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Research Article

Growth and Physiological Response of *Jatropha Curcas* to Different Concentrations of Iron in Soil

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Abstract

Iron is an essential micronutrient which plays a vital role in the growth of plants but if supplied in excess quantity, it causes toxicity which is detrimental for plant survival. *Jatropha curcas* being non edible and hardy crop was explored for its potential to tolerate higher concentrations of iron for pytoremediation of iron contaminated soil. A pot experiment was conducted where the seedlings of *J. curcas* were treated with different concentrations of Fe (1000, 2000 & 3000 mg/kg) supplied as $FeSO_4.5H_2O$ to assess its growth and physiological response. There was significant decline in growth parameters viz. root length, plant height, fresh and dry weight of stem and root at higher Fe concentrations as compared to the control. The relative water content of the leaf also decreased significantly, whereas, the electrolytic conductivity increased significantly at higher concentration of Fe. There was no significant change in chlorophyll content. The carotenoid content showed a significant increase up to 2000 mg/kg Fe treatment and then decreased by 1.5 folds at 3000 mg/kg Fe treatment. The results show that different concentrations of Fe (1000, 2000 and 3000 mg/kg Fe) taken in the present study had adversely affected the growth and physiology of Jatropha seedlings but to fully understand the response of Jatropha to different Fe concentrations a study on the biochemical and molecular level is required.

Keywords: Jatropha curcas, micronutrient, Fe concentrations, growth parameters, toxicity, phytoremediation.

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Introduction

Metallic element with a high specific gravity (typically five times higher than water) is called as a heavy metal. It includes lead (Pb), cadmium (Cd), cobalt (Co), arsenic (As), silver (Hg), Iron (Fe), Manganese (Mn), Molybdenum (Mo), Copper (Cu), Nickel (Ni), Zinc (Zn), chromium (Cr) etc [1]. Some of the heavy metals like Fe, Cu and Zn are essential for plants and animals and are often called as micronutrients [2, 3]. The excess uptake of these micronutrients results in toxic effects [4, 5, 6]. Environmental pollution due to heavy metals has become one of the most dangerous pollution these days. The major problem with heavy metals contamination in soils is that, unlike organic pollutants, they cannot be bio-degraded and therefore remains in the environment for a longer time. Though iron is naturally present in the soil but, the major source of contamination to toxic level is attributed to anthropogenic origin. When taken in excessive quantities, Fe is potentially toxic as it promotes the formation of oxygen based radicals which are able to damage vital cellular constituents [1].

Different plants absorb metals from soil and water to varied extent and accumulate in different tissues [7]. Iron (Fe) is the first rare element recognized as necessary for both plants and animals and it plays an important role in different biochemical and physiological processes [8]. Plant responses to metals are dose dependent. For essential metals like Fe, these responses cover the phases from deficiency -to sufficiency -to toxicity. Iron is involved in plant metabolism so it is a critical element for plant life, even though only 50-100 µg/g dry matter of it is present in plants [9]. Iron toxicity in plants occurs by high Fe^{2+} uptake and translocation by plant tops [10]. Excessive amount of Fe in

plants may cause disturbances in cellular metabolism by inhibiting the activities of several enzymes [11]. When accumulated in high levels, Fe generates hydroxyl radicals by participating in Fenton reaction [12]. Iron toxicity is accompanied with reduction of plant photosynthesis, increase in oxidative stress and ascorbate peroxidase activity resulting in loss of yield [13].

Jatropha curcas L, a hardy perennial shrub belonging to the *Euphorbiaceae* family, of Latin American origin is widespread throughout the tropical regions of the world. Jatropha is a plant that can grow almost anywhere, even on soft, rocky, gravelly, sandy, calcareous, saline and sloping soils. It has low fertility and moisture demand. Jatropha is a non-edible shrub. Many studies show that Jatropha has a marked ability to tolerate a variety of abiotic stresses including heavy metal tolerance. These factors make Jatropha an ideal candidate for phytoremediation of metal contaminated soil. Keeping these points in view, the present study was conducted to evaluate the growth and physiological response of *Jatropha curcas* to different concentrations of Fe in soil in order to determine the limit up to which it can tolerate Fe in the soil and can survive in that soil.

