iJRASET
International Journal For Research in
Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 8 **Issue:** III **Month of publication:** March 2020

DOI:

International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.429
Volume 8 Issue III Mar 2020- Available at www.ijraset.com

Modulatory Effect of Allium Sativum (Garlic Cloves) Extract on Cadmium-Induced Oxidative Stress in Rice Seedlings

Tugbobo O. S[1], Idowu K. S[2], Awonegan P. A[3], Fayose T. S[4], Onemayin K. J[5]
[1, 2, 3]*Department of Science Technology, Federal Polytechnic, Ado-Ekiti, Nigeria*
[4, 5]*Department of Mathematics and Statistics, Federal Polytechnic, ado-Ekiti, Nigeria.*

Abstract: The modulatory effect of garlic cloves extract on cadmium-induced oxidative stress was evaluated in growing rice seedlings raised in sand and clay soils. The growing rice seedlings were cultured under 250µM cadmium chloride ($CdCl_2$) and 500µM Allium sativum aqueous extract for a period of two months. Uptake and distribution pattern of proxidant (Cd^{2+}) with possible induction of oxidative stress and alteration in enzyme activity in the rice shoots and roots were assessed. Results revealed higher significant ($P<0.05$) increase in thiobarbituric acid reactive species in rice seedling roots grown between 15-60 days. Besides, marked increase in antioxidant enzyme activity was observed in cadmium chloride treated seedlings, where the shoots maintained higher defensive enzyme activity than shoots. Results obtained from this research further affirm that heavy metals are potential threat to growing rice plants and could be possibly prevented by garlic extract.
Keywords: Rice-seedlings, cadmium chloride, thiobarbituric reactive species, oxidative stress.

I. INTRODUCTION

High levels of heavy metals in the soil normally result in oxidative damage to plant tissues directly or indirectly by triggering generation of elevated level of reactive oxygen species. This in turn causes damage to biological macromolecules and cytochemical compositions of the plant (Halliwell, 1994). The free radicals are usually superoxide radicals (O_2^*) hydroxyl radicals (OH*), hydrogen peroxides e.t.c. that are released as by-products during membrane-linked electron transport reaction and cellular metabolic processes (Becana et al., 2000). Cadmium exists naturally in soil and water while plants absorb the metal from the soil via the roots (Erik et al., 1999). The relationship between plants and most heavy metals in the soil depends on variable factors such as nature of chemical forms of the element in the soil, properties, climate as well as plant species (Godbold and Kettner, 1991). Reports have documented that increased lead level in the soil environment inhibits seed germination, stunts seedling growth and development thus, resulting in low yields (Kastori and Petrovic, 1992). Oxidative stress occurs whenever there is imbalance between the production of reactive oxygen species and the biological system's ability to readily detoxify the reactive intermediates produced or failure to repair the damage (Tanaka et al., 1985). Myriad of studies on garlic cloves have reported high level of organosulphur and phenolic compounds inherent in the cloves which from research findings are anti-oxidative stress. Hence, the present research is designed to evaluate the modulatory effect of garlic extract on Cd^{2+}-induced oxidative stress in growing rice seedlings.

II. MATERIALS AND METHODS

A. Collection and Preparation of Samples

Soil and garlic cloves samples were collected simultaneously from five South-Western states of Nigeria, namely Ogun, Ondo, Oyo, Osun and Ekiti. Two locations in Ekiti named Igbimo and Emure villages were chosen as case study for the research based on highest cadmium levels found in their soils after a thorough and comprehensive soil test. In addition, 500g of powdered sample of garlic cloves was extracted via maceration for 48hrs using method described by (Berln and Schaller, 2008).

