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Cadmium Uptake, Accumulation and Tolerance in Hydroponic *Brassica chinensis* (Pak Choi)

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Abstract | Cadmium (Cd) is a very poisonous and non-essential heavy metal which is easily absorbed by plants. The metal makes living organisms susceptible to its acute and chronic toxicity through plant food. Similarly, Cd hinders the growth of plants by causing the loss of chlorophyll, which in turn negatively impacts the activities of photosynthesis. Therefore, this current study aims to evaluate the effects of Cd on the germination behaviour, morphology, growth and development of Pak Choi plants. The sterilized Pak Choi seeds were germinated on a soaked filter paper of a petri dish. The petri dish included seven different treatments of Cd chloride with concentrations of 0, 0.01, 0.05, 0.1, 0.2, 0.3, and 0.4 mM. Each treatment was replicated five times. The germination and growth of the seeds were observed for 15 days. Pak Choi seedlings were also planted under hydroponic conditions to investigate their morphological characteristics, growth and development. After two weeks of growth, the application of $CdCl_2$ was made with four treatments consisting of nutrient solution without $CdCl_2$ (control) and 0.1, 0.5 and 1.0 mM $CdCl_2$-nutrient solution. Two weeks of plant growth were allowed before the experiment was terminated. At the end of the experiment, the chlorophyll content (SPAD), root and shoot length (cm), and fresh and dry weight (g) of plants were quantified accordingly. The increase in $CdCl_2$ concentration led to a significant decrease in the length of root and shoot of germinated seedlings significantly. The seedling's growth of Pak Choi was inhibited three days after germination, and the seedling's height (root and shoot) in higher concentrations of $CdCl_2$ was lower than lower doses of Cd. Chlorosis symptoms in the leaf were visible in hydroponic Pak Choi containing Cd. The growth of Cd-treated hydroponic Pak Choi was adversely affected by Cd treatment. The Cd treatment also reduced SPAD, biomass content (fresh and dry), and length of root and leaves number significantly. It can be concluded that exogenous Cd inhibits the Pak Choi germination and growth under hydroponic conditions.

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Introduction

Cadmium (Cd) is the most important hazardous heavy metal due to its great mobility and exceptional toxicity to living organisms and the environment (Li *et al*., 2009; Liu *et al*., 2018). Cadmium can bioaccumulate and thereafter accumulate in the food chain at a rate that is greater than that of any other trace element due to its higher mobility in soil. Despite such low concentrations, cadmium has been reported to be carcinogenic and highly toxic to living cells (Järup *et al*., 1998; Waalkes, 2000). There are about 13,330 ha of farmlands in China already been polluted by cadmium, which has caused a severe problem for the production of healthy food (Huang *et al*., 2007; ATSDR, 2012). This problem has arisen consequence of the expansion of industrialization and modern agriculture in the country (Haider *et al*., 2021). The exposure of Cd in humans can result in skin cancer, damage to the testicles, dysfunction of the kidneys and liver, and other organs (Shaari *et al*., 2023). In addition, Cd has been correlated to the development of cancer and has been discovered to be phytotoxic at greater doses (Abdullahi *et al*., 2022). Plants have the potential to absorb significant amounts of Cd that have been translocated from polluted soil (WHO, 1992). Therefore, foods that are grown in cadmium-contaminated soils may not be suitable for human and animal consumption, and may even be harmful to their health.

Vegetables are a vital part of a healthy diet since they include protein, carbohydrates, minerals, and vitamins; nevertheless, they are also an important route for heavy metals into the food chain, which can have negative impacts on human health and environment (Kresovich *et al*., 2015). The leafy vegetables can absorb a larger amount of Cd in their edible parts when they are grown in less Cd-contaminated soils (Li *et al*., 2009). Pak Choi is a leafy vegetable commonly known as Chinese cabbage that is grown and consumed throughout the world. It is scientifically classified as *Brassica chinensis* under the Brassicaceae family. For this reason, it is necessary to have strict control over the concentrations of cadmium in Pak Choi, particularly in the parts of the plant that are edible, to make safer food production. The higher amount of cadmium causes growth inhibition (Souza *et al*., 2008), chlorosis (Kubis *et al*., 2024), necrosis (van Doorn *et al*., 2011), reduction of photosynthesis (Lysenko *et al*., 2015) and biomass production (Drava *et al*., 2012).

