Gyanshauryam, International Scientific Refereed Research Journal
© 2021 GISRRJ | Volume 4 | Issue 5 | ISSN : 2582-0095

Effect of Sodium and Cadmium on the Amount of Malondialdehyde Content in the Leaves of Brassica Juncea and Plantago Ovate

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Article Info
Volume 4 Issue 5
Page Number : 166-177

Publication Issue :
September-October-2021

Article History
Accepted : 01 Sep 2021
Published : 15 Sep 2021

Abstract - Heavy metals mixed in the soil spread toxicity in the plants, due to which the growth of the plant decrease or stop, their weight starts decreasing and their leaves decrease, and heavy metals in excess amount can also cause the death of the plant. Some metals like Cu, Mn, Fe, Zn etc. are also essential for plants in limited quantity but they become toxic in excessive quantity, due to which many types of biochemical changes occur in plants. Due to salinity there is water loss in plants and ionic poisoning also occurs in plants. Salinity also causes oxidative stress in plants. When metals such as sodium and cadmium enter plant cells, plants produce a defense mechanism to protect their tissues from these metals. Various types of low molecular weight proteins etc. are involved in this defense mechanism. In this paper we will study how sodium and cadmium affect the amount of malondialdehyde (MDH) in the leaves of Brassica juncea and Plantago ovata.

Keywords :- Heavy Metals, Defense Mechanism of plant, Cd and Na Concentration

Introduction- While essential for plants and used as micronutrients, certain metals including Cu, Mn, Fe, and Zn may exhibit severe toxicity at larger doses. Zn is a microelement that plays crucial physiological roles in plants, but at excessive quantities it can become toxic, causing biochemical and physiological changes that ultimately reduce yield. In contrast to moist terrain, salinity impacts are more obvious in dry and semi-arid environments. Salinity causes ionic toxicity and osmotic stress in plants, which in turn triggers secondary stresses such as nutritional problems and oxidative events. These stresses cause the plants to undergo a number of biochemical and molecular alterations that manifest as morphological and physiological abnormalities (Srivastava et al., 2015). Lowered water potential, ionic stress, and secondary oxidative damage are all caused by high salinity. By reducing CO_2 assimilation, oil and protein synthesis, it significantly restricts the plant's growth and development. Plants that are subjected to salt stress modify their metabolism to adapt to the new environment. The ability of the plant to perceive the stimuli, transmit information, and trigger biochemical changes that regulate the metabolism depends on its ability to survive in these demanding conditions.

Plants have an effective system of stress enzymes and antioxidant non-enzymatic compounds, known as antioxidant system, to handle this oxidative stress. Super oxide dismutase (SOD), one of these enzymes, is the first line of defence against ROS because it converts O^{-2} to oxygen and H_2O_2. From cytoplasm

and chloroplast of cells of plant, peroxidase (POX) scavenges H_2O_2, while catalase (CAT) is another enzyme that converts H_2O_2 to water and oxygen (Gill et al., 2011). Proline is a metabolite that has several uses in plant stress responses. It functions as an osmoprotectant, ROS scavenger, protein-compatible hydrotrope, and regulator of cellular redox status. A number of genes that control the expression of metabolite pools and plant development and growth are regulated by protein through redox signalling (Hayet et al., 2012).

When metal ions of sodium and cadmium passed through biophysical barriers and then enter into plant cells and tissues, then plants launch a number of cellular defence mechanisms to counteract and lessen the harmful effects of heavy metals. The main method for coping with or reducing metal toxicity is biosynthesis of various cellular biomolecules. It contains a variety of low molecular weight protein chelators or metallochaperones, such as organic acids, putrescine, nicotianamine, spermine, glutathione, niacinic acid, phytochelatin and/or metallothionein, Cellular exudates, including phenolic compounds and flavoids, heat shock proteins, protons and contains specific amino acids such as histidine and proline (Sharma et al., 2006). When above system fails to stop poisoning of metal, redox systems of plant cells become out of balance, which increases the production of ROS (Mourato et al., 2012). Plant cells developed a mechanism for defending themselves against free radicals which is known as "antioxidant defence system". This system includes both enzyme-based antioxidants like ascorbate peroxidase (APX), guaiacol peroxidase (GPX), superoxide dismutase (SOD), glutathione reductase (GR), and catalase (CAT) and also nonenzymatic antioxidants like glutathione, ascorbate (AsA) (Rastagoo et al., 2011).

Due to a lack of water, salinity results in oxidative stress. In excess, the latter causes production of ROS, which negatively impact on integrity of biological membranes (Del Rio et al., 2002). As a result, the plant either dies or produces less than before. In various cell compartments such as apoplectic space chloroplasts and mitochondria, salt stress results in accumulation of hydrogen peroxide (H_2O_2) and superoxide radicals (O^{-}_{2}), which in turn lead to an increase in oxidative stress parameters resulting in activities such as protein oxidation and lipid peroxidation reactions (Acosta M. et al., 2017). Ascorbate and glutathione levels are typically significantly more impacted by salt stress in sensitive plants than in tolerant ones. A NaCl-sensitive plantago species saw a 50% drop in the total ascorbate pool in soluble fractions after 15 days of stress (Hernandez et al., 2000).

