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BIOSORPTION OF CADMIUM IN FENUGREEK (*TRIGONELLA FOECUM-GRAECUM*) USING FRUIT PEEL POWDER

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ABSTRACT

The present study is a novel study carried out to assess the use of economical biosorbent material such as peel powder of various fruits (banana, orange, pomegranate) for detoxification of heavy metals such as Cadmium (Cd) in fenugreek (*Trigonella foecum-graecum)*. The efficacy of the fruit peel powder was checked by adding them into pot containing fenugreek seeds along with heavy metal cadmium in different conc. (5mM, 10mM, 15mM and 20mM). Cd toxicity had significant ($p \leq 0.05$) retarding effect on seedling growth, root length, shoot length and various biochemical parameters (total sugar content, protein content and amylase activity). The present data revealed that by adding peel powder (banana, orange, pomegranate), there was significant increase ($p \leq 0.05$) in various biochemical parameters and physiological parameters in fenugreek. Thus, it can be concluded that fruit peels could be one of the novel ways to reduce the effects of toxic metals and increase crop production and yield in a cost effective manner.

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INTRODUCTION

Cadmium (Cd) is one of the heavy metal that has always been of major concern due to its potential toxicity to humans, and also due to its bioaccumulation tendency in the soil–plant system. The general symptoms of cadmium toxicity in plants are growth inhibition, low biomass production, impaired water relations, respiration, photosynthesis and nitrogen metabolism (Tran and Popova, 2013). Cd is introduced into environment through both natural and anthropogenic sources which includes metal mining, melting, plating, batteries, pesticides, oil paint, pigments and textiles and alloys, etc. Various methods of remediating metal polluted soils exist ranging from physical and chemical methods to biological methods (encapsulation, solidification, stabilization, electro kinetics, vitrification, vapour extraction, soil washing and flushing) which are expensive and do not make the soil suitable for plant growth (Marques *et al*., 2009). New approach of biosorption by fruit peel powder has been developed which has advantage of being low cost, easy availability with convenient operation and efficiency. Banana peel seems to be good adsorbent and can be used for removal of chromium, cadmium, and copper ions from aqueous solution. Various studies from the literature have reported the use of fruit peels as a bioabsorbant of various metals from polluted water (Pathak *et al*., 2015). (Mercy and Fenifer, 2014) have reported the utilization of fruit peels such as Pomegranate, Orange, Sweet lime and Banana for the effective growth of plants and higher yield. Since there is no reported study of use of peels in mitigating the effects of heavy metals e.g., Cd, the present study was carried to study the effect the fruit peels in plants in reducing the effect of heavy metal toxicity (Cd) on growth of fenugreek seeds.

MATERIAL AND METHODS

Processing of Fruit peels

Fruit peels of banana, orange, and pomegranate were collected separately from local market and dried in hot air oven at 70 degrees for 3-5 days. The dried fruit peels were powered individually, and stored at room temperature. 2g Peel Powder has been added to 100g soil.

Seed sterilization and sowing

Seeds were procured from local market and sterilized using 0.2% of Bavistin to avoid fungal infection. The seeds were sown in triplicates in pots having normal soil, soil with diff concentration (conc). of Cd (5mM, 10mM, 15mM and 20mM), soil with pomegranates peels, orange peels, banana peels, soil with pomegranates peels, orange peels, banana peels and diff conc. of Cd metal. Fenugreek (*Trigonella foecum-graecum*) plants were harvested after 9 days after examining on regular basis and various morphometeric assays were carried out such as no. of seeds germinated, root, shoot length, etc.

Preparation of Homogenate

The germinated seeds were homogenized in distilled water. The supernatant was collected as a sample for testing of various parameters and debris was discarded.

Estimation of Protein, Total sugar content, α-amylase activity

The protein concentration determination was done by the Lowry protein assay method. Total sugar content was estimated at 620nm using glucose as a standard. α-amylase activity was determined using a colorimetrically method with 3,5-dinitrosalicylic acid (DNS) reagent.

Statistical analysis: Statistical analysis was based on one-way analysis of variance (ANOVA). The effects of heavy metal treatment were considered statistically significant when $p \leq 0.05$.

RESULTS AND DISCUSSION

Seed germination under Cd stress was significantly decreased which could be due to accelerated breakdown of reserved food material in seed embryo. The higher cadmium concentration during germination prevents water uptake and water movement in the embryo axis resulting in low seedling development (Vijayaragavan *et al*., 2011). In the present study, there was significant increase in germination index in cases of seeds treated with the fruit peels (pomegranates peels, orange peels, banana peels). The fruit peels generally contain organic compounds such as cellulose, hemicelluloses, pectin substance, chlorophyll substances which act as strong adsorption of metals (Xiaomin *et al*., 2007).

The germination of seeds despite Cd toxicity could be due to the presence of the proteins involved in chloroplast and photosynthesis function which include oxygen-evolving enhancing proteins; proteins associated with the photosystem I reaction center subunit, Rubisco, and CP47 in the fruit peels (Du *et al*., 2016). Gibberellic acid (GA), present in fruit peels are involved in seed germination, stem elongation, leaf expansion, floral organ development and fruit/seed development (Gupta and Chakrabarty, 2013). Earlier studies have reported the role of Gibberelic acid (GA) in Cd tolerance by increasing mitotic activity, carbohydrates metabolism and the contents of protein and RNA (Asgher *et al*., 2014). GA induced biosynthesis pathway genes could be the mechanism for regulation of plant development as in Cd-treated *Parthenium hysterophorus* plants (Hadi *et al*., 2014).