Cd, a toxic heavy metal that is poisonous and inhibits plant growth, is introduced into the plants and environment through a variety of anthropogenic ways (Yan *et al*., 2009). Cd poisoning may occur as a consequence of interference with plant metabolism uncertainty there is a disruption in the absorption and mobilization of nutrient elements from soil or growing media. Both plant nutrients and Cd compete with one another for the same transporters. The presence of cadmium or other heavy metals consequently leads to a shortage of mineral nutrients (Zhang *et al*., 2005). In terms of the capacity for Cd uptake and translocation, leafy vegetables have a pretty high ability. Several health problems, including cancer and toxicity in organ systems, can be brought on by prolonged exposure to Cd, which can be absorbed through the air, water, food, and soil. Although Pak Choi grows soil that contains low concentrations of Cd, there is nevertheless an accumulation of overlimit Cd quantities in vegetables and fruits. It was estimated that exposure can be caused by the eating of vegetables, which accounts for between 70% and 90% of the total Cd intake by humans (Shaari *et al*., 2023). It is essential to have a solid understanding Cd uptake mechanism, tolerance, and the reduction of its distribution in plants to mitigate the exposure risk to human beings.

Materials and Methods

Cadmium exposure on Pak Choi's seeds

Pak Choi seed's uniform size and healthy were chosen and 10% NaOCl (sodium hypochlorite) solution was used for sterilization. The seeds were placed on a moistened filter paper of a petri dish after repeated wash with distilled water. Seeds were incubated at 26°C for four days to germination. After four days of seed germination, the medium of germination was moistened with seven different concentrations (0, 0.01, 0.05, 0.1, 0.2, 0.3 and 0.4 mM) of cadmium chloride ($CdCl_2$). The Petri dishes containing treated seeds were resumed for incubation in the same environment and condition. The seedling's growth and morphological characteristics were observed and measured every 3 days for 15 days of treatments.

Cadmium exposure on Pak Choi's seedlings under hydroponic

The sterilization of Pak Choi's seeds was done using

10% NaOCl solution before seed germination and transplanting. Four days old germinated seedlings were moved into 5 L plastic tanks containing the following nutrient solution. The nutrient solution contains 1.5 mM Potassium Nitrate (KNO_3), 1 mM calcium nitrate Ca $(NO_3)_2$, 500 µM magnesium sulphate ($MgSO_4$), 250 µM Ammonium dihydrogen phosphate ($NH_4H_2PO_4$), 25 µM 25 µM Ferric-EDTA (Fe-EDTA), 46 µM Boric Acid (H_3BO_3), 9 µM Manganese Chloride ($MnCl_2$), 0.8 µM Zinc Sulphate ($ZnSO_4$), 0.3µM Copper Sulphate ($CuSO_4$), and 0.1 µM Ammonium heptamolybdate Tetrahydrate $(NH_4)_6$ Mo_7O_{24}. The hydroponic nutrient solution pH was maintained at around 6.25. The experimental seedlings were grown for 10 days in a plant growth chamber. The temperature (26 ± 2°C), relative humidity (70-80%), photoperiod (16/8) and photon flux density (400 µ mol m^{-2} s^{-1}) were maintained in the plant growth chamber. On the 11th day nutrient solution was provided with 0.05, 0.2 and 0.4 mM of $CdCl_2$ for 2 weeks along with control (treated with distilled water). The nutrient solution of each plastic tank was replaced every second day to mitigate nutrient depletion. The roots of treated Pak Choi seedlings after harvesting were washed in 5 millimole ice-cold calcium chloride ($CaCl_2$) for 15 mins to remove extracellular Cd according to the procedure described by Rauser (1987). The roots and shoots of harvested seedlings were washed with distilled water and gently blotted on dry paper towels. The collected samples (roots and shoots) were kept separately for further measurement and analysis. Pak Choi plant height, weight (fresh and dry) and leaf number were measured and recorded two weeks after treatment application. The chlorophyll content of the Cd treated and control group was determined using a SPAD-502 meter (Konica Minolta, Japan).

Results and Discussion

The effects of cadmium on the germination behaviour of Pak Choi

The treatment of cadmium significantly decreased germination and after-growth of Pak Choi seedlings (Figure 1). The results showed that shoot length decreased with the cadmium concentrations increased (Figure 2a). Higher concentrations of Cd, such as 0.3 and 0.4 mM, resulted in a much more decreased shoot length. Cadmium concentration delays seed germination and reduces seedling growth (Jun-yu *et al*., 2008; Rahoui *et al*., 2010). It has been reported that exogenous cadmium during germination inhibits water absorption and translocation in the xylem vessels of the embryo which reduces the seedling development and growth (Vijayaragavan *et al*., 2011).

