World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

DIFFERENTIAL RESPONSE OF *BRASSICA JUNCEA* CULTIVARS AGAINST CADMIUM TOXICITY

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Article Received on 31/08/2022 **Article Revised on 20/09/2022** **Article Accepted on 10/10/2022**

ABSTRACT
The pot experiment was conducted taking six varieties of *Brassica juncea* viz. Alankar, Pusa Jai Kisan, Varuna, Sakha, Rohini and Pusa Bold were tested for their comparative growth responses against cadmium through soil under prevailing climatic and soil conditions. The relative change in growth and photosynthetic pigments were correlated with Cd toxicity in terms of Cd accumulation, translocation and proline response when sampled at 60 days after sowing (DAS). Treatment was given in soil as $CdCl_2$ as 10 mg/kg soil. The variety Alankar accumulated highest level of Cd among all the cultivars and Varuna, the lowest. Relative change of growth was most prominent in Alankar (Dry weight lowered by 29%) and Varuna (Dry weight higher by 37%) relative to varietal mean. With the increasing uptake of Cd level in root and shoot (Alankar > Sakha > Pusa Bold > PJK > Rohini > Varuna) photosynthetic pigment declined linearly dependent on genotype, bioconcentration factor and translocation factor of the variety. Trigger in leaf proline level was also correlated with increasing Cd level and root to shoot translocation. All the mustard varieties had shown bio-concentration factor above 1.0 with highest in Alankar and lowest in Varuna suggesting former is good for phytoremediation point of view while latter is better for consumption.

KEYWORDS: Accumulation, Alankar, Cd-salts, Cd-toxicity, Growth, Varuna.

INTRODUCTION

Arable soils are regularly and increasingly being contaminated with toxic heavy metals due to excess use of chemical fertilizers and pesticides. This results in toxic level accumulation of these heavy metals in agricultural crop plants viz. mercury, nickel, lead, cadmium etc. Different salt of these heavy metals have different toxicity in crop plants that also in genotype (-variety) dependent manner. Growth metabolism affected directly by level of heavy metal accumulation and defense activated within plant parts. Cadmium is most readily available heavy metal in agricultural soils enriched by pesticides, fertilizers or factory effluents contaminating the plants. Through them it enters into ecological food chain culminating into intoxication of top consumers including human beings. FAO and WHO (1972) recommends the maximum tolerable intake limit of Cd 1.0-1.2 µg kg^{-1} of average human body weight. Accumulation level of heavy metal depends upon plants' uptake efficiency which in turn further depends upon a number of factors as genotype of plant cultivar, soil characteristics, level of heavy metal contamination in soil, exposure time of heavy metals, type of heavy metal salts and presence of other mineral elements and soil nutrient etc.

MATERIAL AND METHODS

Experimental site

The experiment was conducted in the Botany Department, Tilakdhari College of Jaunpur, Uttar Pradesh (25° 52' N latitude, 82°51' E longitude at an elevation of 187.45 m above the sea level). The plastic pots of 6 in. diameter were filled with acid washed sand were placed in netted house, under ambient environmental conditions of September-February 2014–2015.

Experimental layout and treatments

Seeds of all the six varieties of *Brassica juncea* (L.) Czern & Coss cv. Alankar, Pusa Jai Kisan, RH-30, Varuna, Sakha, Rohini and Pusa Bold were procured from the National Seed Corporation Ltd., New Delhi, India. Healthy seeds of uniform size after surface sterilization with 0.01% $HgCl_2$ solution for 5 min were washed five times with double distilled water (DDW) and tested for percent germination.

Table 1: Chemical characteristics of soil before sowing.