The very hazardous heavy metal cadmium interferes with physiological processes in plants, causes oxidative stress, and damages plant cells. Radical superoxide (O^{2-}) can be protonated to form the hydroxyl radical (OH^{-}) and hydrogen peroxide (H_2O_2), which can destroy cellular membranes by turning fatty acids into hazardous lipid peroxides (Lu et al., 2010). Superoxide radicals, which are produced and the outcome of which, caused oxidative damage to a number of biological components, including lipid, protein, and nucleic acid (Lu et al., 2010). In plants exposed to various concentrations of Cd, such as Solanum lycopersicum, there is an increased generation of H_2O_2, which affects the permeability of cell membranes and induces lipid peroxidation as a result of the increased accumulation of ROS (Nogueirol et al., 2016). In necoly growing plant cells, cadmium poisoning increases lipid peroxidation and fats often by raising malondialdehyde content (MDA). When cells are under Cd stress, more ROS are produced, which can be fatal to cell components and lead to changes in antioxidant systems (Ehsan et al., 2014).

Low molecular mass molecules that are compatible solutes that accumulate in plants serve to replace water in biological reactions rather than obstructing normal biochemical processes (Zhifang et al., 2003). Overproduction of several types of suitable solutes is a considerable plant's physiological response to Cd stress (Ahmad P. et al., 2012). It has been discovered that Cd controls antioxidant metabolism and water interactions to counteract the harmful effects of abiotic stress. The ascorbate-GSH antioxidant system and metabolism of critical elements are affected by cadmium (Dong et. al., 2006). According to Nogueirol et al., (2016) "Oxidative stress is caused by cadmium buildup, as seen by production of ROS like hydrogen peroxide and superoxide anion". However, because Cd is not a redox active metal, oxidative stress may be brought on by both disruptions of GSH metabolism and the removal of redox active metal from proteins. Oxidative stress is the phrase used to describe the stress brought on by excessive ROS production.

The regulation of hormone synthesis in presence of heavy metals demonstrates the critical function that plant hormones play in adapting to abiotic stress, which is engaged in numerous physiological and developmental processes (Shah et al., 2001). Plantago ovata and mustard species are capable of storing adequate levels of Cd in their tissues. According to Singh et al., (2008), cadmium interferes with general and membrane physiology, including oxidative processes and nitrogen metabolism, and decreases the activity of several enzymes (Hasan et al., 2009). In B. juncea plants, GSH content was found to rise at lower Cd concentrations before decreasing at higher concentrations, according to Seth et al., (2012). This impact was more prominent for longer exposure times. It's possible that a supposed buildup of H_2O_2 led to a downregulation of the genes that produce the mRNA for the GR implicated in the synthesis of GSH.

Na and Cd metal contaminants are hyperaccumulated in certain species of Plantago. While superoxide dismutase, peroxidase, catalase, glutathione reductase, ascorbate peroxide, and malondialdehyde exhibit altered biochemical activity at greater soil concentrations, Plantago ovata was discovered to be salt tolerant at lower concentrations (Kahrizi et al., 2012). Even at low soil concentrations, Cd is harmful to P. ovata and B. juncea, however the phytotoxicity of Cd depends on soil Cd content as well as plant species and cultivars. Antioxidant enzymes are changed in P. ovata and B. juncea by cd hyperaccumulation. In plant species, cadmium produces ROS that drastically affect the SOD, POX, and CAT enzymes. In plants, GSH is engaged in a wide range of cellular functions like detoxification of xenobiotics, sequestration of heavy metals and defence against ROS (Foyer et al., 2005). Reduced sulphur is commonly transported and stored in the form of GSH. Particularly GSH's thiol group is well suited for performing broad range of metabolic tasks in both plant species due to its chemical reactivity. GSH play an important role for regulating H_2O_2 level in cells of plant, in some redox signalling pathways, its ratio reduced (GSH) to oxidised (GSSG) form changes throughout breakdown (Miller et al., 2003).

Aim of current study is to assess changes in antioxidant enzymes of Plantago ovate and Brassica juncea plants that had been subjected to salt stress and cadmium stress. A second goal was to determine a correlation between variations in antioxidant enzyme activity and various levels of heavy metals (Na and Cd) in the soil. We look how sodium and cadmium affect the amount of malondialdehyde in the leaves of Brassica juncea and P. ovata.

Material and Methods

From the National Seeds Disposal Center of the Indian Agricultural Research Institute (IARI), New Delhi, India, seeds of P. ovata (L.) and Indian mustard (Brassica juncea L.) are collected. On basis of their quantity and germination quality, seeds were chosen. For consistent germination, seeds surfaces are sterilised with 0.5% sodium hypochlorite and immersed for an entire night in sterile water at 4°C. In the green house under natural day/night circumstances (photo synthetically active radiation >950 mmol $m^{-2}s^{-1}$, temperature 24±2^{0}C, relative humidity 75±4%), the seeds were placed to 30cm diameter earthen pods pilled with 6.0Kg reconstituted soil (Mobin M et al., 2007). For five experimental biochemical investigations, pots were set up in a completely randomised design with two components and four replicates in each. Seeds of P. ovata and Brassica juncea sown in pots and supplied Raukuras nutrients solutions.

TREATMENTS- Five levels of NaCl were added to the soil to provide the salinity treatments: "T_1-0 mM (control), T_2-30 mM, T_3-60 mM, T_4-90 mM, T_5-120 mM, and T_6-180 mM". By adding $CdCl_2$ to the soil, the cadmium concentrations of "control 0 mg /kg dirt, T_1-25 mg/kg soil, T_3-50 mg/kg soil, T_4-100 mg/kg soil, and T_5-200 mg/kg soil" were measured.

DDW (100-200ml) was used to water the test pots every day to keep the sand damp. Every two days, 200ml of the nutrient solution was given to each pot. The following salts were used to create the nutritional solutions for the Raukuva: [A]. (gL^{-1}) NH_4NO_3-8.48, $Ca(NO_3)_2.4H_2O$-16.78, $Mg(NO_3)_2$ $6H_2O$-4.94 and KNO_3-2.28 as a stock solution of micronutrients, [B] Micronutrient supplement are "(mgL^{-1}) H_3BO_4-128.80, $CuCl_2.2H_2O$-4.84, $ZnCl_2$-23.45, $(NH_4)_6$ MO_4O_{24} $4H_2O$-0.83, erric citrate penta-hydrate 809.84. (gL^{-1}) KH_2PO_4-2.67, K_2HPO_4-1.64, K_2SO_4-6.62, Na_2SO_4-0". In order to create the diluted solution that was used on the plants, 200ml of each macronutrient stock solution combined with 100ml of the micronutrient supplement, and then mixture was diluted with DDW to 4.5L. In order to keep the pH at 6.0, a solution of KOH or H_2SO_4 was added.

At 30 and 45 days (DAS) after seed germination, malondialdehyde concentration (in vitro) were measured.

Enzyme Assays- Following the steps of the conventional procedures, activity of malondialdehyde was measured. Small portions of leaf samples were chopped up and suspended in a cysteine dihydroholoride solution and this samples then incubated for 20 minutes at 4^{0}C temp. The leaf fragments were placed in test tubes which contain phosphate buffer (pH 6.8), after being wiped with tissue paper. The tubes were then filled with alkaline bicarbonate solution and bromothymol blue indicator, after that test tubes were incubated at 5^{0}C for 20 minutes. 0.05N HCl was used to titrate the reaction mixture after 0.2 ml of methyl red indicator was added. Then leaf tissue (0.5g) was homogenised in 50 mM phosphate buffer (pH 7.0) with 1% polyvinyl pyrrolidine for testing of antioxidant enzymes. The supernatant is the MDA extraction solution employed as the source after the humogenate was centrifuged at 15000 rpm for 10 min at 4^{0}C.

A fifteen Watt fluorescent lamp had used for illuminating reaction vessel which contain 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 mM nitro blue tetrazolium, 2 mM riboflavin, 0.1 mM EDTA, and 0.50 ml enzyme extract. Then light had turned on to start the reaction, and it was left on for 10 minutes. Turning off the light halted the reaction. One enzyme unit was defined as 50% light inhibition of a measurement.

1. Effect of Salinity on malondialdehyde content in leaves of Plantago ovata

A quick and non-destructive way to identify salt stress-related leaf damage is through visual assessments. Under salt stress, MDA level increased, which may be the cause of increased lipid peroxidation and subsequent cell damage. In Plango ovata, MDA buildup increased as NaCl levels rose. Fewer Plantago species showed less accumulation than other species, which is consistent with other findings (Bor et al., 2003). Pro buildup has adaptive relevance because it reduces the production of free radicals and, as a result, the degeneration of membranes associated with lipid peroxidation during salt stress.

Table-1: Effect of NaCl on Malondialdehyde content in leaves of Plantago ovata

Experimental Group	NaCl treatments	MDA content (□□mole g^{-1} F.W.)	
		30 days	45 days
T1	Control (0)	16.85±0.51	18.87±0.61
T2	30	26.56±0.63	27.98±0.65
T3	60	33.18±0.69	36.19±0.71
T4	90	42.17±0.77	39.93±0.80
T5	120	43.19±0.83	26.72±0.86
T6	180	44.03±0.86	25.06±0.86

Exposure to Na resulted significant increase ($p<0.001$) MDA content in root and leaves of P. ovate in different concentration 30, 60, 90, 120, 180 mM treatment in 30 to 45 days exposures.

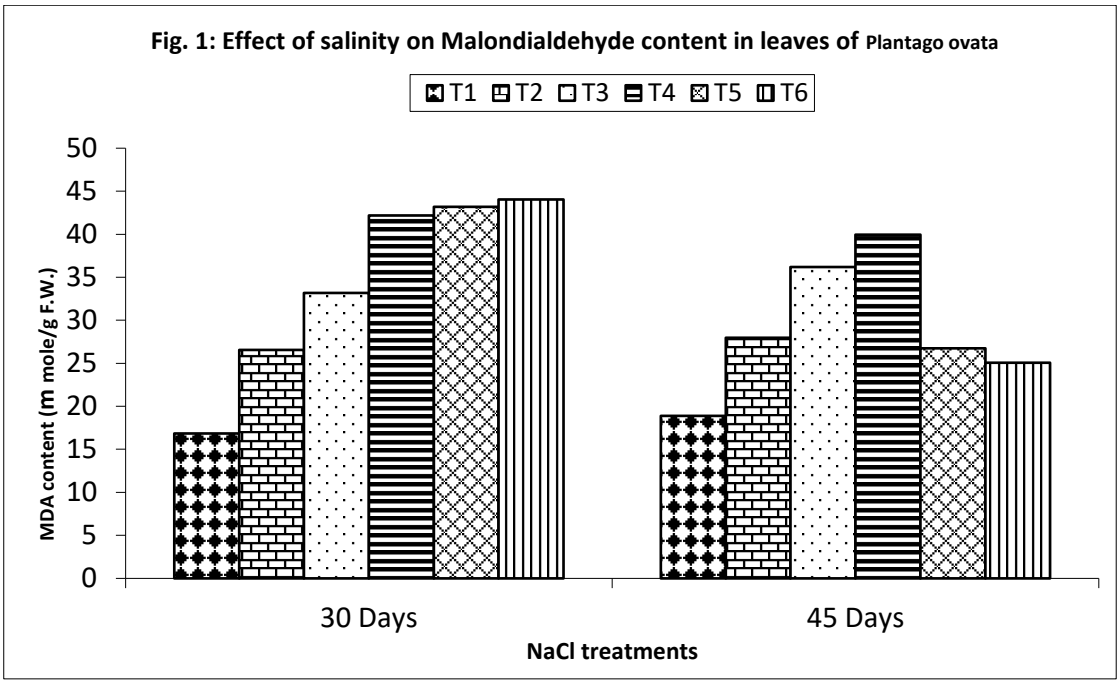


Fig. 1: Effect of salinity on Malondialdehyde content in leaves of Plantago ovata

According to biochemical analyses of the MDA material, all traits grew with rising levels of NaCl concentration. T_6 treatment shows maximum MDA content in leave of P. ovata (44.03±0.86) in comparison to control T_1, (16.85±0.5) n mole.g^{-1} F.W. MDA content increases 57.63%, 96.91%, 150.27%, 156.32% and 161.49% as NaCl concentration increases 30mM, 60mM, 90mM, 120mM and 180mM respectively in 30 days treatments. After 45 days treatment MDA content in fresh leaves of P. ovata increases 48.28%, 91.79%, 111.61%, 41.60% and 32.30% respectively. Experimental data reveal that MDA content in P. ovota leaves increases upto the 90mM concentration again decreases upto 180mM conc. of NaCl in 45 days treatments.

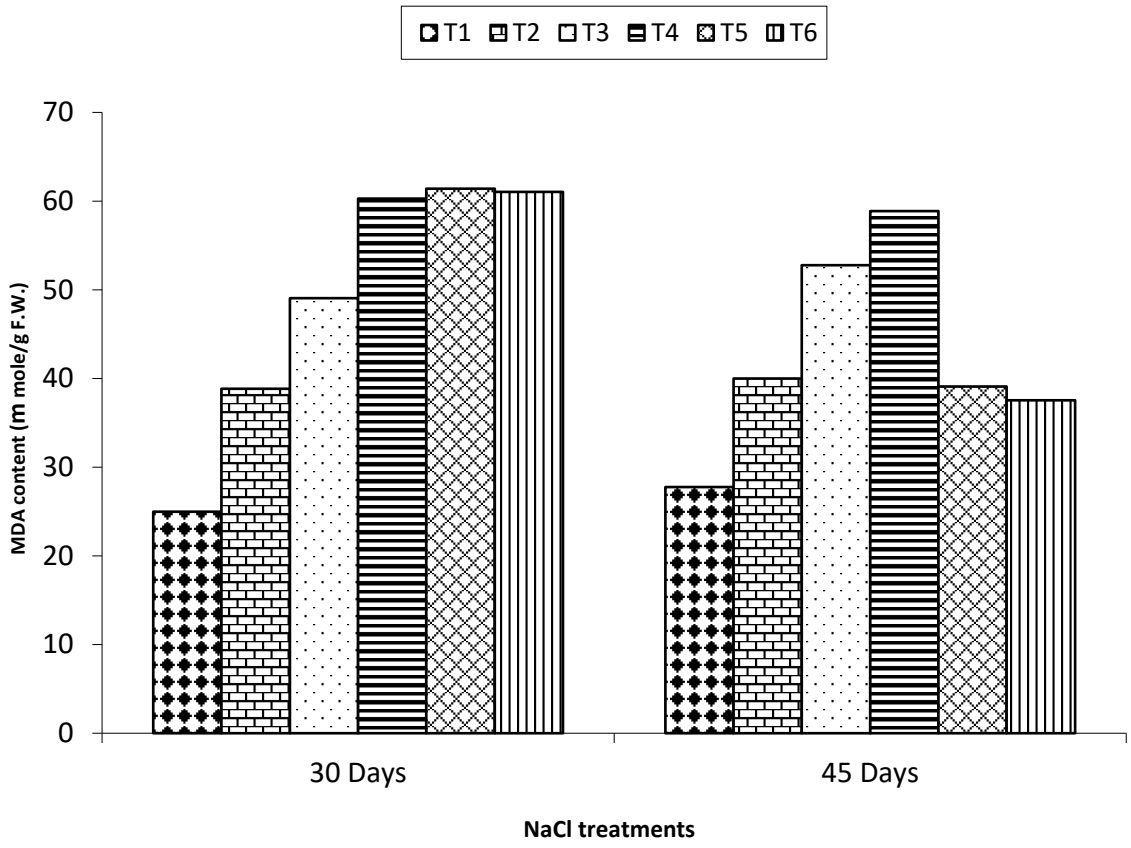
2. Effect of salinity on malondialdehyde content in leaves of Brassica juncea

When non-essential heavy metal sodium (Na) concentrations rise above a certain point, they cause mustard plants to exhibit a variety of harmful reactions. To investigate the impact of Na toxicity in Brassica juncea L., an experiment was carried out. For this study, sodium chloride (0mM, 30mM, 60mM, 90mM, 120mM, 180mM per kg soil) was fed to the soil, and the outcomes demonstrated that it diminished the growth characteristic values and leaf water potential, while it enhance the activities of antioxidant enzymes and MDA content at 30 and 45 day stages of growth. Water potential is impacted by high salt concentration in the soil, which also causes ionic stress and secondary oxidative damage. It affects MDA content, which significantly restricts plant development and growth.

Table-2Effect of NaCl on Malondialdehyde content in leaves of B.juncea

Experimental Group	Concentration of NaCl (mM)	MDA content (□□mole g^{-1} F.W.)	
		30 days	45 days
T1	Control (0)	24.96±0.64	27.76±0.72
T2	30	38.83±0.76	39.98±0.77
T3	60	49.07±0.81	52.78±0.96
T4	90	60.27±1.02	58.87±1.01
T5	120	61.38±1.01	39.08±0.78
T6	180	61.02±1.17	37.53±0.74

Fig. 2: Effect of salinity on Malondialdehyde content in leaves of Brassica juncea



Biochemical study of B. juncea leave revealed that MDA content increase with increasing concentration of salinity in soil in 30 days old mustard plants. MDA content increases 55.57%, 96.60%, 141.47%, 145.91% and 144.47% in T_2, T_3, T_4, T_5 and T_6 treatment respectively. In 45 days old mustard plant's leaves MDA content increases 44.02%, 90.13%, 112.06%, 40.78% and 35.20% in 30mM, 60mM, 90mM, 120mM and 180mM treated mustard plants respectively. This study conclude that the growth reduction and MDA content due to saline content in soil are minimise in long term life span of mustard plants. As a result of numerous metabolic events, the amount of H_2O_2 produced because of the MDA content is constantly rising. On the other hand, it's crucial to remember that none of the treatments found Na in leaves, but despite this, MDA concentration and enzyme activity increased. The ability of H_2O_2 to operate as a systemic and local signalling molecule against oxidative stress brought on by exposure to heavy metals may help to explain this (Zayneb et al., 2015). Additionally, ROS can act as signalling molecules, and H_2O_2 produced as a result of Na stressors may have a moderating impact (Tuleja and Gill, 2010).

3. Effect of Cadmium on malondialdehyde content in leaves of Plantago ovata

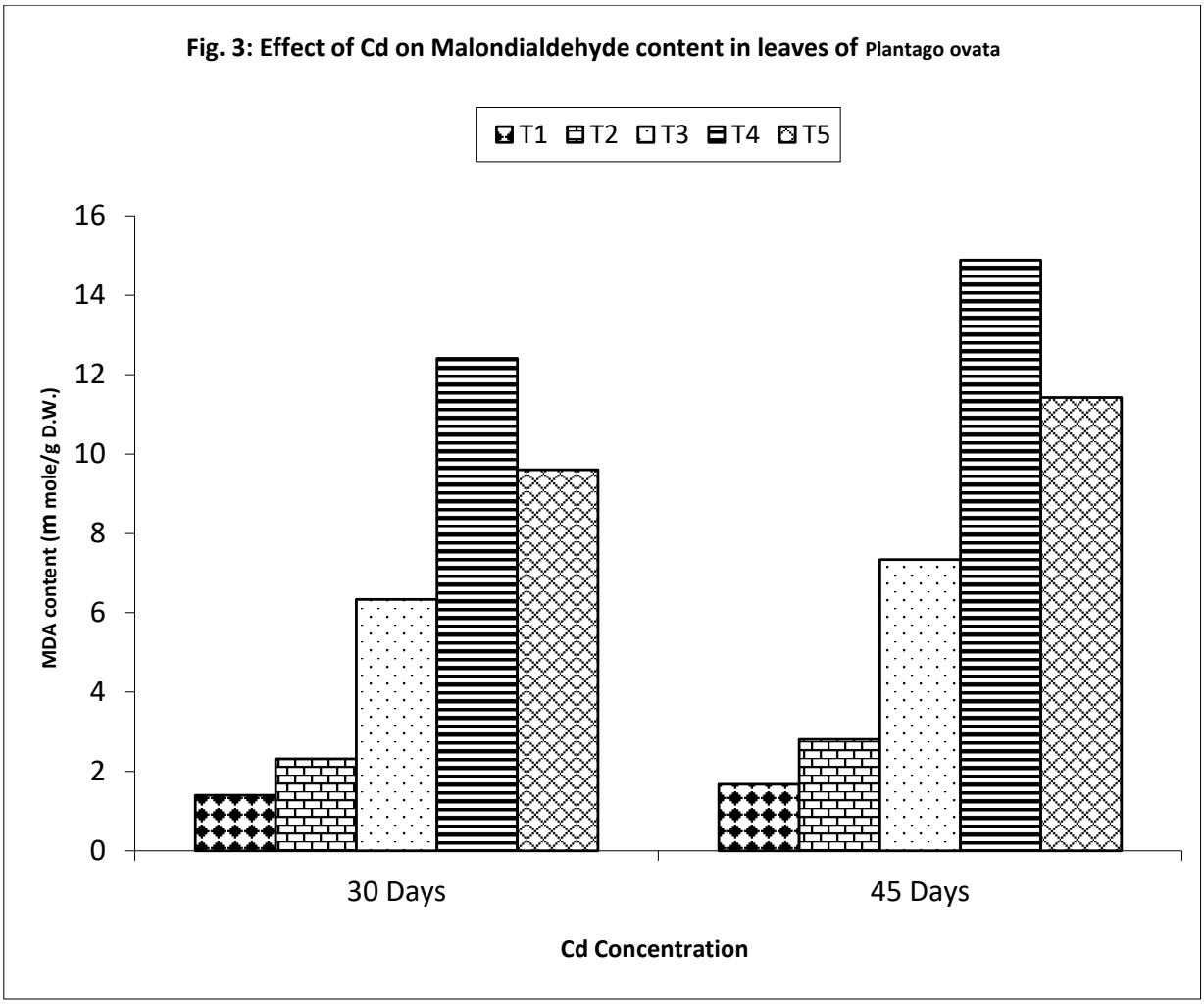
When compared to control plants, P. ovata under Cd stress had a 2.3–6.3 fold rise in malondialdehyde (MDA) concentration, a sign of lipid peroxidation. Plants of P. ovata dramatically reduced yield (21-68%).

Phytohormones are crucial for controlling plant growth under stressful circumstances, such as Cd stress (Hsu et al., 2008). An early effect of Cd stress on plants is an increase in reactive oxygen species formation, which results in cell oxidative damage. For this, plants have developed a number of defence mechanisms against ROS damage, including MDA and other ROS-scavenging enzymes, as well as non-enzymatic components like PPO and TP that are triggered to move Cd stress (Ahmad et al.,2014).

Table-3: Effect of Cd on Malondialdehyde content in leaes of Plantago ovata

Experimental Group	Concentration of Cd in Soil (mg/kg)	MDA content (□□mole g^{-1} D.W.)	
		30 days	45 days
T1	Control (0)	1.4±0.71	1.68±0.72
T2	25	2.32±0.83	2.81±0.87
T3	50	6.34±1.15	7.34±1.22
T4	100	12.41±1.62	14.89±1.68
T5	200	9.60±1.53	11.42±1.49

When P. ovata grow under Cd stress having concentration 25mg, 50mg, 100mg and 200mg kg^{-1} of soil MDA content increased in leaves of P. ovata. Enhancement of MDA-content was 65.7%, 352.8%, 786.43% and 585.71% in 30 days old plants. It seems that MDA content increased rapidly in 100mg/kg^{-1} soil but decreased onwards (Table-3). In 45 days old P. ovata plant MDA content was 67.26%, 336.90%, 786.31% and 580.76% respectively. Our result revealed that high concentration of Cd in soil and long term exposure of plant was not significantly enhance MDA content as previous one. No association was found despite the results showing a time-dependent rise in MDA content (Fig.-3). The MDA content increased during all the tested time periods up to 100 mg/kg soil, then began to fall as the Cd concentrations rose.

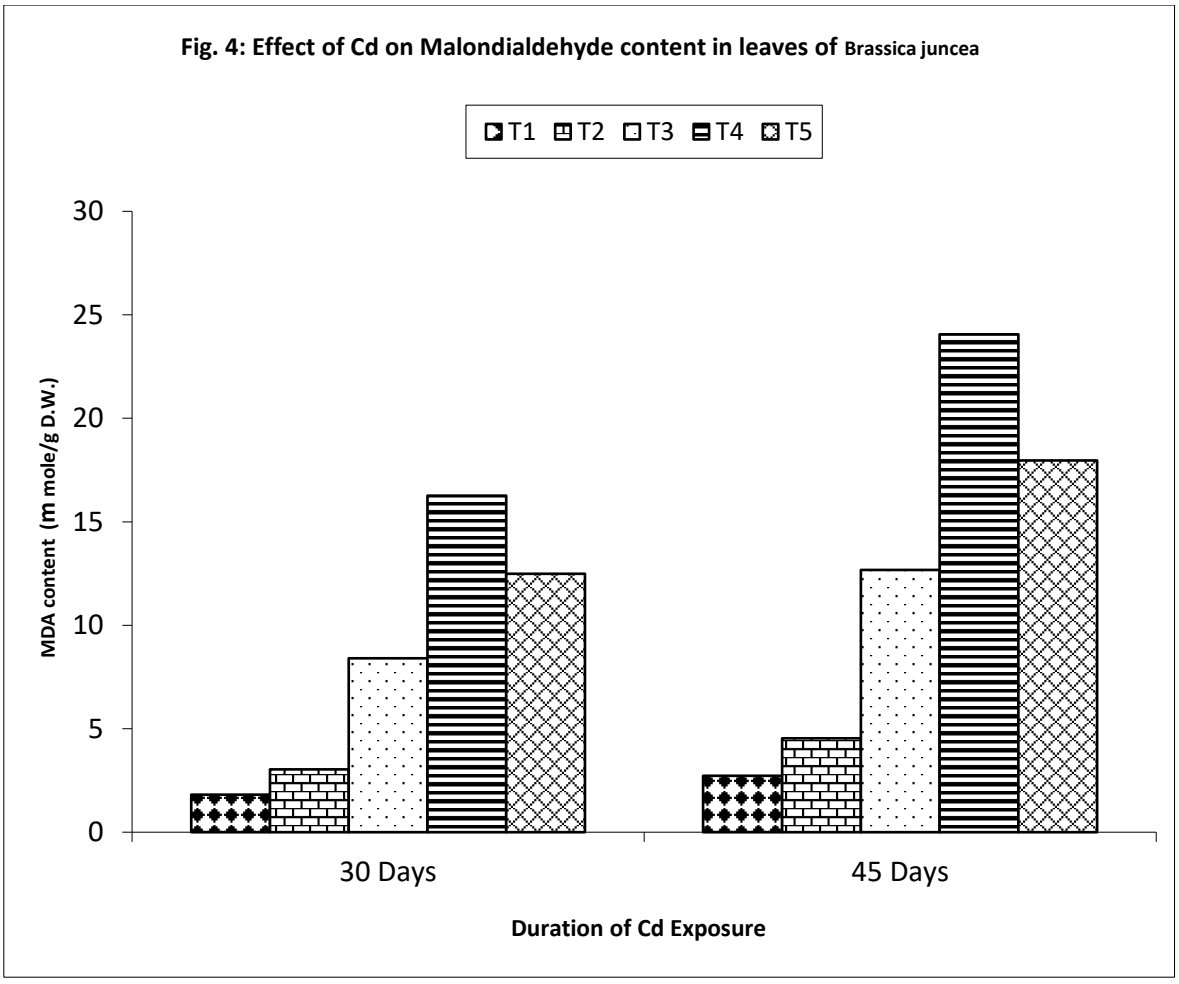


4. Effect of Cd on malondialdehyde content in leaves of Brassica juncea

To investigate the degree of membrane loss by Cd stress and role of Cd in mitigating such detrimental damage, the amount of MDA, a product of lipid peroxidation, was measured in mustard plants treated to various treatments (Fig.4). Due to the Cd stress, the MDA concentration in mustard plants increased by 63.03%, 362.08%, 793.41% and 585.71% after 30 DAT in T_2, T_3, T_4 and T_5 group of plants respectively. MDA content was observed after 45 days was 65.93%, 364.10%, 780.95% and 558.24% in T_2, T_3, T_4, T_5 group respectively in compression to control (T_1). MDA content was increased in group T_2 to T_4 in 30 and 45 days growth of mustard plant and further decreased in T_5 group. Cd-stressed plants received additional Cd, and MDA buildup was considerably reduced (Table-4).

Table-4: Effect of Cd on Malondialdehyde content in leaves of Brassica juncea

Experimental Group	Concentration of Cd in Soil (mg/kg)	MDA content (□□mole g^{-1} F.W.)	
		30 days	45 days
T1	Control (0)	1.82±0.76	2.73±0.87
T2	25	3.04±0.85	4.53±1.01
T3	50	8.41±1.19	12.67±1.22
T4	100	16.26±1.68	24.05±1.67
T5	200	12.48±1.54	17.97±1.62



These findings might point to a heating effect of Cd on mustard plant membranes under Cd stress. Control plants that dop not received Cd as a supplement showed no discernible change in MDA concentration. Plants have evolved a sophisticated antioxidant system in order to counteract and prevent the oxidative damage brought on by biotic and abiotic stress. Our findings suggested that, as evidenced by the elevated levels of

MDA and ROS. ROS may be formed in B. juncea seedlings under Cd stress and contribute to lipid peroxidation. Under Cd stress in plants, ROS scavenging mechanism must be strictly controlled (Sharma et al., 2012).

Conclusion- So It is concluded from the above study that the amount of Malondialdehyde increases gradually due to increase in salinity in the soil in 30 days treatment, but in 45 days treatment as salinity increase and after reaching a maximum concentration, the amount of Malondialdehyde starts decreasing again. Due to high salinity, the yield of plants also decreases. Similarly, when the concentration of Cd was tested, it was found that the amount of Malondialdehyde increases when the amount of Cd is increased up to a certain limit in both treatment (30 and 45 days), but after that it starts decreasing. When the concentration of Cd is 100 mg kg, the amount of Malondialdehyde is maximum and after that the amount of MDA starts decreasing as the concentration of Cd increases.

References

1. Srivastava, P. and Kumar, R. (2015). Soil salinity: a serious environmental issue and plant growth promoting bacteria as one of the tools for its elevation. Saudi J. Biof. Sci. 22: 123-131.
2. Gill, S.S., N.A. Khan, N. Taukeja (2011). Differential cadmium stress tolerance in five Indian mustard (Brassica juncea L) cultivars, an deviation of the role of antioxidant machinery. Plant Signal. Behav. 6: 293-300.
3. Hayat, S., Hayat, Q., M.N. Alyemeni, A.S. Wani, J. Pichtel., A. Ahmad (2012) Role of proline under changing environment: a review. Plant signal Behavi. 7: 1456-1466.
4. Sharma, S.S., and K.J. Dietz (2006). The significance of amino acids and amino acid derivatives molecules in plants responces and adoptation to heavy metal stress. The Journal of Experimental Botany. 57(4): 711-726.
5. Mourato, M., R. Reis and L.L. Martins (2012). Characterization of plants antioxidant system in response to abiotic stresses: a focus on heavy metal toxicity. In Advances in selected plant Physiology Aspects. G. Mantanaro and B. Dichio Eds. Australis, 23-24.
6. Rastgoo L., A Alemzadeh and A. Afsharifar (2011). Isolation of two nobel isoferms encoding zinc and copper transporting PIB- AT Pase from Gouan (Aeluropus littoralis) plant osmic Journals 4(7): 377-383.
7. Acosta-Motos, J.R. Hernadezy, J.A. Alvorez, S. Barba E., G. Sanchez Blance M.J. (2017) Long term resistance mechanisms and irrigation critical threshold showed by Eugenia myrtifolia plants in response to saline reclaimed water and relief capacity. Plant Physiol. Biochem. 111: 244-256.
8. Hernandez J.A., Jimenez A. Mullineaux P.M., Sevillei F. (2000) Tolerance of Pea (Pisum sativum) to long term Balt stress is associated with induction of antioxidant defences plant cell Environ. 23, 853-862.
9. Lu Y, Li X, He M., Zhao X., Liu Y, Cui Y, Pan Y, Tanti (2010). Seedling growth and antioxidative enzymes activities in leaves under heavy metal stress between two desert plants: a perennial (Peganum harmala and an annual (Halogenton glomeratus) grass. Acta Physiologiae Plantarum 32: 538-590.
10. Nogueirol CR, Monteiro, F.A., Gratao PL, de Alcantara de Silva B.K., Azevedo R.A. (2016). Cadmium application in tomato: nutritioinal imbalance and oxidative stress. Water air and soil Pollution, 227: 210.

11. Ehsan S., Ali S, Noureen S, Mohmood K., Farid M., Ishaque W, Shakoor MB. Rizwan M. (2014). Citric acid assisted phytoremmediation to Cd by Brassica napus. Ecotoxical. Environ. Safe 106: 164-172.
12. Zhifang G., Loescher WH (2003). Expression of a mannose 6 phosphate reductase in Arabidopsis that enhances salt tolerance and induces biosynthesis of both mannitol and glucosy-manitol dimmer. Plant cell Environ. 26: 275-283.
13. Ahmad P, Ozturk M, Gucel S (2012). Oxidative damage and antioxidant induced by heavy metal stress in two cultivars of mustard (Brassic juncea) plant Fresenius Environ. Bull. 21: 2953-2961.