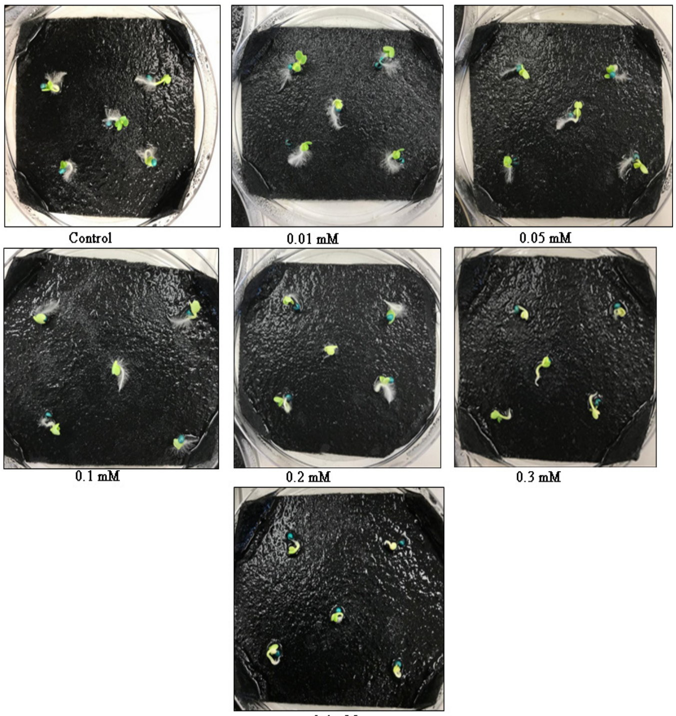

Figure 1: *The effect of cadmium on the seed germination of Pak Choi (at day 3). CdCl2 at 0.4 mM concentration reduced seed germination significantly.*

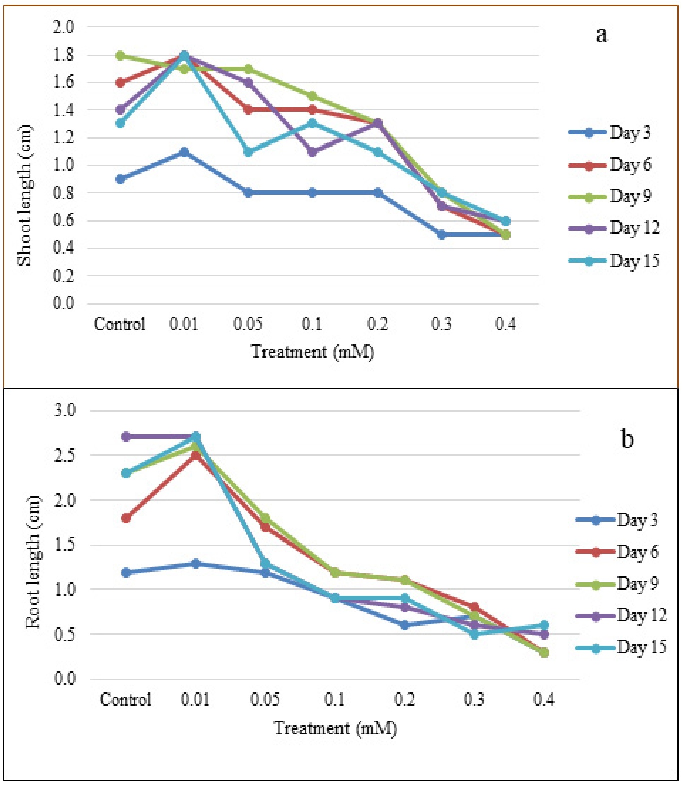

Figure 2: *The effects of different concentrations of cadmium on (a) Shoot length and (b) root length of Pak Choi Grown in hydroponic conditions.*

The exogenous growth-promoting chemicals also can regulate plant growth and development, leaves and fruit formation (Khandaker *et al*., 2018). This failure of water uptake phenomenon is associated with germinative metabolism disorders. In this study, Cd treatments significantly affect the length of seedlings' roots. Treated seedling's root length decreased with the cadmium chloride ($CdCl_2$) concentration increase (Figure 2b). The results showed that root and shoot lengths show some degree of tolerance to cadmium treatments. However, the growth of the shoot was more sensitive to cadmium concentration compared to root growth. The negative impacts of cadmium on root and shoot lengths may be due to PS II activity impairment, inhibition of cell division, enlargement, photosynthesis, respiration, water uptake, and normal cell activities. All these inhibitory activities resulted in poor growth and low biomass production. Roots limited elongation under the cadmium treatments resulted in decreased cell wall components synthesis, damaged Golgi apparatus, and inhibited and disrupted polysaccharides metabolism in the area of root cap and cell division. It was also found that cadmium treatment induced root morphological changes such as reduced number of side roots, fragility and browning.

