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Ameliorating Effect of Fertilizers on Biochemical Characteristics of *Vigna radiata* Treated with Hexavalent Chromium

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The aim of the present study is to compare the ameliorating potential of Cr toxicity in *Vigna radiata* by Farm Yard manure (FYM), NPK, Panchakavya (PK) and Vermicompost (VC). Pot experiment was selected and potassium dichromate was used as the source of chromium. Test group soil was contaminated with 30 mg/kg chromium. The effect of used fertilizers on antioxidant enzymes and pigments were analyzed. The ameliorating potential of fertilizers were compared by providing chromium alone and chromium with fertilizers. The antioxidant enzymes increased in chromium treated plants and slightly decreased by the application of fertilizers. Fertilizer application reduces the chromium accumulation in roots and shoots slightly and prevents oxidative damage. The plant pigments was much affected by Cr but brought back to normalcy by fertilizers. The ameliorating potential of NPK is much better than other fertilizers which accumulates moderate level of Cr in shoot and root but alleviates its effects.

Key words: Hexavalent chromium, *Vigna radiata*, Pigments, Antioxidant enzymes.

From the commencement of the industrial revolution, pollution of the biosphere by toxic metals has accelerated rigorously. Flaming of fossils fuels, smelting and mining of metalliferous ores, fertilizers, municipal wastes, pesticides and waste water irrigation are the most important sources of metal pollution. Contamination of soil and groundwater by the heavy metals leads to chief environmental and human health problems. Plant metabolism is also affected depressingly by the heavy metals (Singh and Agrawal, 2010). Although several heavy metals function as micronutrient at lower concentration, these are dangerous for plants at higher concentration (Shah *et al*., 2008)

Chromium (Cr) is not a vital factor for any metabolic process (Katz and Salem, 1994). Cr is present in some oxidation states, like Cr^{2+} to Cr^{6+} in natural environments. Trivalent and hexavalent states are the steadiest and common in the earthly environment. Both Cr^{3+} and Cr^{6+} are toxic and produce oxidative damage (Panda and Parta, 2000). In leather tanning Cr^{3+} is routinely used. The availability of more oxygen oxidizes Cr^{3+} into Cr^{6+}, which is more soluble and extremely lethal in water than other forms. Generally, most Cr^{6+} added to soil is rapidly reduced to the inert form Cr^{3+} by numerous agents among which humic compounds, sulfides and plant and microbial activity. Sethunathan *et al.,* (2005) revealed that soil microorganisms also contribute to the reoxidation of Cr^{3+} to Cr^{6+} and therefore, both Cr oxidation states should be regarded harmful for the environment. Cr phytotoxicity can result in degradation of nutrient balance, pigment status, enrichment of activity of antioxidant enzymes and

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stimulation of oxidative stress in plants (Panda and choudhury, 2005). Cr produces OH ions from H_2O_2 in its hexavalent state (Vajpayee *et al.,* 2000).

There is little information about the response of growth to Cr toxicity under different fertilizers. Four fertilizers were used to nullify the Cr toxicity on *Vigna radiata*. *Vigna radiata* is protein rich and the most important crop in India. Here we report the influence of different forms of fertilizers on pigment content, antioxidant enzyme activity and Cr concentration in the Cr-stressed green gram plant.

MATERIALS AND METHODS

Under uniform conditions of light, soil and water pot culture experiments were conducted. Pots were divided into 8 groups of two pots each and filled with sterilized soil. 30 mg/Kg concentration of chromium was added to the required test groups. Equal numbers of *Vigna radiata* seeds (15) were sowed in each pot and after germination the number of plants reduced to 8 plants per pot.

Four different fertilizers (Farm Yard manure (FYM), NPK, Vermicompost (VC) and Panchkavya (PK)) were selected to analyze the alleviating effect of chromium toxicity for *Vigna radiata*. PK was diluted to 10g in 1 L before use. FYM, NPK, VC and PK were purchased from Saradha Nursery, Kumbakonam and NPK from commercial fertilizer shop. 10 Kg pots were filled with soil mixed with different fertilizers as follows, 500mg/pot FYM, 20:20:10 NPK (10g), 200g/pot VC and 100 ml diluted PK/pot. Plants were irrigated regularly with water and removed carefully on 30th day and physical and biochemical parameters were analyzed. For analysis plants were randomly selected from two pots of each group.

