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RESEARCH ARTICLE

Effect of hexavalent chromium on yield and biochemical components of *Tagetes erecta* (L.) under Arbuscular mycorrhiza treatment

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ABSTRACT : Degradation of natural environment as particularly soil ecosystem infected from various industrial chemical compounds are discharged into fertile soil and infected seriously on total ecological community of living biosphere. Soil deceit at the confluence of diverse natural systems, soil pollution can be spread to other parts of the natural environment by groundwater, for instance, percolates through the soil and can carry the soil pollutants into streams, rivers, wells and drinking water. Food plants growing on polluted soil may consist of harmful levels of pollutants themselves, and this can be passed on to the animals and people who eat them. The present study concludes that *Tagetes erecta*, (L.) could grow under hexavalent chromium polluted soil of Vellore district and applied different concentration of VAM treatment (Arbuscular mycorrhiza) such as, Control (without VAM treatment), 5g, 10g, 15g, 20g and 25g VAM / kg of soil for reclamation of Cr [VI] infected soil.

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Key Words :
Environment, Industrialization, Ecosystem, Hexavalent chromium, Soil Pollution, VAM

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The environment comprises all living and non-living organisms occurring naturally on earth surface for interaction of all living things depending upon each other. Environment has been constantly interacting with plants, animals, soil, water temperature and light. The pleasing environment provides the favorable conditions to development of growth and reproduction of living organism. Environmental pollution has become a consequent effect of global phenomenon, which has demanded attention from all over the countries. Industrialization, Population explosion, Deforestation and Unplanned urbanization were reflected in varying stages of pollution from soil, water and air (Singh *et al*., 1985) Soil pollution by heavy metals are a global problem causing vast areas of agricultural land to become non-arable and hazardous for both wildlife and human population (Cheng, 2003). Hexavalent state of chromium above at (0.8 to 16 µg/kg) higher concentration in soil conceded as heavy metal and gives adverse impact on environment, irreversibility in nature, cause destruction of total biosphere and resulting to reduction of ecosystem (Straif *et al.*, 2009). Chromium related chemical compounds were handled from chrome plating, paints, leather tanning, corrosion inhibition, steel production, and wood preservation. In the presence of Manganese oxide and Ammonium dichromate etc., on leather industry used exuberance level at highest temperature, Cr(III) oxidizes into

Cr (VI), which is more soluble in water and more toxic than other Cr forms (Dai *et al*., 2009). Bio-toxicity of Cr [VI] ramified to assign the harmful effects of heavy metals to the body when consumed above the bio-recommended limits. Although individual metals exhibit specific signs of their toxicity, the following have been reported as general signs correlated with, chromium, lead, arsenic, mercury, zinc and copper deliver poisoning effects of gastrointestinal (GI) disorders, hemo-globinuria causing a rust–red colour to stool, paralysis, vomiting, convulsion, depression, and pneumonia when volatile vapors and fumes of hexavalent chromium compounds are inhaled at above 0.07–1.1 ng/m3 recommended level of concentration.(McCluggage, 1991). In these points of view phytoremediation technology were used and followed by using *Tagetes erecta*, (L.), grow under Cr [VI] polluted soil with treatment of VAM (Control, 5g, 10g,15g,20g, and 25gVAM / kg of polluted soil).

EXPERIMENTAL METHODOLOGY

Seed collection and preparation :

The economically important ornamental seed crop of *Tagetes erecta* , belonging to the family Asteraceae was selected for the present investigation. This ornamental plant species seeds were obtained from Department of Floriculture, Tamil Nadu Agricultural University, and Coimbatore. Healthy and viable seeds of *Tagetes erecta* were surface sterilized with 0.1 per cent Mercuric chloride for 2. minutes and washed thoroughly with tap water followed by distilled water. The seed preparation experiment were conducted in the Environmental Biology laboratory, Department of Botany and Annamalai University.