Cadmium on growth and morphology of hydroponic Pak Choi

Our results showed that the control treatment produced the highest fresh weight of Pak Choi shoots (2.60 ± 1.06 g), followed by 0.05 mM ($CdCl_2$) treatment (1.67 ± 0.45 g), 0.2 mM ($CdCl_2$) treatment (1.00 ± 0.28 g) and lastly 0.4 mM $CdCl_2$ treatment (0.69 ± 0.29 g). As shown in Figure 3a, the decreasing trend of the fresh shoots can be observed with an increment of $CdCl_2$ concentration. The reduction of Pak Choi shoot weight in treated plants was statistically significant compared to the control group. The same pattern can be seen in the fresh weight of roots of Pak Choi (Figure 3b) with control treatment (8.54 ± 0.052 g), 0.05 mM ($CdCl_2$) treatment (5.21 ± 0.057 g), 0.2 mM ($CdCl_2$) treatment (4.62 ± 0.063 g) and lastly 0.4 mM $CdCl_2$ treatment (4.03 ± 0.069 g). Control showed the highest fresh weight of Pak Choi root followed by 0.05, 0.2 and 0.4 mM of $CdCl_2$ treatments. Higher concentrations of cadmium may cause a reduction of plant biomass, reduce plant growth and negatively affect plant physiology (Grifferty and Barrington, 2000).

After harvesting of Pak Choi Plants, the root and shoot length were measured as indicators of plant growth and development. Cadmium treatments significantly reduced the shoot length (Figure 3c). The highest shoot length was recorded in the control treatment, whereas, the lowest root was found in plants grown in 0.2 mM $CdCl_2$ treatment. The control portrayed the longest root measurement (17.40±5.69 cm) compared to other Cd-treated plants (Figure 3d). On the other hand, the decreasing trend of shoot length was determined with 0.05 mM ($CdCl_2$) treatment (8.74 ± 5.77 cm), 0.2 mM $CdCl_2$ treatment (5.74 ± 3.27 cm) and 0.4 mM ($CdCl_2$) treatment (6.48 ± 1.40). The lowest root length was recorded in 0.2 mM ($CdCl_2$) treatment. It has been reported that a lower concentration of cadmium retards the root growth and development without toxic effects in leaves, however, moderate or higher concentrations of cadmium retard the root growth and cause bioaccumulation in leaves (Prasad, 1995). A higher concentration of cadmium in plants reduces root and shoot growth, and causes chlorosis and leaf rolling (Santtr *et al*., 2013).

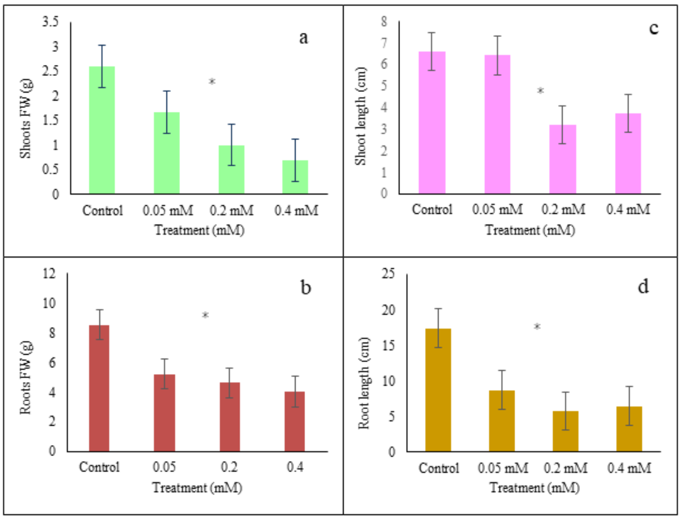

Figure 3: *Fresh weight of Pak Choi shoot (a) and root (b), and the length of (c) shoot and (d) root as affected by different treatments of Cd. * Denote significant difference among treatments.*

