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Research article

Investigation of bisphenol A (BPA) effects on germination and development of wheat and chickpea

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Abstract

Bisphenol A (BPA) is a chemical compound used in the production of various plastics. Its effects on health have been the subject of publications and scientific debate. The current study was carried out to reveal the effects of bisphenol A at various concentrations on seed germination and seedling development of wheat and chickpea plants. At the first step, seeds of wheat and chickpea were planted in sterile petri dishes and imbibed with 0, 1, 5, 10, 20, 40 and 50 mg L^{-1} Bisphenol A concentrations. Germination percentage, vigor index, radicle length, and plumula length were calculated. In the second step, the seeds were first germinated in sterile petri dishes, and seedlings were exposed to the same BPA concentrations. In addition to seedling development measurements, chlorophyll, carotenoid contents, and phenolic and flavonoid changes were analyzed. Stomatal aperture status in wheat seedlings was also monitored. The effect of BPA concentrations varied greatly depending on the plant species. Likewise, their effects on germination and development stages are highly variable. Root and stem lengths decreased due to increasing BPA concentrations. Regarding the effects of BPA on development, 40 and 50 mg L^{-1} concentration applications caused an increase in chlorophyll in wheat and a significant decrease in chickpea plants. Phenolic and flavonoid values showed differences depending on the application dose. It was noticed that their amounts increased significantly at concentrations higher than 20 mg L^{-1}. The cadmium toxicity effect varied depending on the seed species and cadmium concentration. While 1 and 5 mg L^{-1} applications did not cause a negative effect on germination and development, it caused inhibitory effects at high concentrations. BPA concentration in nature is increasing day by day. These findings provide invaluable information on the underlying effects and concentration limit of BPA on crop growth.

Keywords: *Bisphenol A; endocrine disruptors; germination; seedling; toxicity*

1. Introduction

Bisphenol A [2,2-bis (4-hydroxyphenyl) propane] is a widely used chemical compound that was first synthesized by Russian chemist Alexander Dianin in 1891 and is one of the highest-volume chemicals produced worldwide since the 1950s. BPA has been reported to be used primarily to produce polymer materials such as epoxy resins, polycarbonate plastic, and polysulfone resins. For this reason, its presence has been found in all environments, including the hydrosphere, lithosphere, and even the atmosphere (Tsai, 2006). The use of BPA in polycarbonate and epoxy resins results in the release of BPA into the environment through wastewater treatment organism effluents (Melcer and Klečka, 2011). Endocrine-disrupting chemicals, such as bisphenol, have the potential to disrupt human, animal, and plant normalcy. These substances can enter plants through their roots and the surrounding air, where they can impede the function of certain hormones and enzymes. Numerous investigations revealed that these chemicals have a detrimental impact on a variety of plant physiological functions, including germination, photosynthesis, etc. (Saraswat et al., 2024).

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Worldwide production of BPA is constantly increasing and will reach 6.2 million tons (MT) in 2020. According to estimates, the amount of BPA is expected to reach 7.1 MT by 2027 (Abraham and Chakraborty, 2020). Although bisphenol and its derivatives have been detected in all environments, many studies have been carried out on different organisms related to BPA. Bisphenol types have good stability, permanence, and bioaccumulation and show similar structure and properties (Chen et al., 2016). Increasing exposure time to BPA causes the formation of brown spots on wilted leaves in *Arabidopsis thaliana* and broad beans; it has also been reported that it causes a decrease in the number of leaves and aggravation of local necrosis in lettuce (Ferrara et al., 2006; Qiu et al., 2013). Also, tomatoes accumulate large amounts of BPA in their roots and transfer it to their branches, but not all BPA undergo biotransformation and detoxification, so its toxic effect continues. (Ferrara et al., 2006).