Texture	Sandy loam
p.H.(1:2.5 Soil:Water)	7.1
CEC (cmol Kg^{-1}soil)	16.3
EC (1:2.5 Soil:Water) (dSm^{-1})	0.48
Organic carbon (%)	0.849
NO_3-N (mg kg^{-1} soil)	312
K^+ (cmol Kg^{-1}soil)	17.0
Ca^{2+} (cmol Kg^{-1}soil)	3.5
Na^+ (cmol Kg^{-1}soil)	1.0
Mg^{2+} (cmol Kg^{-1}soil)	19.91
Carbonate (mg kg^{-1} soil)	18.61
Bicarbonate (mg kg^{-1} soil)	110.65

Seeds of all six varieties were sown in pot soil (Table 1) amended with $CdCl_2$ as 10 mg/kg soil and allowed to grow. Seeds were sown at the rate of 8 seeds per pot and thinned one week after germination. Each treatment had five pots as replicate where three plants per pot were maintained. Irrigation was done with tap water as and when required. The plants were sampled at 60 DAS to study the growth characteristics and Cd accumulation.

METHODOLOGY

The potted plants were allowed to grow. Mustard plants after 60-days growth were uprooted with intact roots to assess the growth characteristics.

1. Growth analysis

The length of root and shoot were measured using a meter scale while fresh and dry mass of roots and shoots were measured with electronic balance. For analyzing the dry mass the uprooted plants (root and shoots) were placed in an oven at 80^0C for 72 h wrapping in butter paper. The dried plants were then weighed to record plant dry mass. The area of leaf was ascertained by gravimetric method. The leaf area of randomly selected leaves from each variety was determined by tracing its outline on graph sheet and counting the squares covered by leaf on graph paper.

2. Chlorophyll a, b and Caroteonoid level in leaf

Leaf chlorophyll a, b and carotenoid level was estimated in finely cut fresh leaves. Samples extracted from acetone were read at 663 and 645 nm, using spectrophotometer (Mackinney, 1941). These pigments were calculated using following equations and expressed in mg Kg^{-1} of fresh tissue.

$$\text{mg Chl-a kg}^{-1}\text{ tissue} = 12.7\,(A_{663}) - 2.69\,(A_{645}) \times \frac{V}{1000 \times W}$$

$$\text{mg Chl-b kg}^{-1}\text{ tissue} = 22.9\,(A_{645}) - 4.68\,(A_{663}) \times \frac{V}{1000 \times W}$$

$$\text{Carotenoid content} = 7.6\,(\text{O.D.}_{480}) - 1.49\,(\text{O.D.}_{510}) \times \frac{V}{d \times W \times 1000}$$

Where,

A = absorbance at specific wavelengths; V = final volume of chlorophyll extract in 80% acetone (10 ml); W = fresh mass of tissue, used for extraction (0.5 g); d = length of light path = 1 cm

3. Leaf proline content

To determine leaf proline content in fresh tissue (Bates et al., 1973) samples were extracted in sulphosalicylic acid. An equal volume (2 mL) of glacial acetic acid and ninhydrin solutions was added to it. The samples were heated at 100^0C on water bath. After cooling in ice bath 5 mL of toluene was added. The absorbance of aspirated toluene layer was read at 528 nm, on a spectrophotometer (Spectronic-20D, Milton Roy, USA). Amount of proline was calculated using standard curve from pure proline (range 0.1-36µmol) and expressed on fresh mass basis of sample.

$$\text{µmoles of proline/g tissue} = \frac{\text{µg proline/ml}^2 \times \text{ml}^2\text{ toluene}}{115.5\text{ (mol mass of proline)}} \times \frac{5}{\text{g (sample)}}$$

4. Cd accumulation in root and shoot

The root and shoot samples were placed for 10 min in ice cold 5 mM $CaCl_2$ solution to displace extracellular Cd, rinsed with DDW and then oven dried (Meuwly and Rauser, 1992). Cd concentration in tissues was estimated after digesting the samples in nitric acid:perchloric acid

(3:1, v/v). Cd concentration was determined by an atomic absorption spectrophotometer (Spectronic-20D, Milton Roy, USA).

