iJRASET
International Journal For Research in Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 7 **Issue:** VI **Month of publication:** June 2019

DOI: http://doi.org/10.22214/ijraset.2019.6342

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International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.177
Volume 7 Issue VI, June 2019- Available at www.ijraset.com

Impact of Lead Stress on the Growth, Biochemical and Enzymatic Characters of Red Gram (Cajanus cajan (L.) Millsp.)

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Abstract: ***A nursery experiment was conducted to study the impact of lead stress on the growth, biochemical and enzymatic characters of red gram (Cajanus cajan (L.) Millsp.). The plants grown with higher concentration of lead adversity affected the growth, biochemical and enzymatic characters. The results indicated that up to 4mM of lead facilitates the growth and development of red gram but beyond this concentration there was a gradual decrease in growth and development of plants. The reduction of growth may be due to changes in their physiological and biochemical activities especially when the heavy metal involved does not play any beneficial role towards the growth and development of plants.***
Keywords: heavy metal, lead, red gram, growth, biochemical, enzyme

I. INTRODUCTION

The term 'heavy metal' refers to any metallic element that has a relatively high density and is toxic or poisonous even at low concentration[1]. Heavy metal is a general collective term, which applies to the group of metals and metalloids with atomic density greater than 4g/cm^3 or five times or more, than water[2]. Based on their solubility under physiological conditions, 17 heavy metals may be available for living cells and of importance for organism and ecosystems[3]. Among these metals, Fe, Mo and Mn are important as micronutrients. Zn, Ni, Cu, V, Co, W and Cr are toxic elements with high or low importance as trace elements. As, Hg, Ag, Sb, Cd, Pb and U have no known function as nutrients and seem to be more or less toxic to plants and microorganisms[4].

Soil contamination with heavy metals becomes a widespread problem that affects the environment worldwide. Heavy metals occur naturally in soil as a result of human activity such as traffic, paint and many other non-specific civilian sources[5],[6]. Heavy metals are major environmental pollutants due to their ecological, evolutionary, nutritional and environmental consequences[7]. Heavy metals are toxic to almost all living organisms and can stay in nature for very long period of time. It affect human and plant metabolic activities.

Lead (Pb) is a silvery-white highly malleable metal. At normal environmental conditions, this metal is presented in the solid state; it is dense, ductile and very soft with poor electrical conductivity when compared to most other metals. Pb is rarely found in native form in nature but it combines with other elements to form a variety of interesting and beautiful minerals[8]. Lead is a heavy metal of anthropogenic origin and accumulates in soils, sediments, water and extremely persistent in the environment. Pb has no biological function and it is toxic to living organisms even at low concentrations[9].

Naturally, Pb is present in soil, sea water, lakes and rivers. Besides natural sources, exhaust fumes of automobiles, chimneys of factories, mining, effluents from storage battery, smelting of Pb ores, fertilizers, additives pigments, metal plating and pesticides are also major sources of Pb[10]. It is also a component of lead batteries, rubber, paints, metal products and dusts[11]. The environment of pollution by toxic metals arises as a result of various industrial activities and has turned these metal ions into major health issue[12]. Soil pollution with heavy metals is a universal problem, leading to agricultural losses and hazardous health effects as metals enter the food chain.

Heavy metal can infect soils in town areas, Pb deriving from galvanized metals, rubber production batteries, petrol, steel mill residues and paints. The phytotoxicity of the heavy metals because of industrial pollution has serious implications in soil degradation. This may diminish both the quality and productivity of plants. Heavy metals show a discrepancy according to their role in metabolic functions. Plants act in response to heavy metal ion stress in different ways including exclusion and chelation, expression of stress protein genes[13]. Plants are primary victims of heavy metal toxicity. Heavy metals-rich soil disturbs nutrients homeostasis in plants and depresses plant growth by affecting the uptake and distribution of certain nutrients in plants[14]. Plants possess unique defense mechanisms like phytochelations and antioxidant enzymes which protect physiological process from

damage by toxic metals or oxidative stress caused by various metals. Among antioxidant enzymes, catalase (CAT), ascorbate peroxidase (APX) and peroxidase (POD) are considered the most important[15] to have protective roles against oxidative damage. Non-essential heavy metlas (lead, cadmium, Mercury and silver) enter the cell and limit cellular functions by generating reactive oxygen species (ROS).