Experimental design

Control - Soil without fertilizer	Test control	- 30 ppm Cr polluted soil
Group I Control - Soil + FYM	Group I Test	- 30 ppm Cr polluted soil + FYM
Group II Control - Soil + NPK	Group II Test	- 30ppm Cr polluted soil + NPK
Group III Control - Soil + PK	Group III Test	- 30ppm Cr polluted soil + PK
Group IV Control - Soil + VC	Group IV Test	- 30ppm Cr polluted soil + VC

Fresh leaf sample (0.5 g) of 30 days old plants of each group was treated with 5 ml of 80% acetone and centrifuged at 3000 rpm for 10 min. The supernatant was collected separately and the pellet was re-extracted with 80 % acetone until the extract became colorless. All the supernatants were pooled and made up to a known volume with 80% acetone. Then the absorbance of the pigment was measured at 663 nm 645 nm for Chlorophyll (Chl) - a and Chl-b, respectively using spectrophotometer (Hitachi Model-U 2001 Japan). Then Chl a, b and total Chl were calculated (Arnon, 1949).

Proteins were isolated by homogenizing 500 mg of fresh leaves in 10 ml of 50 mM prechilled Tris-HCl (pH 8.0) and centrifuged at 10,000 rpm for 15 min at 4°C and an aliquot from the supernatant was mixed with an equal volume of ice cold 10% trichloroacetic acid (TCA; w/v) and incubated at 0°C for 1 h to precipitate the proteins. The protein pellet was collected by centrifugation at 5,000 rpm for 15 min at 4°C and dissolved in 1 M NaOH. Protein content was estimated by the procedure of Lowry *et al*., (1951) with BSA used as a standard using colorimeter.

Cr induced toxicity and alleviation activity of fertilizers was measured by estimating the anti oxidant enzymes. The whole plant is taken for analysis. The role of antioxidant enzymes is to fight against the radicals formed during stress condition**.** 0.5g of sample were ground with 3.0ml of potassium phosphate buffer, centrifuged at 2000g for 10 minutes and the supernatants were used for the assay of Super Oxide Dismutase (SOD) by following the Kakkar›s method (Kakkar *et al,*. 1963). A 20% homogenate was prepared in 0.1M phosphate buffer (pH 7 for CAT and6.5 for POD) from the various parts of the plant, clarified by centrifugation and the supernatant was used for the assay of Catalase (CAT) by the method of Luck (Luck, 1963) and for Peroxidase (PER) by Reddy's method (Reddy *et al.,* 1995).

Plants were uprooted from the pots with the help of fine jet of water causing minimum damage to the roots, washed thoroughly with

distilled water, and blotted dry. Different plant parts were separated manually cut in small pieces and oven dried (80 deg C). The oven-dried samples were ground and digested in HNO_3 (70%) using Microwave Digestion System MDS 2000 and metal contents were estimated using atomic absorption spectrophotometer. (APHA, 1990)

RESULTS AND DISCUSSION

The chlorophyll (a, b, and total) content in leaves reduced significantly (ANOVA $p<0.05$) as compared to control (Table 1). Analysis of the leaf tissue of *V. radiata* plants treated with mercuric chloride showed the inhibitory effect with reverence to chlorophyll a, chlorophyll b and total chlorophyll level as compared with control. Cr (VI) toxicity severely affects the size of peripheral part of the antenna complex. So that there was a decline in the chlorophyll a and b ratio (Shanker *et al.,*2004). Furthermore, the activity of α-aminolaevulinic acid dehydratase might be impaired, which further leads to reduced photosynthetic pigments under Cr toxicity (Rai *et al.,* 2004). Distribution and degradation of the proteins of the peripheral part by Cr may decrease chlorophyll b. Accumulation of protochlorophyll due to the strong inhibition of chlorophyll synthesis has been observed in *Lycopersicon esculentum Mill*, grown under mercury medium (Nidhi gauba *et al.,* 2007). High activity of chlorophyllase has been observed in metal treated plants which makes degradation and reduction of the Chl level . The decline may also be attributed to poor supply of Mg

Table 1. Effect of different fertilizers on pigment content of *Vigna radiata* under various experimental conditions

Groups	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophyll (mg/g)
Control Water	1.3333 ± 0.1271	1.1667 ± 0.0857	2.5 ± 0.0939
Test ControlChromium	0.7 ± 0.0939	0.55 ± 0.0878	1.5833 ± 0.6241
Group I ControlWater + FYM	1.9333 ± 0.1084	1.3167 ± 0.079	3.2333 ± 0.1837
Group I TestCr + FYM	1.5667 ± 0.1434	0.8167 ± 0.1032	2.4 ± 0.0939
Group II ControlWater + NPK	2.2 ± 0.0939	1.4833 ± 0.1031	3.6833 ± 0.1807
Group II TestCr + NPK	1.9 ± 0.115	1.2 ± 0.0664	3.1 ± 0.115
Group III ControlWater + PK	1.8 ± 0.0939	1.05 ± 0.0575	2.85 ± 0.1101
Group III TestCr + PK	1.4 ± 0.0939	0.7333 ± 0.0542	2.1333 ± 0.0857
Group IV ControlWater + VC	1.65 ± 0.0575	0.9 ± 0.1756	2.55 ± 0.1848
Group IV TestCr + VC	1.2 ± 0.0664	0.6333 ± 0.0857	2.6667 ± 0.1838

Values are mean ± standard deviation. N = 6. $P< 0.05$

Table 2. Effect of different fertilizers on the uptake of chromium by *Vigna radiata*

Groups	Chromium in shoot (µg/g dry wt)	Chromium in root (µg/g dry wt)
Control water	-	-
Test Control Chromium	79.3333 ± 7.6735	228.3333 ± 10.8791
Group I Control Water + FYM	BDL	BDL
Group I Test Cr + FYM	96.8333 ± 87	265 ± 25.6
Group I I Control Water + NPK	BDL	BDL
Group II Test Cr + NPK	111.6667 ± 77	282 ± 23.6
Group III Control Water + PK	BDL	BDL
Group III Test Cr + PK	103.1667 ± 9.5589	232.3333 ± 11.4512
Group IV Control Water + VC	BDL	BDL
Group IV Test Cr + VC	95.6667 ± 5.6967	226.3333 ± 3.7349

Values are mean ± standard deviation. N = 6. $P<0.05$

and Fe to the leaves (Dheeba *et al.,* 2012, Gregor and Lindberg, 1986).

At 30 days after metal treatment, in fresh leaves of *V.radiata* of test control plants, the activity of all antioxidant enzymes increased due to Cr (VI) contact compared to that of control (Fig.1, 2 & 3). Fertilizers treated plants showed significant difference in enzyme activity when compared to test control. Treatment of fertilizers helps in reducing the toxicity. The result reveals that SOD Activity of group III test is almost nearer to control plants. Water and fertilizer treated plants showed lesser SOD activity than control group (116U/g). PER activity of all chromium and fertilizers treated test plants are almost same (60 U/g). NPK and FYM application in Cr treated plant showed the same results on SOD activity (131.6 U/g). Catalase activity was much higher in chromium treated test control plants which indicate the stress (446 U/g). In general Cr induces toxicity and fertilizers nullify the effect of heavy metal. The activity of all antioxidant enzymes were increased under Cr stress and reduced in plants which received fertilizers. From the results the order of efficacy is NPK<FYM<PK<VC (Fig1).

One of the common effects of biotic and abiotic stresses is generation of free radicals. Heavy metals are greatly enhances reactive oxygen species (ROS) production and that implicates stress condition (Dietz *et al.,* 2006). Cr is a toxic metal that can generate ROS which damage plants. Plants exposed to Cr have disruption of cellular and structural functions. Catalase is a heme containing enzyme which produces water and oxygen from hydrogen peroxide in peroxisomes (Panda and Choudhury, 2005). The hyperactivity of catalase in pea plants has been noticed in a concentration dependant manner. In the same way, Rai *et al.*, (2004) reported an increased activity of catalase in *Oscimum tenuiflorum* treated with Cr.

Level of protein was reduced by heavy metal application (18.8 mg/g). The total protein content of control plants found to be 24.2mg/g. There was a 25% reduction in Cr treated plants when compared to control. In general, mean protein content of plants received Cr and fertilizers (higher to lower): PK<NPK<VC<FYM. (Fig 4) It is clear that supply of PK and NPK helps in maintaing

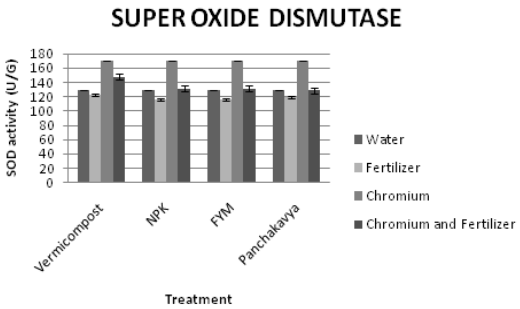

Fig. 1. Effect of chromium and fertilizers on SOD activity of experimental plants

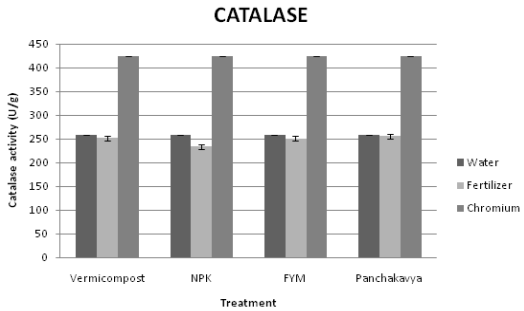

Fig. 2. Effect of chromium and fertilizers on Catalase activity of experimental plants

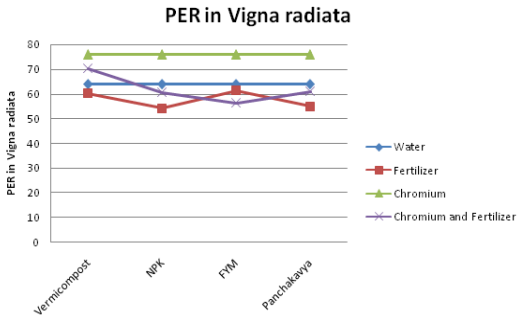

Fig. 3. Effect of chromium and fertilizers on peroxidase activity of experimental plants

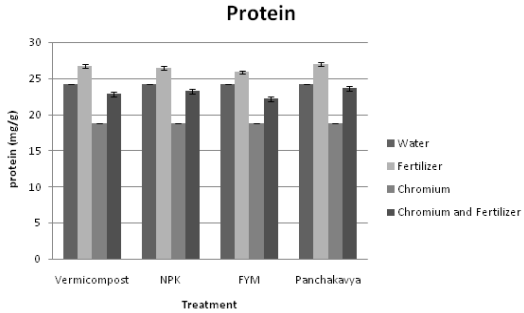

Fig. 4. Effect of chromium and fertilizers on protein content of experimental plants

protein level, may be due to the enhanced supply of nitrates. Same results were reported in *Zea mays* and *Vigna radiata* treated with Cr and effective microbes (Dheeba *et al.,* 2013).

The soluble protein in agricultural crops was reduced by heavy metals (Hemlatha *et al.,*, 1997) as observed in this study. The decrease in protein level is caused either by a reduced biosynthesis or by an increased disintegration of proteins to amino acids (Todd and Arnold, 1961). Vallee and Ulmer have reported that heavy metal ions have strong attraction for side chain ligands of proteins. So that enzyme activities and other functions are affected by heavy metal ions (Valle and Ulmer, 1972). A decline in the quantity of proteins was observed in seedlings of *Zea mays* at all concentrations of mercury (Kallimuthu and Sivasubramanian, 1990). Likewise, a concentration dependent decrease in soluble protein content over the control was observed in the shoot of *Albizia lebbek* (Tripathi and Tripathi, 1999).

Different plant parts and species differ in chromium accumulation (Mehrdad lahouti and Peterson, 1979) resemble with the present study. Roots accumulate greater Cr followed by leaves. Plants which received Cr alone accumulate 228.33 µg/g dry weights in roots and 79.33 µg/g dry weight in shoots, shows high oxidative damage. But the plants supplied with Cr and NPK accumulates 282 and 111.6 µg/g dry weight in roots and shoots respectively. (Table 2, Plate 2) Even though group II test plants accumulates large amount of Cr the toxicity caused by it is nullified with the help of NPK. The order of Cr accumulating capacity of fertilizers is NPK>FYM>PK>VC (Table 3). At the higher levels of Cr exposure, the significant amount of Cr was present in roots. Same results were reported (Sharma *et al.,*2003). High precipitation of Cr in root cells may cause low level of Cr in shoot. Cr was mainly accumulated in roots and poorly transported from root to other organs. Cr used the apoplastic transport across the

Effects of fertilizers on phytoremediation potential of vigna radiata

Plate 1. Chromium toxicity and alleviation by fertilizers on experimental plants

root surface. High level of Cr in root might be due to the adsorption on to the cell walls and in low level in shoot due to retention and transportation of small amount to the upper parts of plant by xylem (Vazquez *et al.,* 1987).

CONCLUSION

The present investigation revealed that Cr causes oxidative damage and affect the growth of *Vigna radiata.* Among four fertilizers used NPK accumulates Cr and nullify its effect on plant growth. The efficiency of fertilizers is found to be NPK>FYM>PK>VC in nullifying the metal toxicity in *Vigna radiata.*

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