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RESEARCH ARTICLE

MORPHOLOGICAL RESPONSES OF *SORGHUM BICOLOR* (L.) MOENCH TO ELEVATED LEVELS OF SOIL APPLIED ZINC.

P. Usha Shri.
Department of Botany, St.Ann's college for Women, Mehdipatnam, Hyderabad – 28, Telangana State, India.

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Abstract

Zinc fertilizers and pesticides are widely used to increase crop production in agricultural practice, which over the years led to elevated levels of zinc in the soil. Zinc is a micronutrient involved in wide variety of physiological processes and represented in all the six enzyme classes. Seeds of *Sorghum bicolor* (L.) Moench cultivar CSH 14 are selected for the experiment and the plants were grown in pots for fifty days. 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. Sampling was done at different stages of plant growth (Viz., 15, 30 and 50 days) for measuring various morphological and growth parameters like root and shoot length, fresh weight, dry weight, phytotoxicity, tolerance index and relative growth index. Significant reduction in different growth parameters was observed in *Sorghum* plants at all stages of plant growth with increase in zinc treatment.

Introduction:-

Modern agricultural ecosystems are 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. To increase crop production, use of zinc fertilizers and pesticides is a common agricultural practice, which over the years led to elevated levels of zinc in the soil. heavy metal accumulation in soil is also due to application of soil amendments by compost refusing and nitrate fertilizers (Ross, 1994). Industrial pollutants, smelting, mining, military activities, fuel production and agricultural chemicals are some of the anthropogenic activities that lead to contamination (Wong 2003; Del Rio *et al*., 2006; Clemente *et al*., 2007; Jadia and Fulekar, 2009). Heavy metal ions play essential roles in many physiological processes. Two major functions of essential heavy metals are participation in redox reaction and are integral part of several enzymes (Nagajyoti, **;2010** Golubovic and Blagojevic, 2012).

Zinc is the 23rd most abundant element on earth with atomic number 30 and specific gravity of 7.14 g cm^{-3}. It is the only transition metal which is represented in all the six enzyme classes (Broadley *et al*, 2006). Zinc is a micronutrient involved in wide variety of physiological processes (Broadley *et al*., 2007). It is required in small but critical concentrations to allow several key plant physiological pathways to function normally (Mousavi *et al*., 2011; Yousefi *et al*., 2011).

Corresponding Author:- P. Usha Shri.
Address:- Department of Botany, St.Ann's college for Women, Mehdipatnam, Hyderabad – 28, Telangana State, India.

Zinc is one of the most widely used metals in the world and the most prevalent metal contaminants in industrialized countries (Reena Singh *et al*., 2011; Subhashini *et al*., 2013). The four most important sources of zinc in soil were estimated to be smelter, sludges and wastes, mine tailings, coal and bottom fly ash, and the discharge of commercial products such as fertilizers (Sanders *et al.*, **;1986** Kumar, 2013). Significant fraction of zinc release from tire rubber debris was reported by Alloway, **;2004** Gualtieri *et al*., 2005; Kawachi *et al*., **2009** .

The present study is focused on the morphological changes displayed by *Sorghum bicolor* plants under conditions of stress induced by zinc excess.

Materials and Methods:-

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. 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.

Soil was collected from a local nursery, 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 of ten such doses were given during the experimental period. Plants treated with water served as control. 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 measurement of various morphological and growth parameters.

Morphological parameters:-

Root and shoot length: The seedlings were separated into roots and shoots after sampling and the length of each part was measured using a scale. Shoot height was measured from the junction of the root to the tip of the tallest leaf.

Percent phytotoxicity: was calculated using the formula of Chou and Lin, (1976).

Tolerance indices (TI): TI of the mean root length and shoot length against each concentration was calculated following Baker *et al*., (1994).

Fresh weight: The plants of both treated and control *Sorghum bicolor* were collected at each sampling stage and gently separated into roots and shoots and their fresh weight was recorded immediately.

Dry weight: After recording the fresh weight of the roots and shoots, they were kept in small labelled paper covers and were dried in a hot air oven at 80˚C for 48 hrs. till constant dry weights were obtained.