Root length and shoot length was significantly reduced ($p \leq 0.05$) with varying conc of Cd (5mM, 10mM, 15mM, 20mM) as compared to control. This could due to the binding of Cd to root epidermal membrane which affects the functioning of transporter proteins either through direct binding to the ion transporters or via membrane assisted ROS production. Inhibition of root length can also be attributed to the inhibition of mitosis, reduced synthesis of cell wall components, damage to golgi apparatus and changes in the polysaccharide metabolism (Berkelaar and Beverley, 2000).There was significant increase in root length at all conc. of Cd with fruit peels which could be due to the ability of fruit peels to mitigate ROS through its antioxidant capacity (Khalili *et al*., 2012) or due to its ability to chelate with various metals and ultimately their removal. The presence of Cytokinins (CKs) in fruit peels could be another reason of increased root length as kinetin enhanced the antioxidant capacity, photosynthetic activity, chlorophyll fluorescence characteristics and overall growth against Cd stress in egg plant (Singh and Prasad., 2014). The auxins in fruit peels might increase the content of hemicellulose which retains Cd^{2+}in root cell wall and hence prevents the translocation of Cd^{2+} from roots to shoots (Zhu *et al*., 2013). Plant growth was significantly affected due to decrease in shoot/root ratio which could due to reduced chlorophyll content and inhibition of leaf photosynthesis (Chugh and Sawhney, 1999). Cd treatment induced the inhibition of cell growth due to the formation of stronger cross binding between pectin molecules in the cell wall and reduction in the size of the intercellular space. In maize roots, Cd induced the incorporation of lignin in to cell wall of roots resulting in increase in cell wall rigidity and reduction in cell wall expansion. (Degenhardt and Gimmler, 2000). The peel treatment significantly increased the root/ shoot of the plant grown in varying conc. of Cd which could be due to presence of cell wall metabolism enzymes such as xyloglucan endotransglucosylase, pectin lyases and pectin methyl esterase which are otherwise needed for ripening of fruits (Du *et al*., 2016). α-amylase is the major enzyme involved in the initial degradation of starch into more soluble forms.

There was a significant increase ($p \leq 0.05$) in alpha- amylase activity of plant under Cd as compared to control. In the present study, the increase in α- amylase activity observed earlier in sugarcane leaves at increasing conc. of metal like Ni (Misra *et al*., 2010) could be plant defence mechanism against metal toxicity. Increase in amylase activity could result in increased mobilization of glucose and fructose to growing embryo axis and accelerating the germination of seed. There was a significant increase ($p \leq 0.05$) observed in alpha- amylase activity of plants grown using fruit peels extract(pomegranate, orange, Banana) at all metal conc. i.e. 5mM, 10mM, 15mM, 20mM as compared to metal control plants (Fig 1).The presence of phytohormones like gibberellins in fruit peels intiates the formation of α- amylase activity in aleurone layer which could be one of the reasons for increased alpha-amylase activity of plants grown using fruit peels (pomegranate, orange, Banana). There was a significant decrease ($p \leq 0.05$) in protein level in Cd metal stressed plants at different metal concentration as reported earlier (Kaur *et al*., 2017). Cd cause damage to soluble proteins, photosynthetic proteins and reduce synthesis of proteins. There was no significant change occurs in protein content in plants which are grown using fruit peels extract at low conc. (5mM) but at higher conc. (20mM) increase in protein content was observed.

The fruit peels may have induced synthesis of stress proteins such as enzymes involved in Krebs cycle, glutathione and phytochelatin biosynthesis and some heat shock proteins to resist metal stress (Tran *et al*., 2013).

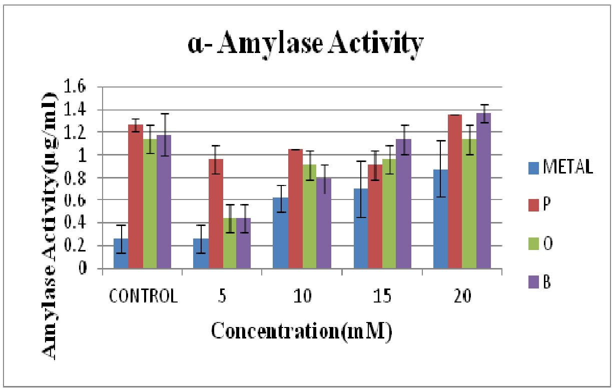

Fig 1:- The figure depicts that there was a significant increase in α-amylase activity of plant grown under different Cd metal concentration such as 5mM, 10mM, 15mm, 20mM as compared to control. Further there was increase in α-amylase activity of plants grown using fruit peels extract (pomegranate, orange, Banana) at all metal conc. i.e. 5mM, 10mM, 15mM, 20mM as compared to control and metal control plants

There was a significant increase ($p \leq 0.05$) in Total sugar content in metal control plant as compared to control which could be due to the generation of ROS and free radicals after Cd stress is given to plants. The sugar helps in the removal of free radicals generated during stressed conditions by acting as a signal molecule to stimulate antioxidant defence mechanism. There was a significant increase ($p \leq 0.05$) observed in plants grown using fruit peels extract (pomegranate, orange, Banana) at high metal conc. as comp are to metal control plants. Thus it can concluded that fruit peels have endogenous stress hormones such as ABA, ethylene, etc, which may transform stress related signals into gene expression molecules (Santner, 2009) needed for appropriate adaptation to Cd toxicity. The phytohormones and antioxidants present in fruit peels play a vital role in the detoxification of heavy metals by preventing the update of Cd ions and also improving plants growth metabolism. There is paucity of literature to fully understand the role of in fruit peets. Cd stress tolerance. Therefore, further research is required for a better understanding of the interactions among Cd and fuit peels in soil-plant system.

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