Figure 4a shows the leaf number of treated and untreated Pak Choi Plants. The finding demonstrates that 0.05 mM of $CdCl_2$ treatment produced the highest leaf number (11.40±1.67) compared to the control with a value of 11.20±0.83 leaf number. The differences were statistically insignificant. In this study, the leaf number of the Pak Choi Plant was decreased as the concentration of $CdCl_2$ increased. The control treatment produced a leaf number of (11.20±0.83) followed by 0.2 mM and 0.4 mM $CdCl_2$ treatments with a value of 9.80±1.48 and 8.20±1.10, respectively

(Figure 4a). Besides nutrient absorption, the number of leaves indicates the photosynthesis capacity of the plants. Maybe a very low concentration of cadmium did not produce significant effects on photosynthesis and plant growth. That, 0.05 mM of $CdCl_2$ concentration treatment has a slightly higher mean number of leaves compared to the control, is probably due to cadmium tolerance in plants and adaptation. However, a higher concentration of cadmium produced significant effects on leaf number (Raziuddin *et al*., 2011). Plants can easily accumulate cadmium in all parts of the soil or growing media which causes chlorosis, leaf epinasty, inhibit photosynthesis and stunted growth (Benavides *et al*., 2005; Tewari *et al*., 2008).

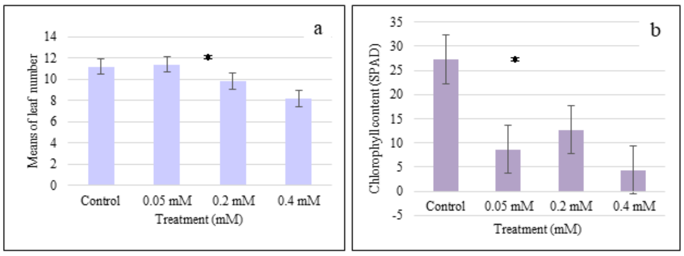

Figure 4: *The leaf number (a) and chlorophyll content (b) of hydroponic Pak Choi as affected by different concentrations of cadmium. * Denote significant difference among treatments.*

Figure 4b shows the graphs of chlorophyll content for each treatment after two weeks of cadmium exposure. The highest leaf chlorophyll content was found in the untreated group with a value of 27.26 ± 3.57 SPAD, followed by 0.2 mM and 0.05 mM $CdCl_2$ treatments with 12.67 ± 3.36 SPAD and 8.63 ± 4.48 SPAD, respectfully. Whereas, the treatment 0.4 mM $CdCl_2$ treatment produced the lowest amount of chlorophyll 4.35 ± 2.69 SPAD. Aggarwal *et al*. (2012) also reported that Cd, Cu, Cr, Hg and Zn decrease the chlorophyll content of various crop plants. The high redox potential of heavy metals produces inhibitory effects on the reductive steps of biosynthetic pathways of photosynthetic pigments which decline the chlorophyll content in leaves (Gouia *et al*., 2004; Correa *et al*., 2006). Cadmium causes chlorophyll loss, negatively affects photosynthesis and inhibits plant growth (Chen *et al*., 2012).

Conclusions and Recommendations

The study reported that the cadmium chloride ($CdCl_2$) treatment does inhibit the seed germination, and decreased the growth and development of hydroponic Pak Choi. Most of the parameters were affected significantly by the different concentrations of $CdCl_2$. It also found that Pak Choi seeds can tolerate a lower concentration of cadmium (0.01 mM – 0.05 mM) and perform normal germination. From this study, it is concluded that higher concentration of cadmium (> 0.2 mM $CdCl_2$) produced significant negative effects on seed germination, length of root and shoot, root and shoot fresh weight, leaves number, biomass content and chlorophyll biosynthesis in hydroponic conditions. Cadmium also induces mineral nutrient deficiencies by taking imbalanced nutrients from the growing media.

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Novelty Statement

The study provides essential information on cadmium toxicity towards Pak Choi seed germination and seedlings growth under hydroponic conditions.

Author's Contribution

Mohammed Moneruzzaman Khandaker: Data analysis, manuscript writing and editing.
Nuratiqah Emran: Performed the experiment.
Nurul Elyni Mat Shaari and Ali Majrashi: Review and editing.
Arba Aleem: Revised the manuscript.
Zanariah Mohd Nor: Editing the manuscript.

Conflict of interest
The authors have declared no conflict of interest.

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