B. Experimental Protocol

The research was conducted under careful adherence to guidelines and ethical norms of USAD. The rice seeds were grown in the soils of the two locations Igbimo and Emure. The rice seeds were surface sterilized with 0.1% sodium hypochlorite solution for 10min and then rinsed with distilled water. The seedlings were raised in clay (Igbimo-area) and sand (Emure-area) cultures respectively in plastic pots devoid of cadmium or related heavy metals. The pots received respective treatment solutions and were carefully maintained under control for seeedling growth and development in a biological oxygen demand and optimum relative humidity as well as 12hrs photoperiod. The pots were divided into four groups (A, B, C, D) in each location. Pots in group A contained seedling treated with distilled water only and served as positive control. Rice seedlings in pots B were treated with 250µM cadmium chloride only, seedlings in pots C received 250µM with 500µM crude garlic extract simultaneously [1:1]. Rice seedlings in group D pots were treated with 500µM crude garlic extract only.

C. Determination of Cadmium [Cd^{2+}] in Rice Seedlings

Fresh root and shoot samples of growing rice seedlings were surface sterilized with 1M HCl and 1mM Na^2EDTA for surface-bound cadmium and then dried in an oven at 70^0C for 5-days. Dried sample collected were ground to a fine powder where 0.5g each was digested in 5:1 (v/v) HNO_3/H_2O_2 5.0mL in a microwave oven (UN18300 Uniflex Co. Japan). Digested samples were dissolved in de-ionized distilled water and cadmium content was estimated using atomic absorption spectrophotometer (Z5010, Hitachi High-Technologies, Japan).

D. Determination of Lipid Peroxides

The level of lipid peroxidation products was determined using modified method of Heath and Packer (2008). Fresh root and shoot samples of the rice seedlings were ground in 0.25% thiobarbituric acid and in 10% trichloroacetic acid using mortar and pestle. The mixture was heated at 95^0C for 30min, cooled in ice bath and centrifuged at 10,000xg for 10min. the absorbance of the supernatant was read at 532nm. The concentration of lipid peroxides and thiobarbituric acid reactive species as well as oxidative-modified proteins of the seedlings were quantified and evaluated as total thiobarbituric acid reactive species (TBARS) expressed as nmol/g fresh weight, using extinction coefficient of $155mM^{-1}cm\text{-}^{1}$.

E. Determination of Inhibitory Potential of Crude Garlic Extract

The inhibition and prevention of lipid peroxides from rice seedling roots and shoots by crude garlic extract was determined using method described by Belle et al, (2004). Roots and shoots were ground in cold saline (1/10w/v) with up-and –down strokes in mortar and pestle. The homogenate was centrifuged for 10min at 10,000xg to obtain supernatant and was also incubated with cadmium chloride and garlic extract at variable concentrations and with de-ionized water at total volume 300µL at 37^0C for 1hr. The color reaction was monitored by adding 200µL, 250µL and 500µL each, 8.1%sodiumdodecylsulfate (SDS), acetic acid at pH 3.4 and 0.6% thiobarbituric acid (TBA) respectively. The reaction mixture solution was incubated at 97^0C for 1hr and absorbance was read at 532nm.

F. Evaluation of Glutathione Reductase Activity

Glutathione reductase activity was determined according to Schaedle and Bassham (1997). 200mg of fresh root and shoot of rice seedlings were homogenized using chilled mortar and pestle in 5ml of 50mM Tris-HCl buffer (pH 7.6). The homogenate was centrifuged at 22000xg for 30min at 4^0C and the supernatant was used for the enzyme assay. The reaction mixture in a total volume of 1mM GSSG, 3mM $MgCl_2$ and 200µl enzyme extract were assayed and the activity of the enzyme (GR) was monitored with absorbance read at 340nm.