Materials and Methods

Plant material and copper concentrations

Healthy and mature seeds of *Jatropha curcas* strain DARL-2 (Accession no. 569095) identified by DIBER (DRDO) Field Station at Pithoragarh, Uttarakhand, were used in the present study. The seeds were soaked overnight in 0.1% (w/v) Bavistin, washed several times under running tap water before sterilizing it with 70% (v/v) ethanol followed by three washes of sterile water. After sterilization the seeds were germinated on moist filter papers in petridishes. After germination (radical protrudence), seedlings of uniform age and size were transplanted into pots filled with equal amount of autoclaved mixture of sand and soil (1:1) in each pots. Ferrous sulphate ($FeSO_4.5H_2O$) solution was added in the pots to obtain the final Fe^{+2} concentrations of 1000, 2000 and 3000 mg/kg soil. In control no external Fe was supplied. Each treatment comprised of five pots with three seedlings each. Watering of both control and treated pots were done at regular intervals in such a manner that the saturation point of soil filled in pots was established to avoid leaching and water stagnation.

Observations recorded

After an exposure period of 120 days growth parameters like plant height, root length, total number of leaves, fresh and dry weight of root and stem were recorded for plants in each treatment.

Physiological parameters

Electrolytic conductivity (EC)/Leaf membrane damage (MD)

For measuring EC, EC meter (WTW, Germany) was used. Leaf pieces (~1.0 cm^2) were taken and washed with distilled water then they were dipped in 20 ml sterilized distilled water and incubated for 24 hrs with intermittent shaking. EC_1 was recorded at the end of incubation. EC_2 was recorded after autoclaving at 121ºC for 20 min. MD was calculated using the formula [14]:

$$MD\ (\%) = (EC_1 / EC_2)\ X\ 100$$

Relative water content (RWC)

For determining RWC, second leaf from three independent plants per treatment was taken and the fresh weight (FW) was recorded immediately. After 24 hrs of saturation with deionized water the turgid weight (TW) was recorded. Dry weight (DW) was determined after drying the leaves for 48 hrs in the hot air oven at 70 °C. The RWC was calculated as:

$$RWC\ (\%) = [(FW-DW)/ (TW-DW)]\ X100$$

Total chlorophyll and carotenoid content

For determining total chlorophyll and carotenoid content, 100 mg leaf sample was ground in acetone (80% v/v) with a pre-chilled mortar and pestle. After homogenization, the mixture was filtered and the volume was adjusted to 10 ml with the cold acetone. The absorbance of the extract was recorded at 645, 663, and 470 nm using a spectrophotometer (UV-Vis Dual Beam, Labomed.inc) and the pigment content were calculated [15].

Statistical analysis

For analysis of variance (ANOVA), Cropstat for Windows (7.2.2007.2 module), developed by the Biometrics unit, IRRI, Philippines was used. Experiments were laid out in Completely Randomized Design (CRD). The treatment means (n=3) were compared by least significant difference (LSD) test ($p \leq 0.05$).

Results

No visual symptoms of deformity in morphology like stunted growth, bending of stem, leaf curling or puckered leaf were observed in the plants treated with different concentrations of Fe. There was no significant change in the opening of cotyledons (**Figure 1**) and emergence of true leaf (**Figure 2**) up to 2000 mg/kg Fe as compared to the control but at 3000 mg/kg Fe, these parameters declined significantly. As compared to the control a significant decrease was observed in the growth parameters viz. root length, shoot length, fresh and dry weight of stem & fresh and dry weight of root (**Table 1**) with increasing concentration of Fe. The root length did not change significantly up to 2000 mg/kg Fe as compared to the control but at 3000 mg/kg Fe, a significant decrease was observed in the root length. The plant height declined significantly at different concentrations of Fe as compared to the control (Table 1). At 3000 mg/kg Fe, the plant height decreased by 2.5 folds as compared to the control. The fresh and dry weight of stem and root also decreased significantly at different concentrations of Fe. At 3000 mg/kg Fe, fresh weight of stem decreased by 4.5 folds and the dry weight of stem decreased by 5.4 folds as compared to the control (Table 1). As compared to the control, the fresh and dry weight of root decreased by 2.8 folds and 2.0 folds respectively at 3000 mg/kg Fe (Table 1).