II. MATERIAL AND METHODS

The seeds of red gram (*Cajanus cajan* (L.) Millsp.) were procured from Tamil Nadu Agriculture Research Center, Kovilpatti, Tamilnadu. In the nursery experiment, the lead stress was given to plants as lead acetate solution at the concentration of 2mM, 4mM, 6mM, 8mM and 10mM. Initially, 1M lead acetate solution was prepared as a stock solution and then the respective concentrations were prepared from the stock solution. The red gram seeds with uniform size, colour and weight were chosen for the experimental purpose and surface sterilized with 0.1% $HgCl_2$ for 1 minute and thoroughly washed with distilled water 3-5 times. Seeds were pre-soaked for 12 hours in distilled water and were sown in sterilized soil mixture. The soil mixture was prepared by mixing black soil, red soil and sand in the ratio of 1:1:1. The heavy metal was treated to the potted plants after the formation of first leaf in the red gram seedlings. The respective concentration of metal was given on every alternate day.

In the treated and untreated control plants, the growth characters such as shoot length, root length, fresh and dry weight were analyzed. The biochemical characters such as chlorophyll[16], soluble sugar[17], soluble protein[18], free amino acids[17], proline[19] and leaf nitrate[20] were analyzed. Further, the enzymatic characters such as nitrate reductase activity[21], peroxidase[22] and catalase[23] were also analyzed. The data were reported as mean ± SE and in the figure parentheses represent the percent activity. Values are expressed as means ± standard deviation of three independent data.

III. RESULTS AND DISCUSSION

A. *Impact of Lead on the Growth Characters of Red Gram*

The lead stress significantly reduced the growth characters of red gram such as shoot length, root length, fresh and dry weight. The impact was varied with the concentration of lead. The treatment of metal ions as lead acetate significantly decreased the shoot length of red gram with increasing the concentration of lead. The reduction of shoot length was varied from 8% to 51%. The results revealed that the maximum reduction was observed in the plants treated with 10mM concentration of lead. Like shoot length, same result was noticed in the root length also. The extreme reduction in the root length was noticed in the highest concentration (10mM) of metal. The treatment of lead significantly decreased the shoot as well as root length of red gram seedlings. It is directly affected the fresh weight and dry weight of red gram also. The results showed that the reduction effect was ranged from 13 % to 74%. The dry weight of red gram was gradually reduced with increasing the concentration of lead from 2mM to 10mM than control plants. The maximum reduction of plant dry weight was 84% at 10mM concentration of lead (Table I). The visual general symptoms of Pb toxicity are fast inhibition of root growth, under developed growth of the plant, blackening of root system and chlorosis. Pb inhibits photosynthesis, let downs mineral nutrition and water balance, enzyme activities. These disorders upset normal physiological activities of the plant. At high concentrations Pb finally may lead to cell death[24]. Lead enters the plants via their roots and alters their morphological, physio-biochemical and molecular mechanisms[25]. It damages cell membranes and suppresses photosynthetic capacity and stomatal conductance of the plant by inhibiting biosynthesis of chlorophylls, activity of Rubisco and water uptake[26]. The growth development, fresh biomass and growth tolerance index of root, shoot and leaves were negatively affected by increasing levels of Pb concentrations in tomato seedlings. Similar results were obtained by some other studies at the calculated Pb concentrations: root, shoot and leaf growth; fresh and dry biomass is greatly reduced in *Zea mays*[27].

B. *Impact of Lead on the Biochemical Characters of Red Gram*

The heavy metal treatment significantly reduced the growth characters of red gram and this effect was directly affected the biochemical characters of red gram such as chlorophyll, soluble sugar, soluble protein, amino acid, proline and leaf nitrate content. Among them, the chlorophyll, total soluble sugar and total soluble protein content were drastically reduced with increasing the concentration of lead. In case of amino acid, proline and leaf nitrate, there was a significant increase was observed. Under nursery condition, the treatment of lead gradually reduced the total chlorophyll content of red gram seedlings with increase the concentration of lead. The maximum reduction was occurring 10mM concentration of lead when compared to control plants and other treatments. Like chlorophyll content, same trend was noticed in the total soluble sugar content of red gram. From the results, a reduction of about 42% in the total soluble sugar content over control plants was observed in 10mM concentration of lead. There was a gradual decrease in protein content of red gram seedlings from 2mM to 10mmM concentrations. The results showed that the protein content was reduced to 54% at 10mM concentration of lead when compared to the control plants (Table II).

The free amino acid content was increased at 10mM concentration of lead to 37% over the control plants. When protein content was degrading during heavy metal treatment, the amount of free amino acids was significantly increased. A significant increase in the amino acid content directly increased in the proline content of treated plants. The result showed that the proline content was increased to 140% over the control plants at 10mM concentration of lead. The application of lead significantly increased the leaf nitrate content of red gram. The results clearly indicated that the nitrate content of treated plants at 10mM concentration of lead was found to be increased to 65% over than control plants (Table III).

The process of photosynthesis is unfavourably affected by Pb toxicity. Plants exposed to Pb ions show a decline in photosynthetic rate which results from partial chloroplast ultra structure, restrained synthesis of chlorophyll, plastoquinone and carotenoids, obstructed electron transport, inhibited activities of Calvin cycle enzymes, as well as deficiency of CO_2 as a result of stomatal closure. *Ceratophyllum demersum* plants when grown in aquatic medium containing $Pb(NO_3)_2$ showed distinct changes in chloroplast fine structure. Leaf cells of such plants showed a reduction in grana stacks together with a reduction in the amount of stroma in relation to the lamellar system as well as absence of starch grains. Pb treatment also changes the lipid composition of thylakoid membranes[28].

The concentration of lead significantly reduced the total concentration of protein. This quantitative decrease in total protein content is the result of several lead effects: acute oxidative stress of reactive oxygen species (ROS) [29], protein utilization by plants for the purposes of lead detoxification and diminution of free amino acid content[30]. Plants have been shown proline accumulation under environmental stress[31]. It has been often suggested that proline accumulation may contribute to osmotic adjustment at the cellular level and enzyme protection stabilizing the structure of macromolecules and organelles. Increase in proline content may be either due to de novo synthesis or decreased degradation or both[32].

C. Impact of Lead on the Enzymatic Characters of Red Gram

The application of lead at various concentrations significantly affected the growth as well as biochemical characters of red gram. These effects were directly reflected in the enzymatic characters of red gram that related to the plant metabolism of red gram. The lead stress in red gram seedlings decreased the *in vitro* nitrate reductase activity (NRA). The activity was decreased from 2mM to 10mM than control plants. The reduction was higher at 10mM than other treatments. The activity was reduced to about 42% when compared to the control plants at 10mM of lead. In red gram seedlings, catalase activity was gradually increased with increasing concentration of lead (8% to 97%). The plants treated with 10mM concentration of lead showed that 97% increased in catalase activity. Like catalase activity, same trend was noticed in the peroxidase activity also. The peroxidase activity was increased with increasing the concentration of lead. The results showed that 10mM concentration of lead significantly increased the peroxidase activity (189%) over control plants (Table IV). Plants maintain their life cycles under the various environmental conditions such as oxidative stress induced by heavy metals. Accumulation of metal ions in plants causes the formation of free radicals and stimulates the antioxidative defense systems. Such ascorbate peroxidase (APX), peroxidase (POD) and superoxide dismutase (SOD). SOD are investigated in the leaves and roots of tomato cultivated under the heavy metal-induced stress. The activities of APX, POD and SOD exhibited remarkable induction with the treatment of Cd, Cu and Pb (10, 20 and 50 ppm) in the leaves of tomato compared to control plants except for 50 ppm Pb[33]. Plants have enzymatic mechanisms related with defense system such as catalase, glutathione reductase, glutathione *S*-transferase, peroxidase and superoxide dismutase, which remove, neutralize and scavenge the free radicals[34]. Oxidative stress damage the equilibrium between ROS and antioxidant systems and antioxidant enzymes react with the free radicals. These enzymes play the main protective role in the elimination of free radicals. Superoxide radical is scavenged with SOD and H_2O_2 decomposition is achieved by APX and catalase (CAT). Another well-known enzyme is POD related with stress, alterations in POD activity have been accepted to be associated with a wide range of physiological processes in response to environmental conditions. The coordinated function of these enzymes can help to maintain the oxidant and antioxidant status of plant cells [35]. The exposure of *Solanum lycopersicum* to heavy metals caused to behave differently in the activity of enzymes depending on the tissue assayed; the application of Cd, Cu and Pb globally enhanced the activities of APX, POD and SOD which are clear responses given by the leaves of tomato, while their activities fluctuated depending on the heavy metal types and concentration in roots[36]. The direct interaction of heavy metals with cellular components can initiate a verity of metabolic responses and finally leading to a shift in the development of the plant. For metal toxicity, this stress point is reached at the toxic threshold level of the metal in the tissue. Above this level the physiological state of the cell will be irreversibly changed. This change is reflected by an increase in activity of certain enzymes. Heavy metal ions accumulate in different parts of plant after they are absorbed by the root system, resulting in retardation of plant growth. This could be due to their interference with the activities of a number of enzymes essential for normal metabolism and developmental processes[37].

IV. CONCLUSION

In the present study, the heavy metal stress (lead) adversely affected the growth, biochemical and enzymatic characters of red gram. Lead stress significantly reduced the growth characters of red gram irrespective of all the concentrations but the impact was higher in 10mM. Lead stress also reduced considerably the biochemical characters of red gram such as chlorophyll, soluble sugar and protein content. But free amino acids, proline and leaf nitrate content significantly increased with increasing the concentration of lead. The reduction of protein content directly correlated with increase in the accumulation of free amino acids content. The increased level of leaf nitrate directly correlated with decreased level of NR activity. The impact of lead on NR activity increased the available nitrate in the leaves. From the results, it is also clear that lead stress significantly increased the activity of certain enzymes such as catalase, peroxidase and nitrate reductase. From the present study, it is clear that the excess amount of lead when absorbed by plants become toxic to the plants. The toxic effects reflected in the growth, biochemical and enzymatic characters of red gram. To protect the plants from the metal stress, plant itself produced higher level of protective enzymes like peroxidase and catalase.

V. ACKNOWLEDGEMENT

The authors are thankful to the Management and the Principal of Ayya Nadar Janaki Ammal College, Sivakasi, Tamil Nadu for providing laboratory facilities and financial assistance to carry out this research work.

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Table I: Impact of Lead on the Growth Characters of *Cajanus cajan* (L.) Millsp.

S. No	Growth Parameters	Control	2mM	4mM	6mM	8mM	10mM
1.	Root Length (cm)	13.5 ±0.033 (100)	12.6 ±0.057 (93)	10.4 ±0.034 (77)	8.7 ±0.120 (64)	7.1 ±0.058 (53)	5.6 ±0.033 (41)
2.	Shoot Length (cm)	39.1 ±0.033 (100)	35.9 ±0.034 (92)	30.5 ±0.066 (78)	27.4 ±0.152 (70)	22.6 ±0.067 (58)	19.2 ±0.102 (49)
3.	Fresh Weight (g)	2.84 ±0.012 (100)	2.47 ±0.003 (87)	1.93 ±0.008 (68)	1.68 ±0.003 (59)	1.17 ±0.005 (41)	0.75 ±0.006 (26)
4.	Dry Weight (g)	0.67 ±0.003 (100)	0.53 ±0.006 (79)	0.46 ±0.005 (68)	0.32 ±0.005 (48)	0.25 ±0.006 (37)	0.11 ±0.028 (16)

Table II: Impact of Lead on the Biochemical Characters of *Cajanus cajan* (L.) Millsp.

S. No	Biochemical parameters	Control	2mM	4mM	6mM	8mM	10mM
1.	Total chlorophyll (mg/gLFW)	3.81 ±0.017 (100)	3.39 ±0.058 (89)	2.73 ±0.007 (72)	2.20 ±0.012 (58)	1.33 ±0.017 (38)	0.78 ±0.003 (20)
2.	Carotenoid (mg/gLFW)	2.92 ±0.007 (100)	2.64 ±0.006 (90)	2.06 ±0.017 (71)	1.72 ±0.026 (59)	1.41 ±0.020 (48)	0.68 ±0.005 (23)
3.	Soluble sugar (mg/gLFW)	5.47 ±0.085 (100)	5.15 ±0.018 (94)	4.72 ±0.036 (86)	4.22 ±0.061 (77)	3.56 ±0.029 (65)	4.67 ±0.028 (76)
4.	Soluble protein (mg/gLFW)	6.17 ±0.011 (100)	5.81 ±0.045 (94)	5.16 ±0.017 (84)	4.67 ±0.028 (76)	3.82 ±0.058 (62)	2.81 ±0.054 (46)

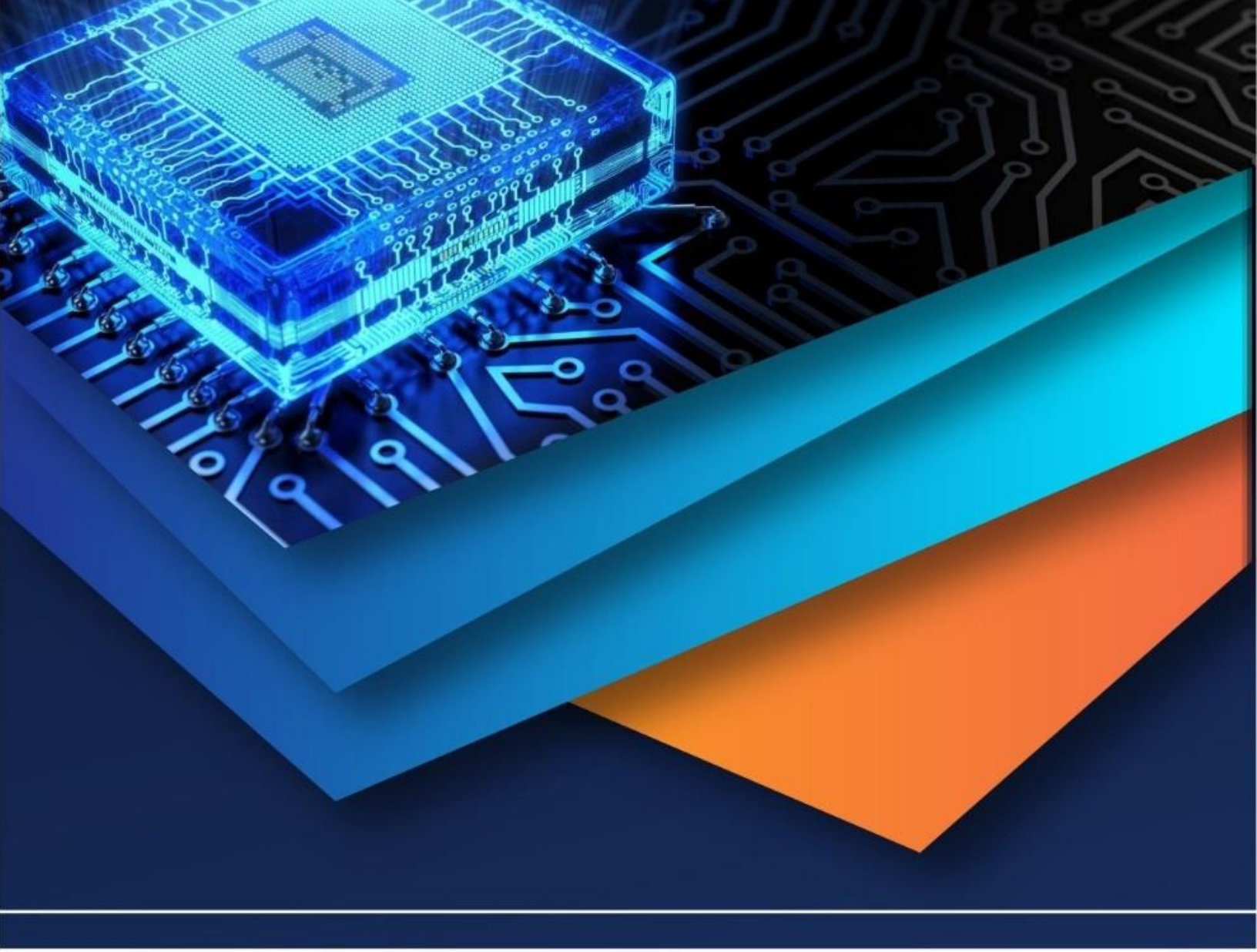
Table III: Impact of Lead on the Biochemical Characters of *Cajanus cajan* (L.) Millsp.

S. No	Biochemical parameters	Control	2mM	4mM	6mM	8mM	10mM

1.	Amino acids (µ mole /gLFW)	7.82 ±0.020 (100)	8.27 ±0.112 (106)	8.70 ±0.153 (111)	9.55 ±0.008 (122)	10.26 ±0.070 (131)	10.71 ±0.116 (137)
2.	Proline (µ mole /gLFW)	3.26 ± 0.015 (100)	3.90 ± 0.011 (120)	4.62 ± 0.024 (142)	5.92 ± 0.020 (182)	6.63 ± 0.008 (203)	7.82 ± 0.012 (240)
3.	Leaf Nitrate (µ mole /gLFW)	6.93 ±0.012 (100)	7.53 ±0.020 (109)	8.76 ±0.017 (126)	9.44 ±0.032 (136)	10.80 ±0.017 (156)	11.45 ±0.024 (165)

Table IV: Impact of Lead on the Enzyme Activities of *Cajanus cajan* (L.) Millsp.

S. No	Enzyme activities	Control	2mM	4mM	6mM	8mM	10mM
1.	NR activity (µ mole /g LFW)	8.84 ±0.020 (100)	8.56 ±0.045 (97)	7.72 ±0.015 (87)	6.87 ±0.025 (78)	5.61 ±0.025 (63)	5.16 ±0.030 (58)
2.	Catalase activity (µ mole /g LFW)	4.71 ±0.012 (100)	5.23 ±0.088 (108)	6.92 ±0.015 (146)	7.19 ±0.005 (152)	8.31 ±0.060 (181)	9.28 ±0.014 (197)
3.	Peroxidase activity (µ mole /g LFW)	2.64 ±0.020 (100)	3.72 ±0.008 (141)	4.63 ±0.020 (175)	5.37 ±0.011 (204)	6.14 ±0.005 (233)	6.93 ±0.017 (289)



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