Preparation of VAM treatment (Arbuscular mycorrhiza) :

The different concentrations of Control (Without VAM), 5g,10g,15g, 20g and 25 g VAM /kg of Cr[VI] polluted soil were prepared and they were used for seed treatment of Hexavalent chromium polluted soil in Vellore district, Tamil Nadu. The VAM was obtained from Department of Microbiology, Tamil Nadu Agricultural University, Coimbatore.

Experimental plot preparation :

The field was thoroughly ploughed two times before sowing. The entire field was irrigated for two days before seed sowing. The selected ornamental plant species seeds were sown with a spacing of 12.5 cm × cm in Hexavalent chromium polluted soil. The water irrigated for twice a day in experimental plot.

Field experiment :

The field experiment were conducted from Hexavalent chromium affected soil of Ranipet area, Vellore district. Field was constructed in Completely Randomized Block Design (CRBD). And there are two sets of experiment were carried out in two seasons such as summer and rainy season. *Tagetes erecta* seeds (15) were sown in the each row of seed bed and gives various treatment of VAM (Arbuscular mycorrhiza) such as Control, 5g, 10g, 15g, 20g and 25 g VAM / kg^{-1} soil. The plants were allowed to grow up to 30 to 90 DAS interval. The controlled plant rows consider as without VAM treatment and other rows of plant species treated as VAM.

Estimation of Biochemical components :

Estimation of chlorophyll :

Five hundred milligram of fresh leaf was ground in a pestle and mortar with 20 ml of 80 per cent acetone. The homogenate was centrifuged at 3000 rpm for 10 minutes. The supernatant was saved and the pellet was re-extracted with 5 ml of 80 per cent acetone each time, until it became colorless. All the supernatants were pooled and utilized for chlorophyll estimation. Absorbance was measured at 645 nm and 663 nm used spectrophotometer (Arnon, 1949).

The chlorophyll content was determined by using formula :

$$\text{Chlorophyll "a" } (\text{mg g}^{-1}) = \frac{12.7 \times A663 - 2.69 \times A\ 645}{a \div 1000 \div W} \div V$$

$$\text{Chlorophyll "b" } (\text{mg g}^{-1}) = \frac{22.9 \times A\,645 - 4.68\ \times A\,663}{a \div 1000 \div W} \div V$$

$$\text{Total chlorophyll } (\text{mg g}^{-1}) = \frac{20.2 \times A\,645 + 8.02 \times A\ 663}{a \div 1000 \div W} \div V$$

Estimation of catalase activity :

One gram of plant sample (root, stem and leaf) was homogenized in 10ml of 0.1M sodium phosphate buffer pH 7 and centrifuged at 4ºC for 10 min at 10,000 rpm. An aliquot of 1 ml of the supernatant of the enzyme extract was added to the reaction mixture containing 1 ml of 0.01M H_2O_2 and 3 ml of 0.1 M sodium phosphate

buffer. The reaction was stopped after an incubation of 5 min at 20ºC by adding 10 ml of 1 per cent H_2SO_4. The acidified medium without or with the enzyme extract was titrated against 0.005N $KmnO_4$ and catalase activity was expressed as moles of H_2O_2 utilized g^{-1} fr.wt.min^{-1} (Machly and Chance, 1967).

Estimation of peroxidase activity :

One gram of fresh plant sample (root, stem and leaf) was homogenized with 20 ml of ice cold extraction medium containing 2mM $MgCl_2$, 1mM EDTA, 10 mM α-mercaptoethonol, 7 per cent PVP and 10 mM sodium metabisulphate. The homogenized was strained through two layers of cheese cloth and centrifuged at 10,000 rpm for 15 min. The supernatant was made upto 20 ml with the same buffer and it was used as the source of enzyme. Assay mixture of peroxidase contained 2 ml of 0.1 M phosphate buffer pH 6.8, 1 ml of 0.001M pyrogallol, 1 ml of 0.005M hygrogen peroxidase and 0.5ml of enzyme extract. The solution was incubated for 5 min at 25ºC after which the reaction was terminated by adding 1 ml of 2.5 N sulphuric acids. The amount of purpurogallin formed was determined by reading the absorbance at 420 nm against a blank prepared by adding the extract after the addition of 2.5N sulphuric acids. The activity was expressed in unit = 0.1 absorbance $minute^{-1}$ mg^{-1} protein (Kumar and Khan, 1982).