5. Bio-concentration Factor (BCF) and Translocation Factor (TF)

The BCF and TF of HMs in the six varieties was calculated by the following formulas:

$$BCF = \frac{\text{Metal concentration in shoot/root (mg/kg DW)}}{\text{Metal concentration in contaminated soil (mg/kg DW)}}$$

$$TF = \frac{\text{Concentration of metal in shoot/root (mg/kg DW)}}{\text{Concentration of metal in corresponding soils/root (mg/kg DW)}}$$

Bio-concentration factor is the ration of concentration of metal in shoot parts to metals of contaminated soil. While the translocation factor was calculated by dividing the heavy metal concentration in shoots by roots and roots by soil.

5. Statistical analysis

The experiment was conducted according to simple randomized block design. Each treatment was replicated five times and three plants per pot were maintained where each pot was considered as a replicate. Treatment means were compared by the analysis of variance using R ver. 3.1.0 for Windows. Least Significant Difference between treatment means was calculated at 5% probability level ($p < 0.05$).

RESULTS

1. Growth parameters

At 60 DAS $CdCl_2$; 10 mg kg^{-1} administered through the soil resulted in relative change of growth (length, fresh mass, dry mass of root and shoot and leaf area) parameters in the mustard varieties at 60 DAS (Table 1 and 2). The relative decline of root and shoot lengths (13% & 14%) and fresh masses (31% & 30%) was maximum in Alankar followed by Sakha while Varuna recorded increased relative lengths (18% & 13%) and fresh masses (35% & 37%) in root and shoot. Similarly, relative change in root dry mass was noticeable, and it declined by 29%, while in Varuna it increased by 37%, relative to mean. Therefore, cadmium affected root and shoot ratio in all mustard varieties. Cadmium at the concentration of 10 mg kg^{-1} declined the leaf area by 18% in Alankar while it was relative increase by 21% in Varuna with mean.

2. Level of leaf photosynthetic pigments

The cadmium-stress ($CdCl_2$; 10 mg kg^{-1} of soil) brought about the relative change in photosynthetic pigments level i.e. chlorophyll-a, chlorophyll-b and carotenoid level, as compared to the mean (Figures 1a,b & c). However, the maximum decline in given photosynthetic pigments was found in Alankar (20%, 18% & 14%), while that of Varuna registered 23%, 17% & 25% increase at 60 DAS as compared to their means, respectively.

3. Proline content

It is evident from Fig. 1D that soil amended with Cd (10 mg $CdCl_2$ kg^{-1}) caused a sufficient change in the proline content in a genotype dependent manner. In Varuna it is 10% lesser than varietal mean. Contrarily Alankar registered 13% increase of proline level in leaves at 60 DAS.

4. Cadmium accumulation in root and shoot

Fig. 2a and b show an differential trend of Cd accumulation in root and shoot tissues with the treatment of $CdCl_2$ in the soil (10 mg $CdCl_2$ kg^{-1}). Shoot comparatively accumulated lesser quantities of Cd than root in both the mustard varieties. The relative increase in the Cd accumulation was higher in Alankar and Sakha than Varuna at 60 DAS. Varuna accumulated 23 µg and 18 µg Cd and Alankar 46 µg and 33 µg Cd g^{-1} of root and shoot dry mass, respectively, at 60 DAS. Rest of the varieties viz. Pusa Jai Kisan, RH-30, Sakha, Rohini and Pusa Bold, accumulated Cd near to varietal mean.

5. Bio-concentration Factor (BCF) and Translocation Factor (TF)

All the mustard varieties had shown root bio-concentration factor above 1.0. The Cd concentration factor in root was the function of genotype. The increasing trend of BCF (Fig. 2c) was Alankar > Pusa Bold > Sakha > PJK > Rohini > Varuna. However, the Cd translocation factor from Root to shoot reflected minimum translocation in Sakha while maximum that of Rohini followed by Varuna (Fig. 2d).

Table 2. Effect of Cd (10 mg/kg soil) on different mustard varieties *Brassica juncea* var. Alankar, Pusa Jai Kisan (PJK), Varuna, Sakha, Rohini and Pusa bold on length (cm) and fresh mass (g) of root and shoot at 60 days after sowing (DAS).

