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SELECTION OF CANOLA (*BRASSICA NAPUS*) BASED ON AGRONOMIC AND PHYSIOLOGICAL RESPONSES FOR BETTER GROWTH UNDER CADMIUM TOXICITY

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Abstract

Cadmium (Cd) is one of the potential toxins for plants. It disturbs the physiological and biochemical processes in plants, resulting in poor plant growth and yield of crops. As the complete removal of Cd toxicity from soil is quite difficult, scientists are working on selecting such tolerant varieties. These varieties cannot only provide the optimum crop yield but also can play an important role in the removal of Cd from the soil. Considering the importance of canola, a current study was planned to screen the Cd tolerant, moderate, and susceptible canola variety. For that total, 15 varieties were sown in a hydroponic experiment using different toxicity levels of Cd, i.e., tap water (no Cd), 0.2, 0.4, 0.6, and 0.8 mg/L. Results showed that CON-II and CON-III performance was significantly better for improvement in shoot length, root length, seedling fresh and dry weight than sandal canola, rainbow, and Oscar at all levels of Cd toxicity. A significantly improved chlorophyll content also validated the better tolerance of CON-II and CON-III over sandal canola, rainbow, and Oscar. The highest antioxidant activity and electrolyte leakage at 0.8Cd was noted in AARI canola, Oscar, sandal canola, and rainbow, which showed that these varieties were susceptible to Cd toxicity. In conclusion, CON-II and CON-III were observed as tolerant, sandal canola, rainbow, and Oscar were found susceptible while remaining as moderate canola varieties against Cd toxicity. More investigations are suggested at the pot and field level to declare the best tolerant, moderate, and highly susceptible canola varieties again Cd.

Key words: Heavy metals, Canola, Growth attributes, Antioxidant activity, Chlorophyll contents.

Introduction

Heavy metals are elements with high atomic weights that can be toxic to humans, plants, and soil when they accumulate in high concentrations (Khairiah *et al.*, 2004; Ali & Khan, 2018). The most common heavy metals that can cause toxicity include lead, mercury, cadmium, arsenic, and chromium (Awan *et al.*, 2022). Among all heavy metals, cadmium (Cd) is a heavy metal that is toxic to both humans and the environment (Azhar *et al.*, 2019; Li *et al.*, 2019; Wang *et al.*, 2023). It is widely distributed in the environment and can be found in soil, water, and air. It is particularly concerning because it can accumulate in the body over time, and even low levels of exposure can cause long-term health problems (Wang *et al.*, 2023). Cadmium is released into the environment through various industrial activities, such as mining, smelting, and refining metals like zinc, lead, and copper (Rahi *et al.*, 2022). These activities can contaminate soil, water, and air with cadmium (Wang *et al.*, 2023).

It can also accumulate in agricultural soils due to the use of fertilizers, sewage sludge, and irrigation water containing cadmium (Randhawa *et al.*, 2014). Crop uptake of cadmium in these soils can result in food contamination and human exposure (Genchi *et al.*, 2020). When humans consume food grown in cadmium-contaminated soil, they can be exposed to heavy metal accumulating in the body over time (Jinadasa *et al.*, 2015). Long-term exposure to cadmium has been linked to various health problems, including kidney damage, osteoporosis, and an increased risk of cancer (Genchi *et al.*, 2020).

Furthermore, exposure to cadmium can reduce plant growth and result in smaller plant size, reduced shoot and root growth, and impaired nutrient uptake (Sanità di Toppi & Gabbrielli, 1999; Zafar-ul-Hye *et al.*, 2018). Cadmium can also cause chlorosis, a yellowing of the leaves, by interfering with chlorophyll production (Das *et al.*, 1997). Reduced photosynthesis and cellular damage can further impair plant health, productivity, and quality. Moreover, cadmium toxicity can reduce seed production and viability, negatively affecting crop yields (Fang *et al.*, 2013; Abbas *et al.*, 2017). On the other hand, once plants absorb cadmium, it can be transferred to humans through the food chain (Das *et al.*, 1997).

In different crops, canola is an important oilseed crop cultivated worldwide and has significant economic and food importance (Daun, 2011; Wanasundara *et al.*, 2017). Canola seeds are a rich source of high-quality oil used for a wide range of food and industrial applications (Rempel *et al.*, 2014). The oil from canola seeds is low in saturated fat and high in monounsaturated and polyunsaturated fatty acids, which are heart-healthy and can help to reduce the risk of chronic diseases such as cardiovascular disease and diabetes (Rempel *et al.*, 2014; Wanasundara *et al.*, 2017).

Canola oil is also used for biodiesel production, a renewable and environmentally friendly alternative to traditional fossil fuels (Issariyakul *et al.*, 2008). The cultivation of canola for biodiesel production has the potential to reduce greenhouse gas emissions and promote sustainable energy production (Issariyakul & Dalai, 2010). In addition to its economic importance, canola cultivation plays an important role in global food security. Canola seeds are used as a feedstock for animal feed, essential for producing meat, dairy, and other animal products (Wanasundara *et al.*, 2017).

Selecting cadmium-tolerant varieties is an important technology because cadmium contamination in soil is a persistent problem and difficult to eliminate. Therefore, it is important to identify canola varieties that can grow in contaminated soils and produce low-cadmium seeds, which can help to ensure food safety and protect human health. However, there is a knowledge gap in selecting cadmium-tolerant canola varieties, as the mechanisms underlying cadmium tolerance in canola are not fully understood. That is why the current study was planned to select the tolerant, moderate, and susceptible canola varieties. This study will be helpful to growers for the achievement of maximum yield of canola under variable levels of Cd toxicity.

Material and Methods

An experiment using the hydroponics technique was carried out in the laboratory of Institute of Botany, Bahauddin Zakariya University Multan, Punjab, Pakistan to explore the resistant, moderate, and sensitive canola varieties based on their growth attributes under varying levels of cadmium toxicity.

Canola varieties: In preparation for the experiment, 15 types of canola were sourced from the Ayub Agriculture Research Institute, namely Dunkled, Super Raya, Oscar, Rainbow, Punjab Canola, Legend, AARI Canola, AC Exul, CON-II, Cyclone, Faisalabad Canola, CON-III, Super Canola, Sandal Canola, and Shiralee. To ensure the quality of the seeds used, a manual screening process was conducted to remove any damaged ones before the start of the experiment.

Cadmium solution development: As per treatment plant $CdSO_4$ (Sigma-Aldrich, Batch Number: MKCS8778, MDL Number: MFCD00010923, Color: White, Form: Powder) was used for maintenance of 0.2, 0.4, 0.6 and 0.8 mg Cd/L of solution. For control, tap water was used in which Cd was not detectable.

Treatment plan: The treatment plan includes: control (no Cd and 1000 ml of tap water only), 0.2Cd (0.2mg Cd (0.37mg of $CdSO_4$) per 1000 ml of tap water), 0.4Cd (0.4mg (0.74mg of $CdSO_4$) Cd per 1000 ml of tap water), 0.6Cd (0.6mg Cd (1.11mg of $CdSO_4$) per 1000 ml of tap water) and 0.8Cd (0.8mg Cd (1.48mg of $CdSO_4$) per 1000 ml of tap water).

Sowing and incubation conditions: Ten sterilized petri dishes were used for sowing the seeds for each of the three replicates, and sterilized filter papers were placed between the seeds to ensure the best possible germination conditions. The petri dishes were kept in an incubator at a constant temperature of 25°C ± 3°C for the entire experiment. To facilitate the germination and growth of the seeds, the humidity within the incubator was maintained at 70% throughout the experiment.

Table 1. Pre-experimental characteristics of tap water.

Attribute	Unit	Value
pH	-	0.68
EC	dS/m	0.23
Carbonates	meq./L	0.00
Bicarbonates		2.49
Calcium + Magnesium		1.91
Chloride		0.1
Sodium	mg/L	27
Cadmium		Not detectable

Data collection and harvesting: The number of seeds that sprouted on the third and seventh day after sowing were counted to determine the germination. The first harvest was carried out 15 days after sowing, during which three healthy seedlings were selected from each petri dish. The length of the roots and shoots were measured using a standard measuring scale. In contrast, the fresh weights of the roots and shoots were measured using an analytical-grade balance. The shoot and root samples were then dried in an oven for 48 hours at 65°C, after which their dry weights were determined by re-weighing them using an analytical-grade balance (Mills & Jones, 1991).