Estimation of polyphenol oxidase activity :

Assay mixture for polyphenol oxidase contained 2 ml of 0.1 M phosphate buffer (pH 6.0), 1ml of 0.1M catechol and 0.5ml of enzyme extract. This was incubated for 5 min at 25ºC, after which the reaction was stopped by adding 1 ml of 2.5 N sulphuric acids. The absorbance of the purpurogallin formed was recorded at 495 nm.The enzyme activity was expressed in units. One unit is defined as the amount of purpurogallin formed, which raised the absorbance by 0.1 min^{-1} under the assay condition (Kumar and Khan, 1982).

Uptake and accumulation of hexavalent chromium in Tagetes erecta :

Bioconcentration factor (BCF), translocation factor (TF) and accumulation factor (AF) indicates the efficiency of plant species accumulated a metal into tissue from the contaminated environment it's calculated as follows (Chakroun *et al*., 2010).

$$\text{BCF} = \frac{\text{Average metal concentration in whole plant tissue (mg kg}^{-1})}{\text{Metal added in soil (mg kg}^{-1})}$$

$$\text{Accumulation Factor (AF)} = \frac{\text{Metal concentration in plant tissue}}{\text{Metal concentration in soil}} \times 100$$

$$\text{Translocation Factor (TF)} = \frac{\text{Metal concentration in plant shoot}}{\text{Metal concentration in root}}$$

EXPERIMENTAL FINDINGS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Chlorophyll contents (mg g^{-1} fresh weight) :

The degradation of chlorophyll molecules occurred through increased rate of chlorophyllase activity due to higher concentration of heavy metal (Lead) increased the rate chlorophyllase in stress condition. (Sharma and Dubey, 2005 and Saadet, 2013). The effect of hexavalent chromium with treatment of different concentration of VAM (Control,5,10,15,20 and 25 g kg soil) revealed photosynthetic pigments like chlorophyll 'a', chlorophyll 'b' and total chlorophyll content in *Tagetes erecta* under hexavalent chromium stress represented in Fig. 1. There was a gradual reduction of chlorophyll content with increased concentration of VAM treatment at 25 g VAM/ kg hexavalent chromium polluted soil for all respective day interval of 30, 60 and 90 DAS. The highest content of chlorophyll a (0.451, 0.534 and 0.513), chlorophyll 'b' (0.391, 0.426 and 0.411) and total chlorophyll (0.842, 0.960 and 0.924) was noted at 20 g VAM/kg of soil for all the sampling days whereas the lowest content of chlorophyll a (0.314, 0.348 and 0.326) chlorophyll 'b' (0.210, 0.248 and 0.213) and total chlorophyll content

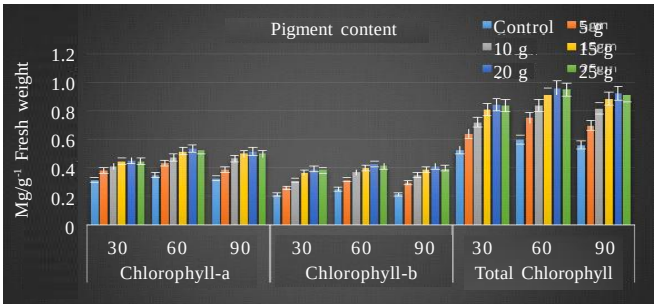

Fig. 1 : Effect of various treatment of Arbuscular mycorrhiza at hexavalent chromium polluted soil on Pigment content (mg g^{-1} fresh weight) of *Tagetes erecta*, (L.). Values shown are mean ± SE for five replicate field experiments

(0.524, 0.596, 0.557) was recorded at control plants (without Arbuscular mycorrhiza) for respective sampling days of 30, 60 and 90DAS.

