STUDY ON THE EFFECT OF ZINC ON BIOMASS PRODUCTION AND PROTEINPROFILE OF *ZEA MAYS* L. SEEDLINGS

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***Abstract*- Present study was carried out to evaluate the effect of different concentrations of zinc (1, 5 and 10 mM) on biomass production and protein profile of maize seedlings for 72 hours. The maximum seedling growth was observed in 1 mM ZnSO4 treatment and germination rate showed a gradual reduction in higher concentrations. The inhibitory effect of Zn toxicity on coleoptile and coleorhiza growth was proportioned to the higher concentrations of Zn. Electrophoretic analysis of the protein fractions revealed that during germination up to 3 days, the intensities of bands slightly increased in 10 mM concentration. Results presented here strongly indicates that in spite of significant morphological changes, the protein distribution pattern of *Zea mays* remains unaltered due to Zn toxicity and 10 mM concentration imposes no toxicity in the metabolism of seedlings.**

***Keywords*: Biomass, *Zea mays*, Growth regulators, Electrophoretic analysis**

INTRODUCTION

Zinc is an essential micronutrient for plants, which remains in ionic form. Zinc plays a key role as a structural constituent or regulatory co-factor of a wide range of different enzymes and proteins in many important biochemical pathways and these are mainly concerned with: carbohydrate metabolism, both in photosynthesis and in the conversion of sugars to starch, protein metabolism, auxin (growth regulator) metabolism, pollen formation, the maintenance of the integrity of biological membranes, the resistance to infection by certain pathogens [1]. Even though a small amount of Zn is required for plants (5–100 mg/kg) but if enough of this element is not available, the plants suffer physiological stress resulting from the incompetenceof several enzyme systems and other metabolic actions interrelated to Zn [2]. During the process of seed germination, zinc absorbed from the nutrient soil mostly get retain in the root, but a portion is absorbed to the parts above the ground parts of the seedlings.

Maize (*Zea mays* L.) is a staple food for millions of individuals worldwide. It is an orthodox seed which belongs to family Poaceae and is a C4 plant. Maize seeds provide a large numberof micro as well as macronutrients. Maize is known as an indicator plant for the evaluation of Zn deficiency of a soil [3]. Maize can grow in light (sandy), medium (loamy), and heavy (clay) soils. Maize is very important because of good source of minerals, vitamins, fiber and oil present in maize. It possesses Anti-allergic, Astringent, Anti angina, Anti-hypertensive, Anti- lithiasis, Anti-diarrheal, anti-prostatitis, anti-tumor, anti dysentery, anti gonorrheal and diuretic properties. Maize seeds contain large amounts of a few classes of storage proteins that are used during early germination as a source of reduced nitrogen [4]. Maize seeds contain ~10% proteins and ~70% of them are classified as storage proteins [5]. Based on their solubility in different solvents, endosperm proteins are divided into four groups: albumins, globulins, glutamines, and prolamins. The storage portion called zeins, made up > 60% of total proteins. Based on the molecular weight, zeins can be divided into four subfamilies, α (19 and 22 kDa),γ (50, 27, and 16 kDa), β (15 kDa), and δ (18 and 10 kDa) [6].

Even though Zn plays an important role as a mineral nutrient, the beneficial roles of the metal have been studied in many plants [7][8]. But the effect of zinc on germination pattern and protein content distribution in the seedlings has not yet been studied in crop plants in detail.

MATERIALS AND METHODS

Collection of seeds:

Mature dry seeds of maize were purchased from the local market and the healthy seeds were selected, then surface sterilized by immersing in 5% (v/v) sodium hypochlorite for 10 minutes and rinsed three times in sterile water, sun dried and used for the experiments.

Standardized concentrations for optimal growth and inhibitory effect:

Concentrations such as 1 mM, 5 mM and 10 mM weighed $ZnSO_4$ salt was dissolved in glass distilled water and were used for the experiment. Glass distilled water was used as the control.

Experiment layout (Petri dish culture):

The seeds collected as described above were thoroughly washed with glass distilled water. In order to break the seed dormancy, trial experiments were conducted with distilled water for getting uniform and cent percent germination. Uniformly sprouted ten seeds were placed in each of 9 cm sterilized Petri dishes lined with Whatman No. 1 filter paper and 5 ml each of treatment solution (1 mM, 5 mM and 10 mM $ZnSO_4$) and glass distilled water (control) were added to appropriate petri dishes containing mechanically

scarified seeds and kept under dark for germination at 25 ± 2°C. After sprouting the seeds were exposed to continuous light (45µ E m2s -1) at 25 ± 2°C. A treatment solution in each petri dish was changed after every 24 hrs. and fresh solution (5 ml each) was added. Each experiment was replicated a minimum of five times.

