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Research Paper

EFFECT OF DIFFERENT CONCENTRATION OF MANGANESE ON PHYSIOLOGICAL AND BIOCHEMICAL PROPERTIES OF WHEAT PLANTS GROWN IN DEGRADED SOIL

RAHUL VERMA, AMIT KUMAR SINGH, SWATI SINGH and AFREEN NAAZ

Plant Nutrietion and Stress Physiology Lab,
University of Lucknow, Lucknow
India.

Abstract

Wheat (*Triticum aestivum* L. variety: HD-2967) plants were grown in degraded soil with different concentration of Manganese viz; (Deficient, 10µm, 50µm, 100µm) in the form of $MnSO_4$ under controlled glass house conditions and were analysed for different physiological parameters viz; plant height, leaf area and RWC. Plants showed maximum growth in 10µM of Manganese supply but Manganese absent (00.00µM) or deficient level of Manganese sulphate supply (50µM) and toxic level (100µM), showed poor plant growth, with Hoagland treatment. Manganese plays very important and effective roles in plant growth and cellular metabolisms like 'oxidation and reduction processes', and transport of electrons in photosynthesis process. Manganese also plays an important role in the activities of various enzymes like Catalase, Peroxidase and Carotenoid. Biochemical analysis of enzymes like Catalase, Peroxidase were also analysed and found that their activities were greater in 10µM of Manganese supplied plants than the others.

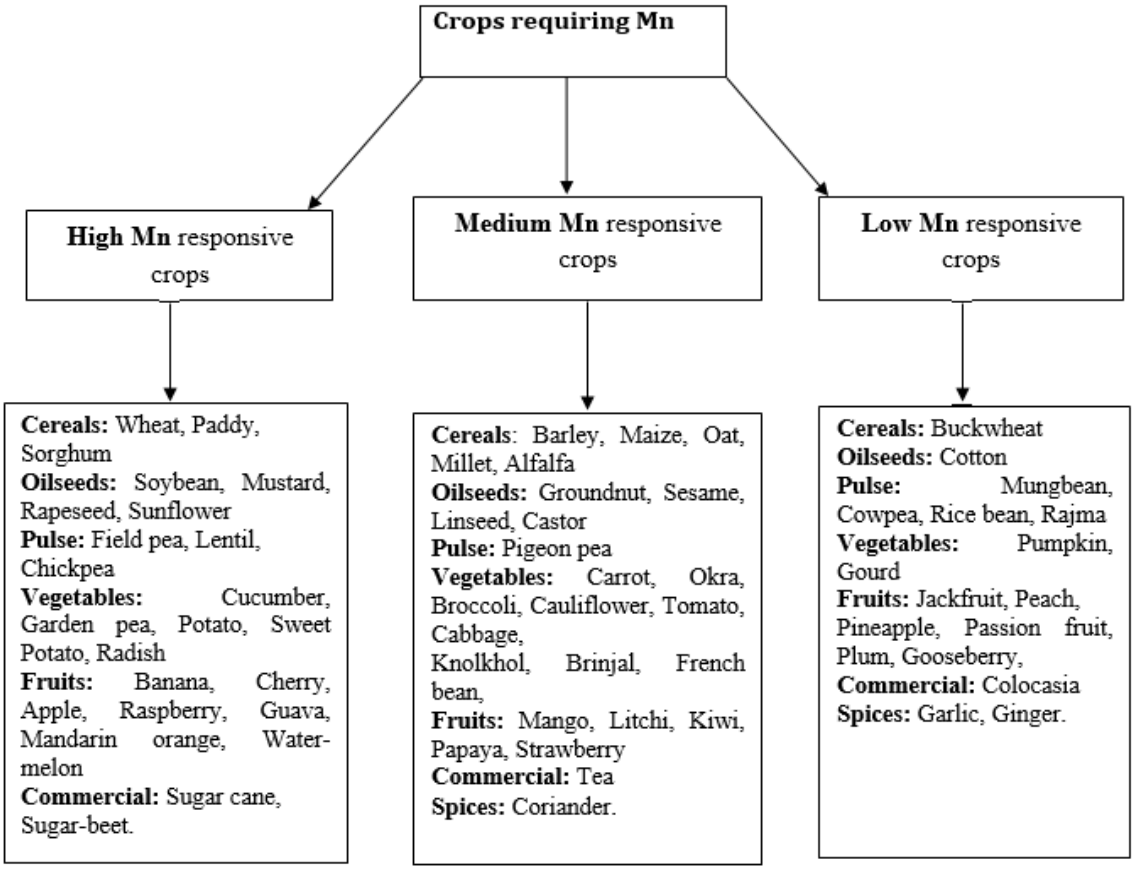
Key words: Wheat, Plant height, Leaf area, Catalase and Peroxidase etc.

INTRODUCTION

Manganese (Mn) is one of the essential micronutrients required for growth of plants [Foy *et al.,* 1978], humans and animals. It is essential for reproduction, carbohydrate/lipid metabolism, brain functioning [Greger, 1999] and neurotransmitter synthesis [Golub et. al. 2005] in humans and animals. Deficiency of Mn in humans may cause anorexia, weakness, and apathy (Huang *et al.,* 1989). More than 30% of the world's population is affected by the micronutrient's deficiencies. And manganese is the eleventh most abundant element forming the earth crust and its concentration is about 0.1% [Emsley, 2003]. It is found along with the other minerals contain elements such as

oxygen, carbon, silicon, sulfur, and chlorine [Turekian & Wedepohl, 1961]. Manganese in the soil ranged from 20 to 3000 ppm. Divalent nature of manganese (Mn^{2+}) is absorbed by clay minerals and organic materials (humus) and divalent form manganous is more stable and absorbed by plants [Turekian & Wedepohl, 1961; Malakouti& Tehrani, 1999]. Manganese is mainly absorbed in the form of Mn (II) in plants and Mn (II) is translocated as a free divalent cation in xylem from root to shoot in different plants. Divalent nature of Manganese (Mn (II)) is absorbed by clay minerals and organic materials (humus) and divalent form Manganous is more stable and it is absorbed by plants [Turekian & Wedepohl, 1961; Malakouti& Tehrani, 1999]. Manganese occurs in the form of exchangeable Manganese, Manganese oxide, Organic Manganese, and component of Ferro-Manganese silicate minerals in the soil. Manganese ion (Mn (II)) size is similar with the Magnesium (Mg (II)) and it can be substituted as being co-factor or activator of many enzymes [Sharma, 2006; Führs *et al.,*, 2010; Pandey, 2020]. Manganese reactions with soil are quite complex [Yang & Deng, 2008]. Thus, the dynamics of Manganese in soil are mostly represented by concept of balance of soluble form of Mn (II) and insoluble Mn-oxides (MnOx) [Gupta, 1986; Sharma, 2006; Yang & Deng, 2008]. Therefore, in plants Manganese play an important role in redox process and Manganese is also serves as electron storage for delivery to the chlorophyll reaction centers [Millaleo *et al.,*, 2010].

Manganese is the most important and essential micro-nutrient that, relative to iron-oxide, is required by plants after Manganese in large quantity. Manganese application to the plant in many ways through the soil application, foliar sprays, and seed treatments and so on. Nonetheless, every method has its own pros and cons. For instance, soil-applied micronutrients may get fixed within soil and may thus become unavailable for the plants [Christensen 2005]. Difficulty in the uniform application of small quantity of micronutrients [Ryan *et al.,* 2013] and its rapid conversion to plant unavailable oxidized forms within soil [Reuter *et al.,* 1973] is some of the other problems associated with Mn soil application. Crops can be divided into three major groups based on the Mn requirement, such as high Mn responsive, medium Mn responsive and low Mn responsive [Brouder *et al.,*, 2003] an schematic figure is given below.



The deficient level of Manganese in soils is responsible for the drastic differences in their range and their ability to grow crop species and cultivars. Generally, the critical tissue Mn requirement lies between 10-20 mg Mn kg-1 for most plant species. Soybean, for example, is a high Mn-requiring plant and its root nodule Bacteroids use 'nicotinamide-adenine di-nucleotide (NAD)'-malic enzyme activated by Mn to obtain energy from the plant. In order to release fixed nitrogen from root nodules into leaves and growing pods, Mn dependent enzymes are needed [Winkler *et al.*,, 1985]. The concentration of Mn in rice stems and leaves increased with the rise in Mn levels [Alam, 1985]. The application of Mn substantially increased the consumption of Mn in wheat plants, suggesting that the requirement of Mn for wheat is higher than for other crops [Fageria and Baligar, 1997].

Mostly, Mn-deficient plant exhibits small chlorotic patches in the interveinal areas of the upper middle leaves. At severity these patches increase in size and form chlorotic strips in the monocot crops (barley, rice etc.). While in di-cots, chlorotic mottling in leaves has been described [Sharma, 2006]. At severe deficiency conditions,

interveinal areas turn necrotic with the development of reddish-brown coloration, and sometimes followed by splitting of lamina [Agarwala & Sharma, 1979].

Manganese is found in different oxidation forms (+2, +3, +4,+6, +7), and the most stable biological oxidation state is Mn^{+2} significant to biochemical responses of plants have been reported [Sharma, 2006; Schmidt *et al.,*, 2015]. However, redox cycling of oxidation states is +2, +3, and +4 present in the rhizosphere of plants [Sharma, 2006]. Some metallo enzymes in plants have been identified as Mn-SOD, oxalate-oxidase [Sharma, 2006; Whittaker *et al.,*, 2007; Broadley *et al.,*, 2012], and involve in photo-system II are common Mn co-ordination geometries in plants cellular metabolism [Pandey, 2020]. Also, it is present in proteins in octahedral, square pyramidal and tri-gonal bi-pyramidal and tetrahedral forms of Mn have fast exchange legend capacity, can easily replace by other divalent ions, like as Ca, Mg, Fe, Co, Zn, and Cu etc. [Schmidt *et al.,*, 2015;Pandey, 2020]. Among the transition of biologically relevant elements, Mn^{+2} ions are the hard to borderline ion have large density and low polarization prefer for the coordination with hard legends like as negative charged oxygen atoms in the group of asparagines', carboxylase, and glutamine as well as the polar oxygen atoms within carbonyl group of imidazole ring in histidine is another important legend for Mn^{+2} [Sharma, 2006; Broadley *et al.,*, 2012].

Manganese involved in photosynthesis, biosynthetic pathways of amino acids, in regulation of nitrogen metabolism, protection of cellular components etc. in plants [Chen *et al.,*, 2006; Sharma, 2006].It catalyzes oxygen evolution in biological system. It is a metal co-factor in the oxygen evolving complexes (OEC) in the higher plants for photosynthesis process [Sharma, 2006; Mousavi *et al.,*, 2007]. Manganese is co-factor or constituents of many metalloid-enzymes in most of the plants involve in various biosynthetic pathways [Burnell, 1988; Chen *et al.,*, 2002; Pandey, 2018], about 30 types of enzymes in cellular metabolism of plants such as the activation of phenylalanine ammonialyase in the shikomic pathway; Golgi localized glycosyl transferases (localized in Golgi bodies), de-carboxylase and de-hydrogenase in the tri-carboxylic acid [Hoganson *et al.,*, 1993; Sharma 2006; Pandey, 2018; Pandey, 2020]. And the most-well-studied function in plant metabolism that depends on Mn is the water-splitting reaction in PSII, which is the first step of photosynthesis. This process requires the tetra-Mn cluster Mn_4O_5Ca to split two water molecules into four electrons, four protons, and molecular O_2 [Bricker *et al.,*, 2012].

MATERIAL AND METHODS

Wheat (*Triticum aestivum* L. variety: HD-2967) plants were grown in the glass house under controlled conditions. Soil for experiment was collected from an area near to banks of river Gomti near Gosaiganj, Lucknow. Soil sample was filtered with the help of sieved and the filtered soil sample was filled in mud pots and used polythene bags in between soil sample and mud pots to stop drainage of water from soil sample to mud pots. The composition of nutrient solution excluding 8 mM Ca $(NO_3)_2$, 4 mM KNO_3, 2 mM $MgSO_4$, 1.33 mM NaH_2PO_4, 0.1 mM Fe EDTA, 0.33 mM H_3BO_3, 1.0 µM $CuSO_4$, 1.0 µM $ZnSO_4$, 0.1µM Na_2MoO_4, 0.1 M NaCl, 0.1µM $CoSO_4$, 0.1 µM $NiSO_4$, and Mn supplied at four level (Mn absent, 10 µM, 50 µM, and 100 µM) in form of $MnSO_4$.

After some time, germination of Wheat seedling, wait for 25 to 30 days for proper growth of Wheat seedlings, Hoagland's nutrient solution including Mn at above prescribed level were supplied daily. Plant was examined on daily basis for changes in growth parameters and visible symptoms of Mn-Deficient and high level were recorded. After 50 to 60 days, collect the plant sample from mud pots and send the plant sample for laboratory to analysed physiological and biological parameters of collected sample.

Chlorophyll and relative water content were estimated in comparable of young and fresh leaves. Chlorophyll extraction in 80% of acetone solution and centrifugation at 5000rpm and measured on spectrophotometrically Perkin Elmer UV/VIS Lambda Bio 20 [Lichtenthaler, H.K., 1987].

Relative water content was analyzed, firstly cut the leaves in disc shaped with the help of cork borer and filled these leaf parts in glass distill water for 4 hours at 5^oC. Thereafter, leaf sample are placed in oven at 55^oC for 24 hours, and reweight again for dry weight. The relative water content (RWC) was calculated by the formula: RWC = (F.W– D.W/ T.W– D.W) × 100.

Catalase (CAT) was extracted by homogenization of fresh leaf tissue in ice cold distilled water (1:10) with mortar and pestle. The reaction mixture containing0.005 M H_2O_2 and 0.025 mM phosphate buffer (pH- 7.0) was incubated with 1 ml of suitably diluted enzymes extract at 25°C for 5 min. The reaction was stopped with 2 ml 2N H2SO4 and mixture was titrated against 0.1 N $KMnO_4$ [Euler, H.,Vin and Josephson, K., 1927].

Peroxidase (POD) was extracted by homogenization of fresh leaf tissue with ice cold glass distilled water in a clean, chilled motor and pestle. The homogenate was stained through four layer of muslin cloth. The reaction mixture for POD contained 2 ml of 0.1µ MPO_4 buffer (pH- 6), 1ml of 0.01 % H_2O_2 and 1 ml of 0.5 % p-phenylenediamine. The reaction was started by adding 1 ml of enzyme extract to mixture. After 5 minute incubation at 25°C, the reaction was stopped with 2 ml 4N H_2SO_4. Optimal density was measured at 485 nm change in OD, was calculated [Luck, M., 1963].

For Proline extraction, fresh leaves were homogenized in sulpho-salicylic acid. After filtration suitable aliquot was taken with ninhydrin reagent and glacial acetic acid and boiled for 1 hour. The color was extracted in toluene and read absorbance at 520nm [Bates, L. S., Weatherly, P. E., 1962].

For analysis the Hydrogen peroxide, chilled leaf tissue was homogenized in chilled acetone and homogenate was filtered. The filtrate was brought to volume by water to adjust ratio of acetone in water 2:1. Take suitable amount of aliquot; add 2.5ml of hydrogen per-oxide and water add 0.5ml of titanic tetra chloride and 1ml of concentrated ammonia solution and centrifuge the aliquot at 10000 rpm for 5 minutes. Precipitate was solubilized in 5N of 4ml H_2SO_4, read the absorbance at 415 nm by using spectrophotometer [Brennan and Frenkel, 1977].

For Assay of Ascorbate peroxidase Plant material was homogenized in 50 mM phosphate buffer pH 7.0 containing1% insoluble polyvinyl pyrrolidone (w/v) at 4°C with mortar and pestle (0.1gfresh weight mL-1 buffer) filtered through four layers of muslin and centrifuged at 15,000 rpm for 10 minutes. The supernatant obtained was designed as crude enzyme extract.50 mM phosphate buffer (pH 7.0), 0.5 mM ascorbate,0.1 mM EDTA, 0.1 ml enzyme extract, add 0.1 mM H_2O_2 to total volume 3 ml, decrease in absorbance was recorded at each 30s interval for 3 minutes at 290nm in UV spectrophotometer [Nakano and Asada, 1981].

Table1: Effect of different concentration of Manganese supply on physiological parameters of Wheat plant like as: plant height, Leaf area and relative water content of Wheat (*Triticum aestivum L.* var., HD 2967) plants.

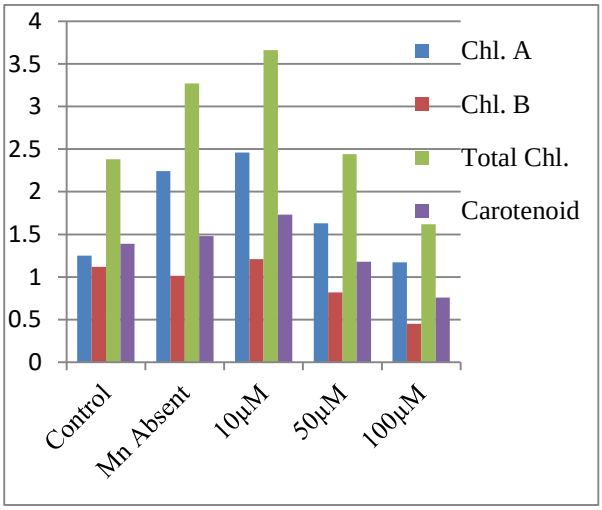
Physiological parameters	**µM Manganese supply**				
	Control	**00 µM**	**10 µM**	**50 µM**	**100 µM**
			Unit Cm (After 60 days)		
Plant height	46.9	51.23(+9.23)	55.3(+17.91)	52.17(+11.23)	49.28(+5.07)
			Unit Cm^2per plant(After 60 days)		
Leaf Area	11.36	18.17(+59.94)	25.92(+12.16)	16.34(+43.83)	14.28(+25.70)
			Unit mg (After 60 days)		
R.W.C.	90.35	91.19(+0.91)	96.06(+6.31)	94.93(+5.06)	92.68(+2.57)

Table 2: Effect of different levels of Manganese supply on the concentration of chlorophyll and Carotenoid in Wheat (*Triticum aestivum* L. var., HD 2967) plants.

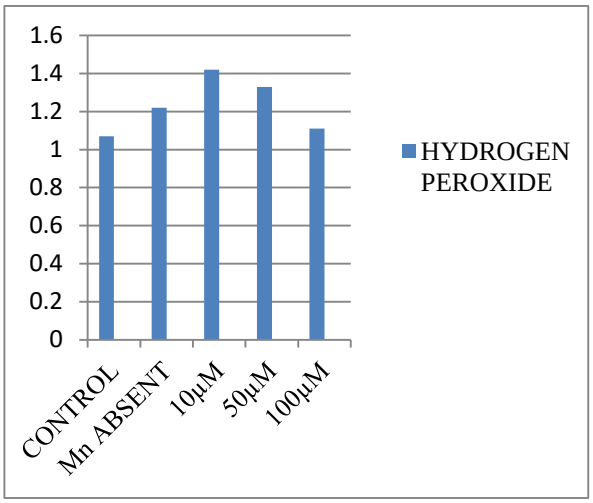
	µM Manganese supply				
	Control	**00 µM**	**10 µM**	**50µM**	**100 µM**
			mg g^{-1} fresh weight		
Chlorophyll Chl. A	1.25	2.24	2.46	1.63	1.17
Chl. B	1.12	1.01	1.21	0.82	0.45
Total Chl.	2.38	3.27(+37.39)	3.66(+53.78)	2.44(+2.52)	1.62(-31.93)
Carotenoid	1.39	1.48(+6.47)	1.73(+24.46)	1.18(-15.10)	0.76(+45.23)

Table 3: Effects of different concentration of Manganese sulphate on the activities of Hydrogen peroxide, Catalase, Peroxidase, Ascorbate peroxidase, and Proline in leaves of wheat (*Triticum aestivum* L. var., HD 2967) plants.

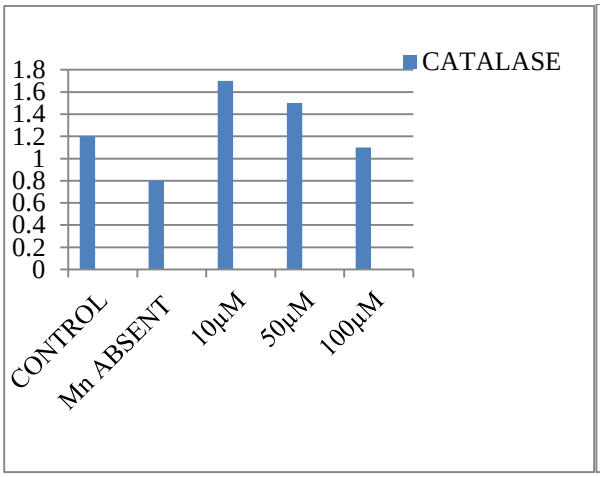
Enzyme	µM Manganese supply				
	Control	00 µM	10 µM	50 µM	100 µM
			µ mol 100mg $^{-1}$ Fresh weight		
Hydrogen peroxide	1.07	1.22(+14.01)	1.42(+32.71)	1.33(+24.29)	1.11(+3.73)
			µ mol H_2O_2 decomposed mg^{-1} Protein weight		
Catalase	1.2	0.8(-33.33)	1.7(+41.66)	1.5(+25.00)	1.1(-8.33)
			Units mg^{-1} Protein		
Peroxidase	0.425	0.380(-10.58)	0.53(+25.41)	0.49(+15.52)	0.416(-2.11)
			µ mol Ascorbate oxidized mg^{-1} protein		
APX	35.35	37.76(+6.81)	52.56(+48.68)	40.06(+13.32)	32.53(-7.97)
			µ mol g^{-1}Fresh weight		
Proline	3.55	4.66(+31.26)	1.83(-48.45)	2.83(-20.28)	4.106(+15.66)



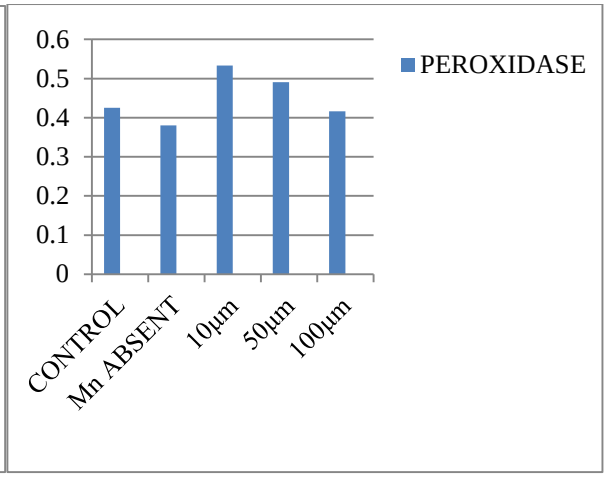
GRAPH-1



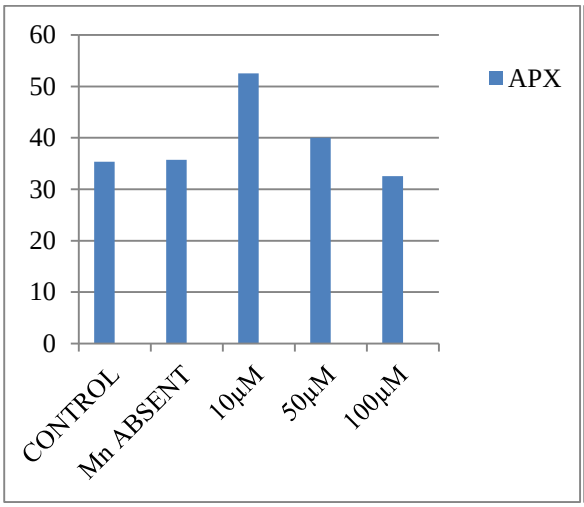
GRAPH-2



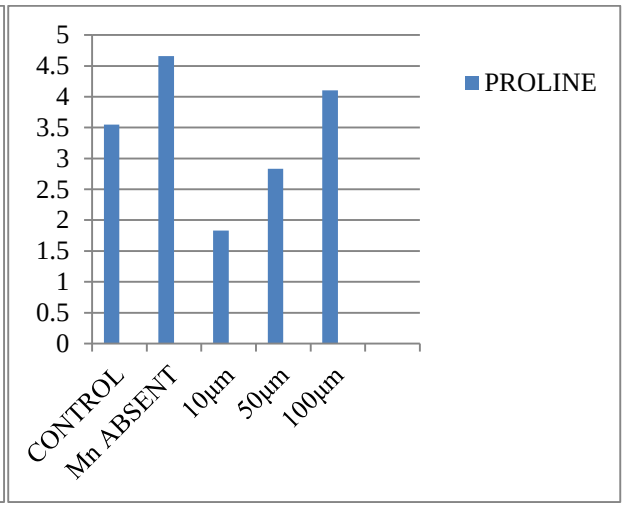
GRAPH-3



GRAPH-4



GRAPH-5



GRAPH-6

NOTE- Graph -1 represents the higher content of chlorophyll at 10 µM of Manganese sulphate.

Graph-2 represents the hydrogen peroxide concentration at 10 µM of Manganese sulphate.

Graph-3 represents the higher activity of enzyme Catalase at 10 µM than the other level.

Graph-4 represents the higher activity of enzyme Peroxidase at 10 µM than the other level.

Graph-5 represents the higher activity of APX at 10 µM compare to other Mn level.

Graph-6 represents the lower level activity of Proline at 10 µM of Mn sulphate level.

RESULTS AND DISCUSSION

The optimal growths of Wheat (Triticum aestivum L. var., HD 2967) plants were observed at 10µm Manganese sulphate. On the other hand the wheat plants received less than 10µm and more than 10 µM of Manganese supply showed, decrease in the plant growth, relative water content and leaf area of plants. Deficient concentration of Manganese supply resulting in the growth retardation of plants and significant decline in plant height and leaf area. Minimum plant growth and relative water content was observed in deficient level (Manganese absent) and so on (50 µM & 100 µM). Plant growth and height showed abnormal pattern in wheat plant, i.e., 10µm Manganese supply showed normal and maximum plant height and minimum plant height in deficient level (Mn absent) and so on.

The value of relative water content (RWC) decreased significantly in the leaves of Manganese deficient (Mn Absent) as compared to 10 µM of Manganese supply and the value of relative water content was also decreased in 50µm towards 100 µM of Manganese supply. This result showed that the Mn deficient plants show susceptibility to drought stress because Mn deficiency reduced the waxy content that increased transpiration water loss and lower water use efficiency. The value of relative water content is the probably the most appropriate measure of plant water status in terms of the physiological consequences of cellular water deficit in plant leaves. And the value of relative water content was optimum in 10 µM and the least value was observed in 100µM of wheat sample. The plant height after 60 days, maximum plant height was recorded in 10 µM of wheat plant and minimum was 100 µM, and the leaf area was also maximum in 10 µM and minimum in 100 µM of Manganese supply

In this experiment the concentration level of chlorophyll in leaves of wheat plant was decreased significantly with decreasing and increasing the concentration of Manganese from deficient (Mn absent) to 100µm. These results clear the important and critical role of Manganese ion as a co-factor for photosynthesis light dependent reactions. The Carotenoid concentration was also decreased in Manganese deficient (Mn Absent) supply of wheat plant as compared to 10µm of solution. Lower chlorophyll and Carotenoid concentration in plant leaves is indicator of senescence, stress and damage of plant tissue and the photosynthetic apparatus, expressed by faster breakdown by lower level of chlorophyll presence.

After the treatment period, i.e., 15 to 20 days the catalase enzyme activity was reduced in Manganese deficient (Mn absent) plants and showed the inability of the plants to overcome the oxidative damage. The optimum catalase [Evler and Josephson, 1927] enzyme activity was in 10µm of Manganese supply and it also showed maximum plant growth. The optimum concentration of Catalase enzyme was observed in 10µM of Manganese ion supply of wheat plant and least concentration was present in deficient (Manganese absent) of Manganese supply.

The activity of Peroxidase in wheat plants showed very critical and important role of Manganese ion as a co-factor in non-specific enzymes. The activity of Peroxidase [Nakaro and Asada, 1981] was optimum in 10µm of Manganese supply and lowest activity was detected in Manganese deficient (Mn absent) level of supply and so on in 50µm and 100µm. Peroxidase enzyme catalysis the oxidation of a substrate by removal of hydrogen which combines with hydrogen peroxide. The activity of Peroxidase enzymes also affects the degradation of hydrogen peroxide, removal of toxic compounds, defense against insect herbivore and some other stress related problems in plants.

The activity of Proline enzymes was increased in Manganese deficient (Mn absent) of Wheat plant and lowest concentration in 10µM of Manganese supply after 15 to 20 days of Hoagland solution supply. The activity of Proline also indicates the water stress in Manganese deficient plants.

In this experiment the activity of Ascorbate Peroxidase depended on catalyzing conversion of hydrogen peroxide into water by using an ascorbate as a specific electron donor. The activity of Ascorbate peroxidase in 10µM of Manganese supply in wheat plant is optimum level and the minimum activity was recorded at 100µM of Manganese supply.

CONCLUSION

The study was performed in degraded and non-irrigated soil collected from areas near to Gomti River at Gosaiganj in Lucknow district. Our study comes on conclusion that, the degraded or non-irrigated soils near the Gomti River may have low quantity of Manganese than the other soils. The Manganese itself found responsible for the optimal growth of wheat plants used in experiment when supplied at optimal level i.e. 10 µM. The Manganese deficiency badly affected the plant growth, metabolic activities and

reproductive yield. Manganese stressed or Manganese deficiency induced water stress and weakens the complex defense mechanism in response to oxidative stress of plant metabolism. The toxicity of Manganese also affected plant's optimal growth and reproductive yield. Hence for better yield of crop and for a strong defense system against Abiotic stresses among plants, the optimal supply of Manganese is recommended.

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