Relative growth index (%) (RGI): was calculated according to the method of Paliouris and Hutchinson, (1991).

Statistical analysis:-
The data is represented as mean values ± S.E. Data is 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:-

Root and shoot length:-
The effect of Zn on root length of *Sorghum bicolor* is depicted in Table 1. The root length of the plants was determined on the fifteenth day, thirtieth day and fiftieth day of growth. In fifteen day old plants, it was observed that Zn treatment caused a linear decrease in root length. The recorded decrease was 9.5% at 1.5mM of Zn treatment whereas at 9.5mM the decrease was 64.04%. In thirty day old plants the same trend was observed. The decrease in root length was highest (63.53%) at 9.5 mM. In fifty day old plants the percent reduction was 18.9% at 1.5mM and 59.95% at 9.5 mM of zinc. The root length showed a significant ($P < 0.05$) decrease with increase in zinc concentration and age of the plants.

Table 1:- Effect of soil applied zinc on root length (cm plant^{-1}) of *Sorghum bicolor* Moench (CSH 14) at different stages of plant growth.

Zinc added to the soil (mM)	Sampling days		
	15	**30**	**50**
Control	32.60 ±1.45	36.2 ± 0.63	40.2 ± 0.72
1.5	29.50 ± 0.33	30.2 ± 0.64	32.6 ± 0.41
3.5	27.8 0± 0.41	25.4 ± 0.46	29.2 ± 0.58
5.5	25.60 ±1.29	22.6 ± 0.78	24.1 ± 1.12
7.5	13.75 ± 0.21	17.3 ± 1.06	20.5 ± 0.21
9.5	11.72 ± 0.64	13.2 ± 0.34	16.1 ± 1.06

Data expressed as mean ± SE

The effect of Zn on shoot length of *Sorghum bicolor* is depicted in Table 2. A significant ($P < 0.05$) decrease in the shoot length was observed with increase in Zn concentration at all stages of plant growth. In fifteen day old plants, it was observed that Zn treatment caused a linear decrease in shoot length. The recorded decrease was 4.8% at 1.5mM of Zn treatment whereas at 9.5mM the decrease was 47.02%. In thirty day old plants the decrease was 13.96% at 1.5mM and 46.6% at 9.5mM of Zn treatment. In fifty day old plants the percent reduction was 16.70% at 1.5mM and the decrease was maximum (49.17%) at 9.5mM of Zn. In general the decrease in root length was more as compared to the shoots.

Table 2:- Effect of soil applied zinc on shoot length (cm plant $^{-1}$) of *Sorghum bicolor* Moench (CSH 14) at different stages of plant growth.

Zinc added the to soil (mM)	Sampling days		
	15	**30**	**50**
Control	18.5 ± 0.28	35.8 ± 0.24	42.5 ± 0.57
1.5	17.6 ± 0.88	30.8 ± 0.52	35.4 ± 0.40
3.5	15.2 ± 0.75	28.2 ± 0.60	34.6 ± 0.46
5.5	14.0 ± 0.57	24.0 ± 0.66	26.4 ± 0.42
7.5	11.6 ±0.33	20.5 ± 0.76	23.3 ± 0.52
9.5	9.8 ± 0.16	19.1 ± 0.49	21.6 ± 0.33

Data expressed as mean ± SE.

Root and shoot fresh weight:-
The fresh weight of the roots and shoots was determined in fifteen days, thirty days and fifty days old plants. The effect of Zn on fresh weight of roots in *Sorghum bicolor* is shown in Table 3.

In fifteen day old plants, it was observed that Zn treatment caused a gradual decrease in root fresh weight. The recorded decrease was 19.13% at 1.5mM of Zn treatment and at 9.5mM the decrease was 39.13%. In thirty day old plants a similar trend was observed. The decrease in root fresh weight was 17.24% at 1.5mM of Zn treatment where as it decreased to 45.86% at 9.5mM. In fifty day old plants the reduction was 8.82% at 1.5mM and at 9.5mM of Zn treatment, a maximum decrease of 52.35% was recorded. The decrease in root fresh weight was highly significant at P < 0.05 at all stages of plant growth with increase in zinc treatment and age.