BPA and its derivatives harm not only human health but also the soil's microbiological and biochemical equilibrium, plant growth, and plant development (Zaborowska et al., 2023). BPA is quickly absorbed by plants from water through their roots, where it is then converted to several glycosidic molecules. Plants glycosylate BPA to increase the parent compound's estrogenic properties. Peroxidase and polyphenol oxidase are two oxidative enzymes that are intimately linked to the metabolism of BPA. According to a study on rice plants, BPA can be absorbed from the roots of seedlings quickly and transferred to their leaves (Noureddin et al., 2004). In another study it was reported that BPA sorption has a high potential to cause damage to the entire plant (Ferrara et al., 2006).

BPA has concentration-dependent effects on plant germination and development, with both low and high quantities having either promoting or inhibitory effects (Pan et al., 2013). The effects of BPA on plants are generally concentrated in its effects on enzymes, mineral uptake, pollen tube formation, microorganisms, and nucleic acids. The known effects of BPA in plants are increased respiratory enzyme activity and mineral element absorption in roots (Nie et al., 2015), changes in chlorophyll synthesis and effects on stomatal opening (Jiao et al., 2017), cell division and growth (Adamakis et al., 2013), increased levels of reactive oxygen species (ROS), lipid peroxidation, and suppression of root and seedling elongation (Pan et al., 2013; Wang et al., 2015; Zhang et al., 2016). According to the literature, plants detoxify BPA through a variety of metabolic pathways, and exposure to BPA triggers the signaling of ROS (Babu et al., 2013; Tian et al., 2014).

Germination is an important step, as it is the starting point of plant life and is affected by all environmental conditions and chemicals. Among organisms, plants are one of the most affected by environmental effects due to their sessile characteristics (Eskin et al., 2013). Increasing use of BPA in the developing world will increase the number of living organisms exposed to it. The effects of pesticides, herbicides, or similar chemicals should be evaluated in terms of human and environmental health.

Wheat and chickpea plants are among the most highly productive and widely cultivated crops in the world, so the study presented aims to determine the effects of different BPA concentrations on germination and seedling growth.

2. Materials and methods

2.1. Seed selection and BPA solutions

Wheat (*Triticum vulgare* L. Doğu-88 genotype) seeds used in the study were obtained from Van Yuzuncu Yil University, Faculty of Agriculture, and chickpea (*Cicer arietinum* L.) seeds were obtained commercially. BPA (Sigma-Aldrich) in crystal form was preferred for our study. First, a stock solution was prepared at a concentration of 50 mg L^{-1}, and other concentrations (1, 5, 10, 20 and 40) were diluted from the stock solution. 80 µl of absolute ethanol (Merck) was used to completely dissolve BPA. The same amount of ethanol was added to the internal control group.

2.2. Germination assay

Sterile glass petri dishes were used for the germination application. Two layers of sterile blotting paper were placed in the petri dishes. Unharmed, uniform 10 wheat and 6 chickpea seeds were placed separately in each petri dish. BPA solutions were added at application concentrations (1, 5, 10, 20, 40 and 50 mg L^{-1}) to each of the Petri dishes containing the seeds. BPA solutions were used: 6 mL for wheat and the last 10 mL for chickpeas. Ethanol-containing water was used for the internal control group, and pure water was used for the external control group. All petri dishes were covered with parafilm to prevent solution evaporation. The germination study was started under controlled conditions (24°C temperature, 65% humidity, 8/16 photoperiod).

2.2.1. Germination percentage and vigor index

To evaluate the effects of BPA on seed germination; germination percentage, inhibition/stimulation status, and vigor index values were calculated according to the formulas:

Germination percentage:

$$Germination\ \% = \frac{Germinated\ Seede}{Total\ seed} * 100$$

Stimuli/inhibition:

$$BPA\ \frac{inhibition}{stimulation}\ \% = \frac{Germiantion\ of\ Sample - \text{Control}}{Control} * 100$$

(-indicate inhibition, + stimulation)

BPA seed vigor index = Germination percentage * total length

2.3. Seedling assay

For the seedling effect of BPA, wheat and chickpea seeds were pre-germinated in sterile petri dishes. The germinated seeds were transferred to jars containing two layers of sterile blotting paper. Hoagland medium was added to the jars to continue the development phase (Hoagland and Arnon, 1950). For each 50 mL of Hoagland's solution, 2.42 mg Iron (III) chloride (Merck) and 75 mg EDTA (Sigma-Aldrich) were added. The germinated seeds were allowed to acclimate for one day. At the end of the period, different concentrations (0, 1, 5, 10, 20, 40 and 50 mg L^{-1}) of BPA were applied to the seedlings. Glass jars were sealed with parafilm. Seedling development was observed for seven days in appropriate light and temperature (24°C temperature, 65% humidity, 10/14 light period).