Germination percentage

To determine the germinability of seeds, a sample consisting of 50 seeds were soaked in water. After 5-6 hrs. excess water was decanted and the seeds were kept for germination in Petri dishat room temperature. Number of sprouted seeds were counted and germination percentage was calculated for a period of 72 hours. Seeds were considered to be germinated with the emergenceof the radical. The rate of germination percentage was estimated by using a modified Timsons' index of germination velocity according to Khan and Ungar (1997) as stated below:

$$\text{Germination percentage (GP)} = \frac{\text{No. of seeds germinated}}{\text{Total no. of seeds}} \times 100$$

Sampling: seedling analysis

After 72 hours of growth, treatments and control seedlings were sampled and recorded the morphological data such as length of coleorhiza and coleoptile. Fresh weight of the seedling components (coleorhiza, coleoptile and endosperm) were prepared by cutting the seedling parts from randomized samples of 10-20 seedlings. These samples were kept for dry weight determination. The coleorhiza, coleoptile and endosperm of individual seedlings were cut and pooled and composite samples were kept separately. Weighed samples of each component prepared as explained above were kept at -20°C for SDS-PAGE studies.

Dry weight determination:

Samples were taken in a pre-weighed container and after taking fresh weight; these containers were kept in an oven at 100ºC for 1 hour until a constant weight was obtained.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Preparation of protein sample: For the preparation of crude protein extracts of maize, 200 mg each from control, 1 mM, 5 mM and 10 mM of maize composite (coleorhiza, coleoptile and endosperm), kept at -20°C were weighed and homogenized in a chilled glass mortar and pestle with 400 µl of 0.2 M Tris-HCl buffer (pH 8). The homogenates were centrifuged at 3000 rpm for 10 minutes. For analysis by SDS-PAGE, 25 µl supernatant from control and each concentrations, and 10 µl protein markers were pipetted out into individual vials. To each of these vials 15 µl of sample loading buffer (Tris buffer of pH 6.8, SDS, β Mercaptoethanolwhich reduces disulphide bonds, sucrose/glycerol to increase the density and bromophenol blue as tracking dye) was added, and the vials were heated in boiling water bath for 5 minutes to denature the protein sample.

Electrophoresis: Marker and the prepared protein samples were added to electrophoretic wells and the order in which the samples have been loaded is noted. Set the voltage at 100 V and switch on the power supply until the dye front reaches 0.5 cm above the bottom of the gel. Resolution of the protein bands is gently increased by applying the samples onto a short stacking gel on top of the separating gel. Differences in pH and composition between these two gels cause the sample to be concentrated into narrow bands by isotachophoresis. As the samplesmigrate through the separating gel, proteins get resolved depending on their molecular weights. Electrophoresis is stopped when the dye front reaches the bottom of the gel.

Visualization of proteins: The gel after electrophoretic run was separated and washed with water by transferring into a tray. Then the gel was inserted into staining solution (20 ml Ezee blue) for overnight , and finally the gel was inserted into de-staining solution until the bands appeared and image analysis was performed [9]. The tray was placed on a rocker intermittently every 10 to 15 minutes for uniform staining and washing. Generally proteins are colorless and hence cannot be visualized directly. So suitable dyes are used to visualize them. Identification of protein bands was done by comparing with similarly process marker protein along with the samples.

Statistical analysis

All the experiments/treatments were repeated minimum for five times and mean values are calculated and mean values was taken. Standard Error was calculated from mean values and (S.E) standard deviation (SD).