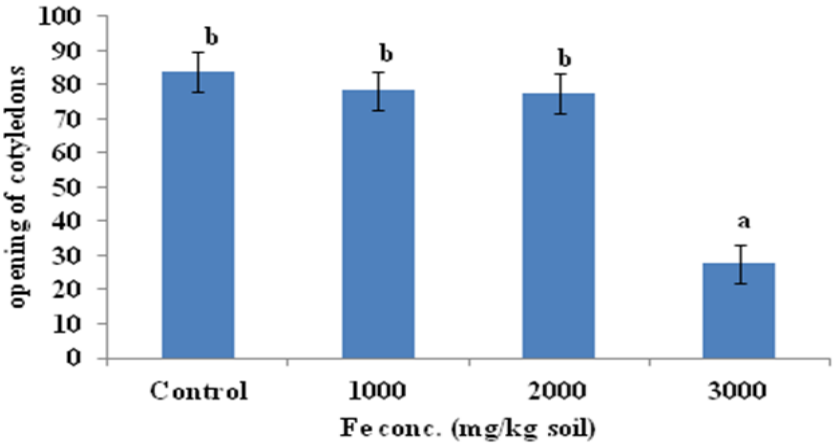

Figure 1 Effect of different concentrations of Fe on opening of cotyledons. Different letters indicate significant differences at $p \leq 0.05$, as determined using Least Significant Difference (LSD) test. Error bars indicate SE of three treatment means

In the present study, no significant difference was observed in the RWC at 1000 mg/kg Fe, but at higher concentrations (>1000 mg/kg Fe) significant decrease in the RWC was observed as compared to the control (**Figure 3**). Also, a significantly higher electrolytic conductivity was observed at different Fe concentrations as compared to the control (**Figure 4**). At 3000 mg/kg Fe, the electrolytic conductivity of leaf tissue leachates was 1.2 folds as compared to the control (Figure 4). The total chlorophyll content did not change significantly at different Fe

concentrations as compared to the control. However, the carotenoid content increased up to 2000 mg/kg Fe and then significantly decreased at 3000 mg/kg Fe treatment (**Table 2**).

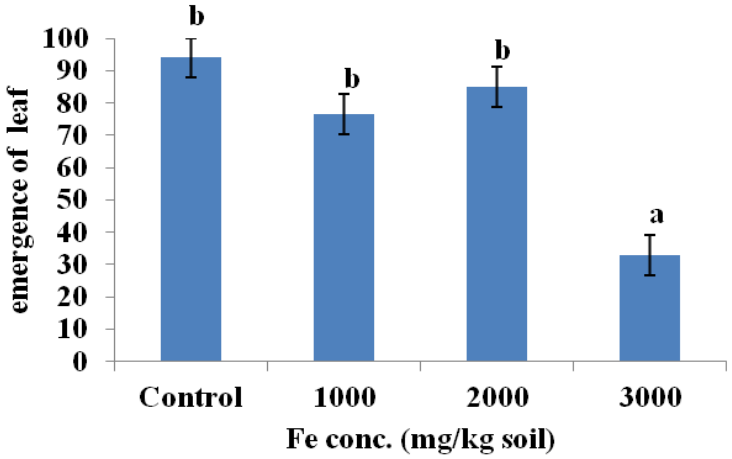

Figure 2 Effect of different concentrations of Fe on emergence of leaf. Different letters indicate significant differences at $p \leq 0.05$, as determined using Least Significant Difference (LSD) test. Error bars indicate SE of three treatment means

Table 1 Effect of different concentrations of Fe on growth parameters of *J. curcas*.

$FeSO_4.5H_2O$ (mg/kg soil)	Root length (cm)	Plant height (cm)	Total leaves	Stem fresh weight (g)	Stem dry weight (g)	Root fresh weight (g)	Root dry weight (g)
Control	8.17^{b}	16.60^{d}	5.0	4.70^{d}	1.21^{b}	0.51^{c}	0.13^{c}
1000	8.20^{b}	12.27^{c}	5.0	2.75^{c}	0.53^{a}	0.32^{b}	0.07^{b}
2000	8.23^{b}	8.53^{b}	5.0	1.75^{b}	0.36^{a}	0.31^{b}	0.07^{b}
3000	7.30^{a}	6.73^{a}	5.0	1.04^{a}	0.23^{a}	0.18^{a}	0.06^{a}
SE	0.21	0.33	0.0	0.10	0.07	0.02	0.00
LSD (5%)	0.72	1.16	0.0	0.36	0.23	0.06	0.01

*The values marked with different letters are significantly different from each other at $p \leq 0.05$, as determined using Least Significant Difference (LSD) test.

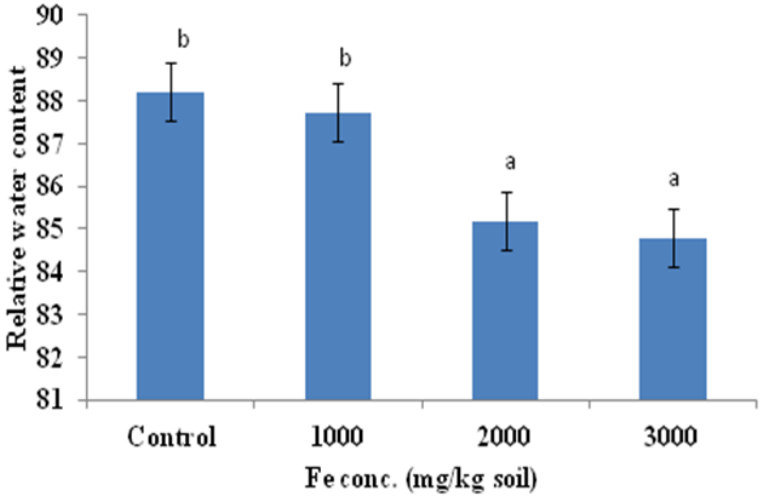

Figure 3 Effect of different concentrations of Fe on relative water content of leaf. Different letters indicate significant differences at $p \leq 0.05$, as determined using Least Significant Difference (LSD) test. Error bars indicate SE of three treatment means

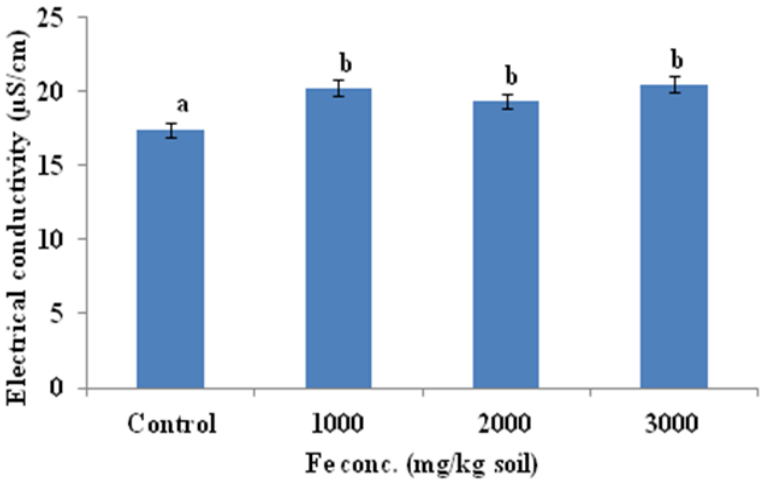

Figure 4 Effect of different concentrations of Fe on electrical conductivity of leaf. Different letters indicate significant differences at $p \leq 0.05$, as determined using Least Significant Difference (LSD) test. Error bars indicate SE of three treatment means

Table 2 Effect of different concentrations of Fe on photosynthetic pigments of *J. curcas*.

$FeSO_4.5H_2O$ (mg/kg soil)	Chl (a + b) (mg/gFW)	Carotenoids (mg/gFW)
Control	32.75^a	1158.44^b
1000	34.69^a	1269.26^c
2000	34.57^a	1243.93^c
3000	34.53^a	774.13^a
SE	1.00	16.15
LSD	3.45	55.87

*The values marked with different letters are significantly different from each other at $p \leq 0.05$, as determined using Least Significant Difference (LSD) test.

Discussion

Iron in soil can occur either in the divalent ferrous ion (Fe^{+2}) or trivalent ferric ion (Fe^{+3}) states but plants can absorb only the ferrous ion (Fe^{+2}) and this is the reason that in most of the Fe metal toxicity studies, the Fe is added as a salt solution ($FeSO_4$) to the growth medium so that the results may conservatively represent effects of Fe in its most bio-available form, and not those that have undergone geochemical processes, valence changes, sorption/desorption and other processes that are expected in an environmental soil matrix [16]. Symptoms of Fe toxicity are expressed differently in different plants. For example, in flax excess Fe causes dark green foliage and stunted root and shoot growth [17], in rice excess Fe causes brown spots on the lower leaves [18]. Iron toxicity in tobacco produces brittle, tender, dark brown to purple leaves with poor burning qualities and flavor [19, 20] while in navy bean; it is associated with zinc deficiency producing black spots on the leaves [21]. In the present study the absence of any morphological toxicity at higher concentrations of Fe indicates the tolerance of *Jatropha curcas* to these concentrations of Fe in soil.

The concentrations of Fe taken in the present study (1000, 2000 & 3000 mg/kg) were decided on the basis of previous work (results not shown) done with lower concentrations (upto 750 mg/kg Fe) in which no difference in terms growth was observed in the plants as compared to the control and the plants at 750 mg/kg Fe were as good as the control plants in terms of the growth parameters. So, it was decided to study the response of the plant towards higher concentrations of Fe.

Examination of the results of the present study showed that as compared to the control at all the concentrations of Fe the various growth parameters were significantly declined except the number of leaves. Many researchers also have reported Fe toxicity as a problem in plants, particularly in rice [22, 23]. However, the threshold of toxicity due to Fe varies in different plants. In an aquatic reed species (*Phragmites australis*), Fe above a total concentration of 1 mg/l causes growth inhibition [24] whereas the effective concentration of Fe for duckweed (*Lemna minor)* is 3.7 mg/l.

Relative water content (RWC) is the appropriate measure of plant water status in terms of the physiological consequence of cellular water deficit. RWC is a major factor which determines the ability of a plant to tolerate any stress. The present study indicates no significant change in RWC of the plants at 1000 mg/kg Fe as compared to the control plants which signifies that Jatropha can osmotically adjust to this concentration of Fe in soil and can maintain its turgor. Being a redox active metal Fe participates in the redox reaction in cells and results in the formation of $O^{2\bullet-}$ and subsequently in H_2O_2 and $^{\bullet}OH$ production via the Haber-Weiss and Fenton reactions [25]. The increased electrolytic conductivity at different concentrations of Fe as compared to the control indicates that membrane damage has occurred. Our result is supported by the work of [13], who also found that Fe toxicity causes damage to the membranes due to lipid oxidation in *Hydrilla*. Heavy metals cause membrane damage through various mechanisms, including the oxidation of and cross-linking with protein thiols, inhibition of key membrane protein such as H^+-ATPase, or causing changes in the composition and fluidity of membrane lipids [26]. In order to assess the impact of environmental stress in plants, chlorophyll content is often measured. Changes in pigment content are linked to visual symptoms of plant illness and photosynthetic productivity [27]. Fe has the ability to lose or gain electrons and because of this property it works as a co factor for enzymes involved in a wide variety of oxidation-reduction reactions like photosynthesis, respiration, hormone synthesis, DNA synthesis, etc [1]. Results of the present study showed an increase in the total chlorophyll and carotenoid content with increasing concentration of Fe, though the increase was not significant. Similar types of results were obtained in pea plants where excess concentration of Fe caused upto 28% increase in the pigment concentrations.

Previous study suggest that *Jatopha curcas* has a high potential to tolerate and accumulate a high concentration of heavy metals including Fe and it can be a good option for phytoremediation of multi metal contaminated sites [28]. Growth of different strains of *Jatopha curcas* on iron rich wasteland soil suggested that the clone BTP-N can effectively grow in Fe rich wasteland site exhibiting vigorous growth and is able to remove the excessive iron from soil [29].

Conclusion

The results of the present study indicates that 1000 mg/kg Fe in soil has no negative effect on root length, total number of leaves, RWC and total chlorophyll content but the fresh and dry weight of root and stem was significantly declined. Moreover, the absence of any visual toxic symptoms in Jatropha seedlings up to 3000 mg/kg Fe may warrant its deployment in iron contaminated soil where edible plants cannot be grown. Though no visual symptoms were observed but it may affect flowering and fruiting at maturity stage. Its phytoremediation use can be justified as it is a non-edible crop and can accumulate higher concentrations of iron. The role of enzymatic and non–enzymatic antioxidant defence mechanisms of Jatropha plant towards Fe toxicity will form a part of our future study.

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References

[1] Rout GR, Sahoo S. Role of Iron in plant growth and metabolism. Rev. Agricul. Sci. 2015; 3: 1-24.

[2] Wintz H, Fox T, Vulpe C. Responses of plants to iron, zinc and copper deficiencies. Biochem. Soc. Trans. 2002; 30: 766-768.

[3] Reeves RD, Baker AJM. Metal-accumulating plants. In: Phytoremediation of Toxic Metals: Using Plants to Clean Up the Environment (Raskin I, Ensley BD, eds.). 2000; 193-229. Wiley. New York.

[4] Hall JL. Cellular mechanisms for heavy metal detoxification and tolerance. J. Exp. Bot. 2002; 53: 1–11.

[5] Blaylock MJ, Huang JW. Phytoextraction of metals. In: Phytoremediation of Toxic Metals-Using Plants to Clean Up the Environment (Raskin I, Ensley BD, eds.). 2000; 53-70. Wiley. New York.