2.3.1. Determination of photosynthetic pigment contents

Plant materials crushed in liquid nitrogen were transferred to 80% acetone (Merck) and homogenized in a Sonicator (Wiggen Hauser) for 1 minute. The samples were centrifuged at 4000 rpm and the supernatants were transferred to clean falcon tubes. 2 mL of 80% acetone was added and readings were performed on the spectrophotometer (Shimadzu). Pigment content measurements were made according to Arnon (1949). The amounts of photosynthetic pigments were calculated in mg g-L according to the formulas:

Chlorophyll-a = [A663x12.70 - A645x2.49] [(V/1000)*W]

Chlorophyll-b = [A645x22.90 - A663x4.68] [(V/1000)*W]

Total chlorophyll = [A645x20.2 + A663 x 8.02] [(V/1000)*W]

Carotenoid = [A480 + A663x0.114 - A645 x 0.638/112.50] [(V/100)*W]

2.3.2. Total phenolic and flavonoid contents

Total phenolic contents were measured using the Folin-Ciocalteu reagent (Sigma-Aldrich) (Dalar et al., 2012). 25 µL of plant extract was mixed with 125 µL of Folin-Ciocalteu (1: 10, v/v) reagent and shaken for 3 minutes. 125 µL, 6% Na_2CO_3 (Merck) was added, and the microplate was shaken for 10 minutes. At the end of the period, absorbance values were measured at 600 nm. Results were expressed as mg gallic acid (GA) equivalent/g according to the gallic acid standard (Merck) curve (Dalar and Konczak, 2013).

For total flavonoid values, 25 µL plant extract was mixed with 125 µL ultra-distilled water and 7.5 µL 5% $NaNO_2$ (Merck) (1:20 w/ v). After incubation for 5 minutes, 15 µL of 10% $AlCl_3$ $6H_2O$ (Sigma-Aldrich) was added. Samples were incubated at room temperature for 6 min, then 50 µL of 1 M NaOH was added and mixed thoroughly until pinkish color appeared. Results were expressed as mg Rutin (R) E/g according to the rutin standard curve. Analyses were performed in three replicates (Akbay et al., 2003).

2.4. Statistical analysis

Experiments were carried out via three replications. The average value ± standard deviation was used to present the results. Using Minitab software, the acquired findings were analyzed using one-way analysis of variance (ANOVA) at a significance level of $p < 0.05$.

3. Results

3.1. Effect of BPA on germination

BPA application caused variable responses in the germination of wheat and chickpea seeds. The responses exhibited different effects depending on the plant species and concentration. While germination inhibition in wheat seeds started at a concentration of 20 mg L^{-1}, in chickpea seeds it started at a concentration of 10 mg L^{-1}. Although it caused a slight inhibition in chickpea seeds at 5 mg L^{-1}, the seeds were not affected by BPA concentrations of 1 and 5 mg L^{-1} (Fig. 1A-B).

When the inhibition percentages of BPA concentrations were examined, it was determined that there was a significant difference between chickpea and wheat seeds. In wheat seeds, 4% inhibition was detected at 20 mg L^{-1}, 5% at 40 mg L^{-1}, and 9% inhibition at 50 mg L^{-1} concentration. In chickpea seeds, 11% inhibition was observed at 20 mg L^{-1}, 28% at 40 mg L^{-1}, and 33% inhibition at 50 mg L^{-1} concentration.