Varieties	Root length (cm)	Shoot length (cm)	Root Fresh Mass (g)	Shoot Fresh Mass (g)
Varuna	2.02 ±0.11	4.12 ±0.03	2.87 ±0.037	33.82 ±0.61
Rohini	1.66 ±0.09	4.74 ±0.02	3.02 ±0.012	30.33 ±0.67
PJK	1.44 ±0.10	4.48 ±0.01	3.02 ±0.009	27.49 ±0.76
Sakha	1.13 ±0.11	4.21 ±0.01	3.07 ±0.009	25.45 ±0.87
Alankar	1.04 ±0.11	4.02 ±0.01	2.97 ±0.009	22.88 ±1.07
Pusa Bold	1.55 ±0.14	4.56 ±0.02	3.01 ±0.015	27.67 ±0.88
Mean	1.47	4.36	2.99	27.94

Note- Values are mean of three replicates followed by ±Standard Errors

Table 3. Effect of Cd (10 mg/kg soil) on different mustard varieties *Brassica juncea* var. Alankar, Pusa Jai Kisan (PJK), Varuna, Sakha, Rohini and Pusa bold on dry mass (g) of root and shoot dry mass (g), shoot/root ratio and leaf area (cm^2) at 60 days after sowing (DAS).

Varieties	Root Dry Mass (g)	Shoot Dry Mass (g)	Root/Shoot Ratio	Leaf Area (cm^2)
Varuna	2.02 ±0.11	4.12 ±0.03	2.87 ±0.037	33.82 ±0.61
Rohini	1.66 ±0.09	4.74 ±0.02	3.02 ±0.012	30.33 ±0.67
PJK	1.44 ±0.10	4.48 ±0.01	3.02 ±0.009	27.49 ±0.76
Sakha	1.13 ±0.11	4.21 ±0.01	3.07 ±0.009	25.45 ±0.87
Alankar	1.04 ±0.11	4.02 ±0.01	2.97 ±0.009	22.88 ±1.07
Pusa Bold	1.55 ±0.14	4.56 ±0.02	3.01 ±0.015	27.67 ±0.88
Mean	1.47	4.36	2.99	27.94