III. RESULTS

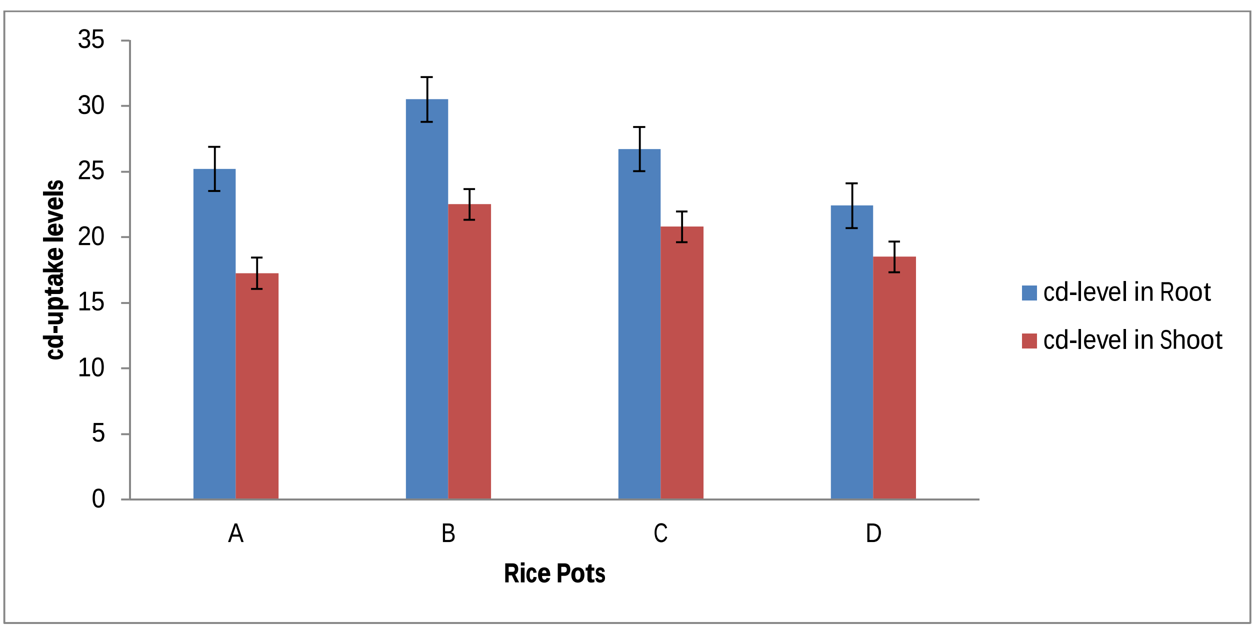


Fig.1: Uptake of Cd2+ by growing rice seedlings at Igbimo-Ekiti for period of 60-days.