Among the BPA applications, the highest inhibition value was observed in chickpea seeds at a concentration of 50 mg L^{-1}. Interestingly, 50 mg L^{-1} application in wheat seeds caused 9% inhibition, while more than 3 times inhibition was detected in chickpea seeds at the same concentration (Fig. 1C-D).

While there was no significant difference in germination inhibition between 20 and 40 mg L^{-1} concentrations in wheat seeds, a significant inhibition difference was detected between both applications in chickpea seeds.

Similar effects were seen in vigor index values depending on the germination rate. While the 10 mg L^{-1} concentration in wheat seeds did not cause inhibition, it caused a significant decrease in the vigor index value. It was determined that the vigor index level decreased due to the germination inhibition observed in chickpea seeds at a concentration of 5 mg L^{-1}. Interestingly, 1 mg L^{-1} BPA application caused to be higher than the control group for the vigor index values in both wheat and chickpea seeds. In addition, even though increasing BPA concentration in chickpeas reduced the germination percentage, no significant difference could be detected between the vigor indices of 40 and 50 mg L^{-1} applications (Fig. 2A-B).

3.2. Effect of BPA on seedling

3.2.1. Effect on root elongation

When the effects of BPA on root lengths in wheat seedlings were examined, it was determined that 1, 5, and 10 mg L^{-1} BPA applications stimulated root development at a much higher rate compared to the control group, and there was a significant difference, especially between the 1 mg L^{-1} application and the control group. It was observed that 40 and 50 mg L^{-1} applications had a negative effect on root development.

The application of 1 mg L^{-1} concentration of Bisphenol A in the root length of chickpea seedlings increased the root length significantly, but no significant difference was observed in all other concentration groups. It was determined that all BPA applications except 20 mg L^{-1} caused the root length to be longer than the control (Fig. 3 A-B)

3.2.2. Effects on pigment concentration

In terms of chlorophyll values, it was determined that BPA affected chickpea seedlings more than wheat seedlings. In all applications of BPA, chlorophyll values were lower than the control. An increase in chlorophyll values was detected in 10 and 20 mg L^{-1} applications in chickpea seedlings. While there was no significant difference between control and application values in wheat seedlings, it was calculated that the chlorophyll values of the 20 mg L^{-1} BPA application had the highest value (Fig. 4A).

Carotenoid values were similar to chlorophyll values. Unlike the chlorophyll ratios, no decrease was observed in chickpea seedlings at 1 and 5 mg L^{-1} concentrations. Again, the