Note- Values are mean of three replicates followed by ±Standard Errors

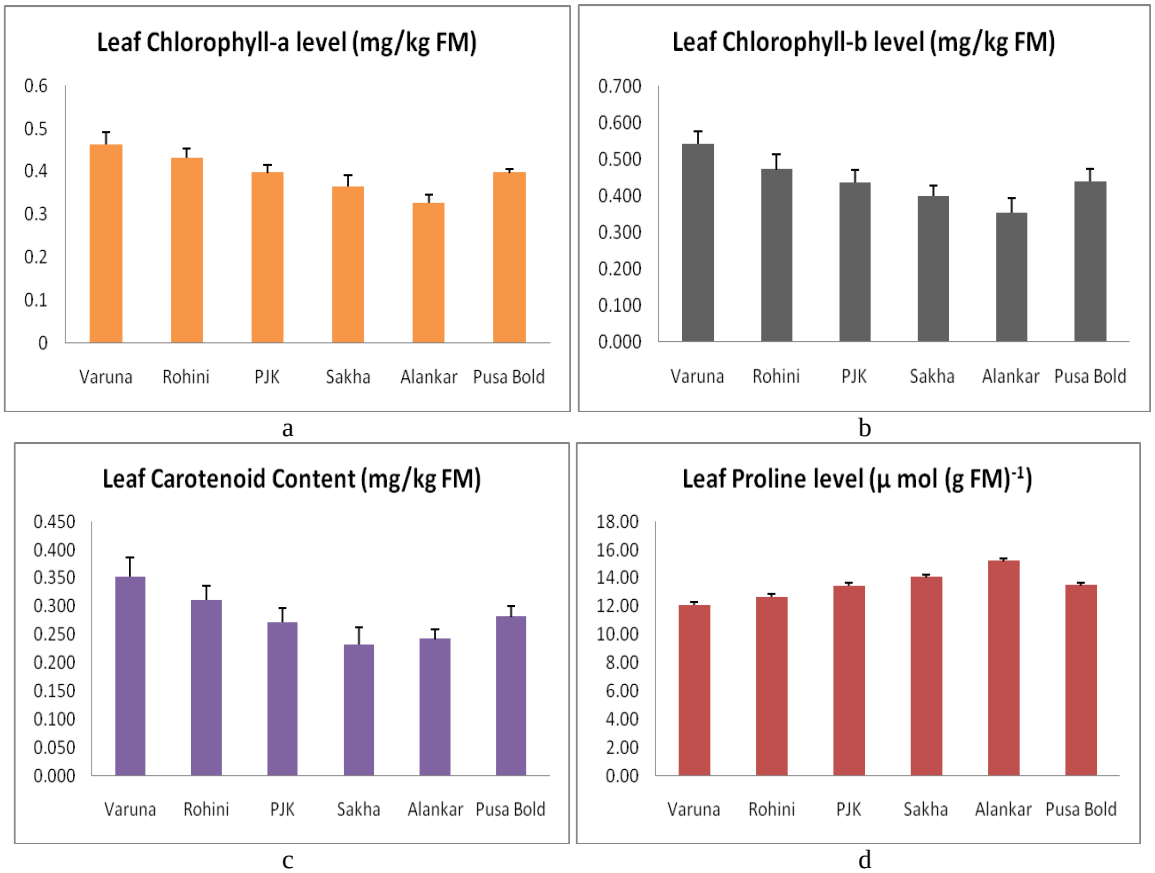

Figure 1. Effect of Cd (10 mg/kg soil) on of different mustard varieties *Brassica juncea* var. Alankar, Pusa Jai Kisan (PJK), Varuna, Sakha, Rohini and Pusa bold on (a) leaf chlorophyll-a level (mg/kg FM), (b) chlorophyll-b

level (mg/kg FM), (c) leaf carotenoid content (mg/kg FM) and (d) leaf proline level (µ mol (g FM)$^{-1}$) at 60 days after sowing (DAS).

Note- Graph values are mean of three replicates; bars on graphs show ±Standard Errors

