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RESEARCH ARTICLE

ALTERATIONS IN THE NUCLEIC ACID AND PROTEIN CONTENT IN *SORGHUM BICOLOR* PLANTS UNDER ZINC EXCESS

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Abstract

Trace metals like zinc are natural components of the environment but, elevated and potentially toxic levels sometimes occur. The use of zinc fertilizers and pesticides is a common agricultural practice, which over the years lead to elevated levels of zinc in the soil.In the present investigation seven-day old *Sorghum bicolor* plants were treated with five different concentrations of zinc solution and sampling was done at different stages of plant growth to estimate the nucleic acid and protein content. The results revealed a significant reduction in DNA, RNA and proteins in the plants growing at higher concentrations of zinc treatment as compared to control. Toxic levels of heavy metal ions induce several cellular stress responses and damages different cellular components such as membranes, proteins and DNA.

Introduction:-

Domestic effluents possibly constitute the largest single source of elevated metal values in rivers and lakes. They may consist of untreated or mechanically treated waste waters. The use of detergents creates a possible pollution hazard, since common house hold detergent products can affect the water quality. Anginoet al., (1970) found that most enzyme detergents contained trace amounts of the elements Fe, Mn, Cr, Co, Zn, Sr and B. Urban runoff presents a serious problem of heavy metal contamination. Bradford, (1997) revealed that urban storm water runoff has long been recognized as a major source of pollutants to surface waters. Studies by Bolter et al., (1974) indicate that lead is leached by humic and other acids, thus increasing its availability for runoff rather than percolation into the upper soil layer. Animal manure enriches the soil by the addition of Mn, Zn, Cu and Co and sewage sludge by Zn, Cr, Pb, Ni, Cd and Cu (Verkleji, 1993).

Agricultural ecosystems are also contaminated by metals, originating from atmospheric pollution, pesticide applications and contamination by chemical fertilizers, and irrigation with wastewater of poor quality or addition of sewage sludge. The use of zinc fertilizers and pesticides is a common agricultural practice, which over the years lead to elevated levels of zinc in the soil. The application of phosphate fertilizers to the agricultural soil has led to increase in Cd, Cu, Zn and As, (Zarcinaset al., 2004). Fertilizers, sewage sludges and animal wastes used as fertilizers, pesticides and irrigation water are cause of contamination (Grasmanis and Leeper, 1966; Gibson and Farmer, 1983; Ross, 1994).

Trace metals are natural components of the environment, but, elevated and potentially toxic levels occur sometimes. In acid soils, elevated levels of soluble metals (particularly Al or Mn) may occur, whilst high concentrations of other metals (such as Cu or Pb) may be present in sites contaminated by agriculture, mining, industry, or transport.

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Zinc is commonly found in the earth's crust, and natural releases to the environment can be significant. Zinc is one of the most widely used metals in the world and the most prevalent metal contaminants in industrialized countries (Boardman and McGuire, 1990; Raymond and Okieimen , 2011; Reena Singh et al., 2011; Subhashiniet al., 2013). Zn has been found in the highest concentrations of all of the heavy metals found in industrial waste (Boardman and McGuire, 1990). Zinc is often present in soils and grasses as a result of atmospheric deposition (Dale and Freedman, 1982; Alloway, 2008). Hazardous waste sites are additional sources of zinc in soil (Watschke and Mumma, 1989; Bastaet al., 2005; Khan et al., 2008; Zhang et al., 2010; Raymond and Okieimen , 2011;Vandana, 2011).

The total Zn concentration in soil usually ranges from 10 to 300 mg kg^{-1}, the average being ∼50 mg kg−1 (Mortvedt, 2000); however, it can reach thousands of mg kg^{-1} in severely Zn contaminated soils (Kiekens, 1990). The response of plants to elevated soil zinc has generated a substantial literature, Zinc toxicity in crops is far less wide spread than Zn deficiency.

The present study is focused on understanding the variation in the nucleic acid content and proteins in *Sorghum bicolor*plants under conditions of stress induced by zinc excess.

Materials and Methods:-

The experimental plant, *Sorghum bicolor* (L.) Moench cultivar CSH 14 belongs to the family Poaceae (graminae). *Sorghum* is one of the important crops for food security in semi-arid and arid regions of the world due to high nutritional quality. Certified seeds of *Sorghum bicolor* L. Moench cultivar CSH 14 were obtained from the National seed corporation, Hyderabad. Seeds with uniform size were chosen for experimental purpose.

The soil was collected from a local nursery. The soil was air dried and sieved through 2mm sieve to discard the non soil particles. Earthen pots of 20cm diameter and 25cm height were selected for growing the plants. Each pot was filled with 3kg of air-dried soil.

Seeds were surface sterilized with 0.001M mercuric chloride for two minutes and thoroughly washed with water several times. Ten sterilized seeds were sown in each pot. All the pots were watered to field capacity daily. Plants were thinned to a maximum of three seedlings per pot after a week of germination.

The seven-day old plants were treated with five different concentrations of zinc solution viz: 1.5, 3.5, 5.5, 7.5 and 9.5mM taken as zinc sulphate. Different concentrations of zinc solution (300 ml) were given once in two days to the field capacity, total ten doses were given during the experimental period. Plants treated with water served as control. Two doses of soil application of NPK solution was prepared was given to the plants on 25^{th} and 35^{th} day of growth. The plants were grown under natural photoperiod. During the growth period plants were regularly monitored for any morphological changes and phytotoxicity symptoms. Each treatment including the control was replicated six times.

The plant samples were collected at fifteen-day intervals approximately viz: 15, 30 and 50 days. The plants were first removed from the soil, the entire plant with roots and shoots were put under constant flow of water to remove the soil particles and exogenous contaminants adhered to the plants. The water droplets were blotted dry with help of blotting paper. Sampling was done in the early hours for the estimation of macromolecules like DNA, RNA and Proteins.

Nucleic Acids Extraction and Estimation

Extraction Method

Nucleic acids and protein were extracted by the method of Ogur and Rosen, (1950).

2.5 ml of ethanol homogenate was taken and to this 5 ml of 10 % ice cold trichloro acetic acid was added. This was centrifuged at 4000 rpm for 10 min and supernatant was discarded. To the pellet 5 ml of 3:1 ethanol ether mixture is added and centrifuged at 4000 rpm for 10 min. The supernatant was discarded. Ethanol ether washing was repeated thrice. To the precipitate, 5 ml 0.5 N NaOH was added and mixed well and left for incubation at 37^0C for 18 hours. Then the mixture was centrifuged at 4000 rpm for 10 min. and the supernatant was used for the estimation of RNA which was present in a hydrolyzed form and the precipitate was used for the estimation of DNA. To the supernatant equal volume of 10% TCA was added and centrifuged at 4000 rpm for 10 min.

Estimation of D N A
Method of Burton, (1956) was used for the DNA estimation.To 1.5 ml of perchloric acid extract, 3 ml of diphenylamine reagent was added. The tubes were kept in a water bath and maintained at 70°C for 20 min and then cooled. The colour development was read at 600 nm on a Systronics Spectrophotometer. A standard calibration curve was prepared by using known concentrations of calf thymus DNA. The DNA content was expressed in mg DNA gr^{-1} fresh weight of the leaf tissue.

Estimation of RNA
RNA was estimated by the method of Schinder, (1957).To the 2 ml nucleic acid fractions, 3 ml of orcinol reagent and 0.5N perchloric acid was added and heated on a water bath for 20 min at 90°C and then cooled. The colour development was read at 660 nm. A standard calibration curve was prepared by using know concentrations of purified RNA. The RNA content was expressed in mg RNA/ gram fresh weight of the leaf tissue. For blank 3 ml of 0.5 N perchloric acid and 3 ml of orciol reagent was taken.

Estimation of Protein
Protein was estimated by the modified method of Lowry, (1951).To 1 ml of the protein extract, 5 ml of reagent C was added and the mixture was incubated for 8 to10 min and then 0.5 ml of reagent D was added. The mixture was incubated for another 20 to 30 min. The colour developed was read at a wavelength of 750 nm. The protein standard was estimated by using BSA as a stock solution. Protein content was expressed in mg of protein/g of fresh leaf tissue. For blank 1 ml of 0.1 N NaOH and 5 ml of reagent D was added.

Statistical analysis
The data were represented as mean values ± S.E from three replicates. Data was subjected to two - way ANOVA to know significance between metal treatment and exposure duration for the morphological and biochemical parameters under zinc excess at 5% ($P < 0.05$) significance.

Results:-

The effect of zinc on DNA content in *Sorghum bicolor* leaves is furnished in Fig.1. DNA content was estimated in the leaves at different stages of plant growth.

In fifteen-day, old plants, DNA content decreased linearly with increase in zinc concentration. The maximum decline was at 9.5mM of zinc.

In thirty and fifty-day old plants also a similar trend was observed. Maximum decline of 81.8% and 88.2% at 9.5mM of zinc was observed in thirty and fifty days of plant growth respectively.

The DNA content decreased significantly ($P <0.05$) with increase in zinc concentration and age of the plants.

Ribonucleic acid (RNA)
The effect of zinc on RNA content in *Sorghum bicolor* leaves is furnished in Fig.2. RNA content was estimated in the leaves of *Sorghum bicolor* at different stages of plant growth.

A gradual decline in the RNA content at elevated concentrations of zinc was observed in fifteen and thirty-day old plants. Maximum decrease of 18.5% and 47% was observed at 9.5mM zinc in fifteen- and thirty-day old plants respectively.

In fifty-day, old plants, as the zinc concentration increased the RNA content gradually decreased. Highest decrease of 54% at 9.5mM of zinc was recorded.

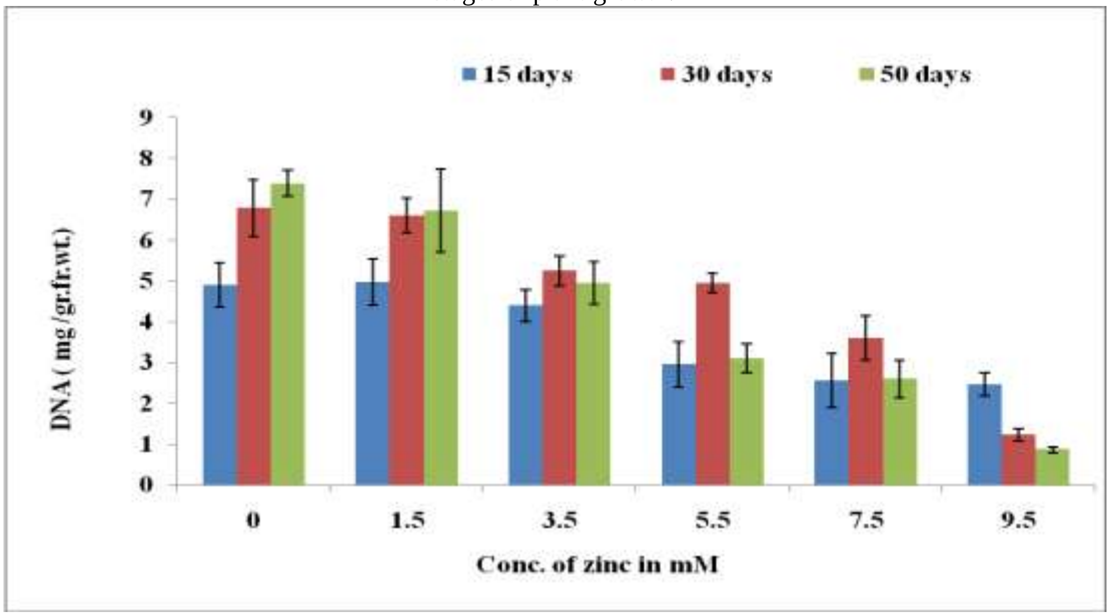
RNA recorded significantly ($P < 0.05$) lower values both, with increase in zinc concentrations and the age of the plants.

Soluble proteins
The effect of zinc on soluble protein content in *Sorghum bicolor* leaves is represented in Table 1.

Protein in the leaves of *Sorghum bicolor* was estimated at different stages of plant growth. Highly significant ($P < 0.05$) reduction was observed with increase in zinc treatment and increase in age of the plants.
In fifteen-day, old plants, a gradual decrease in the protein was noted with increase in zinc treatment. Maximum percent reduction of 58% was noted at 9.5mM of zinc.

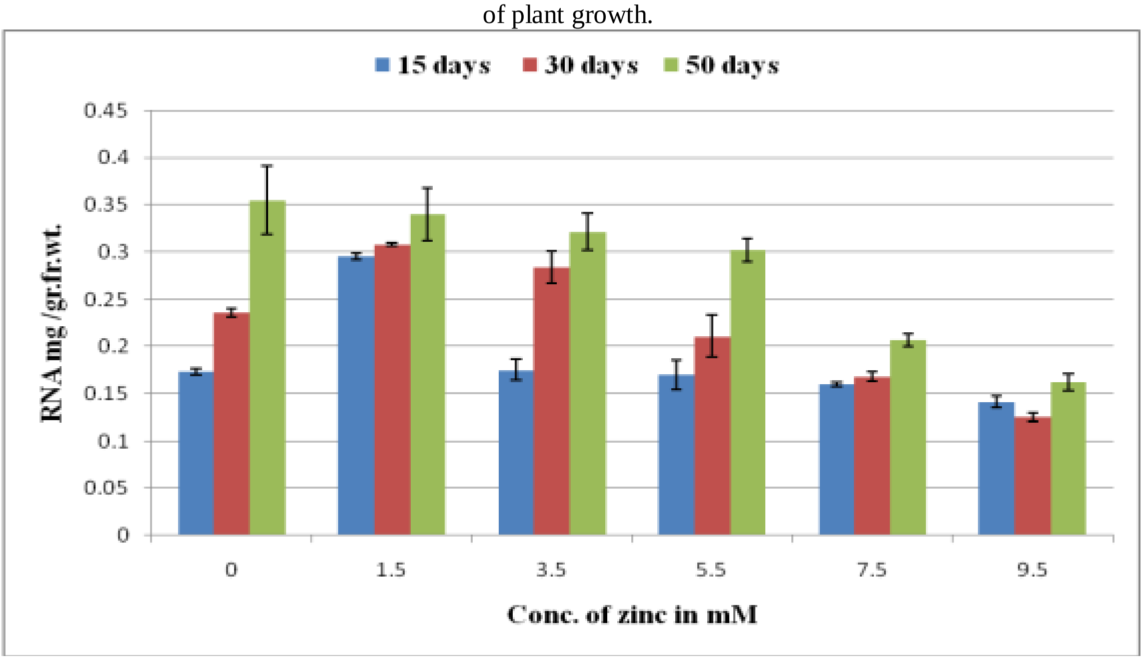
In thirty- and fifty-day old plants a slight increase in protein content at 1.5mM of zinc was observed and at all other concentrations the protein content recorded lower values. 30% reduction was seen at 9.5mM in thirty-day old plants and percent decrease was 9.8% at 9.5mM of zinc in fifty-day old plants.

Fig. 1:- Effect of zinc on DNA (mg / gfw) in the leaves of *Sorghum bicolor* (L.) Moench (CSH 14) at different stages of plant growth.



Vertical bars represent ± SE. Zinc treatment **, Sampling days** (** Significant at $P < 0.05$).

Fig. 2:- Effect of zinc on RNA (mg / gfw)in the leaves of *Sorghum bicolor* (L.) Moench (CSH 14) at different stages of plant growth.



Vertical bars represent ± SE. Zinc treatment **, Sampling days** (** Significant at $P < 0.05$).

Table 1:- Effect of zinc on Soluble protein (mg / gfw) in the leaves of *Sorghum bicolor* (L.) Moench (CSH 14) at different stages of plant growth.

Zinc added to the soil (mM)	Sampling days		
	15	30	50
Control	11.00 ±1.20	14.00 ± 0.05	17.30 ± 0.02
1.5	9.00 ±1.52	16 .00 ± 0.01	18.00 ± 0.02
3.5	7.90 ± 0.63	11.26 ± 0.00	20.00 ± 0.03
5.5	7.86 ± 0.69	11.10 ± 0.04	15.30 ± 0.23
7.5	5.30 ± 0.60	10.66 ± 0.001	13.83 ± 0.12
9.5	4.60 ± 0.00	9.73 ± 0.02	11.60 ± 0.88

Data expressed as mean ± SE, (n = 3). Zinc treatment **, Sampling days** (** Significant at $P < 0.05$).

Discussion:-

The plant genome is very stable but its DNA might get damaged due to exposure to abiotic and biotic stress (Tutejaet al., 2009). It has been reported that OH^- is most reactive and cause damage to all component of the DNA molecule damaging both the purines and pyrimidine bases and also the deoxyribose backbone (Halliwell and Gutterigde, 1999). Some metals like Mn, Cu, Zn, Mo and Ni are essential for normal growth and development of plants, and at appropriate concentrations function as cofactors and or required for structural and catalytic components of proteins and enzymes (Moustakaset al., 1994; Nedelkoska and Doran, 2000). However, toxic levels of heavy metal ions induce several cellular stress responses and damages different cellular components such as membranes, proteins and DNA (Patraet al., 1998; Waisberget al., 2003; Jimi et al., 2004).

Decline in the nucleic acid content was observed in the leaves of *Sorghum bicolor* with increase in soil zinc treatment at all stages of plant growth. The DNA content reduced at all stages with increase in zinc treatment and the lowest content was recorded at 9.5mM in fifty-day old plants. Decrease in the nucleic acid content was reported earlier by many workers; in submerged aquatic plants under heavy metal stress (Jana and Choudhuri, 1984), in Phaseolus vulgaris (Neelofer Hamid et al., 2010), in wheat cultivars (Kumar et al., 2012).

Endogenously generated ROS (reactive oxygen species) can damage to DNA is known as spontaneous DNA damage which is produced by reactive metabolites (OH^-, O_2^- and NO^-). Plants under metal stress are known to experience oxidative stress as the result of ROS. ROS is capable of damaging almost all macromolecules including DNA (Tutejaet al., 2001; Agarwal et al., 2020), causing mutations and genetic defects (Tutejaet al., 2009). Reduced efficiency of DNA synthesis, weaker DNA protection from damaged chromatin protein (histone) and increased deoxyribonuclease (DNase) activity have been reported for Cd, Cu, Cr, Ni, Pb, Hg, Pt and Zn (Prasad and Strzalka, 2002). Decline in the synthesis of DNA, RNA and proteins may be due to decrease in sugars as suggested by Espenet al., (1997).

Elements such as Cu, Ni, Cd and Pb have been reported to decrease RNA synthesis and to activate ribonuclease activity leading to further decrease in RNA content (Schmidt, 1996).

RNA content in the leaves of *Sorghum bicolor* decreased at all stages of plant growth with increase in soil zinc treatment. However, in the early stages of plant growth (15 and 30 days) slight increase in the RNA content was observed at 1.5 and 3.5 mM of zinc. Similar decreased content of RNA was reported in Phaesolus vulgaris under Pb (Neelofer Hamid et al., 2010), and in wheat cultivar at high concentration of Zn and Cu (Kumar et al., 2012). When a cell homeostasis is affected by stress ROS production increases to the point where it can damage cellular components and ultimately lead to cell death. ROS can affect lipids, proteins, carbohydrates and DNA (Valkoet al., 2005; Moller et al., 2007).

Protein content in the leaves of zinc treated *Sorghum bicolor* decreased with increase in soil zinc level. However, at 1.5mM in Thirty-day old plants and at 1.5mM and 3.5mM in fifty-day old plants higher values of protein were recorded as compared to control. Highest protein content was observed at 3.5mM of zinc in fifty-day old plants, further increase in zinc treatment showed a decrease in protein content at all days of sampling. The reduction in protein in*Sorghum bicolor* under zinc stress could be due to reduced RNA synthesis. Reduced RNA content in the present investigation might have reduced the protein content, since RNA is required for the process of protein synthesis through transferring the amino acids into protein synthesis centers as suggested by Udovenkoet al., (1971).

The results obtained in this study are in corroboration with the results of Vijayarengan, (2012), for zinc treated radish. Nag et al., (1981) observed a similar trend due to heavy metal like Cu, Zn, Hg, and Cd in rice. Khudsaret al., (2004) suggested that changes in protein content and in protein profile can be found as a consequence of the stress induced by toxic metal enhancing catabolism of some metabolites, such as chlorophyll, protein and RNA. Abiotic stress may inhibit synthesis of some proteins and promote other (Ericson and Alfinito, 1984) with a general trend of decline in the overall protein content. Protein content under heavy metal influence may also be affected due to enhanced protein hydrolysis or induced lipid peroxidation and fragmentation of proteins due to toxic effect of ROS resulting in decreased concentration of soluble protein (Melnichuk et al., 1982; Palma et al., 2002)

Proteins can also suffer oxidation by ROS, causing certain enzymes to lose its catalytic functioning. One of the more susceptible targets in proteins is thiol groups, the oxidation of which leads to protein denaturation and loss of functional conformation (Moller et al., 2007). Protein oxidation also leads to production of carbonyl groups and to increased rate of proteolysis as the damaged proteins are targeted by proteolytic enzymes (Palma et al., 2002).

The protein content normally declines under heavy metal stress (Bhattacharya and Choudhuri, 1994). The decrease in soluble protein content under zinc toxicity and deficiency has been reported by Tavallaliet al., (2009) in Pisatschio seedling. Many studies have reported decrease in soluble protein content under heavy metal influence in Vigna and Hydrilla (Bhattarcharya and Choudhuri, 1994), in Helianthus annus (Kastoriet al., 1992), in Lemna minor (Mohan and Hosetti, 1997), in Lemnapolyrrihiza (Palma et al.,2002), in Brassica juncea (John et al., 2009) and in Phaseolus vulgaris (Neelofer Hamid et al., 2010).

Conclusions:-

ROS are unavoidable byproducts of normal cell metabolism. However, various environmental stresses if prolonged disrupt the cellular homeostasis and enhance the production of ROS causing exacerbating damage to cellular components. In the present studydecrease in DNA resulted in a decrease in RNA content that could have led to the observed decrease in the protein content in the leaves of *Sorghum bicolor*plants.Nucleic acids (DNA and RNA) content was observed to be low in the plants under zinc treatment. The decrease could be related to the damage caused to the macromolecule under zinc stress and also could be due to the activity of nucleases which enhance degradation of these macromolecules.To sum up, in spite of high concentration of zinc the plants seemed to be better adapted at 1.5 and 3.5 mM of soil applied butat elevated levels (7.5 and 9.5mM) of zinc the production of ROS seems to have exceeded the capacity of the plants defense mechanisms, causing toxicity as a result of oxidative stress resulting in a decline in the nucleic acid and protein content.

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