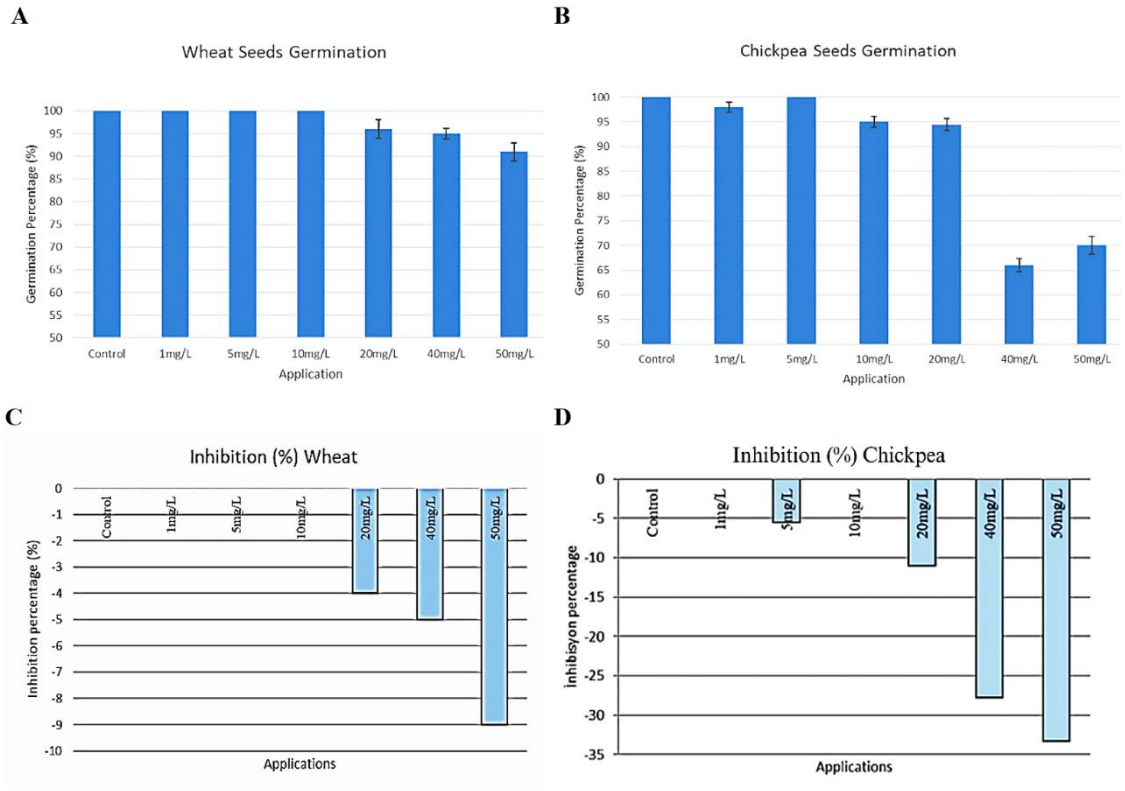

Fig 1. Effects of BPA on seed germination (A) Wheat seeds germination percentage, (B) Chickpea seeds germination percentage, (C) Wheat seeds germination inhibition, (D) Chickpea seeds germination inhibition.

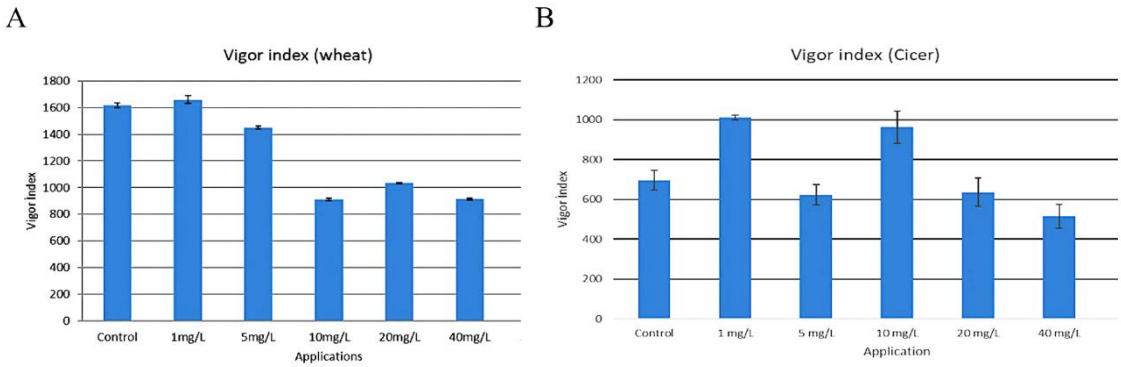

Fig 2. The effects of BPA applications on vigor index (A) wheat, (B) chickpea.