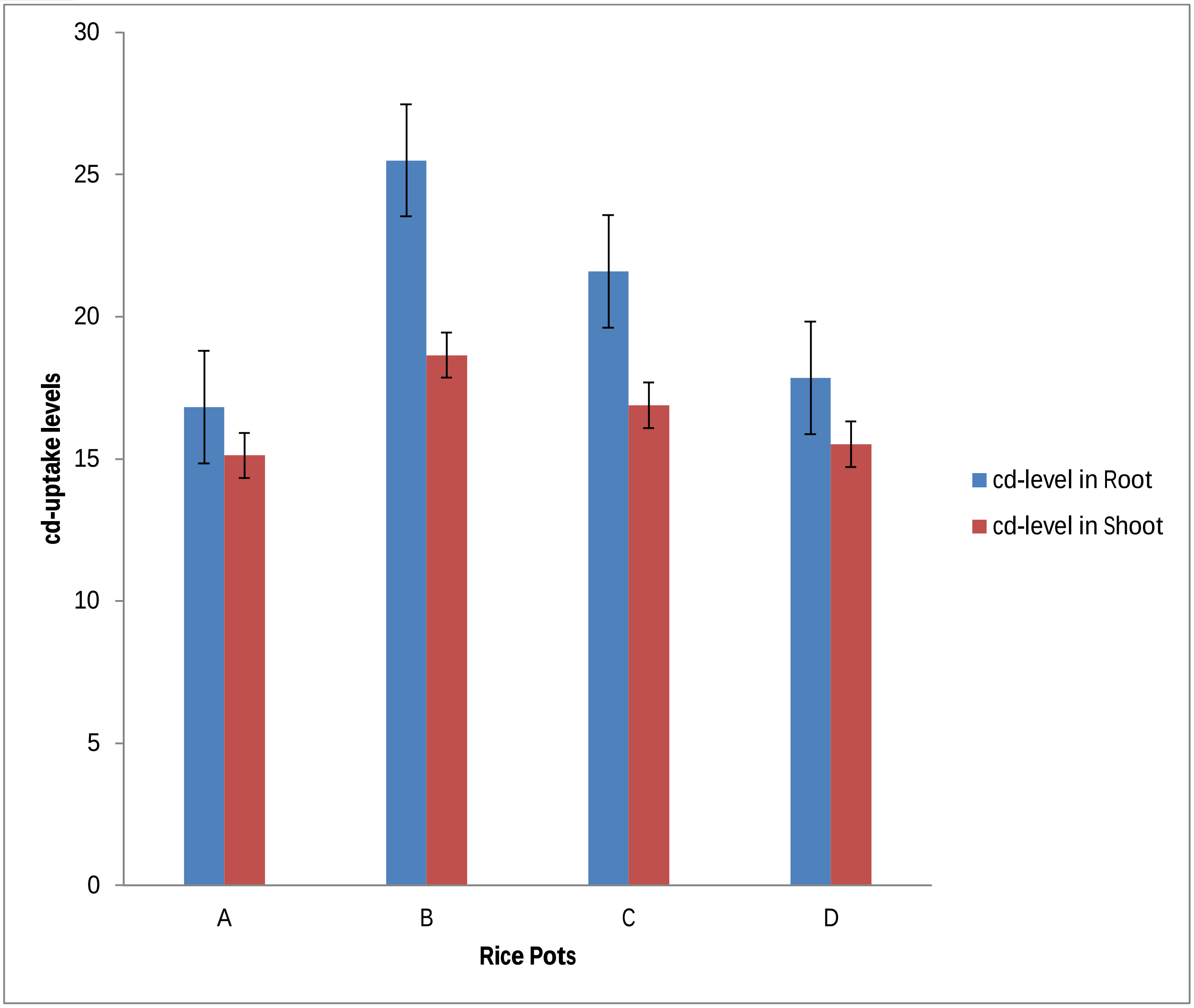


Fig.1: Uptake of Cd2+ by growing rice seedlings at Emure-Ekiti for period of 60-days.

Table 1: Levels of total lipid peroxides in root and shoot of growing rice seedlings

Age of seedlings (days)	Treatment	Total lipid peroxides (nmol/g)	
		Root	Shoot
15	Control	15.16±1.23	10.97±1.23
	250µM Cd^{2+}	51.61±2.79	30.39±1.51
30	Control	21.42±2.27	17.52±2.29
	250µM Cd^{2+}	76.45±3.73	60.32±3.12
45	Control	28.55±4.78	22.42±3.91
	250µM Cd^{2+}	88.55±4.78	75.81±3.94
60	Control	32.58±1.59	27.33±1.25
	250µM Cd^{2+}	91.63±4.48	79.45±3.76