Table 3:- Effect of soil applied zinc on root fresh weight (g plant^{-1}) of *Sorghum bicolor* (L.) Moench (CSH 14) at different stages of plant growth.

Zinc added to the soil (mM)	**Sampling days**		
	15 days	**30 days**	**50 days**
Control	0.230 ± 0.008	0.29 ± 0.008	0.34 ± 0.005
1.5	0.186 ± 0.002	0.198 ± 0.002	0.31 ± 0.005
3.5	0.176 ± 0.002	0.177 ± 0.004	0.26 ± 0.005
5.5	0.165 ± 0.004	0.17 ± 0.005	0.179 ± 0.003
7.5	0.150 ± 0.007	0.163 ± 0.001	0.173 ± 0.002
9.5	0.140 ± 0.005	0.157 ± 0.002	0.162 ± 0.002

Data expressed as mean ± SE.

The effect of Zn on fresh weight of shoot in *Sorghum bicolor* is depicted in Table 4. In fifteen day old plants a linear decrease in shoot fresh weight was observed with Zn treatment. The recorded fresh weight showed a decrease of 1.7 % whereas at 9.5mM of Zn treatment the decrease was 26.7%. In thirty day old plants similar trend was detected. With a decrease of 27.3% at 9.5mM. In fifty day old plants the fresh weight showed a reduction of 49.69% at 9.5mM of Zn treatment. The decrease observed was significant with increase in Zn treatment at all sampling days.

Table 4:- Effect of soil applied zinc on shoot fresh weight (g plant $^{-1}$) of *Sorghum bicolor* Moench (CSH 14) at different stages of plant growth.

Zinc added the to soil (mM)	**Sampling days**		
	15 days	**30 days**	**50 days**
Control	0.583 ± 0.003	0.633 ± 0.009	0.821 ± 0.017
1.5	0 566 ± 0.016	0.603 ± 0.002	0.790 ± 0.050
3.5	0.446 ± 0.046	0.501 ± 0.010	0.611 ± 0.011
5.5	0.393 ± 0.031	0.423 ± 0.024	0.586 ± 0.021
7.5	0.346 ± 0.04	0.381 ± 0.100	0.501 ± 0.006
9.5	0.316 ± 0.01	0.336 ± 0.018	0.412 ± 0.018

Data expressed as mean ± SE.

Root and shoot dry weight:-

The dry weight of roots and shoots was determined on fifteenth day, thirtieth day and fiftieth day of plant growth. The effect of Zn dry weight of roots in *Sorghum bicolor* is represented in Table 5. In fifteen day old plant, root dry weight decreased with increase in zinc treatment. The recorded dry weight decrease was 10.52% at 1.5mM and at 9.5mM the dry weight reduction was 1.31%. In thirty day old plants the reduction in dry weight was 53.3% at 9.5mM. In fifty day old plant at 1.5mM reduction was 20.58% and at 9.5mM of zinc treatment decrease was (54.41%). The decrease was significant (P < 0.05) with increase in zinc concentration and age of the plants.

Table 5:- Effect of zinc on dry weight of root (mg plant^{-1}) in *Sorghum bicolor* (L.) Moench (CSH 14) at different stages of plant growth.

Zinc added to the soil (mM)	**Sampling days**		
	15	**30**	**50**
Control	0.038 ± 0.001	0.045 ± 0.001	0.068 ± 0.001
1.5	0.034 ± 0.001	0.037 ± 0.002	0.054 ± 0.001
3.5	0.032 ± 0.003	0.035 ± 0.001	0.046 ± 0.001
5.5	0.028 ± 0.001	0.032 ± 0.003	0.037 ± 0.001
7.5	0.026 ± 0.001	0.026 ± 0.001	0.033 ± 0.002
9.5	0.018 ± 0.001	0.021 ± 0.002	0.031± 0.002

Data expressed as mean ± SE.

The effect of Zn on dry weight of shoots in *Sorghum bicolor* is presented in Table 6. The dry weight of shoot was determined at different stages of plant growth which showed significant decrease with increase in Zn concentration and growth period. A linear decrease in shoot dry weight was observed in fifteen day old plants. Maximum decrease of 48% was recorded at 9.5mM. In thirty day old plants the decrease at 1.5mM was 12.5% whereas at 9.5mM of Zn treatment the recorded decrease was 50%. In fifty day old plants significant decrease of 50.8% at 9.5mM of Zn treatment. The percent reduction in fresh and dry weights of roots was more when compared to the percent reduction in fresh and dry weights of shoots.

Table 6:- Effect of zinc on dry weight of shoot (mg plant^{-1}) in *Sorghum bicolor* (L.) Moench (CSH 14) at different stages of plant growth.

Zinc added to the soil (mM)	**Sampling days**		
	15	**30**	**50**
Control	0.101 ± 0.011	0.112 ± 0.001	0.126 ± 0.001
1.5	0.094 ± 0.001	0.098 ± 0.013	0.109 ± 0.006
3.5	0.080 ± 0.001	0.086 ± 0.002	0.104 ± 0.002
5.5	0.062 ± 0.005	0.064 ± 0.006	0.068 ± 0.010
7.5	0.060 ± 0.001	0.062 ± 0.004	0.064 ± 0.004
9.5	0.053 ± 0.003	0.056 ± 0.001	0.058 ± 0.002

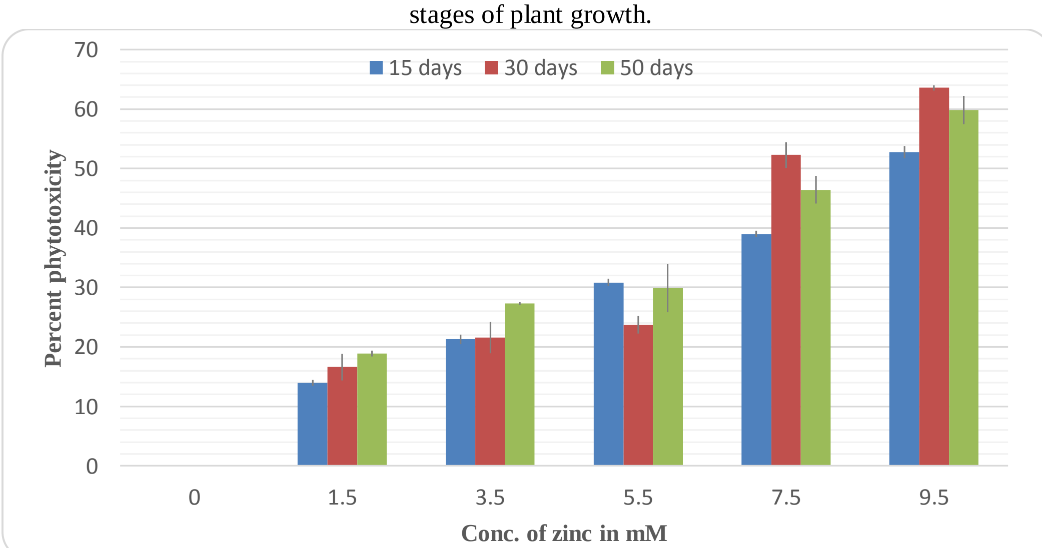
Data expressed as mean ± SE.

Percent Phytotoxicity:-

The effect of zinc on percent phytotoxicity of roots and shoots in *Sorghum bicolor* is depicted in Fig.1 and Fig. 2. for roots and shoots respectively. The percent phytotoxicity of the roots and shoots was determined at different stages of plant growth and was observed to be highly significant with increase in externally applied soil zinc.

In fifteen day old plants, the percent phytotoxicity in roots showed a linear increase with increase in Zn treatment. The recorded phytotoxicity significantly increased to 64.04%, 63.3% and 59.9% in fifteen, thirty and fifty day old plants respectively at 9.5mM of Zn treatment. In general, it was observed that in the older plants, percent phytotoxicity recorded was low.

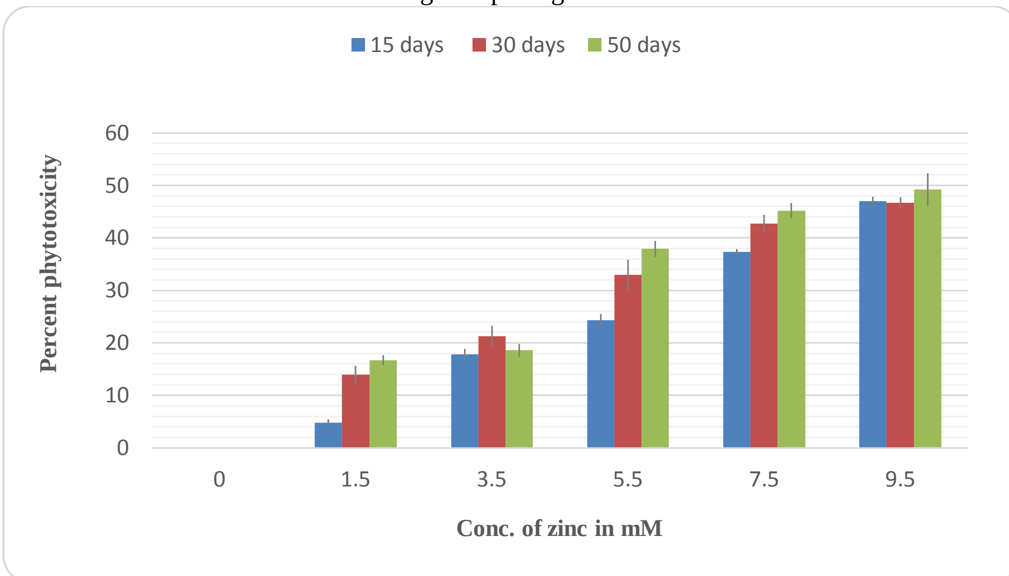
Fig. 1:- Effect of zinc on percent phytotoxicity (%) in roots of *Sorghum bicolor* (L.) Moench (CSH 14) at different stages of plant growth.



Vertical bars represent ± SE

In shoots a significant ($P < 0.05$) linear increase in percent phytotoxicity was observed with increase in zinc treatment. The recorded increase in percent phytotoxicity in fifteen day old plants at 9.5mM was 47.02%. In thirty day old plants, at 9.5mM of zinc treatment it increased to 46.64% and in fifty day old plants it enhanced to 49.18%. The percent phytotoxicity of roots was more as compared to the shoots of *Sorghum bicolor*.

Fig. 2:- Effect of zinc on percent phytotoxicity (%) in shoots of *Sorghum bicolor* (L.) Moench (CSH 14) at different stages of plant growth.



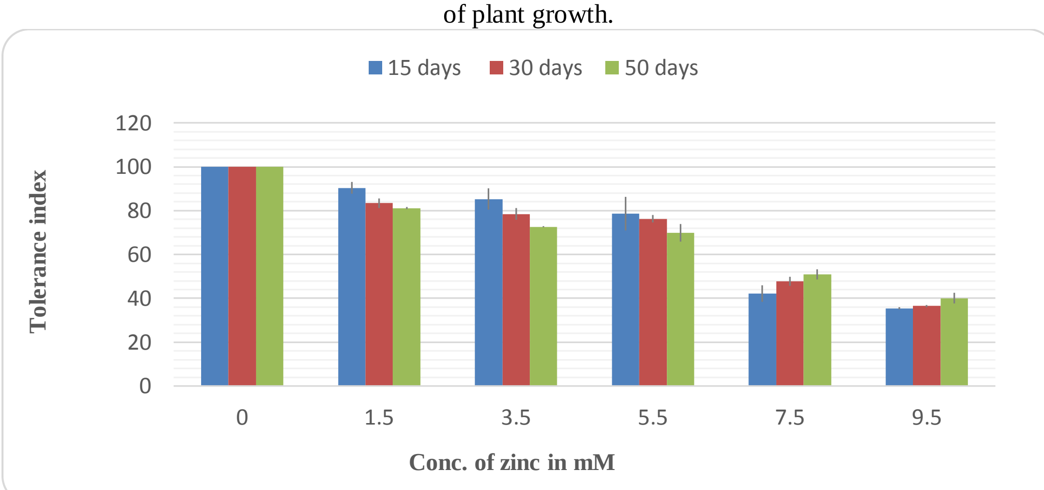
Vertical bars represent ± SE

Tolerance Index (TI):-

The effect of zinc on tolerance index of roots and shoots in *Sorghum bicolor* is depicted in Fig.3 and Fig. 4. respectively. The Tolerance index of the plants was determined on the fifteenth day, Thirtieth day and the fiftieth day of plant growth.

Tolerance index of the roots and shoots decreased consistently with increase in Zn treatment. Maximum reduction in TI in roots and shoots was observed in thirty day old plant at 9.5 mM of zinc treatment.

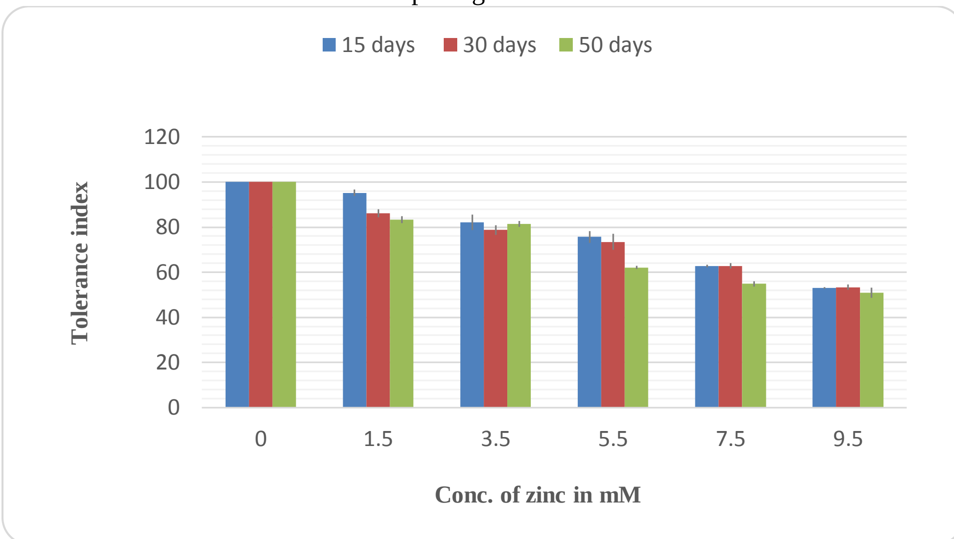
Fig 3:- Effect of zinc on tolerance index (%) of roots in *Sorghum bicolor* (L.) Moench (CSH14) at different stages of plant growth.



Vertical bars represent ± SE

Tolerance index of shoots showed significant ($P < 0.05$) decrease both with increase in zinc treatment and growth period (age of the plants). The tolerance index of the shoots was more as compared to the tolerance index of the roots.

Fig 4:- Effect of zinc on tolerance index of Shoot in *Sorghum bicolor* (L.) Moench (CSH 14) at different stages of plant growth.



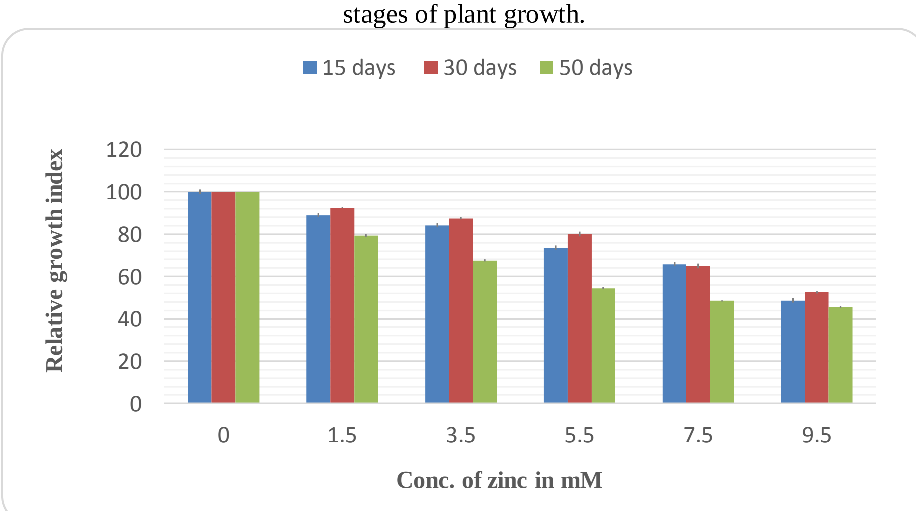
Vertical bars represent ± SE

Relative growth Index (RGI):-

The effect of Zn on the RGI of roots in *Sorghum bicolor* is represented in Fig.5. RGI was determined at different periods of plant development and significant ($P < 0.05$) decrease was observed at different growth periods and with increase in zinc concentration.

In fifteen day old plants, as concentration of Zn increased the RGI decreased. The RGI at 9.5mM of Zn the observed value was 48.68% (51.32% reduction). In thirty day old plants The RGI progressively declined at elevated levels of Zn and the value recorded at 9.5mM was 52.4% with a reduction of 47.5%. In fifty day old plants marked decline in RGI of 45.58% was observed at 9.5mM (54.42% reduction).

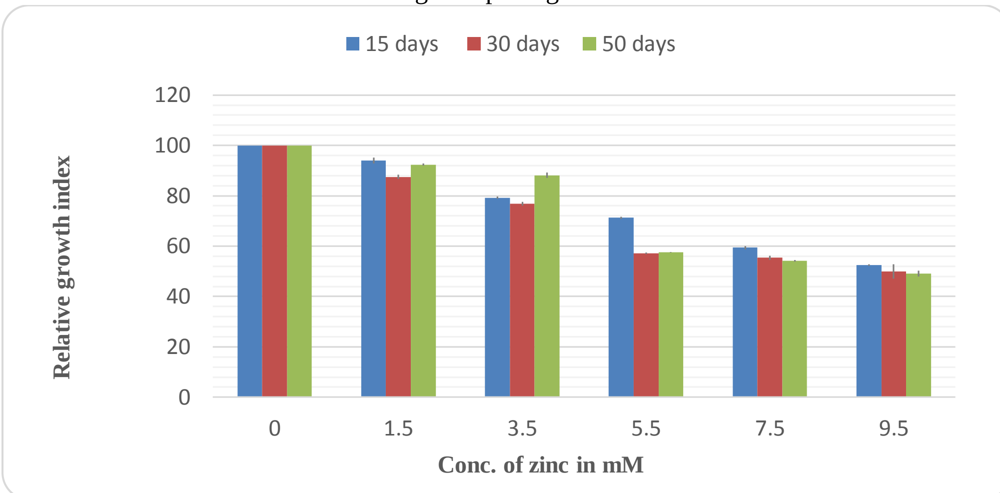
Fig 5:- Effect of zinc on relative growth index (%) of roots in *Sorghum bicolor* (L.) Moench (CSH 14) at different stages of plant growth.



Vertical bars represent ± SE

The effect of Zn on the RGI of shoots in *Sorghum bicolor* is shown in Fig. 6. In fifteen day old plants, the RGI showed a gradual decrease with increase in Zn treatment. At 9.5mM it was 47.53% reduction. 50% and 50.85% reduction was recorded at 9.5mM in thirty and fifty day old plants respectively.

Fig 6:- Effect of zinc on relative growth index (%) of shoots in *Sorghum bicolor* (L.) Moench (CSH 14) at different stages of plant growth.



Vertical bars represent ± SE

Discussion:-

Plants are known to develop different strategies to cope with heavy metal stress. Some use an avoidance strategy to reduce trace element assimilation while others use internal defense mechanisms to cope with the increasing levels of the toxic species (reactive oxygen species) and to allow them to grow despite the presence of variable concentrations of trace elements, but the threshold concentrations as well as the different response mechanisms strongly depend on plant species and on the type of metal. Visible effects of toxicity hence depend not only on the concentration and duration of application of the heavy metals, but also on the age of the plant and relative levels of other macro and micro nutrients.