Catalase activity (minutes^{-1} mg^{-1} protein) :

The toxic effects of high reactive oxygen intermediate to catalase synthesis were found to be increased. The stimulation of catalase synthesis by excess of heavy metals might be due to increased the synthesis of protein mobility of catalase and iron porphyrin (Prasad *et al*., 1999). That can be compared with earlier reports of Bhattacharya and Choudhuri (1994) in *Vigna* and rice Battacharjee and Mukherjee (1996) and Shah *et al.* (2001) in rice, Wu and Zhang (2002) in barley, Skorzynska *et al.* (2004) in *Arabidopsis thaliana*, Vijayarengan (2013) in Cymopsis tetragonolaba under zinc treatment and Stancheva *et al.* (2014) in *Ocimum basilicum* and *Origanum vulgare* under Pb, and Zn treatment. The catalase activity of *Tagetes erecta* on root, stem and leaf at various treatment of control 5, 10, 15, 20 and 25 g VAM/ kg of soil in different stages of growth (30, 60 and 90 DAS) was showed in Fig. 2. The minimum values of catalase content on root (0.891, 1.399 and 1.024), stem (1.171, 1.867 and 1.691) and leaf (1.943, 1.986 and 1.497) was observed in 20 g VAM treatment/kg of soil for all the sampling days. There was a gradual reduction in the catalase activities with increased concentration of VAM treatment like 5,10,15 and 20 g VAM/kg of soil. Highest catalase content of root (2.816, 2.973 and 2.938), stem (2.948, 3.041 and 2.962) and leaf (3.164, 3.248 and 2.973) was noted. The highest catalase activity was recorded

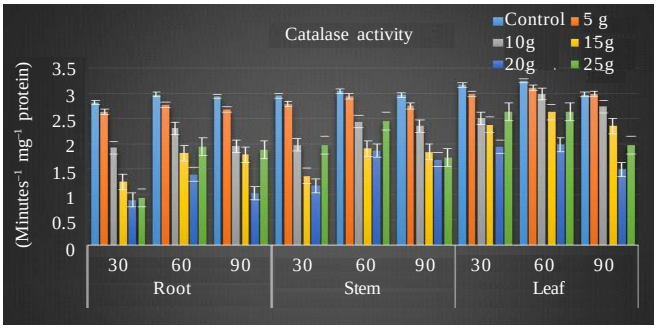

Fig. 2 : Effect of various treatment of Arbuscular mycorrhiza at hexavalent chromium polluted soil on Catalase activity (minutes^{-1} mg^{-1} protein) of Tagetes erecta, (L.). Values shown are mean ± SE for five replicate field experiments

in without treatment of Arbuscular mycorrhiza for control plants of all the sampling days.

Peroxidase activity (minutes $^{-1}$ mg^{-1} protein) :

The peroxidase activity showed a similar trend as that of the catalase activity of *Tagetes erecta* on root, stem and leaf at various treatment of control, 5, 10, 15, 20 and 25 gVAM/ kg of soil in different stages of plant growth (30, 60 and 90 DAS) were showed in Fig. 3. The minimum values of peroxidase content on root (0.196, 0.281 and 0.264), stem (0.283, 0.318, and 0.272) and leaf (0.372, 0.384 and 0.377) were noted in 20 g VAM treatment/kg of soil for all the sampling days. There was a gradual reduction of peroxidase activities with increased concentration of VAM treatment like 5, 10, 15 and 20 g VAM/kg of soil. Highest peroxidase content of root (0.316, 0.394 and 0.387), stem (0.389, 0.437 and 0.394)

Table 1 : Effect of various treatment of Arbuscular mycorrhiza at hexavalent chromium polluted soil on uptake and accumulation (µg g^{-1} dry weight) of *Tagetes erecta*, (L.).