RESULTS AND DISCUSSION

Seed germination

Difference in seed germination percentage due to zinc treatment after 72 hrs is given in Fig 1. The reduction of germination percentage in *Zea mays* seeds indicates the quality of seeds. Since the seeds are purchased from open market, cent percent germination rate cannot be expected mainly due to the lack of proper drying, storage etc. The germination percentage of *Z. mays* seeds in control showed 95%. Zn treatment resulted in a gradual decrease of germination percentage proportionate to the concentration increases from 1 mM to 10 mM with a minimum of 30 to 36% growth. The reduced germination rate is found to be due to either the damage of testa caused by zinc [10] or by change of permeability of the medium which also impose advisable change in the rate of germination. Recently zinc solutions are used for seed primingto improve nutritional potential of seeds [11]. In the present study Zn solution applied in seedsis moderately more concentration compared to the concentration which normally used for seed priming. So it seems that germination percentage as well as seedling growth are adversely affected by 1, 5 and 10 mM $ZnSO_4$.

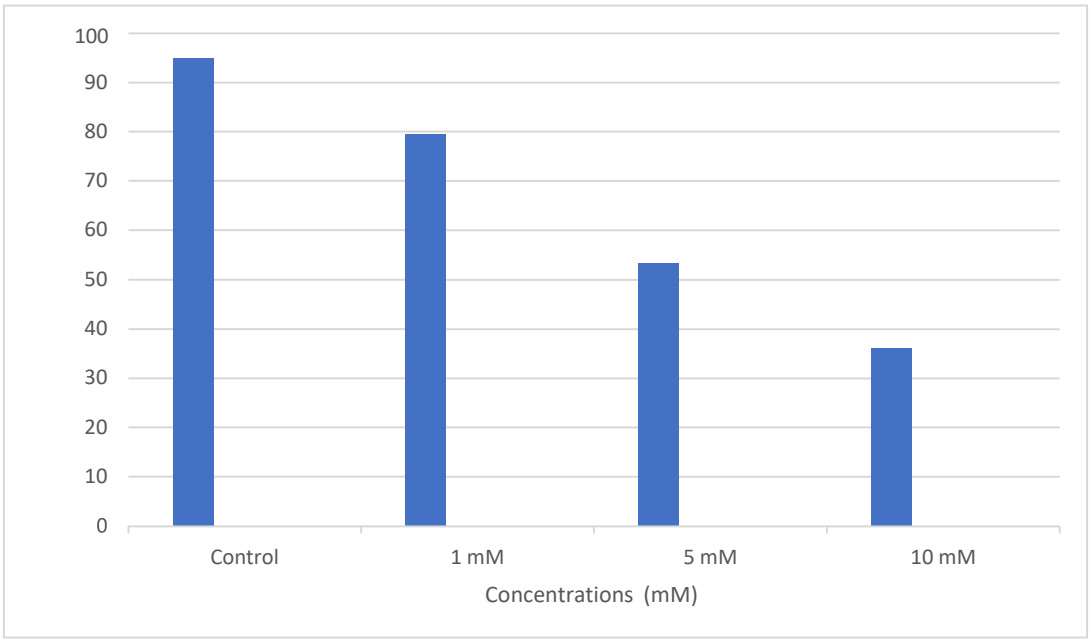

Fig 1: Effect of Zinc on seed germination of *Zea mays*.

Seedling morphology

The degree of sprouting of the seeds decreased, as the Zn concentration increased towards the higher concentration (10 mM). It was demonstrated that many plants sensitive to heavy metals germinate in polluted environment but later stop growing, therefore root growth parameter is more sensitive and useful for determining toxicity criteria [12][13][14]. A few coleorhiza tips in control showed slight brownish color and dark brown color was observed in 2 or 3 seedlings of lower concentrations (1 mM and 5 mM). In higher concentration (10 mM) the color of coleorhiza ranges from light to dark maroon, root hair development was not observed and splitted coleorhiza appeared. The length of coleorhiza was inhibited in higher concentration (10 mM) and root tip did not developed further. This may be due to the over accumulation of Zn in coleorhiza and will not transfer further because Zn in higher concentration is toxic to plants.

Coleoptile showed green color in all the three concentrations but the intensity of green color increased towards 10 mM (Fig 5). ATP synthesis, chloroplasts activity and photosynthesis will decline as the concentration of Zn increases in plants [15]. The expansion of the endosperms increased as the concentration increases. This might happened because, a higher concentrationof Zinc is toxic to seedling growth so the nutrients essential for their growth and development are not utilized and will remain in the endosperm itself. These results demonstrated that this metal ($ZnSO_4$) significantly influence changes in the morphology of *Zea mays* seedlings.

Seedling growth

Changes in coleorhiza and coleoptile length of Zn treated seedlings of *Zea mays* (Fig 2) showedvariations in comparison with the control. Maximum seedling growth was noticed in 1 mM concentration with an increase in coleoptile and coleorhiza length (Fig 5b). This was due to the utilization of Zn by maize seeds, since it is an essential element for their growth and metabolism. A similar promotion of growth was found in maize plant treated with relatively lower level (0.28 ppm) of Zn nano particles (<100 nm) as compared to normal $ZnSO_4$ [16]. An inhibition of growth mainly the length of coleorhiza and coleoptile was observed in the seeds treated with 10 mM $ZnSO_4$ (Fig 5d). Due to easy uptake and translocation of zinc from rootsto shoots and its accumulation in vegetative and generative parts, too high concentration of this element in the soil solution is harmful to plants and which results in stunted growth [17].

Growth as dry weight of coleorhiza and coleoptile of *Zea mays* seedlings treated with Zn is shown in Fig 3. There showed a sharp decline in the dry weight of coleorhiza and coleoptile as the concentration increased. But in the case of maize plant the highest dry matter weight of coleorhiza and coleoptile was recorded at 0.5 ppm Zn through ZnO nano particles [16].

Growth as dry weight of endosperm of *Zea mays* seedling is shown in Fig 4. From the figure itis clear that a slight increase in dry weight was noticed when the concentration of Zn treatment exceeded. Dry weight of endosperms in the lower concentration (1 mM) was noticeably greater than control. Maximum dry weight was observed in higher concentration (10 mM).

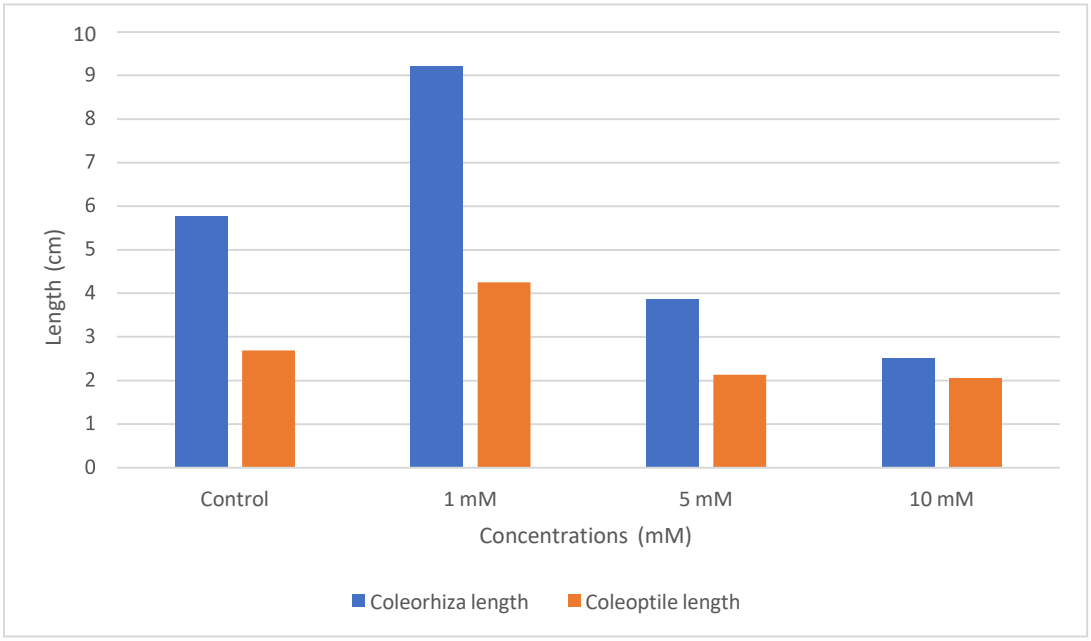

Fig 2: Effect of Zinc on growth of *Zea mays* seedling parts after 72 hours of germination.

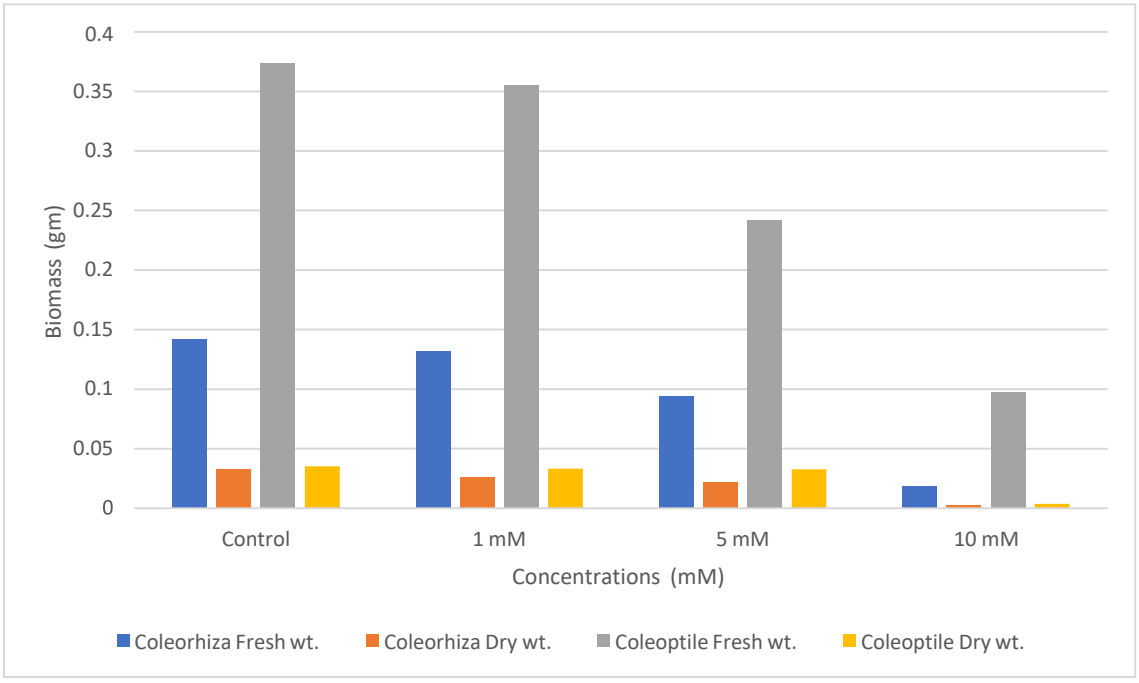

Fig 3: Effect of Zinc on coleorhiza and coleoptile biomass of *Zea mays* after 72 hours ofgermination.

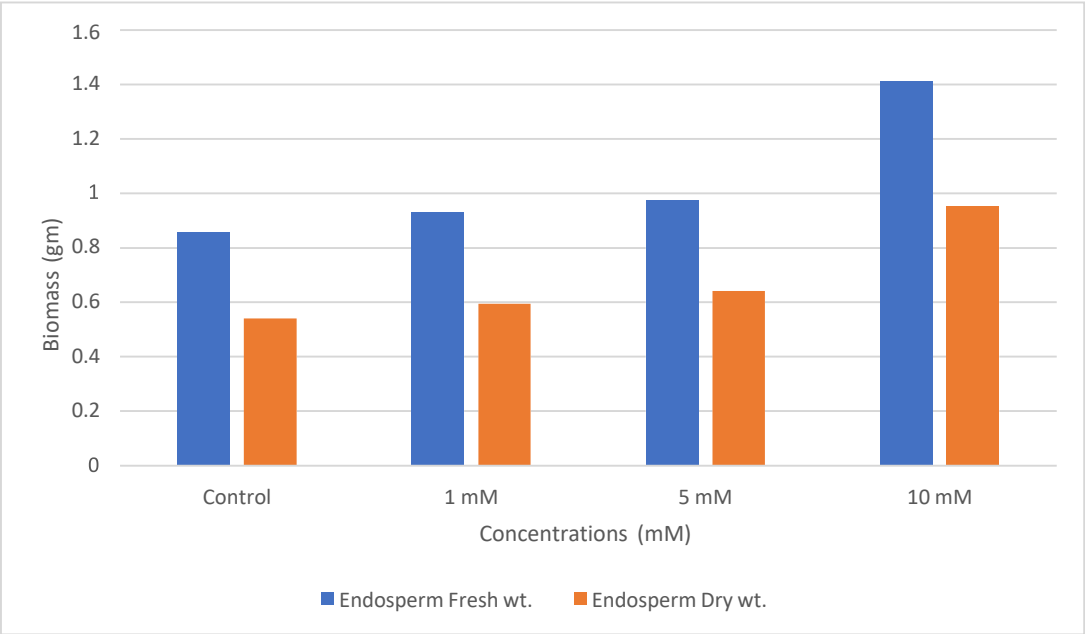

Fig 4: Effect of Zinc on endosperm biomass of *Zea mays* after 72 hours of germination.

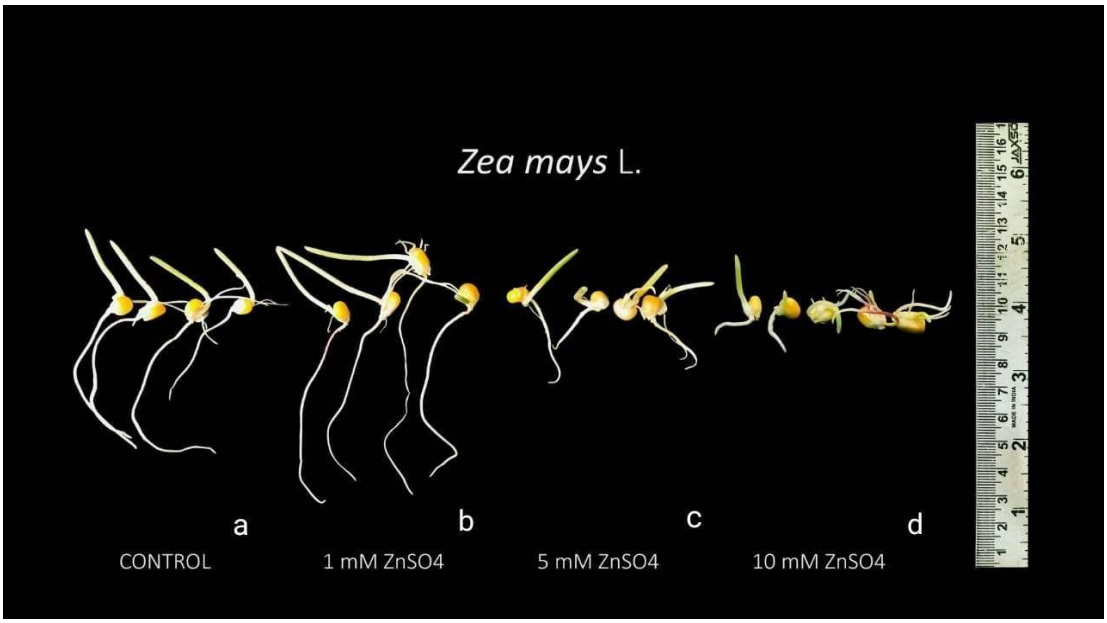

Fig 5: Seed germination pattern of *Zea mays* treated with $ZnSO_4$ after 72 hours.
(a) Control (b) 1 mM $ZnSO_4$ (c) 5 mM $ZnSO_4$ (d) 10 mM $ZnSO_4$.

Analysis of SDS-PAGE

Figure 6 represent SDS-PAGE protein profile of *Z. mays* seedlings treated with 1 mM, 5 mM and 10 mM $ZnSO_4$ (lane 3, 4 and 5) respectively and that of the control (lane 2). The results exhibited no distinct alterations compared to control. The marker has proteins of molecular weight: 66; 43; 29 and 14 kDa (lane 1). Total number of bands detected was 4 in control, 1 mM, 5 mM and 10 mM. Maize seedlings treated with 10 mM $ZnSO_4$ (lane 5) noticed minute changes compared to the control. The intensity of protein isolate close to marker of molecular weight 66 kDa was more clear. Also the intensity of last 2 bands in lane 5 showed slight increase. The higher intensities indicate that proteins of similar molecular mass were expressed due to the treatment with Zn. In general protein bands of the treatment and control were feebly colored indicating the low protein content of *Zea mays*. This observation is the agreement witha review which states that *Z. mays* containing less than 12% of protein whereas carbohydrate content is 76% in dry seeds [18]. Quantitative changes are insignificant as shown by protein profile (Fig 6) of the Zn treated and control seedlings. In Maize kernels at least 15 different endopeptidases are detectable during the first 3 days of germination. Nevertheless significant quantitative changes in the protein content are not observed [18].

Seedlings of *Z. mays* subjected to Zn at 1, 5 and 10 mM concentrations showed only negligible changes compared to the control.

Zn^{2+} ions are known to involved in the structural and functional properties of stress induced enzyme like SOD [19]. So it seems that in *Z. mays* the Zn ions are involved in the activity of SOD resultantly the toxicity is not shown significantly because one of the important symptoms of metal toxicity in plant is the production of Superoxide which is inactivated by SOD. Irrespective of the significant difference in the concentrations of Zn (1, 5 and 10 mM) toxicity is found to be nullified presumably due to the production of SOD in the activity of which is controlled by Zn^{2+} ions are reported earlier [19].

Another aspect of Zn toxicity in plant is reported to the species which is inhibiting different levels of tolerance to the metal. Zn higher tolerance has been reported in Poaceae and Lamiaceae [20]. It can be presumed that since *Z. mays* belong to Poaceae this plant is tolerant to Zn and hence toxicity symptoms are not seen in the distribution of protein profile in all concentrations (1, 5 and 10 mM) and no difference between the control and treatment. In spiteof the similarity in the protein profile of Zn treated plant and control; morphological features like coleorhiza length and presence of root hair vary significantly towards the concentrationsof Zn and control (Fig 6). Based on the morphological features, *Z. mays* cannot be considered as tolerant to Zn whereas the germination and survival of seedlings occur in all concentrationsof Zn.

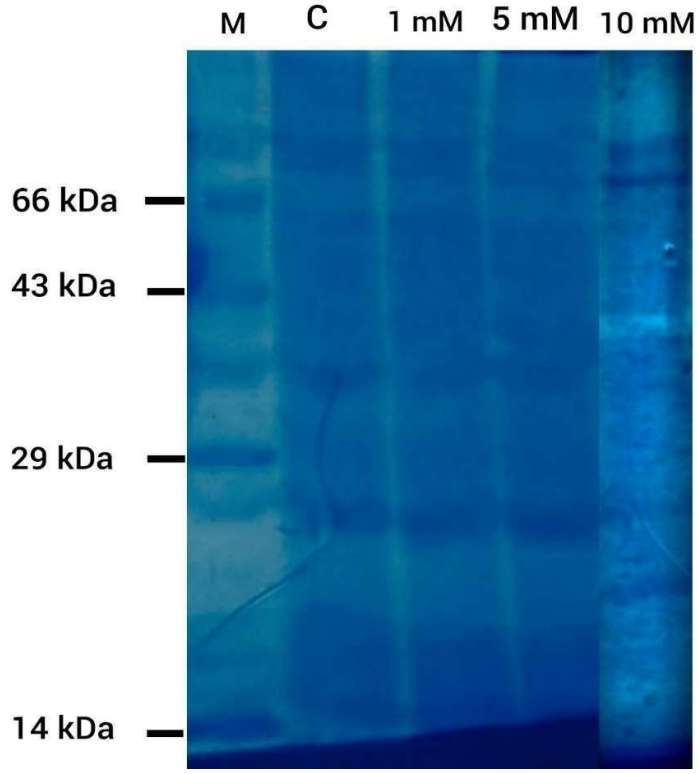

Fig 6: SDS-PAGE Protein Profile of *Z. mays* seedlings treated with control, 1 mM, 5mMand 10 mM $ZnSO_4$ after 72 hours of germination.
Lane 1 – Marker, Lane 2 – Control, Lane 3 – 1 mM concentration, Lane 4 – 5 mMconcentration and Lane 5 – 10 mM concentration.

CONCLUSION

Effect of different concentrations such as 1, 5 and 10 mM solutions of $ZnSO_4$ was investigatedin *Z. mays* seeds purchased from local market. Effect of Zn on *Z. mays* seeds is shown by reduced germination rate and seedling with proportional to the concentrations of Zn. Butseedlings were survived in all concentrations indicating considerable tolerance to Zn presumably a characteristic of Poaceae species which is tolerance towards Zn and variation in concentrations are found to be related to many limiting factors such as deranged metabolismand impaired translocation of seed reserve metabolites for healthy seedling establishment. SDS- PAGE revealed only insignificant variations in the distribution of protein bands in terms of intensity and band width revealing the absence of Zn toxicity even at 10 mM concentration. It can be concluded that in spite of Zn toxicity resulting in significant morphological changes, protein distribution pattern of *Z. mays* remains unaltered due to Zn toxicity since *Z. mays* belongs to Poaceae on one hand and concentration (10 mM) imposes no toxicity in the metabolism of seedlings on the other.

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