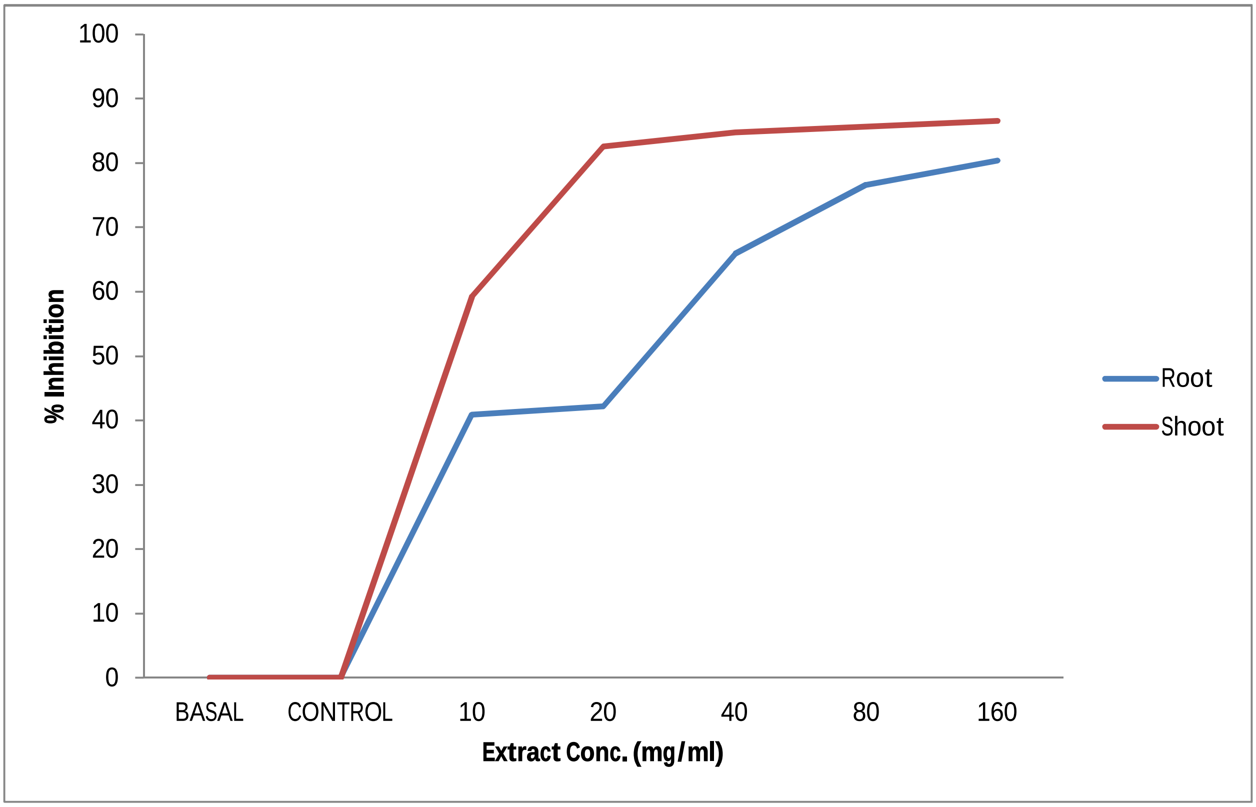


Fig.3: Inhibitory effect of crude garlic extract against Cd^{2+}-induced lipid peroxidation in growing root and shoot of rice seedlings.