Treatments (g kg^{-1})	Metal accumulation in plant (%)	Available metal in soil (%)	(AF) (%)	(TF) (mg/kg of soil)	BCF (mg/kg of soil)
Control	13.10	82.41	11.58	0.704	0.115
5	23.53	74.94	19.05	0.739	0.190
10	35.72	63.67	26.32	0.742	0.263
15	48.12	51.63	32.48	0.761	0.324
20	56.17	43.26	39.67	0.819	0.396
25	47.65	51.72	32.27	0.781	0.322

ANOVA

Source of variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Rows	295.2981	5	59.05962	0.55706	0.731432	2.71089
Columns	22053.84	4	5513.461	52.00382	2.7310	2.866081
Error	2.120406	20	106.0203			
Total	22351.25	29				

and leaf (0.479, 0.512, and 0.498) was observed. The highest peroxidase activity was recorded in without treatment of Arbuscular mycorrhiza of control plants for all the sampling days. Similar type of peroxidase activity due to metal treatment on different plants Radotic *et al. (*2000) on *Spruce* plant, Hegedue *et al. (*2001) in barley, Eapen *et al. (*2013) in *Brassica juncea* and Dikkaya and Ergün (2014) in maize.

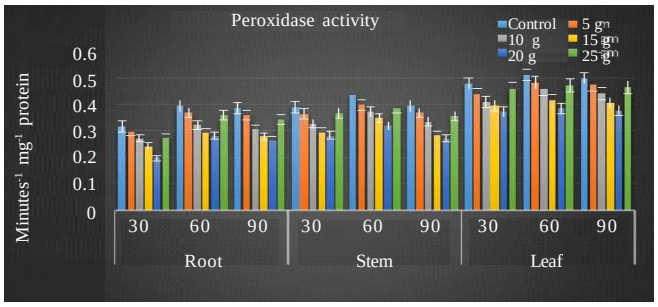

Fig. 3 : Effect of various treatment of Arbuscular mycorrhiza at hexavalent chromium polluted soil on Peroxidase activity (minutes^{-1} mg^{-1} protein) of *Tagetes erecta*, (L). Values shown are mean ± SE for five replicate field experiments

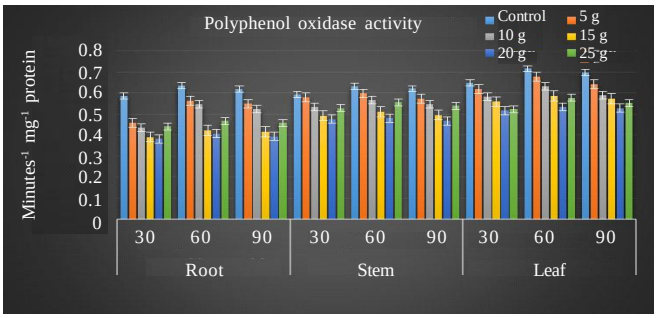

Fig. 4 : Effect of various treatment of Arbuscular mycorrhiza at hexavalent chromium polluted soil on Polyphenol oxidase content (minutes^{-1} mg^{-1} protein) of Tagetes erecta, (L). Values shown are mean ± SE for five replicate field experiments

Polyphenol oxidase activity (minutes^{-1} mg^{-1} protein):

The polyphenol oxidase activity also showed similar trend of catalase and peroxidase activity under Cr[VI] stress of *Tagetes erecta* on root, stem and leaf at various treatment of Control, 5, 10, 15, 20 and 25 g VAM/ kg of soil in different stages of growth (30, 60 and 90 DAS) was shows Fig. 4. The minimum values of polyphenol content on root (0.382, 0.407, and 0.394), stem (0.476, 0.481, and 0.467) and leaf (0.516, 0.534, and 0.529) were noted in 20 g VAM/kg of soil for all the sampling days. There was a gradual reduction in the peroxidase activities with increased concentration of VAM treatment like 5,10,15 and 20 g/kg of soil. Maximum polyphenol content of root (0.586, 0.635, and 0.618), stem (0.593, 0.632, and 0.621) and leaf (0.648, 0.716, and 0.697) was observed. The highest polyphenol activity was recorded in without treatment of Arbuscular mycorrhiza. Increased polyphenol oxidase activity was reported under various metal treatments as reported by Selige (1993) in copper, Kariev (1996) in zinc and nickel.