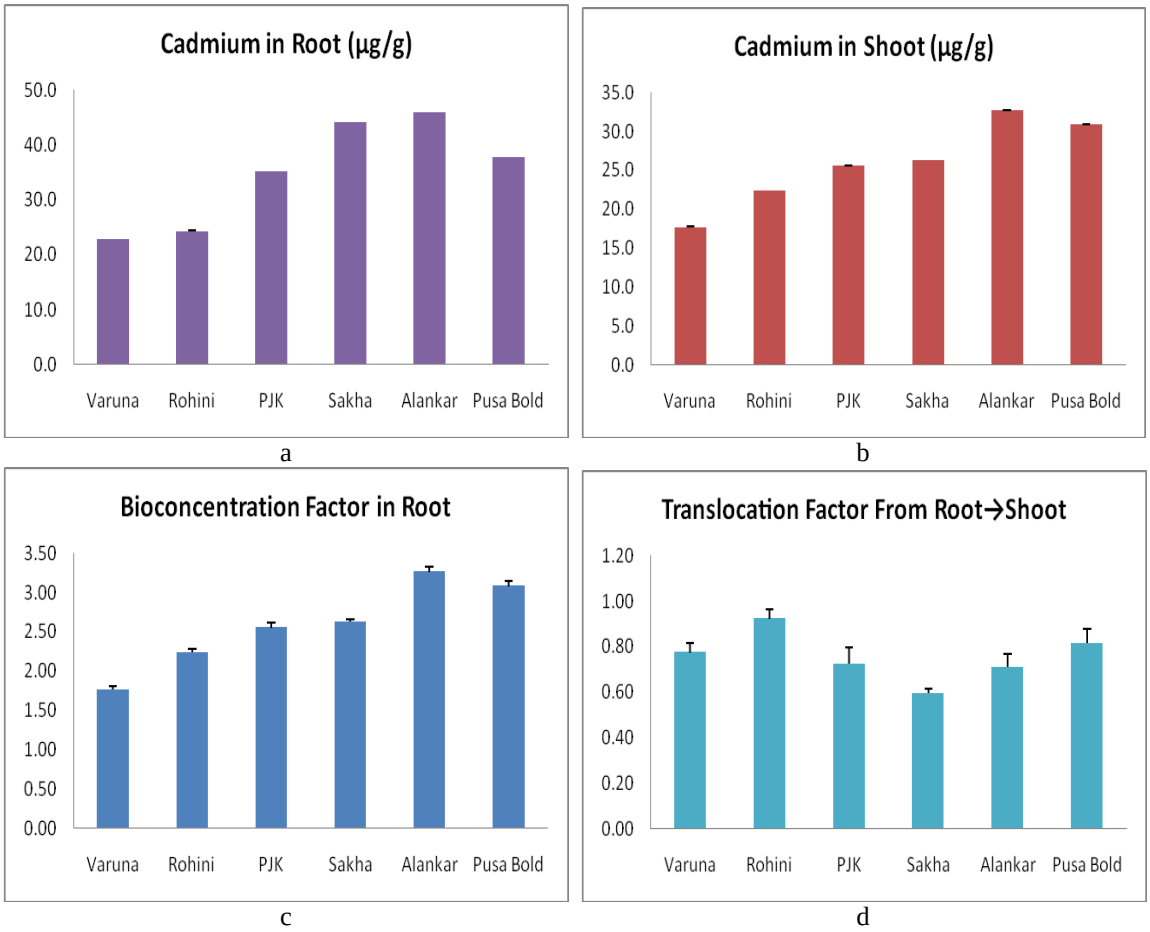

Figure 2. Effect of Cd (10 mg/kg soil) on of different mustard varieties *Brassica juncea* var. Alankar, Pusa Jai Kisan (PJK), Varuna, Sakha, Rohini and Pusa bold on Cd accumulation level (µg/g DM) in (a) root and (b) shoot, (c) Cd bioconcentration factor and (d) Root→ Shoot translocation factor at 60 days after sowing (DAS).

Note- Graph values are mean of three replicates; bars on graphs show ±Standard Errors

DISCUSSION

Soil administered Cd at the early growth stage results in more inhibition as compared to those supplemented at the latter growth stages, thus Cd interferes the metabolism of the young seedlings at the stage, when the plants are in the most active stage of metabolism, affecting the subsequent growth. The study conducted here reveals that the presence of soil Cd adversely affects root and shoot length their ratio, fresh and dry mass and leaf area expansion (Table 2 & 3). In most of arable environmental conditions Cd is readily taken up by roots, consequently translocated to shoot parts. Therefore, root first experience Cd mediated damage (di Toppi and Gabbrielli, 1997). It is channelized through root cortical cells to approach xylem through apoplastic and/or symplastic pathway (Salt et al., 1995) and then makes complex with ligands as organic acids and/or phytochelatins (Salt et al., 1995). Being relatively anaerobic organ roots normally retain Cd and only small part is translocated to shoot system (Cataldo et al., 1983). Cd when reacts with cell wall fibers it increases cross linking between wall components, it is also deposited in the middle lamella, therefore, checking cell expansion and growth (Poschenrieder et al., 1989). Cadmium mediated alteration of membrane properties and leakage results into water loss, thus altering water relation to cause physiological draught (Asada, 1999). In shoot parts e.g. leaves Cd perturbs metabolism by triggering production of reactive oxygen species (Asada, 1999), altering photosynthesis (Krupa et al., 1993, Chaugh and Sawhney, 1999) and nutrient uptake (Obata et al., 1996), ultimately resulting in decreased length, dry mass and leaf area expansion (Table 2 & 3).