[6] Monni S, Salemma M, Millar N. The tolerance of Empetrum nigrum to copper and nickel. Environ. Pollut. 2000; 109: 221-229.

[7] Chambers JC, Sidle RC. Fate of heavy metal in abandoned lead, zinc tailing ponds: I Vegetation. J. Environ. Qual. 1991; 20: 745-748.

[8] Pooladvand SM, Ghorbanli MFS. Effect of various levels of iron on morphological, biochemical, and physiological properties of Glycine max var. Pershing. Iranian J. Pl. Physiol. 2012; 2 (4): 531-538.
[9] Guerinot ML and Yi Y (1994) Iron: Nutritious, noxious, and not readily available. Plant Physiol., 104: 815-820.
[10] Brown, J. C., and W. E. Jones. Manganese and iron toxicities dependent on soybean variety. Commun. Soil Sci. Plant Anal. 1977; 8: 1-15.
[11] Juwarkar AA, Yadav SK, Kumar P, Singh SK. Effect of Biosludge and Biofertilizer amendment on growth of *Jatropha curcas* in heavy metal contaminated soils. Environ. Monit. Assess. 2008; 145: 7-15.
[12] Halliwell B, Gutteridge JMC. Biologically relevant metal ion-dependent hydroxyl radical generation. FEBS Lett. 1992; 307: 108–112.
[13] Sinha S, Guptha M, Chandra P. Oxidative Stress induced by iron in Hydrilla verticillata Royle: response of antioxidants. Ecotoxicol. Env. Safe. 1997; 38: 286–291.
[14] Lutts S, Kinet JM, Bouharmont J. Changes in plant response to NaCl during development of rice Oryza sativa L. varieties differing in salinity resistance. J. Exp. Bot. 1995; 46: 1843-1852.
[15] Arnon DI. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Pl. Physiol. 1949; 24: 1–15.
[16] US-EPA. Ecological Soil Screening Level for Iron. Nov 3 2003; 1-8.
[17] Millikan CR. Effects on flax of a toxic concentration of boron, iron, molybdenum, aluminum, copper, zinc, manganese, cobalt, or nickel in the nutrient solution. R. Soc. Victoria Proc. 1949; 61: 25-42.
[18] Tadano T. Devices of rice roots to tolerate high iron concentrations in growth media. Jpn. Agric. Res. Q. 1975; 9: 34-39.
[19] Perkins HF, Gibson EJ, Bertrand AR. Influence of iron content on quality of cigar wrapper tobacco. Commun. Soil Sci. Plant Anal. 1972; 3: 459-466.
[20] Rhoads FM. Relations between Fe in irrigation water and leaf quality of cigar wrapper tobacco. Agron. J. 1971; 63: 938-940.
[21] Ambler JE, Brown JC, Gauch HG. Effect of zinc on translocation of iron in soybean plants. Plant Physiol. 1970; 46: 320-323.
[22] Howeler RH. Iron induced ringing disease of rice in relation to physicochemical changes in a flooded soil. Soil Sci. Soc. Am. Pro. 1973; 37: 898-903.
[23] Snowden RED, Wheeler BD. Iron toxicity to fen plant species, J. Ecol. 1993; 81: 35-46.
[24] Batty LC, Younger PL. Effects of external Iron concentration upon seedling growth and uptake of Fe and phosphate by the common reed, Phragmites australis (Cav.) Trin ex. Steudel. Annals of Botany. 2003; 92: 801-806.
[25] Gill M. Heavy metal stress in plants: a review. Int. J. Adv. Res. 2014; 2(6): 1043-1055.
[26] Meharg. The role of plasmalemma in metal tolerance in angiosperm. Physiol. Plant. 1993; 88(1): 191–198.
[27] Parekh D, Puranlk RM, Srivastava HS. Inhibition of chlorophyll biosynthesis by cadmium in greening maize leaf segments. Biochemie Physiologie der Pflanzen.1990; 186: 239–242.
[28] Majid NM, Islam MM, Riasmi Y. Heavy metal uptake and translocation by *Jatropha curcas* L. in sawdust sludge contaminated soils. Aus J Crop Sci. 2012; 6(5): 891-898.
[29] Ghavri SV, Singh RP. Phytotranslocation of Fe by biodiesel plant Jatropha curcas L. grown on iron rich wasteland soil. Braz. J. Pl. Physiol. 2010; 22(4): 235-243.

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