Uptake and phyto-accumulation (µg g^{-1} dry wt.) :

The uptake and accumulation of Cr[VI] was increased in different concentration of 5,10, 15 and 20 g VAM treatment /kg of hexavalent chromium polluted soil on *Tagetes erecta* was shows in Fig. 5. The higher accumulation of hexavalent chromium with treatment of Arbuscular mycorrhiza in root (274.9, 399.2, and 392.6), stem (236.9, 293.8 and 284.1) and leaf (183.2, 278.9 and 253.4) was observed at 20 g VAM/kg of soil from different sampling days of 30, 60 and 90 DAS. The minimum accumulation of hexavalent chromium in root (71.7, 118.7, and 121.0), stem (62.30, 94.3, and 98.6) and leaf (41.9, 71.6, and 79.0) was recorded for without treatment of control plants. The gradual reduction of accumulation was noted in increasing concentration of VAM at 25 g /kg of soil for all the sampling days.

Table 2 : Yield parameters of *Tagetes erecta*, (L.) as affected by hexavalent chromium polluted soil with treatment of Arbuscular mycorrhiza (90 DAS)

Treatments (g kg^{-1} soil)	Head	Number of flowers	Seed output plant^{-1}			Total seed weight (gm plant^{-1}) (100 seeds)
			Seeds (Fertile)	Seeds (Unfilled)	Total number of seed	
Control	02.10	37.05	17.34	19.71	37.05	0.79
5	03.71 (+76.66)	54.17 (+46.20)	37.19 (+114.4)	16.98 (-13.85)	54.17 (+46.20) (+19.22)	0.96 (+21.51)
10	05.84 (+178.0)	61.87 (+66.99)	47.06 (+171.3)	14.81 (-24.86)	61.87 (+66.99) (+34.50)	1.25 (+58.22)
15	07.21 (+243.3)	73.92(+99.51)	61.27 (+253.3)	12.65 (-35.81)	73.92 (+99.51) (+43.15)	1.37 (+73.41)
20	12.14 (+478.0)	87.34 (+135.7)	78.14 (+350.6)	09.20 (-53.32)	87.34 (+135.7) (+66.49)	1.54 (+94.93)
25	9.17 (+336.6)	71.12 (+91.95)	59.26 (+241.7)	11.86 (-39.82)	71.12 (+91.95) (-29.84)	1.41 (+78.48)

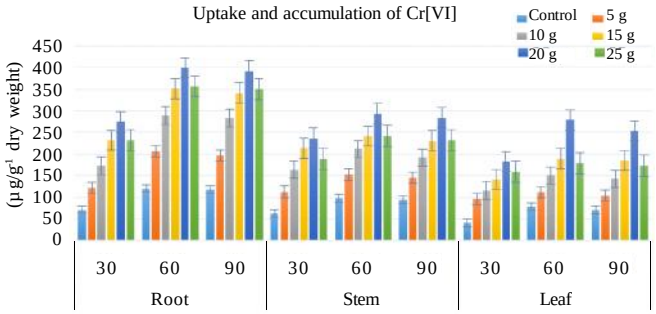

Fig. 5 : Uptake and accumulation of hexavalent chromium (µg g^{-1} dry weight) on *Tagetes erecta* (L). plant parts. Values shown are mean ± SE for five replicate field experiments

Metal accumulation in Tagetes erecta (%) :

The uptake and accumulation of hexavalent chromium was increased with increasing concentration of 5, 10, 15, and 20 g VAM/kg of soil under hexavalent chromium stress was tabulated in Table 1. The minimum hexavalent chromium (13.10%) was accumulated at control plants of without VAM treatment. The maximum accumulation (56.17%) of hexavalent chromium was observed at 20 g VAM (Arbuscular mycorrhiza) treatment/kg of soil. The gradual reduction (47.65) of accumulation of hexavalent chromium was observed in increasing concentration of VAM at 25 g/kg of contaminated soil from all the sampling days.