Transplantation: Afterwards, the remaining seedlings from the petri dishes were transplanted into plastic cups that were 3 inches in diameter and 5 inches in depth containing water. To support the shoots of the seedlings, a rolled filter paper was placed beneath them. The experiment was continued for a total of 30 days after sowing.

Harvesting for antioxidants analysis: During the second harvest, after 30 days of sowing, three healthy seedlings were selected from each petri dish (Mills & Jones, 1991). Fresh leaf samples were collected from these seedlings to analyze total antioxidant activity with DPHH (2,2-diphenyl-1-picrylhydrazyl) using a spectrophotometer (Brand-Williams *et al.*, 1995).

Chlorophyll contents: First, small pieces of plant tissue, typically leaves, were collected and homogenized with 80% acetone using a mortar and pestle. The homogenate was filtered through Whatman filter paper No. 42 to remove debris or insoluble material. The filtrate was collected in a test tube and diluted with 80% acetone to bring the volume 5ml. The absorbance of the solution was measured at two different wavelengths, 663 nm, and 645 nm, using a spectrophotometer (Arnon, 1949). The equation used to calculate chlorophyll content was as follows:

$$\text{Chlorophyll a}\left(\frac{\text{mg}}{\text{g}}\right) = \frac{(12.7 \times \text{A663}) - (2.69 \times \text{A645}) \times \text{V}}{1000 \times \text{W}}$$

$$\text{Chlorophyll b}\left(\frac{\text{mg}}{\text{g}}\right) = \frac{(22.9 \times \text{A645}) - (4.68 \times \text{A645}) \times \text{V}}{1000 \times \text{W}}$$

$$\text{Total Chlorophyll}\left(\frac{\text{mg}}{\text{g}}\right) = 20.2(\text{OD } 645) + 8.02(\text{OD } 663) \times \text{V}/1000\ (\text{W})$$

where W is the weight of leaf samples, and V is the final volume made

Electrolyte leakage: Initially, small portions of plant tissue, usually leaf tissue, were cut and rinsed with distilled water to remove any surface impurities. Next, these tissue pieces were placed in test tubes with distilled water and kept at room temperature for about 2-3 hours to allow for equilibration. The starting conductivity of the solution was then measured with a conductivity meter, denoted as C1, and recorded. After that, the test tubes were immersed in a water bath maintained at 40-50°C for 30 minutes to stimulate electrolyte leakage. Following 30 minutes, the test tubes were removed from the water bath and allowed to cool down to room temperature. Then, the final conductivity of the solution was measured, denoted as C2 (Lutts *et al.*, 1996). The proportion of electrolyte leakage was then calculated with the formula:

$$\text{Electrolyte Leakage (\%)} = \frac{(\text{C2} - \text{C1})}{\text{C1}} \times 100$$

Cadmium analysis: The di-acid digestion method was used to extract Cd from root and shoot samples for analysis. The plant material was initially washed thoroughly with deionized water to remove any surface contamination. The root and shoot samples were then oven-dried separately at 70°C for 48 hours till a constant weight was obtained. The dry weight of the samples was recorded using an analytical balance. Di-acid digestion involves using nitric acid and perchloric acid to dissolve the plant material. The procedure was carried out in a fume hood due to the corrosive nature of the acids. Approximately 0.2 g of dry plant material was weighed and transferred into a digestion tube. Subsequently, 5 ml of di-acid mixture (3:1, v/v) was added to the tube, which was covered with a watch glass. The mixture was allowed to stand for 24 hours to facilitate complete digestion of the plant material. The digestion tube was then heated on a hot plate at 280°C under a fume hood until the solution became clear and colorless. The temperature was increased gradually to avoid any spattering of the acid mixture. After digestion, the solution was cooled and transferred quantitatively to a 50 ml volumetric flask. The flask was then filled to the mark with deionized water (Miller, 1998). Finally, samples were run on a pre-calibrated atomic absorption spectrophotometer to compute Cd in digested samples (Hanlon, 1998).

Statistical analyses: The data were subjected to standard statistical analysis procedures (Steel *et al.*, 1997). To compare the treatments, a two-way analysis of variance (ANOVA) was conducted, followed by Fisher's least significant difference test (LSD) at a significance level of $p \leq 0.05$. Principal component analysis was used to establish correlations among the canola varieties, Cd toxicity levels, and the studied attributes. The data analysis and graph creation were done using OriginPro2021 software (OriginLab Corporation, 2021).

Results

Germination: For 3rd day of germination, all varieties showed a decrease in percentage germination compared to the tap water treatment. The CON-II variety had the highest germination percentage in tap water at 82.67%. However, it decreased to 81.46%, 80.68%, 79.08%, and 77.69% at Cd concentrations of 0.2, 0.4, 0.6, and 0.8, respectively. Cyclone variety showed the second highest germination percentage of 71.98% in tap water. However, it decreased to 71.61%, 71.49%, 71.09%, and 70.93% at Cd concentrations of 0.2, 0.4, 0.6, and 0.8, respectively. Punjab Canola variety had a germination percentage of 70.06% in tap water, which decreased to 69.66%, 69.40%, 69.15%, and 68.95% at Cd concentrations of 0.2, 0.4, 0.6, and 0.8, respectively. The Legend variety, which had a germination percentage of 66.40% in tap water, decreased to 65.33%, 64.97%, 64.59%, and 64.28% at Cd concentrations of 0.2, 0.4, 0.6, and 0.8, respectively. Super Raya had a germination percentage of 24.67% in tap water and decreased to 21.67%, 18.67%, 15.33%, and 11% at Cd concentrations of 0.2, 0.4, 0.6, and 0.8, respectively. CON-III variety had a germination percentage of 77.21% in tap water, which decreased to 76.75%, 75.94%, 75.23%, and 74.73% at Cd concentrations of 0.2, 0.4, 0.6, and 0.8, respectively. The Oscar, Rainbow, Super Canola, and Sandal Canola varieties showed no germination in tap water and Cd treatments. Shiralaee variety had a germination percentage of 63.76% in tap water, which decreased to 63.46%, 62.88%, 62.49%, and 62.05% at Cd concentrations of 0.2, 0.4, 0.6, and 0.8 respectively. Dunkled variety had a germination percentage of 79.94% in tap water, which decreased to 77.40%, 73.79%, 73.47%, and 72.06% at Cd concentrations of 0.2, 0.4, 0.6, and 0.8 respectively. AC Exul variety had a germination percentage of 53.87% in tap water, which decreased to 53.58%, 53.11%, 52.69%, and 52.24% at Cd concentrations of 0.2, 0.4, 0.6, and 0.8 respectively. Faisalabad Canola had a germination percentage of 35.84% in tap water, which decreased to 35.44%, 35.18%, 34.61%, and 33.98% at Cd concentrations of 0.2, 0.4, 0.6, and 0.8 respectively. Overall, the results suggest that Cd concentrations negatively affect the germination of all varieties (Fig. 1A).

After seven days of germination, the results show a decrease in the percentage of germination for most varieties when compared to tap water. CON-II had a 0.77% decrease when treated with 0.2Cd, a 6.96% decrease with 0.6Cd and an 11% decrease with 0.8Cd. Cyclone showed no change in germination with any of the treatments. Punjab Canola showed no change in germination with any of the treatments. Legend showed a 2.67% decrease with tap water, a 4.11% decrease with 0.2Cd, a 4.11% decrease with 0.4Cd, a 4.11% decrease with 0.6Cd and a 4.11% decrease with 0.8Cd. Super Raya showed a significant decrease in germination with tap water (49%), and the same percentage of decrease with all Cd treatments. CON-III had a 6.99% decrease with 0.4Cd and a 7.71% decrease with 0.6Cd. Oscar had a 50% decrease in germination with tap water and no change with treatments. Shiralaee had a 2.33% decrease with tap water, a 4.11% decrease with 0.2Cd, a 4.11% decrease with 0.4Cd, a 4.11% decrease with 0.6Cd and a 4.11% decrease with 0.8Cd. Dunkled showed a 0.67% decrease with tap water, a 1.77% decrease with 0.2Cd, a 5.06% decrease with 0.4Cd, a 5.71% decrease with 0.6Cd and a 6.42% decrease with 0.8Cd. AC Exul showed no change in germination with any of the treatments. Rainbow had a 40% decrease in germination with tap water and no change with treatments. AARI Canola showed no change in germination with any of the treatments. Super canola had a 2.33% decrease with tap water, a 4.11% decrease with 0.2Cd, a 4.11% decrease with 0.4Cd, a 4.11% decrease with 0.6Cd and a 10.67% decrease with 0.8Cd. Sandal Canola had a 40% decrease in germination with tap water and no change with treatments. Faisalabad Canola showed a 3.33% decrease with tap water, 11.67% decrease with 0.6Cd and 11% decrease with 0.8Cd (Fig. 1B).

