum* treated with lesser concentrations of BPA (2 mg/l) has longer plant height than control. This tendency of growth in plants at lesser concentrations of BPA treatment was also stated in previous studies (Liu *et al.* 2013, Ali *et al.* 2016, Malea *et al.* 2022) who reported increase in growth or stimulative growth effects. The plant height, fresh and dry weights of leaves and stem started to decrease at 5 to 25 mg/l as compared to control.

Photosynthesis results in the production and buildup of biological material and hence the growth and development of plants depends on it. If the pollutants present in the environment damage or alter the photosynthetic processes, growth and development of plants got affected (Kummerov *et al.* 2006, Gui *et al.* 2022). Qiu *et al.* (2013) reported that BPA treated soybean exhibited inhibition of growth because of a reduction in Pn, a precise indication regarding the photosynthetic capability. Therefore, the plant height inhibition that was examined is probably because of the photosynthesis inhibition.

The outcomes of the present investigation revealed that BPA treatment affects indicator of photosynthesis like chlorophyll content. A vital role in photosynthetic process in plants is played by chlorophyll. (Hopkins and Huner 2009, Jiao *et al*. 2017a). Since, chlorophyll is precisely associated with energy, photosynthesis and respiration, it is significant *in vivo* endpoint (Chia and He 1999, Guo *et al.* 2005, Jiang *et al.* 2007). Various studies have revealed that treatment with BPA in plants resulted in enhanced oxidative stress which results in a rise in oxygen radicals (Ali *et al.* 2016, Jiao *et al.* 2017b, Qiu *et al*. 2013, Jianing *et al.* 2022). In case of rice which is grown in hydroponic conditions, treatment with BPA (10 mM or greater) decreased chlorophyll conetent (Ali *et al*. 2016). They reported an enhancement in permeability of cells and damage of root chloroplast cells and stated that BPA treated plants were harmed by oxidative stress, which triggers harm to mitochondria, chloroplasts and plasma membranes because of the accumulation of oxygen radicals. Qiu *et al.* (2013) and Jiao *et al.* (2017a) reported that in hydroponic conditions, there was a decline in chlorophyll contents in soybean. Qiu *et al*. (2013) elucidated that metabolites of BPA travel to leaves and thereby enhance the ROS accumulation in leaf cells, harming thylakoid membrane and interrupt the pigments and the structure of chloroplast. Hence, it is considered that the decline in the chlorophyll levels found in this study is associated with these processes.

The present work showed that BPA can affect the growth indicants and chlorophyll content in a dose related manner. BPA at low concentration (2 mg/l) stimulates the growth in pea seedlings where as BPA at higher concentration exhibited a significant inhibitory influence on the growth of pea seedlings, which is due to the decrease in photosynthesis because of the reduction in the content of chlorophyll. Hence, the outcome of the present study could be utilised in future studies of ecological hazards related to soil due to BPA and managing of BPA with regards to soil.

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