Available hexavalent chromium in soil (%) :

The available hexavalent chromium in soil was decreased with increasing concentration of 5, 10, 15 and 20 g VAM/kg of hexavalent chromium treatment polluted soil was tabulated in Table 1. The minimum available hexavalent chromium (43.26 %) percentage in soil was occurred in 20g VAM/ kg of soil. The maximum available hexavalent chromium (82.41) percentage in soil was observed at without treatment of control plants for all the sampling days.

Accumulation factor (AF) and Bio concentration factor (BCF) was increased with increasing concentration of 5, 10, 15, and 20 g VAM/kg of soil under hexavalent chromium stress was tabulated in Table 1. The minimum AF, and BCF of *Tagetes erecta* were found (11.58, and 0.115) in control treatment of without VAM concentration. The maximum AF and BCF (39.67, and 0.396) were observed at 20 g VAM/kg of soil. The highest (0.819) translocation factor (TF) was recorded in 20 g VAM/kg of soil, whereas below (TF) translocation factor (0.704) was observed at control plants. Similar results were reported by Toman *et al*. (2005) in Pheasant (*Phasianus colchicus*), Manciulea and Ramsey (2006) in lettuce, Tanhan *et al. (*2007) in *Chromolaena odorata*, Pehlivan *et al. (*2008) in sugar beet, Rascio *et al*. (2007) in rice, Amor and Ching (2014) on *Zea mays* plant

*The "F" test values were significant at 1 per cent for the treatment of Arbuscular mycorrhiza level in hexavalent chromium infected soil, sampling day's interaction between treatment and sampling days.

Yield parameters :

The yield parameters of number of heads, number of flowers per plant, fertile seeds, unfilled seeds, total seeds and total seed weight (g $plant^{-1}$) from harvested stage of *Tagetes erecta* were treated with different concentration of Control, 5,10,15,20 and 25 g VAM/kg of soil under effect of hexavalent chromium contaminated soil were tabulated in Table 2. The highest yield parameters (12.14, 87.34, 78.14, 09.20, 87.34, and 1.54) were observed at 20 g VAM (Arbuscular mycorrhiza) treatment/kg of hexavalent chromium polluted soil. The minimum values (02.1, 37.05, 17.34, 19.71, 37.05, and 0.79) were observed at control plants from different sampling days of 30, 60 and 90 DAS. The gradual reduction of yield was noted in increasing concentration of VAM at 25 g/kg of soil for all the sampling days.

Conclusion :

The present study of effect of hexavalent chromium on *Tagetes erecta*, may concluded that, plant species of *Tagetes erecta* grown in Cr[VI] contaminated soil and accumulate highest amount of Cr [VI] in roots followed by stem and leaf under different concentration of Arbuscular mycorrhiza treatments (Control, 5g, 10g, 15g, 20g and 25g VAM/ kg of soil). Chromium accumulation by *Tagetes erecta* affects many physiological processes of enzyme activities and accumulation related to Arbuscular mycorrhiza treatments. Chromium induced oxidative stress were tolerated to *Tagetes erecta* plant through the hyper activity of bio-enzyme defensive system (catalase, Peroxidase, and Polyphenol oxidase activity under chromium stress related to different concentration of VAM treatments). The H_2O_2 formed by the super-oxidation of active oxygen species was quenched by catalase and polyphenol oxidase. However

peroxidase took a little part in super oxidation of H_2O_2 under hexavalent chromium stress and equalized the enzyme activities related to the treatment of VAM. The present study of phytoremediation of Cr[VI] effect on *T. erecta* can be grown in Cr[VI] contaminated soil with treatment of various VAM(Arbuscular mycorrhiza) induced higher accumulation and yield of flower formation whereas compare to without treatment of VAM in control plants.

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