A number of investigators had reported retardation in root and shoot growth as a result of zinc stress. Reduction in growth in *Vigna radiate* and in *Sorghum bicolor* (Balashouri, 1995), *Bacopa monniera* (Ali *et al*., 1999), in two cereal crops (Tomulescu *et al*., 2004), Oryza *sativa* (Zhang *et al*., 2009) and in *Brassica juncea* (John *et al*., 2009). Zinc concentration of 100 – 400 µg g^{-1} soil caused significant decrease in root and shoot growth parameters at different developmental stages of *Artemisia annua* plants (Khudsar *et al* ., 2004). Mirshekali *et al*., (2012) observed a significant decrease in plant growth with increasing zinc concentration in the soil in *Chenopodium album* and *Sorghum bicolor*.

The effect of zinc on inhibition of growth was stronger, in roots than in shoots. Zinc at elevated levels is known to inhibit root growth directly by inhibition of division or cell elongation or combination of both (Fox, 1988). In the present investigation zinc caused drastic reduction in the growth of roots than the shoots.

Toxic effect of zinc was evident from the curtailed growth and biomass reduction in the plants of *Sorghum bicolor*. The root and shoot biomass in terms of dry weight was highly reduced in zinc treated plants compared to control. A negative correlation between biomass and different concentrations of soil applied zinc was observed. Similar decrease in dry weight of root and shoot was also reported by several authors in a number of plants: in soya bean and fenugreek (Aery *et al*., 1994), pigeon pea (Dube *et al*., 2001), in radish (Dube *et al*., 2003) under zinc treatment.

The reduction in biomass yield was more severe at elevated levels of zinc at all stages of growth of *Sorghum bicolor*. Reduction in yield at higher concentration of zinc in the soil was also reported by Mirshekali *et al*., (2012) in *Chenopodium album* and *Sorghum bicolor*.

In the present experiment the root biomass was more severely affected, this could be due to the roots being the first site of exposure to toxic metal. Reduction in dry matter of plants could be due to depletion of moisture content in plant organs and also due to degradation of roots (Joshi *et al*., 1999).

In both roots and shoots of *Sorghum bicolor* the percent phytotoxicity increased with increase in zinc treatment at all stages of plant growth. Commonly metal phytotoxicity is caused as a result of movement of metal from the soil to root system (Foy *et al*., 1978). However at the older stage (50 days) of plant growth the percent phytotoxicity of the shoot decreased as a result of increase in tolerance index

The identification of the toxic concentration of metals and tolerance indices of plant species could be helpful in overcoming various problems associated with metal pollution in any region (Kabir *et al*., 2008).In the present experiment a marked decrease in tolerance index was observed in the roots and shoots with increase in zinc treatment at all stages of plant growth in *Sorghum bicolor* plants. Zinc toxicity and differential zinc tolerance in various plants were reported by Brown, 1979; Symeonidis *et al*., 1985). Shafiq *et al*., (2008), reported reduced tolerance percentage in *Leucaena leucocephala* under elevated levels of Pb and Cd.

Growth parameter in terms of relative growth index (RGI) was markedly affected at higher levels of zinc treatments and decrease in root RGI was more than shoot. Zinc treatment had a negative influence on RGI compared to control at all sampling stages. The reduction of biomass by zinc toxicity could be the direct consequence of the inhibition of chlorophyll biosynthesis (Merskekali *et al*., 2012). Madhava Rao and Sresty (2000), reported decreased RGI at increasing concentration of zinc and nickel in pigeon pea cultivars. Radic *et al*., (2009), reported negative influence of zinc on relative growth rate in *Lemna minor* after fifteen days of treatment.

Conclusions:-

Zinc treatment at all levels decreased the various growth parameters such as length of the roots and shoots which was due to increased phytotoxicity and reduced tolerance indices of the *Sorghum* plants. Tolerance indexes have been useful to characterize plant tolerance. Dry weight of roots and shoots reduced and as a consequence the relative growth index decreased. Approximately fifty percent (Effective concentration, EC_{50}) inhibition in RGI was demonstrated at 5.5mM of zinc in the roots of fifty day old plants. At a concentration of 1.5 and 3.5 Mm of zinc *Sorghum* plants demonstrated tolerance which is an indication that these plants can be grown in soils of moderate zinc toxicity so, can be effectively used for phytoremediation / phytoextraction, which is environmental friendly and sustainable and productive technique to clean up the metal contaminated soils.

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