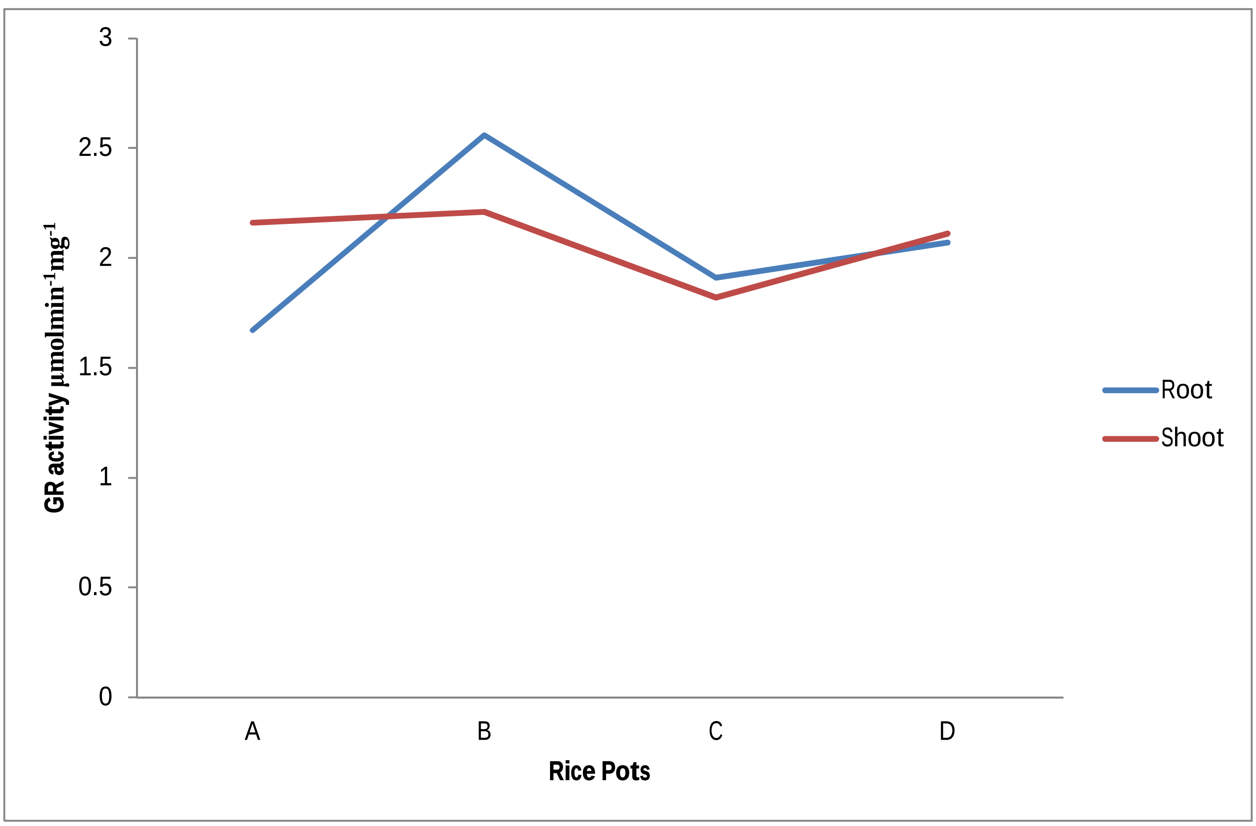


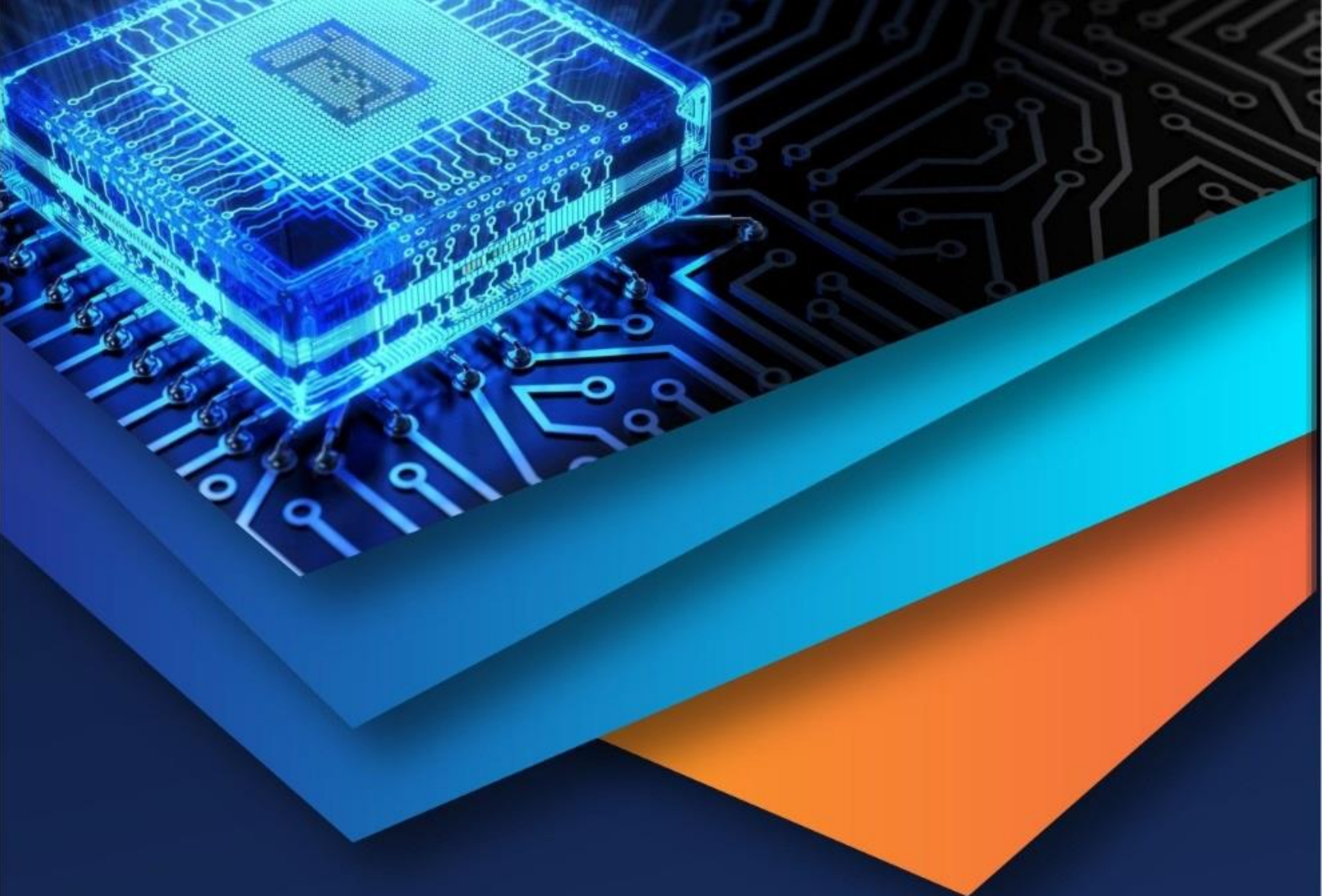
Fig.4: Effect of Cd^{2+} uptake on glutathione reductase (GR) activity in growing rice seedlings