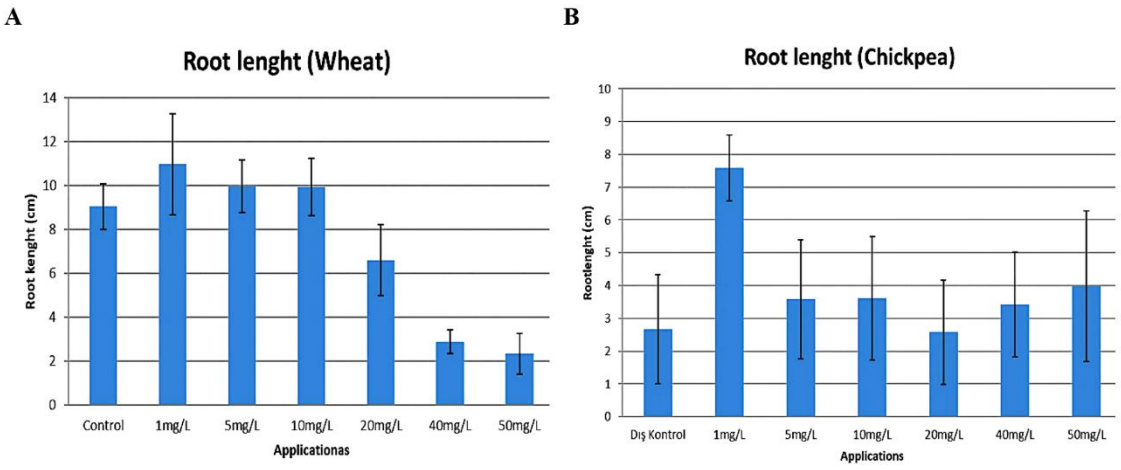

Fig 3. The effects of BPA applications on root length of seedlings (A) wheat, (B) chickpea.

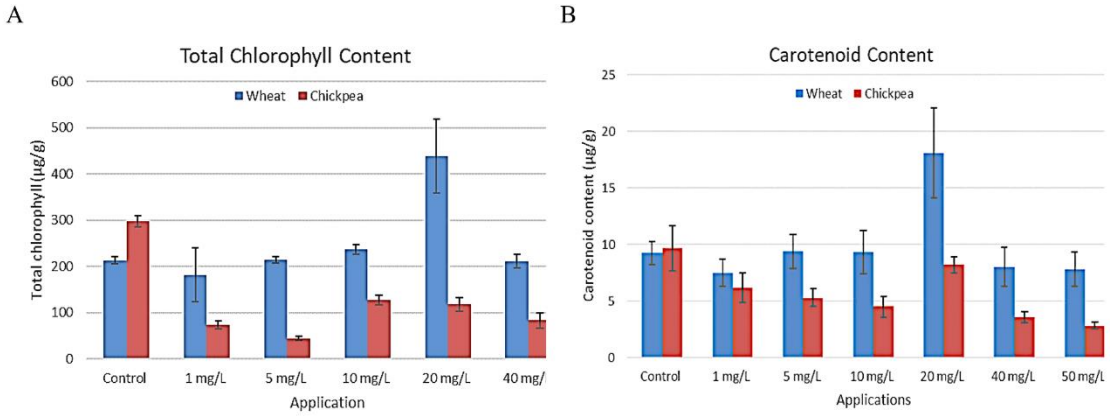

Fig 4. The effects of BPA applications on pigment content (A) total chlorophyll, (B) total carotenoid.

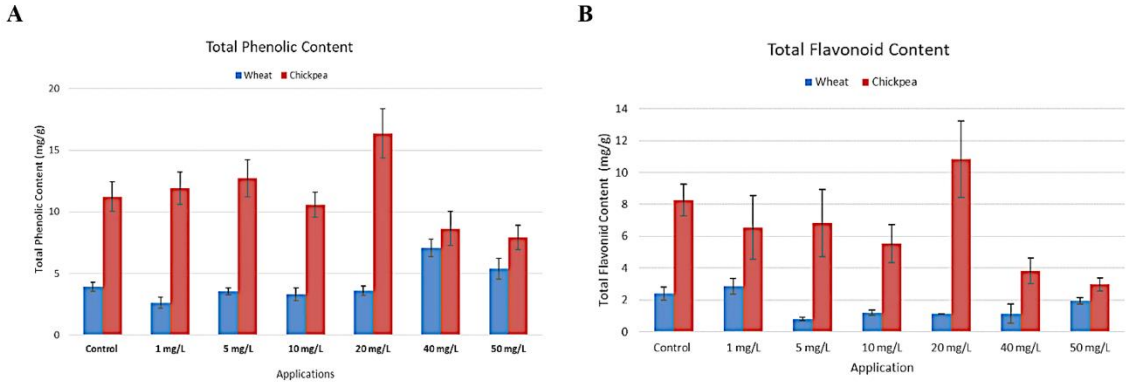

Fig 5. The effects of BPA applications (A) total phenolic content and (B) total flavonoid content.

highest carotenoid values for both wheat and chickpea seedlings were recorded in the 20 mg L^{-1} application (Fig. 4B).