The Cd stress also resulted in an increase in the level of proline in the leaves (Figure 1). The free proline accumulation in response to heavy metal exposure is common indicator among plants (di Toppi and

Gabbrielli, 1997; Prasad, 1995; Costa and Morell, 1994). Its expression in plant's stressed part is regulated by early inducing genes of proline biosynthesis and suppression of down regulation genes of proline synthesis (Kishor et al., 1995). It was reported that increased activity of two of the enzymes (Δ-pyroline-5-carboxylate synthetase and Δ'-pyroline-5-carboxylate reductase) is responsible for proline biosynthesis and a decrease of of proline dehydrogenase, the enzyme that degrades proline in cowpea, under stress (Sumithra and Reddy, 2004). Moreover, in some transgenic plants it was shown that the transcription genes for these enzyme proteins were also over expressed, under stress (Kishore et al., 1995). The proline level induction in the leaves is assumed to be mediated through water loss or Cd mediated physiological drought (Barcelo and Poschenrieder, 1990), though in partial. Contrarily, accumulation of higher level of proline in metal exposed plants could be directly due to metal uptake, rather than to water deficit stress as suggested by Kastori et al. (Kastori et al., 1992). Proline accumulation, as its function, signify the restoration of water ameliorating the water balance (Costa and Morell, 1994) and scavenging the radicals (Smirnoff and Cumbes, 1989)

The level and activity of enzyme chlorophyllase has been enhanced by the Cd this results into the degradation of the chlorophyll level (Reddy and Vora, 1986) and also decrease the synthesis of d-aminolevulinic acid and protochlorophyllide reductase complex (Stobart et al., 1985). Thus, a resultant effect is a decrease in the total content of total chlorophyll (Figure 1). This finding is in conformity with Gadallah (1995).

Cadmium is readily available to roots in agricultural soils; however, its uptake efficiency and root to shoot translocation and accumulation is dependent on species and genotype/cultivar. Mustard is heavy metal accumulator plant. Figure 2 suggested here cultivar dependent accumulation of Cd in root and shoot. Alankar followed by Sakha accumulated highest Cd in roots while Alankar followed by Pusa Bold accumulated highest Cd in shoot tissues. Plants roots are the primary organs which encounters first with the heavy metals and it generally accumulate a greater amount of heavy metal than shoot tissues (Simonova et al., 2007: Bauddh and Singh, 2011). The BCF and TF were also observed in mustard varieties which were grown in soil amended with 10 mg Cd kg^{-1} soil. The ability of mustard plants to tolerate and accumulate heavy metals may be useful for phytostabilization. Both bioconcentration factors (BCF) and translocation factors (TF) can be used to estimate a plant's potential for phytoremediation purpose. These important parameters in the heavy metal uptake studies (Zayed et al., 1998b: Marchiol et al., 2004) are measure of a plants' uptake potential the heavy metals from soils to roots or translocation from root to shoots or other parts (Mattina et al., 2003). As per estimation if the BCF and TF value of plant < 1 is less suitable, and >1 is indicative of a potential of heavy metal hyperaccumulator species (Zhang et al., 2002). If BCF >1 and TF <1 then plant will be suitable for phytostablization (Fitz and Wenzal, 2002: Yoon et al., 2006). All the mustard varieties had shown bio-concentration factor above 1.0 with highest in Alankar and lowest in Varuna. The translocation factor was maximum in Rohini followed by Pusa Bold, though it was below 1.0 for all.

6. CONCLUSION

From the above study it may be concluded that the treatment of mustard varieties (*Brassica juncea* viz. Alankar, Pusa Jai Kisan, Varuna, Sakha, Rohini and Pusa Bold) with cadmium 10 mg kg^{-1} through soil amendment resulted in reduced growth, photosynthetic pigment content and in the leaf, whereas the proline content in leaf increased in correspondence with Cd level. This concentration of Cd staggered the growth in genotype dependent defense particularly early growth stage. Detoxification of heavy metals through defense indicates the utilization of these plants as phytoremediation potential or consumables.

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