IV. DISCUSSION

The results from the experiment showed that highest levels of cadmium uptake (30.50 and 25.50)µmolg^{-1} were obtained generally in roots treated with 250µM $CdCl_2$ in pot B. The lowest cadmium levels (18.50 and 15.52)µmolg^{-1} were equally obtained rice shoot treated with crude garlic extract in pot D in both studied areas. Comparatively, the cadmium highest level observed in pot B rice shoot was much lesser than that observed in root while the lowest cadmium level was observed in pot D. This could be unconnected with the fact that plant roots absorb more heavy metals from the soil than shoots (Ushimaru et al., 1992). Similarly at Emure area, (25.50, 17.85)µmolg^{-1} and (18.65, 15.52)µmolg^{-1} were highest and lowest cadmium levels observed in rice root and shoot respectively and were comparatively lower that cadmium levels observed at Igbimo area. This might be attributed to enhanced metal absorption usually aided by flooded soil (Mayer and Brown, 2010). Besides, cadmium absorption was remarkably reduced in pot D in both areas and this could be due to antioxidative activity of the crude garlic extract against metal absorption. Results also revealed elevated lipid peroxides in both root and shoot of the rice seedlings treated with 250µM cadmium concentration relative to seedling's age and period of growth. The rice roots exhibited higher peroxides level than shoots with (91.63)nmolg^{-1} maximum concentration obtained from roots. This was reasonably possible because roots absorb metals faster and the retained metals do not easily transport and thus, results to slow mobility for prompt uptake by the plant shoots (Goto et al., 1997). This bioaccumulation in roots could enhance Cd^{2+} ability to catalyze one electron (e-) transfer reaction that consequently generates reactive oxygen species. This further produces more thiobarbituric acid reactive species as free radicals that damage the rice plant physiology and as well cause immense cellular assault. However, (86.6%) and (80.4%) were obtained as highest inhibitory potential demonstrated by crude garlic extract against Cd^{2+}-induced lipid peroxidation in rice shoot and root respectively. This could be attributed to extract redox modulatory potential in reducing water soluble cadmium to minimal level in the soil coupled with its antioxidant activity against cadmium concentration gradient prior to onward absorption into the plant tissues (Sanchez-Fernandez et al, 1997). The possible effect on the rice seedlings inherent defensive antioxidant enzymes was clearly exhibited by glutathione reductase (GR). Glutathione reductase activity was higher in both root (2.56)µmolmin^{-1}mg^{-1} and shoot (2.21)µmolmin^{-1}mg^{-1} of rice seedlings treated with cadmium chloride in pot B compared to the control in pot A. Similarly, the GR activity was significantly ($P<0.05$) higher in pot D treated with the extract than tissues of rice seedlings in pot C. This could be attributed to antioxidant potential of the extract which invariably compliments the GR defensive antioxidant activity (Scandalios, 1990). Besides, the elevated GR activity suggests possible involvement of GR in regenerating reduced glutathione (GSH) from oxidized glutathione (GSSG) under acute metal toxicity in order to boost GSH/GSSG ratio, thus increasing total glutathione pool of the rice plants (Noctor and Foyer, 1998). Hence, this study clearly suggests that cadmium toxicity induces lipid peroxidation in rice plants which could be ameliorated by garlic extract while glutathione reductase enzyme plays a vital role in combating oxidative stress in growing rice seedlings.