Shoot and root length: In tap water, CON-II exhibited the longest shoot length at 10.72 cm, followed by Cyclone at 7.51 cm, Punjab Canola at 7.12 cm, Legend at 6.69 cm, and Super Raya at 5.02 cm. The remaining varieties had shoot lengths between 3.31 and 9.84 cm. At a concentration of 0.2Cd, CON-II again had the longest shoot length at 9.98 cm, followed by Cyclone at 7.45 cm, Punjab Canola at 7.08 cm, Legend at 6.64 cm, and Super Raya at 4.97 cm. All varieties except Rainbow had a decreased shoot length compared to tap water. The percentage decrease in shoot length ranged from 4.4% for Oscar to 37.3% for Super Raya. At a concentration of 0.4Cd, CON-II had the longest shoot length at 9.54 cm, followed by Cyclone at 7.38 cm, Punjab Canola at 7.01 cm, Legend at 6.62 cm, and Super Raya at 4.88 cm. All varieties except Rainbow had a decreased shoot length compared to tap water. The percentage decrease in shoot length ranged from 4.6% for AC Exul to 36.9% for Super Raya. At a concentration of 0.6Cd, CON-II had the longest shoot length at 9.27 cm, followed by Cyclone at 7.33 cm, Punjab Canola at 6.97 cm, Legend at 6.58 cm, and Super Raya at 4.80 cm. All varieties except Rainbow had a decreased shoot length compared to tap water. The percentage decrease in shoot length ranged from 6.0% for AC Exul to 42.2% for Super Raya. At a concentration of 0.8Cd, CON-II had the longest shoot length at 9.08 cm, followed by Cyclone at 7.21 cm, Punjab Canola at 6.79 cm, Legend at 6.55 cm, and Super Raya at 4.75 cm. All varieties except Rainbow had a decreased shoot length compared to tap water. The percentage decrease in shoot length ranged from 6.5% for AC Exul to 43.1% for Super Raya (Fig. 2A).

Increasing the concentration of Cd in water is associated with a decrease in the root length of canola varieties. Under 0.2Cd concentration, the root length of CON-II, Cyclone, Punjab Canola, Legend, Super Raya, CON-III, Shiralaee, Dunkled, AC Exul, AARI Canola, Super Canola, Sandal Canola, and Faisalabad Canola decreased by 7.1%, 0.2%, 0.7%, 0.9%, 1.6%, 2.9%, 1.0%, 8.4%, 0.8%, 1.2%, 1.4%, 3.5%, and 2.8%, respectively, compared to tap water. At 0.4Cd concentration, the root length of CON-II, Cyclone, Punjab Canola, Legend, Super Raya, CON-III, Shiralaee, Dunkled, AC Exul, AARI Canola, Super Canola, Sandal Canola, and Faisalabad Canola decreased by 11.4%, 0.5%, 1.6%, 1.4%, 3.4%, 2.4%, 2.2%, 17.4%, 1.6%, 3.1%, 2.8%, 6.3%, and 3.8%, respectively, compared to tap water. For 0.6Cd concentration, the root length of CON-II, Cyclone, Punjab Canola, Legend, Super Raya, CON-III, Shiralaee, Dunkled, AC Exul, AARI Canola, Super Canola, Sandal Canola, and Faisalabad Canola decreased by 13.4%, 3.5%, 2.8%, 2.2%, 5.0%, 4.9%, 3.2%, 20.0%, 2.9%, 4.1%, 4.8%, 7.9%, and 5.5%, respectively, compared to tap water. Under 0.8Cd concentration, the root length of CON-II, Cyclone, Punjab Canola, Legend, Super Raya, CON-III, Shiralaee, Dunkled, AC Exul, AARI Canola, Super Canola, Sandal Canola, and Faisalabad Canola decreased by 14.4%, 5.2%, 3.7%, 2.9%, 6.0%, 5.7%, 4.1%, 21.7%, 4.1%, 4.5%, 6.0%, 11.6%, and 7.5%, respectively, compared to tap water. The extent of decrease varies among the different varieties. CON-II and AC Exul are the most and least sensitive varieties, respectively, to Cd toxicity regarding root length (Fig. 2B).

Seedlings fresh and dry biomass: The results showed that the seedlings grown in tap water had varying fresh biomass levels. The highest fresh biomass was recorded for CON-II with 0.08267 g, followed by Dunkled with 0.07633 g, and the lowest fresh biomass was recorded for Rainbow with 0.02 g. In water spiked with 0.2Cd, the highest fresh biomass was recorded for CON-II with 0.07581 g, followed by Dunkled with 0.06892 g, and the lowest fresh biomass was recorded for Rainbow with 0.02 g. Similarly, in water spiked with 0.4Cd, the highest fresh biomass was recorded for CON-II with 0.07196 g, followed by Dunkled with 0.061 g, and the lowest fresh biomass was recorded for Rainbow with 0.02 g. At a Cd concentration of 0.6, most cultivars showed a decrease in fresh biomass compared to the tap water control. However, the Cyclone, CON-III, and Dunkled cultivars showed increased fresh biomass at this Cd concentration. Finally, at a Cd concentration of 0.8, the fresh biomass of most cultivars decreased compared to the tap water control. The cultivars CON-II and Cyclone had the highest fresh biomass at this Cd concentration, with values of 0.07 g. Rainbow, Super Canola, Sandal Canola, and Oscar again had the lowest fresh biomass, all with values of 0.03 g or lower. Overall, it can be observed that most cultivars' fresh biomass decreased as the Cd concentration increased. However, there were some exceptions to this trend, with some cultivars showing an increase in fresh biomass at certain Cd concentrations. In the case of CON-II variety, the seedling fresh biomass decreased by 8.31% at 0.2Cd concentration, 12.87% at 0.4Cd concentration, 14.91% at 0.6Cd concentration, and 15.39% at 0.8Cd concentration when compared to the Tap Water control. For Cyclone variety, the seedling fresh biomass decreased by 1.12% at 0.2Cd concentration, 1.67% at 0.4Cd concentration, 11.33% at 0.6Cd concentration, and 12.42% at 0.8Cd concentration when compared to the Tap Water control. In the case of Punjab Canola variety, the seedling fresh biomass decreased by 0.29% at 0.2Cd concentration, 2.10% at 0.4Cd concentration, 2.10% at 0.6Cd concentration, and 2.10% at 0.8Cd concentration when compared to the Tap Water control. There was no change in the seedling fresh biomass for Legend variety at any cadmium concentration compared to the Tap Water control. In the case of Super Raya variety, the seedling fresh biomass decreased by 0.83% at 0.2Cd concentration, 3.37% at 0.4Cd concentration, 19.93% at 0.6Cd concentration, and 22.10% at 0.8Cd concentration when compared to the Tap Water control. For CON-III variety, the seedling fresh biomass decreased by 1.43% at 0.2Cd concentration, 1.43% at 0.4Cd concentration, 7.69% at 0.6Cd concentration, and 8.91% at 0.8Cd concentration when compared to the Tap Water control. In the case of Oscar and Shiralaee varieties, there was no change in the seedling fresh biomass at any cadmium concentration compared to the Tap Water control. For Dunkled variety, the seedling fresh biomass decreased by 9.65% at 0.2Cd concentration, 20.08% at 0.4Cd concentration, 20.43% at 0.6Cd concentration, and 23.41% at 0.8Cd concentration when compared to the Tap Water control (Fig. 3A).

In the case of the CON-II variety, a 6.24% decrease was observed in seedlings dry biomass when exposed to 0.2Cd and a 13.73% decrease when exposed to 0.4Cd, 17.65% decrease for 0.6Cd and 18.9% decrease for 0.8Cd. For Cyclone variety, there was a 0.28% decrease in seedling's dry biomass when exposed to 0.2Cd and a 1.34% decrease when exposed to 0.4Cd, 3.57% decrease for 0.6Cd and a 5.26% decrease for 0.8Cd. Punjab Canola did not decrease at 0.2Cd but showed a 4.76% decrease when exposed to 0.4Cd, 4.76% decrease for 0.6Cd and 4.76% decrease for 0.8Cd. For Legend, there was no decrease in any cadmium concentration in seedling's dry biomass. The Super Raya variety showed a 3.63% decrease for 0.2Cd, 6.8% for 0.4Cd, 14.29% for 0.6Cd, and 14.1% for 0.8Cd in seedlings dry biomass. CON-III had a 1.45% decrease for 0.2Cd, 0% decrease for 0.4Cd, 2.54% decrease for 0.6Cd, and 0% decrease for 0.8Cd. The Oscar variety did not decrease in any cadmium concentration in seedling's dry biomass. Shiralaee and AC Exul also showed no decrease in any cadmium concentration. Dunkled showed a 13.08% decrease for 0.2Cd, 16.58% decrease for 0.4Cd, 13.87% decrease for 0.6Cd, and 14.81% decrease for 0.8Cd. Rainbow had a 0% decrease at any cadmium concentration. AARI Canola had a 5.88% decrease for 0.4Cd and 6.25% decrease for 0.6Cd, and 5.88% decrease for 0.8Cd in seedlings dry biomass. Super Canola and Sandal Canola showed no decrease in any cadmium concentration. Faisalabad Canola had a 6.25% decrease for 0.4Cd, 6.25% decrease for 0.6Cd, and 6.25% decrease for 0.8Cd. Overall, the results indicated that exposure to cadmium concentrations had a negative impact on seedling dry biomass, with some varieties being more sensitive than others (Fig. 3B).

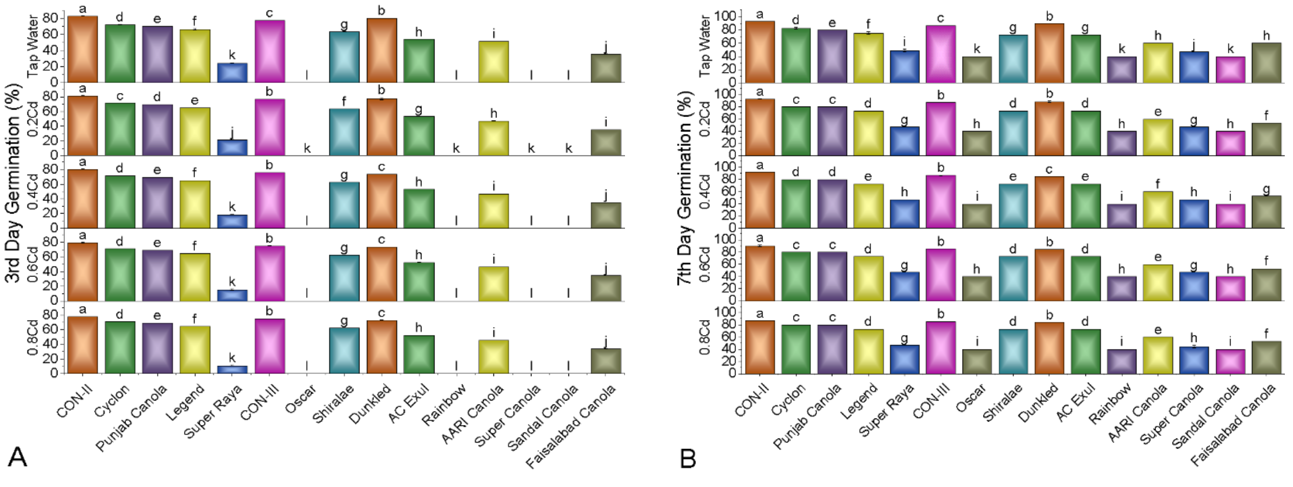

Fig. 1. Effect of different toxicity levels of cadmium (control tap water, 0.2, 0.4, 0.6 and 0.8 µg/g) on 3rd day (A) and 7th day germination (B) of 15 canola varieties. Bars are means of three replicates ± SE. Different letters were obtained by applying Fisher LSD. These letters showed significant alteration at p≤0.05.

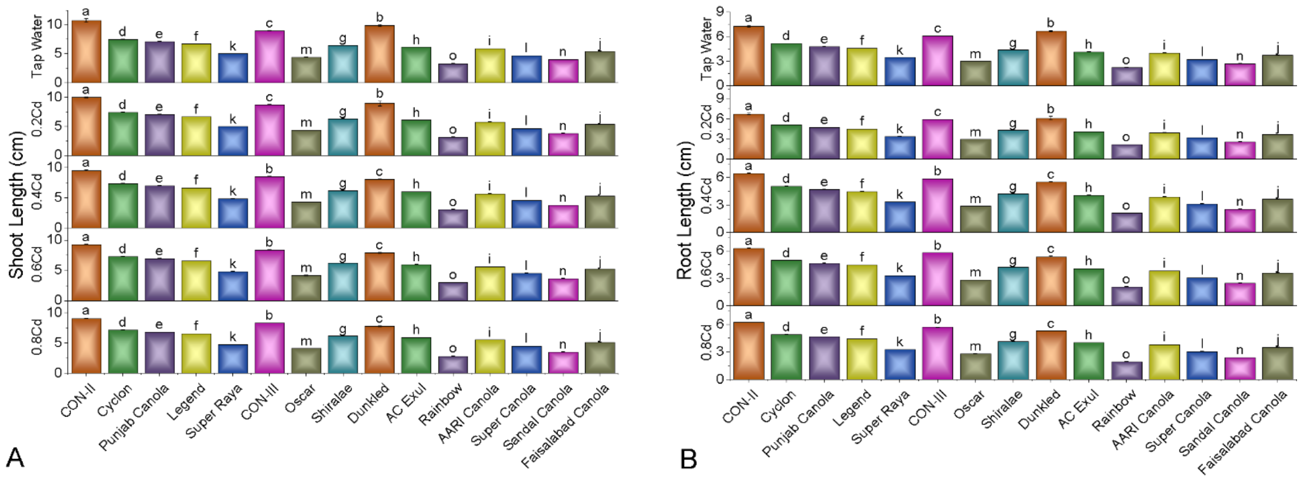

Fig. 2. Effect of different toxicity levels of cadmium (control tap water, 0.2, 0.4, 0.6 and 0.8 µg/g) on shoot length (A) and root length (B) of 15 canola varieties. Bars are means of three replicates ± SE. Different letters were obtained by applying Fisher LSD. These letters showed significant alteration at p≤0.05.

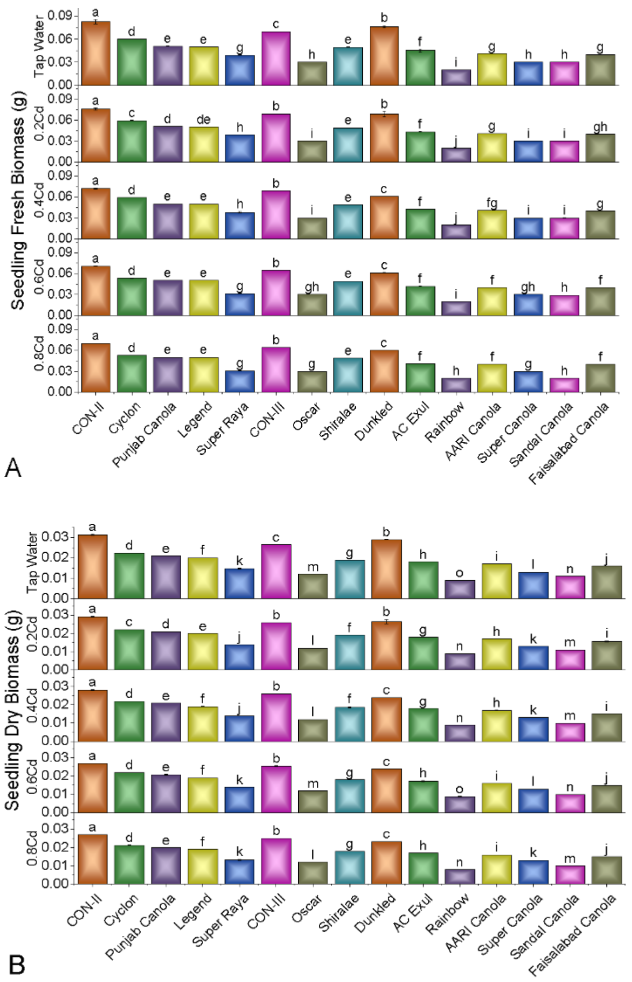

Fig. 3. Effect of different toxicity levels of cadmium (control tap water, 0.2, 0.4, 0.6 and 0.8 µg/g) on seedlings fresh biomass (A) and seedlings dry biomass (B) of 15 canola varieties. Bars are means of three replicates ± SE. Different letters were obtained by applying Fisher LSD. These letters showed significant alteration at p≤0.05.

Chlorophyll contents: Comparing the results of each variety in tap water, it can be seen that CON-II had the highest level of chlorophyll a (1.32 mg/g). In comparison, Rainbow had the lowest 0.55 mg/g. When the plants were exposed to 0.2Cd concentration, there was a decrease in the level of chlorophyll a in all varieties compared to tap water. The percentage decrease in chlorophyll a ranged from 1.07% for AARI Canola to 35.97% for Rainbow. Cyclone, Punjab Canola, and Legend decreased 0.64%, 1.96%, and 4.51%, respectively. On the other hand, Super Raya showed the highest percentage decrease of 2.74%, indicating that it may be more susceptible to cadmium toxicity. When the concentration of cadmium was increased to 0.4Cd, there was a further decrease in the level of chlorophyll a in all varieties. The percentage decrease ranged from 1.5% for CON-II to 47.24% for Faisalabad Canola. Similar to the previous results, Super Raya showed the highest percentage decrease of 6.08%. In comparison, Punjab Canola showed the least decrease of 0.64%. At 0.6Cd concentration, the percentage decrease in chlorophyll a was even greater, ranging from 1.25% for CON-II to 46.25% for Faisalabad Canola. Cyclone showed the least decrease of 4.27%, while Rainbow had the highest with a decrease of 35.62%. Finally, at the highest concentration of 0.8Cd, all varieties showed a significant decrease in chlorophyll a levels. The percentage decrease ranged from 1.68% for CON-II to 48.56% for Faisalabad Canola. Cyclone, Punjab Canola, and Super Raya decreased 3.77%, 1.87%, and 3.77%, respectively. Like the previous results, Rainbow showed the highest percentage decrease of 39.28% (Fig. 4A).

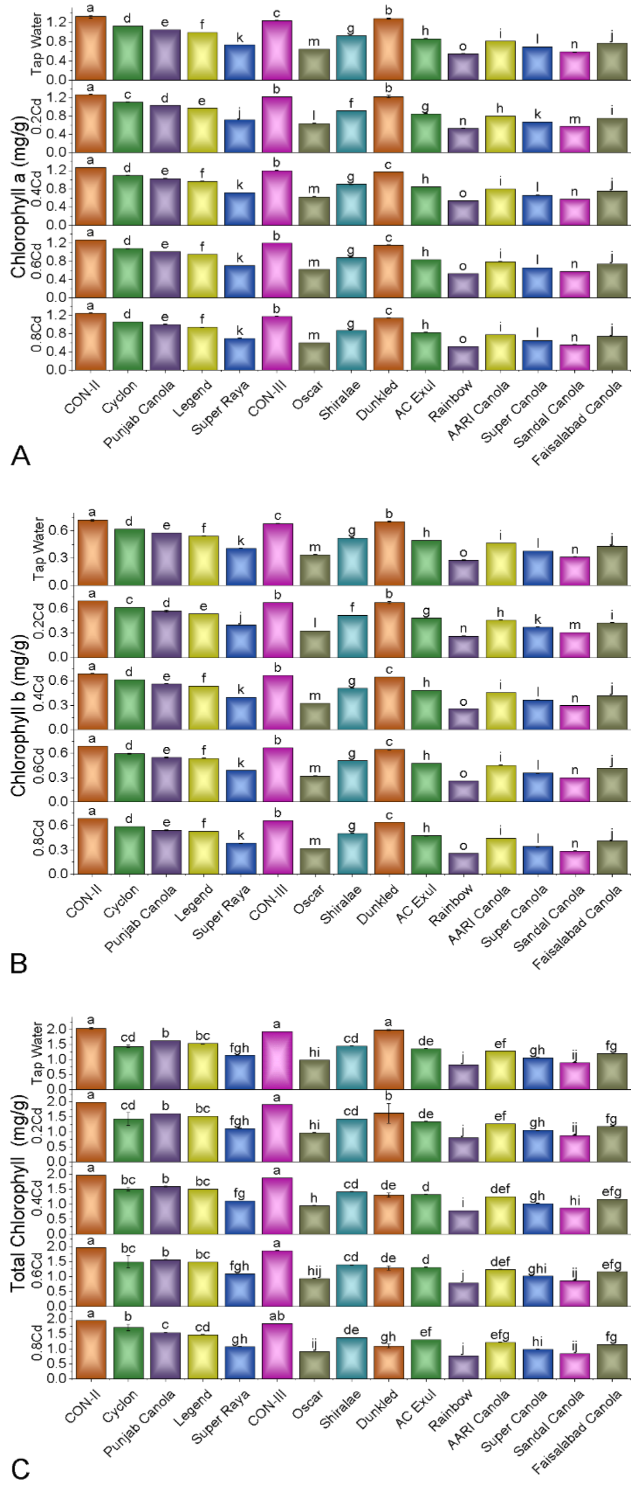

Fig. 4. Effect of different toxicity levels of cadmium (control tap water, 0.2, 0.4, 0.6 and 0.8 µg/g) on chlorophyll a (A) chlorophyll b (B) and total chlorophyll (C) of 15 canola varieties. Bars are means of three replicates ± SE. Different letter were obtained by applying Fisher LSD. These letters showed significant alteration at p≤0.05.

The tap water (control) showed a range of chlorophyll b content from 0.27 to 0.72 mg/g. Among the varieties, CON-II showed the highest chlorophyll b content in tap water, while Rainbow had the lowest. At 0.2Cd exposure, the chlorophyll b content ranged from 0.26 to 0.7 mg/g. The highest content was observed in CON-II and the lowest in Rainbow. The chlorophyll b content decreased in all varieties compared to the tap water control, with a percentage decrease ranging from 2.78% in CON-III to 60.88% in Punjab Canola. For 0.4Cd exposure, the chlorophyll b content ranged from 0.26 to 0.69 mg/g. CON-II had the highest chlorophyll b content, while Rainbow had the lowest. The chlorophyll b content decreased in all varieties compared to the tap water control, with a percentage decrease ranging from 1.96% in CON-II to 63.33% in Punjab Canola. Under 0.6Cd exposure, the chlorophyll b content ranged from 0.3 to 0.69 mg/g. CON-II had the highest chlorophyll b content, while Rainbow had the lowest. The chlorophyll b content decreased in all varieties compared to the tap water control, with a percentage decrease ranging from 0.14% in Legend to 57.7% in Punjab Canola. At 0.8Cd exposure, the chlorophyll b content ranged from 0.33 to 0.69 mg/g. CON-II had the highest chlorophyll b content, while Super Raya had the lowest. The chlorophyll b content decreased in all varieties compared to the tap water control, with a percentage decrease ranging from 4.17% in CON-II to 47.22% in Super Raya. Overall, the chlorophyll b content decreased in all varieties as the level of cadmium exposure increased (Fig. 4B).

The chlorophyll content of the tap water ranged from 0.82688 mg/g in Rainbow to 2.04063 mg/g in CON-II. Oscar showed the lowest chlorophyll content among the varieties at 0.98895 mg/g. Super Raya had the highest content at 1.1388 mg/g. When exposed to 0.2Cd, most varieties showed a decrease in chlorophyll content compared to tap water, ranging from 1.61292 mg/g in Punjab Canola to 0.80521 mg/g in Rainbow. Cyclone greatest reduction was observed, with a decrease of 0.0088 mg/g or 61.59% compared to tap water. Similarly, exposure to 0.4Cd caused a reduction in chlorophyll content, with the greatest decrease in Dunkled by 0.67465 mg/g or 34.02% compared to tap water. In contrast, some varieties, such as AC Exul and Super Canola showed increased chlorophyll content at this concentration. At 0.6Cd, most varieties showed a decrease in chlorophyll content, with the greatest reduction observed in Cyclone by 0.9525 mg/g or 63.72% compared to tap water. In case of each variety, At 0.2Cd, CON-II had a 2.99% decrease, Cyclone had a 0.69% decrease, Punjab Canola had a 1.20% decrease, Legend had a 0.81% decrease, Super Raya had a 1.60% decrease, CON-III had a 0.87% decrease, Oscar had a 1.43% decrease, Shiralaee had a 0.21% decrease, Dunkled had a 18.37% decrease, AC Exul had a 1.16% decrease, Rainbow had a 2.17% decrease, AARI Canola had a 1.23% decrease, Super Canola had a 1.53% decrease, and Sandal Canola had a 1.87% decrease. In case of 0.4Cd, CON-II had a 3.26% decrease, Cyclone had a 4.97% decrease, Punjab Canola had a 2.45% decrease, Legend had a 1.49% decrease, Super Raya had a 2.30% decrease, CON-III had a 2.53% decrease, Oscar had a 3.69% decrease, Shiralaee had a 2.25% decrease, Dunkled had a 34.16% decrease, AC Exul had a 1.60% decrease, Rainbow had a 3.47% decrease, AARI Canola had a 1.56% decrease, Super Canola had a 3.63% decrease, and Sandal Canola had a 2.34% decrease. Under 0.6Cd, CON-II had a 3.47% decrease, Cyclone had a 3.14% decrease, Punjab Canola had a 3.98% decrease, Legend had a 2.80% decrease, Super Raya had a 4.07% decrease, CON-III had a 3.16% decrease, Oscar had a 4.62% decrease, Shiralaee had a 3.93% decrease, Dunkled had a 35.00% decrease, AC Exul had a 3.45% decrease, Rainbow had a 4.94% decrease, AARI Canola had a 2.99% decrease, Super Canola had a 5.04% decrease, and Sandal Canola had a 3.18% decrease. At 0.8Cd, CON-II had a 6.61% decrease, Cyclone had a 7.93% decrease, Punjab Canola had a 6.74% decrease, Legend had a 5.31% decrease, Super Raya had a 7.40% decrease, CON-III had a 5.70% decrease, Oscar had a 7.66% decrease, Shiralaee had a 6.60% decrease, Dunkled had a 39.09% decrease, AC Exul had a 7.44% decrease, Rainbow had a 8.10% decrease, AARI Canola had a 5.70% decrease, Super Canola had a 8.03% decrease, and Sandal Canola had a 5.68% decrease (Fig. 4C).

Electrolyte leakage and total antioxidant activity: The results showed that all varieties experienced an increase in electrolyte leakage under cadmium stress, with the percentage increase varying depending on the concentration of cadmium and the plant's genotype. At 0.2Cd, most varieties showed a relatively small increase in electrolyte leakage compared to tap water. Among the varieties tested, AC Exul exhibited the largest percentage increase of 65%, followed by Punjab Canola and Cyclon, with an increase of 3% and 32%, respectively. In contrast, Super Raya showed no increase in electrolyte leakage at this cadmium concentration. Under 0.4Cd, the percentage increase in electrolyte leakage was generally higher than at 0.2Cd. The most tolerant varieties at this concentration were CON-II, CON-III, and Dunkled, which exhibited a small increase in electrolyte leakage. The most susceptible varieties were Rainbow, AARI Canola, and Sandal Canola, which showed a percentage increase of over 50%. The rest of the varieties had a moderate increase in electrolyte leakage, ranging from 14% in Legend to 53% in AC Exul. In the case of 0.6Cd, the percentage increase in electrolyte leakage was even higher than at 0.4Cd, with most varieties showing a significant increase. Oscar and Shiralaee were the most tolerant varieties at this concentration, with a percentage increase of only 2% and 4%, respectively. The most susceptible variety was Faisalabad Canola, which showed a percentage increase of 35%. The rest of the varieties had a moderate to high increase in electrolyte leakage, ranging from 18% in Legend to 58% in AARI Canola. At 0.8Cd, the percentage increase in electrolyte leakage was the highest among all cadmium concentrations tested. Super Raya and CON-II remained relatively tolerant at this concentration, with a percentage increase of only 1% and 3%, respectively. In contrast, Rainbow, AARI Canola, Sandal Canola, and Faisalabad Canola all showed a percentage increase of over 80%, indicating severe damage to cell membranes. The rest of the varieties had a moderate to high increase in electrolyte leakage, ranging from 16% in Legend to 75% in Oscar (Table 2).

The total antioxidant percentage for tap water ranged from 17% to 48%. Among the tested varieties, Faisalabad Canola had the highest total antioxidant percentage at 48%, while CON-II had the lowest at 17%. At a cadmium concentration of 0.2Cd, the total antioxidant levels ranged from 19% to 60%. Among the tested varieties, Punjab Canola showed the highest increase in total antioxidant percentage compared to tap water at 210%. In comparison, CON-II showed the lowest increase at 12%. Under a cadmium concentration of 0.4Cd, the total antioxidant levels ranged from 20% to 59%. Among the tested varieties, Punjab Canola showed the highest increase in total antioxidant percentage compared to tap water at 195%. In comparison, CON-II showed the lowest increase at 11%. At a cadmium concentration of 0.6Cd, the total antioxidant levels ranged from 20% to 59%. Among the tested varieties, Punjab Canola showed the highest increase in total antioxidant percentage compared to tap water at 185%. In comparison, CON-II showed the lowest increase at 11%. In case of 0.8Cd, the total antioxidant levels ranged from 20% to 56%. Among the tested varieties, Punjab Canola showed the highest increase in total antioxidant percentage compared to tap water at 168%. In comparison, CON-II showed the lowest increase at 12% (Table 2).

Table 2. Effect of different toxicity levels of cadmium (control tap water, 0.2, 0.4, 0.6 and 0.8 µg/g) on electrolyte leakage (A) and total antioxidants (B) of 15 canola varieties.

Varieties	TW	0.2Cd	0.4Cd	0.6Cd	0.8Cd	TW	0.2Cd	0.4Cd	0.6Cd	0.8Cd
	Electrolyte Leakage (%)					Total Antioxidants (%)				
CON-II	25o	27n	27o	27o	27o	17j	19i	19i	19i	19j
Cyclon	33k	35l	36l	36l	38l	24h-j	25hi	25g-i	26g-i	27h-j
Punjab Canola	39j	40k	41k	41k	44k	28g-i	29g-i	29f-h	29f-h	31g-i
Legend	45i	46j	46j	47j	48j	32f-h	32f-h	33e-g	34e-g	34f-h
Super Raya	66e	66e	66e	66e	67e	47b-d	47b-e	47b-d	47b-d	48b-d
CON-III	28m	28m	28n	28n	28n	20ij	20i	20hi	20hi	20j
Oscar	72c	73c	73c	75c	75c	51a-c	52a-c	52ab	53ab	53ab
Shiralae	49h	49i	50i	51i	52i	35e-g	35f-h	35e-g	36ef	37e-g
Dunkled	26n	28m	31m	31m	31m	19ij	20i	22hi	22hi	22ij
AC Exul	32l	53h	54h	54h	54h	23h-j	38e-g	38d-f	38d-f	38d-g
Rainbow	55g	81a	83a	84a	85a	39d-f	58a	59a	59a	60a
AARI Canola	56g	57g	58g	59g	85a	60a	40d-f	40c-e	41c-e	42c-f
Super Canola	61f	68d	69d	70d	70d	43c-e	48a-d	49a-c	49bc	50bc
Sandal Canola	71d	77b	78b	78b	79b	50bc	55ab	55ab	55ab	56ab
Faisalabad Canola	63f	64f	65f	65f	80b	56ab	45c-e	45b-d	46b-d	46b-e

Values are means of three replicates ± SE. Different letter were obtained by applying Fisher LSD. These letters showed significant alteration at p≤0.05

Table 3. Effect of different toxicity levels of cadmium (control tap water (TW), 0.2, 0.4, 0.6 and 0.8 µg/g) on root cadmium concentration (A) and shoot cadmium concentration (B) of 15 canola varieties.

Varieties	TW	0.2Cd	0.4Cd	0.6Cd	0.8Cd	TW	0.2Cd	0.4Cd	0.6Cd	0.8Cd
	Root Cadmium Concentration (µg/g)					Shoot Cadmium Concentration (µg/g)				
CON-II	3.46n	3.63m	3.73l	3.84m	3.90n	1.22a	1.19a	1.17a	1.17a	1.16a
Cyclon	4.63k	4.76j	4.81i	5.00j	5.17k	0.86cd	0.86c-e	0.90bc	0.89b-d	1.03b
Punjab Canola	5.27i	5.32h	5.38h	5.54h	5.99g	0.98b	0.97b	0.95b	0.94b	0.93c
Legend	6.36e	6.75d	7.06c	7.54b	7.80b	0.92bc	0.91bc	0.90bc	0.89bc	0.88cd
Super Raya	11.82a	11.92a	12.02a	12.20a	12.30a	0.68fg	0.67gh	0.67fg	0.65gh	0.65h
CON-III	3.94m	4.05l	4.11k	4.14l	4.20m	1.15a	1.14a	1.12a	1.12a	1.10ab
Oscar	6.53d	6.70d	6.75d	6.80e	6.92e	0.59gh	0.58hi	0.57hi	0.56h-j	0.55ij
Shiralae	4.09l	4.25k	4.41j	4.53k	4.71l	0.87cd	0.86cd	0.85cd	0.83c-e	0.82de
Dunkled	1.85o	2.00n	2.20m	2.24n	2.26o	1.19a	0.97b	0.78de	0.77ef	0.66gh
AC Exul	4.87j	5.00i	5.32h	5.38i	5.41j	0.56h	0.82de	0.81d	0.80de	0.79d-f
Rainbow	7.27b	7.33b	7.35b	7.37c	7.51c	0.78de	0.50i	0.48i	0.48j	0.47j
AARI Canola	5.43h	5.45g	5.52g	5.56h	5.60i	0.47i	0.77ef	0.76d-f	0.75ef	0.74e-g
Super Canola	6.19f	6.26e	6.33e	6.39f	6.45f	0.73ef	0.64gh	0.63gh	0.62g-i	0.61hi
Sandal Canola	6.99c	7.05c	7.11c	7.17d	7.24d	0.59gh	0.54i	0.53i	0.53ij	0.52ij
Faisalabad Canola	5.65g	5.66f	5.72f	5.78g	5.86h	0.51hi	0.72fg	0.71e-g	0.70fg	0.69f-h

Values are means of three replicates ± SE. Different letter were obtained by applying Fisher LSD. These letters showed significant alteration at p≤0.05

Root and shoot Cd concentration: Compared to Tap water, at the lowest cadmium concentration (0.2Cd), there is a slight increase in root cadmium concentration in most of the varieties, except for Dunkled, which shows a decrease. At the next level (0.4Cd), most varieties show a further increase in root cadmium concentration, except for CON-II and Dunkled. At the higher cadmium concentration levels (0.6Cd and 0.8Cd), all varieties show a continued increase in root cadmium concentration. Looking at the percentage increase or decrease compared to Tap water, CON-II shows a 4.91% increase in root cadmium concentration at 0.2Cd, while Cyclon shows a 2.80% increase. Punjab Canola shows a 1.51% increase, Legend shows a 6.13% increase, Super Raya shows no increase or decrease, CON-III shows a 2.79% increase, Oscar shows a 2.60% increase, Shiralae shows a 3.92% increase, Dunkled shows a 7.45% decrease, AC Exul shows a 2.66% increase, Rainbow shows a 0.82% increase, AARI Canola shows a 0.37% increase, Super Canola shows a 1.13% increase, Sandal Canola shows a 2.05% increase, and Faisalabad Canola shows a 0.18% increase. At 0.4Cd, CON-II shows a 7.28% increase, Cyclon shows a 3.00% increase, Punjab Canola shows a 2.27% increase, Legend shows a 10.86% increase, Super Raya shows no increase or decrease, CON-III shows a 3.63% increase, Oscar shows a 2.29% increase, Shiralaee shows a 7.84% increase, Dunkled shows a 7.73% decrease, AC Exul shows a 2.66% increase, Rainbow shows a 0.27% increase, AARI Canola shows a 0.37% increase, Super Canola shows a 2.32% increase, Sandal Canola shows a 1.01% increase, and Faisalabad Canola shows a 1.42% increase. For 0.6Cd, CON-II shows a 10.06% increase, Cyclon shows a 8.15% increase, Punjab Canola shows a 2.85% increase, Legend shows a 11.11% increase, Super Raya shows a 3.44% increase, CON-III shows a 4.06% increase, Oscar shows a 4.43% increase, Shiralaee shows a 7.77% increase, Dunkled shows a 20.27% decrease, AC Exul shows a 9.07% increase, Rainbow shows a 1.04% increase, AARI Canola shows a 1.10% increase, Super Canola shows a 3.54% increase, Sandal Canola shows a 2.45% increase, and Faisalabad Canola shows a 2.65% increase. At 0.8Cd, CON-II shows a 12.43% increase, Punjab Canola shows a 13.67% increase, Legend shows a 22.81% increase, Super Raya shows a 3.42% increase, CON-III shows a 6.60% increase, Oscar shows a 5.86% increase, Shiralae shows a 15.08% increase, Dunkled shows a 22.16% increase, AC Exul shows a 10.16% increase, Rainbow shows a 3.32% increase, AARI Canola shows a 5.71% increase, Super Canola shows a 4.94% increase, Sandal Canola shows a 3.22% increase, and Faisalabad Canola shows a 3.95% increase, all compared to Tap Water (Table 3).

At tap water (TW), the shoot cadmium concentration for different varieties ranges from 0.50 µg/g to 1.22 µg/g. Among the varieties, Super Raya shows the highest shoot cadmium concentration at TW, with 1.22 µg/g. On the other hand, Dunkled has the lowest shoot cadmium concentration at TW, with 0.50 µg/g. When the cadmium concentration is increased to 0.2Cd, the shoot cadmium concentration shows a mixed trend for different varieties. Super Raya and Legend show a slight decrease in shoot cadmium concentration, while AC Exul, Rainbow, and AARI Canola slightly increase in shoot cadmium concentration. The remaining varieties show a similar shoot cadmium concentration compared to TW. At 0.4Cd, shoot cadmium concentration for all varieties increases compared to TW. Among the varieties, Oscar shows the highest increase (35.47%) in shoot cadmium concentration at 0.4Cd, followed by Shiralaee (27.16%) and Super Canola (23.29%). At 0.6Cd and 0.8Cd, shoot cadmium concentration for all varieties continues to increase compared to TW. Among the varieties, Dunkled shows the highest increase in shoot cadmium concentration at 0.6Cd (71.24%) and 0.8Cd (84.48%), followed by Sandal Canola at both concentrations. On the other hand, Super Raya shows the lowest increase in shoot cadmium concentration at both concentrations. Overall, the data shows that increasing cadmium concentrations in the water increased shoot cadmium concentration in most of the canola varieties tested. The extent of the increase varied among the varieties, with some showing higher concentrations than others at lower cadmium concentrations (Table 3).

Principal component analysis: The results of the principal component analysis (PCA) indicate that the first principal component (PC1) accounts for 89.5% of the variance, while the second principal component (PC2) accounts for 4.6% of the variance. The cumulative percentage of variance explained by the two principal components is 94.1%. The variables with the highest positive correlation with PC1 are 3rd Day Germination (11.6) and Shoot Length (0.3), followed by Root Length (0.2), Seedling Fresh Biomass (0.1), Cd Shoot (0.02), Total Chlorophyll (0.004), Chlorophyll a (0.003), and Chlorophyll b (0.002). The variables with the highest negative correlation with PC1 are Electrolyte Leakage (-0.02), Total Antioxidant Activity (-0.01), and Cd Root (-0.001). The variables with the highest loadings on PC1 were 3rd Day Germination (%), Shoot Length (cm), Root Length (cm), Seedling Fresh Biomass (g), and Seedling Dry Biomass (g). These variables were positively correlated with PC1, indicating that they contribute to the same underlying factor. Conversely, the variables with the highest loadings on PC2 were Cd Root (µg/g) and Total Antioxidant Activity (%), which were positively correlated but negatively correlated with the other variables. On the other hand, Cd Root (0.9) has the highest positive correlation with PC2, followed by Cd Shoot (0.3), Total Chlorophyll (0.25), 3rd Day Germination (-0.019), Chlorophyll a (0.017), Seedling Fresh Biomass (0.03), Shoot Length (0.03), Chlorophyll b (0.015), and Root Length (0.03). Electrolyte Leakage (-0.02) and Total Antioxidant Activity (-0.03) negatively correlate with PC2. Overall, the results of the PCA suggest that 3rd Day Germination, Shoot Length, and Root Length are the most important variables in explaining the variation in the data. At the same time, Cd Root and Cd Shoot are important in explaining the variation in the second principal component (Table 4).

Table 4. Eigenvalue, percentage of variance (%), cumulative (%) PC1 and PC2 values obtained by apply principal component analysis on studied attributes.

Principal component number	Eigenvalue	Percentage of variance (%)	Cumulative (%)	PC1 (89.5%)	PC2 (4.6%)
3rd Day germination (%)	11.63203	89.47716	89.47716	0.27772	-0.01913
7th Day germination (%)	0.59895	4.60728	94.08444	0.28622	-0.06295
Shoot length (cm)	0.26547	2.04208	96.12652	0.28978	0.03181
Root length (cm)	0.22688	1.74526	97.87178	0.2897	0.02978
Seedling fresh biomass (g)	0.15129	1.16378	99.03556	0.28775	0.0318
Seedling dry biomass (g)	0.05861	0.45088	99.48644	0.29054	0.01935
Electrolyte leakage (%)	0.02296	0.17662	99.66307	-0.2815	-0.02489
Cd Shoot (µg/g)	0.0175	0.13462	99.79768	0.26604	0.30391
Cd Root (µg/g)	0.01128	0.08679	99.88448	-0.2014	0.91314
Total antioxidant activity (%)	0.00901	0.06931	99.95379	-0.26519	-0.02818
Chlorophyll a (mg/g)	0.00347	0.0267	99.98049	0.29071	0.01743
Chlorophyll b (mg/g)	0.00209	0.01608	99.99657	0.29075	0.01513
Total Chlorophyll (mg/g)	4.45307E-4	0.00343	100	0.27546	0.25348

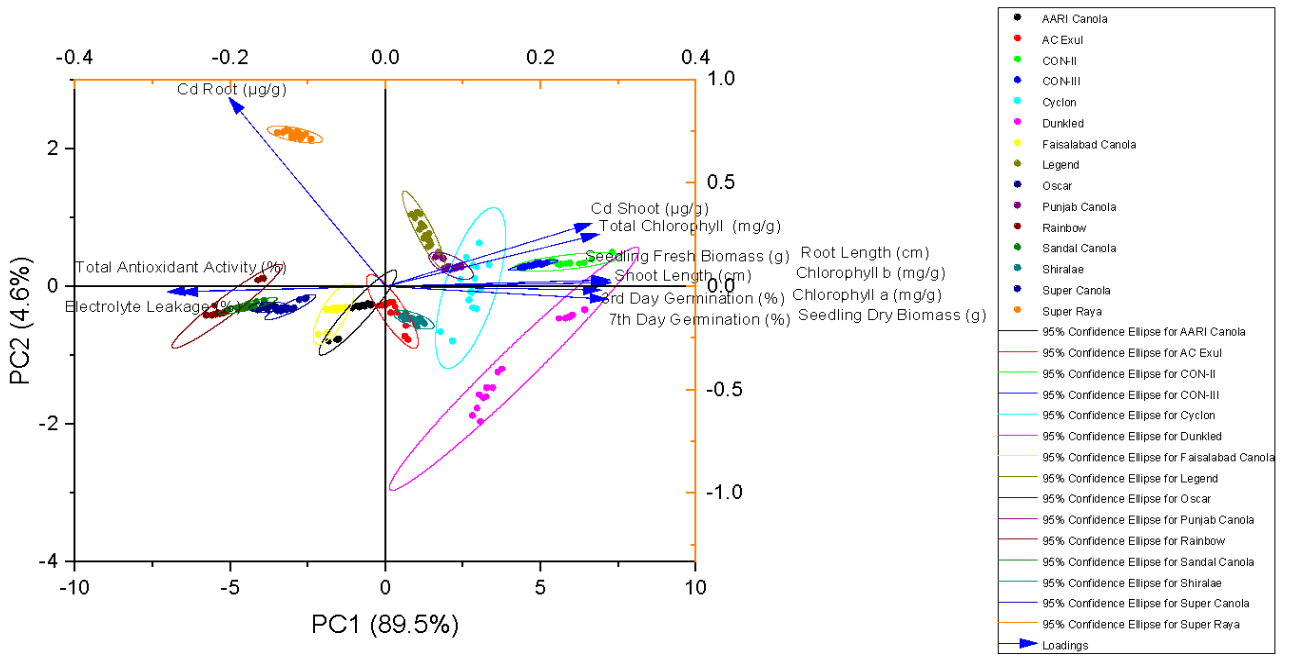

Fig. 5. Principal component analysis showing ellipse and loading for all studied varieties.

Discussion

When plants are exposed to high levels of cadmium, it can accumulate in the roots and shoots, disrupting various physiological and metabolic processes (Zhang *et al.*, 2022). One way cadmium toxicity can decrease plants' shoot and root length is by impairing cell division and elongation (Van Belleghem *et al.*, 2007). Cadmium can interfere with enzyme activity in cell wall synthesis, leading to reduced cell expansion and elongation (Parrotta, 2015). This can result in stunted growth of both roots and shoots. In the cortex of the root, cadmium can disrupt various physiological processes, including nutrient uptake and transport, water balance, and cell division and elongation (Nazar *et al.*, 2012). Cadmium can bind to cellular components and disrupt their functions, leading to cell damage and death (Thévenod & Lee, 2013). This can result in reduced growth and development of the root system. Moreover, cadmium can also affect the structure and function of the xylem and phloem. In the xylem, cadmium can accumulate and cause blockages or narrowing of the vessels, impairing water and nutrient transport from the roots to the shoots. This can result in wilting and reduced plant growth (Mendoza-Cózatl *et al.*, 2008).

In the phloem, cadmium can affect the activity of enzymes involved in sugar transport and metabolism, leading to reduced photosynthesis and carbohydrate production. This can also result in reduced growth and development of the plant (Mendoza-Cózatl *et al.*, 2008). Cadmium can interfere with the activity of enzymes involved in chlorophyll biosynthesis and degradation, leading to imbalances in the production and breakdown of the pigment (Chugh & Sawhney, 1999; Zhang *et al.*, 2020). This can result in reduced chlorophyll content and impaired photosynthesis, which can lead to reduced growth and development of the plant (Chugh & Sawhney, 1999; Zhang *et al.*, 2020). Moreover, cadmium can also induce oxidative stress in plant cells. Cadmium can generate reactive oxygen species (ROS) in plant cells, which can cause damage to cellular structures, including chlorophyll molecules. This can lead to reduced chlorophyll content, impaired photosynthesis, and other physiological and metabolic changes in plants (Cuypers *et al.*, 2010). Additionally, cadmium can also affect the activity of proteins involved in photosynthesis, including the photosystem II (PSII) reaction center (De Filippis *et al.*, 1981). Cadmium can bind to the PSII reaction center and disrupt its function, reducing photosynthetic efficiency and damaging chlorophyll molecules (Yang *et al.*, 2020). The total antioxidant activity (TAA) of plants under cadmium (Cd) toxicity can be explained by various mechanisms. One of the key mechanisms is the activation of antioxidant defense systems in plants, including the upregulation of enzymatic and non-enzymatic antioxidants (Shah *et al.*, 2020).

Conclusion

It is concluded that CON-II and CON-III were tolerant. Sandal canola, rainbow, and Oscar were found susceptible while remaining all as moderate canola varieties against Cd toxicity. Growers are recommended to cultivate CON-II and CON-III in Cd contaminated sites for achievement of better growth and production of canola. More investigations are suggested at the pot and field level to declare the best tolerant, moderate, and highly susceptible canola varieties again Cd. Further investigations at both pot and field levels are warranted to ascertain the most tolerant, moderately susceptible, and highly susceptible canola varieties in response to Cd toxicity.

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