3.2.3. Effects on phenolic and flavonoid content

Wheat and chickpea seedlings exhibit different reactions in their phenolic contents. While a significant increase was observed in wheat seedlings up to 20 mg L^{-1} application, significant decreases were detected at 40 mg and 50 mg L^{-1} applications. However, while no significant change was observed in chickpea seedlings until 20 mg L^{-1} application, significant increases were observed in 40 and 50 mg L^{-1} applications. The highest phenolic substance content was calculated when 20 mg L^{-1} was applied to wheat plants and 40 mg L^{-1} was applied to chickpea seedlings. (Fig. 5A).

Total flavonoid content in wheat seedlings decreased steadily with the increment of concentration, but in the 20 mg L^{-1} application, the flavonoid value was found to be higher than all applications, including the control. While decreases were observed in chickpea seedlings with 1-5 and 10 mg L^{-1} applications, no significant change was observed between other applications. (Fig. 5B).

3.2.4. Stomatal aperture effect on wheat leaves

Stomatal apertures in wheat plant leaves were determined by electron microscopy. Stoma analysis could not be performed on chickpea leaves because they were very poorly developed and had a gold coating problem depending on the leaf form.

In the electron microscopy analysis, it was observed that stomatal aperture was markedly open in the control, 1 mg L^{-1} and 5 mg L^{-1} concentrations of wheat leaves. In other applications, it was observed that the stomata were completely closed, especially in the application of 40 mg L^{-1} BPA (Fig. 6).

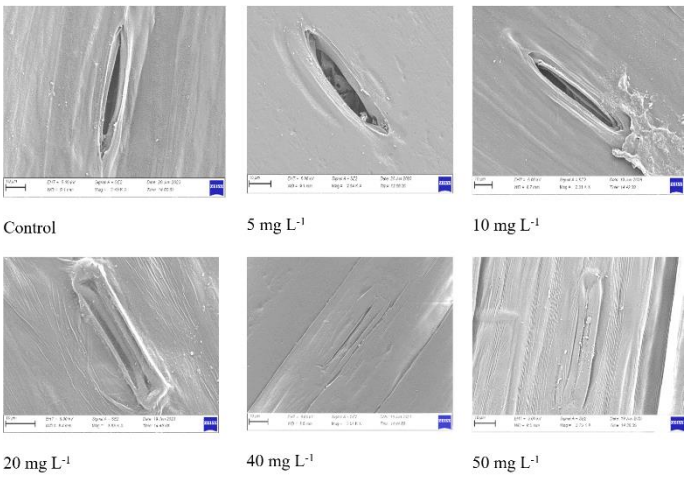

Fig 6. Stress-related stoma images of wheat by electron microscopy.

4. Discussion

Penetration of a radicle from the seed coat and growth into a new plant, known as seed germination marks the beginning of plant life. The germination period is the stage when plants are most sensitive. Therefore, seed germination is one of the established criteria often used to screen plant species for their tolerance to toxicity. According to earlier research, different BPA doses can have varying impacts on the germination of

seeds. BPA has concentration-dependent impacts on plant growth and performance, with low and high BPA concentrations observed to have promotion and inhibitory effects, respectively (Xiao et al., 2020). In our study, different concentrations of BPA caused variable effects on the germination of wheat and chickpea seeds. This may depend on many factors, such as the cotyledon status of the plants, the seed coat, and their genetic potential.

In the current study, it was determined that low doses of BPA did not inhibit germination, while high doses inhibited it. Recent study results revealed that BPA may have a stimulating effect at low concentrations and an inhibitory effect at high concentrations (Pan et al., 2013; Qiu et al., 2013). A BPA concentration of > 50 mg L^{-1} was found to inhibit the germination of seeds of *Arabidopsis thaliana* L. and *Cicer arietinum* L. (Dogan et al., 2010; Tian et al., 2014). This may be connected to greater BPA dosages inhibiting energy metabolism during seed germination (Xiao et al., 2020). In addition, it can be thought that BPA has a similar biological activity to gibberellic acid in low doses and shows a behavior that mimics this hormone. The use of multibiomarkers showed that a reduction in the plant hormone GA_3 is closely linked to the suppression of root growth. The amount of GA_3 was likewise reduced by BPA at the lowest concentration. Following a reduction in GA_3 and inhibition of root growth, H_2O_2 and O_2 generation as well as the activation of antioxidant defenses (Vujčić et al., 2023). Several possibilities have been suggested for BPA to affect seed germination. The first of these is that it has been reported that BPA activates the *PF3* gene of phytochromes with a helix-loop-helix transcription feature in seeds exposed to light during germination. Accordingly, it is suggested that seeds exposed to light generally have higher amounts of phytochrome A. The possible effect of BPA at high concentrations on phytochromes is thought to cause germination inhibition (Hanumappa et al., 1999; Pan et al., 2013).

Low-dose BPA stimulates stem cell elongation and proliferation and has a cytokinin-like effect, which promotes root growth (Terouchi et al., 2004). Also, low-dose BPA exposure promotes stem cell elongation and division, raising the amounts of the hormones gibberellin (GA), zeatin (ZT), and indole-3-acetic acid (IAA) in roots and controlling the growth of primary and lateral roots (Li et al., 2017). While decreases were observed in chlorophyll values in the study, a significant increase was observed, especially at the 20 mg L^{-1} dose. Interestingly, a similar situation was observed in the phenolic and flavonoid contents. This data indicates that the equivalent value of BPA in the seedlings is 20 mg L^{-1} and that the seedlings exhibit the highest response at this concentration. At other high doses (40 and 50 mg L^{-1}), it is seen that the seedlings are damaged and cause metabolic deterioration. BPA in high doses is related to the ROS (reactive oxygen species) effect. Exposure to high doses of BPA can lead to the accumulation of ROS (Ferrara et al., 2006; Tian et al., 2014). The thylakoid membrane and chloroplast structure are oxidized and damaged by the excess ROS, which also lowers the amount of chlorophyll and the efficiency with which light energy is absorbed and converted. These effects all have an impact on photosynthesis (Li et al., 2018). This situation also directly affects the stoma aperture. The stomatal aperture in wheat leaves is quite small at doses of 40 and 50 mg L^{-1}. In a study, it was stated that the possible mechanisms by which carotenoids play a role in reducing BPA stress in tobacco are related. It provided a new strategy to increase the phytoremediation efficiency of plants in BPA-contaminated soil (Fu et al., 2023). Growth indicators and chlorophyll content can be impacted by BPA in a dose-related way. When BPA is present in low concentrations (2 mg L^{-1}), it stimulates the growth of pea seedlings. However, when BPA is present in higher concentrations, it significantly inhibits the growth of pea seedlings. This is because the reduced chlorophyll content causes a decrease in photosynthesis (Siddqiui et al., 2022).

Research has indicated that when plants are exposed to BPA concentrations below 3.0 mg L^{-1}, their development is enhanced. These effects were associated with increased respiratory enzyme activity and mineral element absorption in roots (Ali et al., 2016; Xiao et al., 2019), chlorophyll synthesis (Jiao et al., 2015), and stomatal opening (Jiao et al., 2017). Additionally, improved photosynthetic system II (PS II) efficiency was linked to these effects (Zhang et al., 2015). However, more than 3.0 mg L^{-1} BPA exposure exhibited negative impacts on plant growth, as demonstrated by elevated ROS and lipid peroxidation levels as well as suppression of root and seedling elongation (Pan et al., 2013).

The current study and literature research present that BPA has different toxic effects not only on humans and animals but also on plants. Despite the increasing use of BPA, studies with plants are important for raising the awareness of future generations.

Conflict of interest: The authors declare that they have no conflict of interests.

Informed consent: The authors declare that this manuscript did not involve human or animal participants and informed consent was not collected.

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