REFERENCES

[1] Becana, M., Dalton, D.A., Moran, J.F. (2000). Reactive oxygen species and antioxidant in legume nodules. Plant Physiol. 109: 372-381.
[2] Belle, N.A.V., Dalmolin, G.D., Fonini, G., Rubim, M.A., Rocha, J.B.T. (2004). Polyamine reduces lipid peroxidation induced by different pro-oxidant agents. Brain Res. 1008: 245-251.
[3] Berln, A. and Schaller, K.H. (2008). Modified standard method of plant extraction. Plant Physiol. 25: 665-671.
[4] Erik, M.I., Peak, J.D., Brady, P.V. (1999). Lead absorption in soil. Soil Sc. 164: 28-29.
[5] Halliwell, B. (1994). Free radicals and antioxidant defense system. J. Lab. Clin. Med. 119: 598-620
[6] Heath, R.I. and Packer, L. (2008). Photoperoxidation in isolated chloroplast; kinetics and stochiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 125: 189-198.
[7] Godbold, D. and Kettner, C. (1991). Lead influence on root growth and mineral nutrition of Picea abies seedlings. Journal of Plant Physiol. 139: 95-99.
[8] Goto, I., Koya, O., ikamoto, S. (1997). Rapid arsenic absorption in flooded soil and oxidative stress on seedlings. Plant Sci. 310: 758-764.
[9] Kastori, R. and Petrovic, M. (1992). Effect of excess lead, cadmium, copper and zinc on water relation in sunflower. Journal of Plant Nutrition. 15: 2427-2439.
[10] Mayer, D.T. and Brown, K. (2010). Role of defensive enzymes in development of water impermeable seeds. Journal of Phys. Biochem. 193: 265-275.
[11] Noctor, G. and Foyer, C.H. (1998). Ascorbate and glutathione; keeping active oxygen under control. Annual Review of Plant Mol. Biol. 499: 249-279.
[12] Sanchez-Fernandez, R., Fricker, M., Corben, L.B., White, N.S. (1997). Cell proliferation and hair tip growth of Arabidopsis root under different forms of redox control. Proc. Natl. acad. Sci. 94: 2745-2750.
[13] Scandalios, J.G. (1990). Response of plant antioxidant defense genes towards environmental stress. Advanced Genetics. 28: 11-41.
[14] Schaedle, M. and Bassham, J.A. (2009). Chloroplast glutathione reductase. Plant Physiol. 59: 1011-1012.
[15] Tanaka, T., Kato, T., Nishioka, Y. (1985). Lead burden and oxidative stress effect. Journal of Biol. Chem. 263: 11646-11651.
[16] Ushimaru, T., Shibasaka, M., Tsuji, H. (1992). Development of detoxification system during water absorption by rice seedling's roots. Plant Cell Physiol. 33: 1065-1071.



10.22214/IJRASET

45.98

IMPACT FACTOR: 7.129

IMPACT FACTOR: 7.429

TOGETHER WE REACH THE GOAL

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY