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# Ecophysiological studies of a pulse crop (*Cajanus cajan L.*) with zinc sulphate stress

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**ABSTRACT**

The germination data showed that there is direct impact of Zinc sulphate on the germination of seeds. When the plants were exposed to $ZnSo_4$ stress, at high concentrations it inhibits seed germination, growth and development of seedlings. At a higher concentration i.e., 100 Mg/L, the seed germination is very less than that of control. Both root and shoot growth of seedlings were badly affected with exposure to high concentration of Zinc sulphate. Roots were highly affected and much more reduced than that of shoots. Morphologically, they looked different from normal roots by their colour, shape and size. Effect of different concentrations of Zinc sulphate was visible in different pigment concentrations of leaves. With increase in concentration of the toxicant exposure to the seeds, a decline in chlorophyll-a, chlorophyll b, total chlorophyll contents in the shoots were observed. This was a clear indication of decreases in the plant growth rate and pigment contents that had direct impact on photosynthesis. Conclusively, our result showed that Zinc at higher concentration decreases, chl-a, chl-b and total chlorophyll. This results suggests that the presence of Zinc at higher concentration caused in growth inhibition, a decline in physiological and pigment activities, structural damage etc. However, further research in this regard is required to find out the effects of $ZnSO_4$ on metabolic activities of different plants.

**Keywords:** Zinc Sulphate, Pulse, Cajanas, Pigment, Germination

## INTRODUCTION

Among heavy metals, Zinc is one of the most common toxic substances in the environment. It comes from various environmental sources viz. agricultural land which is influenced by anthropogenic activity, excessive use of agrochemicals, fertilizers and also found in atmosphere as dust, vapour fumes, water and in soil as a mineral. Zinc readily enters into food chain and can subsequently affect the human and animal health. Plants can be exposed either to Zinc by direct administration as antifungal agents, mainly to crop plants through seed treatment or foliar spray, or by accident. The parameters screened were seed germination, seedling growth, relative growth of roots and shoots and pigment concentra-tions.

The absorption of organic and inorganic Zn from soil by plants is low and there is a barrier to Zn and translocation from plant roots to tops. Zn affects both light and dark reactions of photosynthesis.

Zinc has been known as micro and necessary element in plant nutrient and it has an important role in improvement of crop quality and quantity so that ample yield of plant crops including cereal crops, forage crops, industrial crops and pulse crops, are affected by zinc. This element accelerates the process of plant growth and development because its role as a cofactor in tryptophan amino acid biosynthesis (auxin-substrate) and nitrogen, starch and lipid metabolism. Some of these problems are more acute and clearly evident in developing countries where people depend on cereal-based foods for their daily diet and they cannot afford to diversify their meal by adding mineral-rich fruits, vegetables, and meat (Maret and Sandstead, 2006). The availability of soil Zn for pigeon pea from flooded (anaerobic) soil is affected by an additional set of factors including soil redox potential, total sulfur content, and soluble bicarbonate (Impa and Johnson-Beebout, 2012). Thus, a combination of agronomic management practices and genetic approaches are essential to improve the soil health conditions to enhance the root uptake of Zn.

In pigeon pea, direct root uptake, remobilizations from vegetative tissues or combination of both of these two approaches are the main source of Zn in grains (Impa *et al*., 2013). Efficient loading of Zn into grains, especially to the endosperm is most important for Zn bio-fortification of pigeon pea (Waters and Sankaran, 2011) .A continuous supply of Zn to different tissues throughout the life cycle by translocation and phloem remobilization to grains is an important feature of Zn efficient pigeon pea genotypes (Yin *et al*.,2016). The increased root uptake of Zn and root to shoot transfer could not proportionately increase the grain Zn concentrations indicating that internal translocation /re-translocation of Zn from vegetative tissues to grains is the major bottleneck for improving grain Zn concentrations (Yin *et al*., 2016, Stomph *et al*., 2014). Though, a number of physiological studies have been published about Zn-efficient pigeon pea, little is known on how Zn is redistributed and remobilized from vegetative tissues to the grains (Ren *et al*., 2006).

Pigeon pea has also been found to show different levels and patterns of Zn accumulation under high or low Zn conditions and in different pigeon pea ecosystems (Matthias *et al*., 2006, Impa *et al*., 2013, Mabesa *et al*., 2013). There is a variation in the pattern of Zn distribution within pigeon pea grain with the aleurone layer having 25–30 % of the total Zn, and this is the loss during processing, while the endosperm has 60–75 % of Zn, which is retained even after polishing (Hansen *et al*., 2009). The genetic basis of high grain Zn in brown/polished pigeon pea is very complex and a better understanding of the genetic basis is essential for the systematic utilization of pigeon pea germplasm in Zn biofortification programs. The combining ability analysis by diallele crosses involving seven specific pigeon pea varieties with different levels of Zn showed that additive genetic effects were more important for Zn, while the co-efficient of variation (CV) for Zn varied significantly among the entries over the years and locations, indicating significant genotype and environment interactions (G x E) (Sharifi *et al*., 2013).

## MATERIALS AND METHODS

### *Selection of Pulse Cultivars*

*Cajanus cajan*,L.is a common pulse crop in Odisha and is widely cultivated. The seeds of pigeon pea (*Cajanus cajan, L.*) of variety OBGG-52 Durga, were procured from the Pulse Research Institute (CPRI) Ratanpur, Berhampur. The seeds choosen were of uniform size, colour and weight . *Cajanus cajan,* L. is a perennial legume of family Fabaceae and a short duration variety. It is grown during June to July and harvested in the month of October to November. Zinc sulphate ($ZnSO_4$) was used as test chemical which was guaranteed as a reagent from Merck limited, India. First stock solution was prepared by dissolving 1g of test chemical in 1L of distilled water. Different concentrations of the metal were prepared by using distilled water as the solvent from the above stock solution. Different concentrations of solution of 25,50,75,100mg/l and control—were prepared by proportional dilution with distilled water which ~~is~~ were used for various treatments.The seeds were first kept in distilled water for 12 hrs. and then soaked in wet cloth for 12 hrs which led to the germination of seeds. Appearance of plumule or radicle was considered as an index of germination.

### *Germination Studies*

The seeds of *Cajanus cajan L.* showed 90% germination in September-December. For germination studies ice-cream cups were used by making holes at the bottom. Then surface sterilized soil was added upto $3/4^{th}$ volume of cups. 10 numbers of seeds were kept in each cup at uniform distance in all the sets. Respective concentration of the test chemicals 25,50,75,100mg/l, control) was sprayed on the ice-cream cups before adding the seeds to the soil. The cups were incubated in the dark at room temp ($32^0$ Celsius) and then kept under the sunlight. The emergence of radicle or plumule was considered as an index of germination. Seeds were allowed to germinate. Better sprouted and healthy seedlings of 10 days old were used as experimental material. Care was taken to avoid drying and over flooding of test chemical in the cups.

***Morphological Studies***

The growth of plant was evaluated by measuring the shoot and root length of seedlings on 11th day. A 15cm scale was used for the measurements in the experiment of the shoot and root length.

***Estimation of Chlorophyll***

The fresh samples of shoot materials of the 10 days old seedlings were collected. Care was taken for separation of control and treated samples. A known quantity of about 100mg of samples of weighed shoot material was taken in a mortar and pestle and macerated to a paste by adding 80% acetone, stirred thoroughly and centrifuged(10min). The pellet was discarded and the supernatant was kept for chlorophyll estimation. The absorbance of each extract determined in a spectrophotometer at a wavelength of 645 and 663 nm of wavelengths.

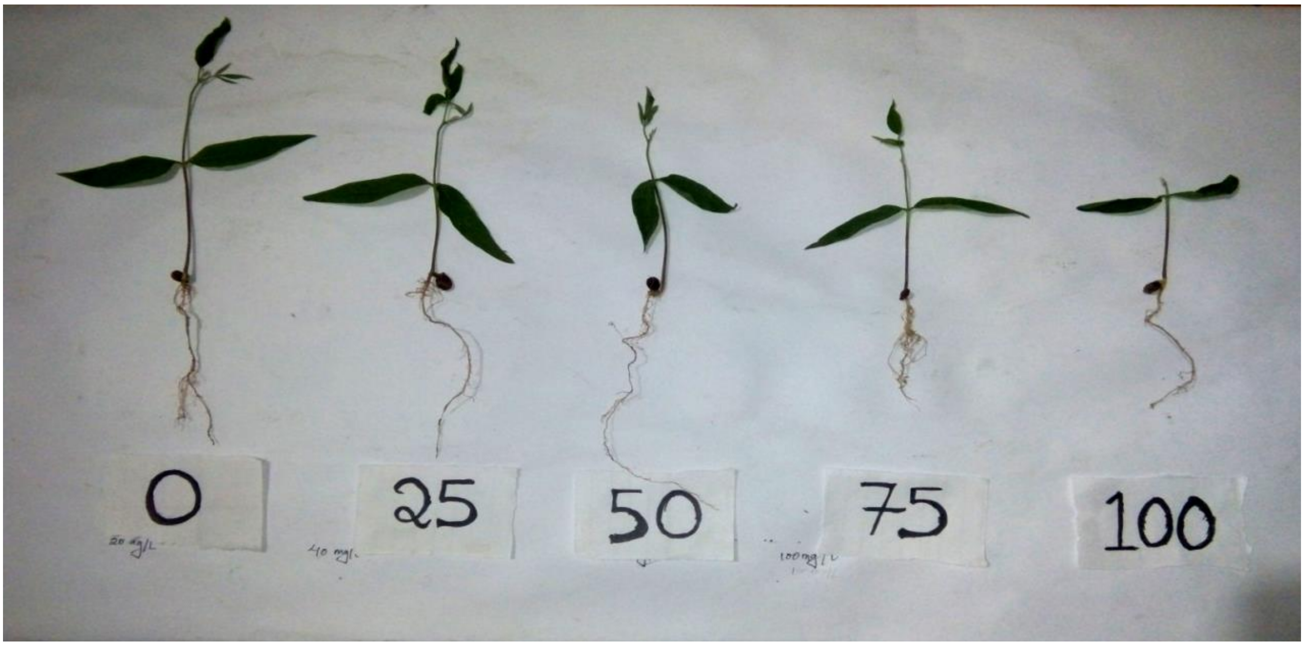
The total chlorophyll (chl), chl-a and chl-b contents ~~was~~ were measured by recording the absorbance of the extract at 645 and 663 nm wavelength and the values were calculated by using the formula given by Arnon [(1949).

**RESULTS**

***Germination Percentage***

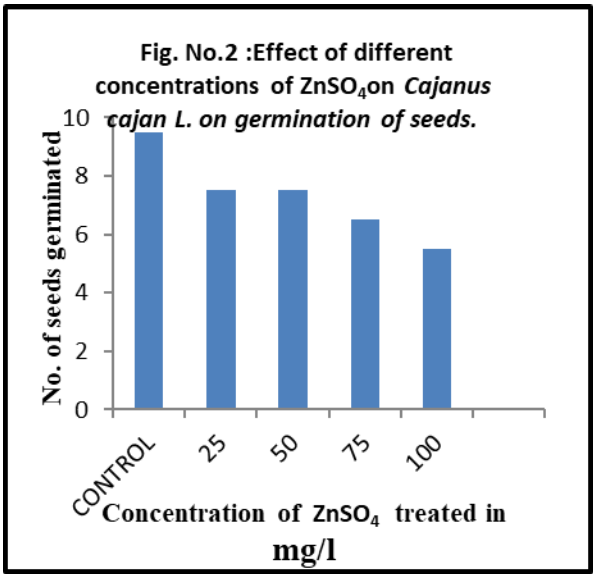
The germination %value of Pigeon pea under different $ZnSO_4$ treatments was calculated. The maximum germination was observed in control (100%). With the increase in the concentration of $ZnSO_4$ the percentage of germination decreased (Figure No.1 and 2).

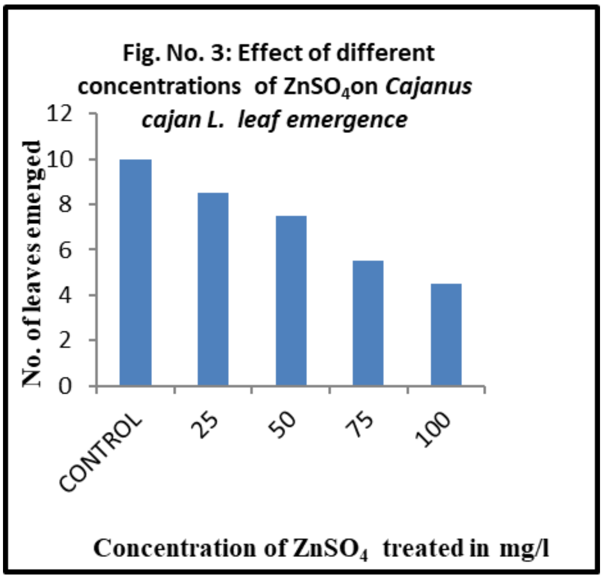

**Figure 1: Germination of pigeon pea (*Cajanus cajan L.*) seeds after 10 days treatment of $ZnSO_4$ under lab condition**

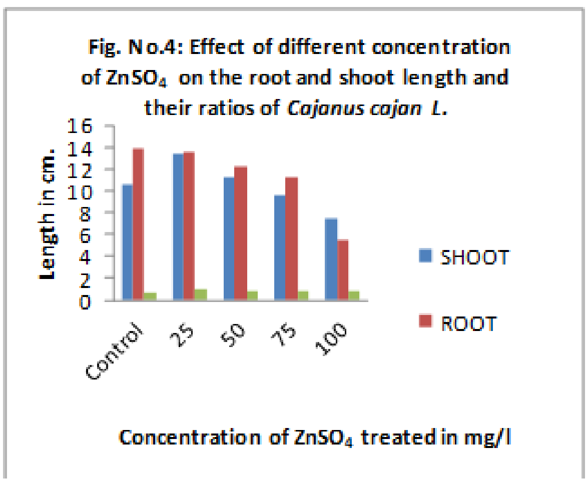


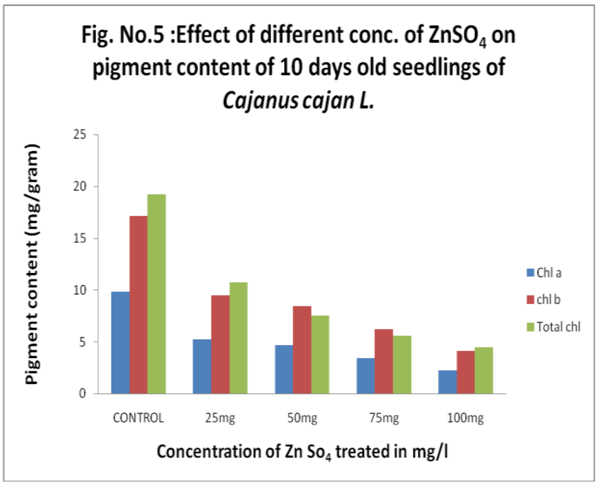

CONTROL 25 50 75 100mg/L

**Fig. 2 : Photograph showing the effect of different concentration of $ZnSO_4$ on the growth of seedlings of (*Cajanus cajan L.*) after 10 days.**



**SEEDLING LENGTH (Shoot and Root)**

The root length decreased with the increased level of $ZnSO_4$.The maximum root length was observed in untreated plants when compared to treated plants and the results obtained were shown the Figure No.4.

The changes in root and shoot length of10 days old seedlings were shown in figure2.The radicle emerged within 48 hrs of incubation in the dark room. The emergence of radicle or plumule was considered as the index of germination. The seeds were exposed to light after germination. In the next day after exposing the seeds to light there was rapid elongation of radicle and plumule. At the end of 92 hrs, most of the seeds showed radicle emergence of primary root and the shoot splits from coleoptiles and emerged as primary leaf. At the end of 10th day the seedlings showed primary root with adventitious roots (nodal roots), a number of rootlets and fully expanded 1st and 2nd leaves in control.

There were visible morphological changes seen in the roots which appeared different from that of the control. There was marked difference in the development of primary roots to adventitious roots. On exposure to higher concentrations beyond 75mg/l, there was a reduction in the primary roots, no lateral roots developed and blackening and browning of root tips. Shoot elongation was affected as we increased the concentrations of test chemical. Chlorosis of leaves, patchy brown spots, necrotic lesions on leaves and eventually senescence of leaves at higher concentrations were observed.

**Pigment contents**

Control plants appeared green and healthy and showed considerable lateral root development. With increase in $ZnSO_4$concentrations, three toxic symptoms were observed viz. decrease in plant height, depressed lateral root development and interveinal chlorosis. Increasing $ZnSO_4$concentrations further to 100mg/l, leaves appeared to totally alleviate chlorosis.

**DISCUSSION**

Seed germination and growth are of vital importance for continuation of plant life. For germination and seedling growth entry of water into seed is a basic requirement to initiate and trigger the intricate sequence of metabolism. At this stage, the seed and seedlings are susceptible to environmental stress as-it resulted in the deleterious effect on germination of seeds and seedling growth. The primary toxicity of heavy metals were shown as altering the catalytic function of enzymes, damaging cellular membranes and inhibiting root growth. The inhibitory action of zinc sulphate in root and shoot lengths might be due to reduction in cell division and toxic effect of heavy metals on photosynthesis.

It has been reported that the first visible damage-due to zinc sulphate is it inhibition of germination with the increase in concentration of $ZnSO_4$ , it also reduces the length of roots and shoots. $ZnSO_4$ applications had also positive effect on plant growth. The lower zinc applications, improved the root system of plants and it may also help the plants to the better absorption of water and other nutrients dissolved in the soil solution. Exposure to Zn can also reduce photosynthesis, transpiration rate, and water uptake and chlorophyll synthesis. Both organic and inorganic Zn have been showed to cause loss of potassium, magnesium, manganese and accumulation of iron. These decreases explain the change in the permeability of cell membrane by compromising its integrity.

The germination percentage of vegetable seeds decreased at all Zinc treatments. For all species, the lowest germination percentage always occurred in highest zinc concentration treatment. It significantly inhibited germination for *Brassica oleracea, B.rapa* and *Spinacia oleracea*. seeds.

Zinc treatment at all concentrations decreased seed germination, shoot length, root length and seedling dry weights. *Cajanus cajan L.* is more sensitive to zinc stress in seedling growth and root elongation than seed germination. A combination of agronomic management practices and genetic approaches are essential to improve the soil health conditions to enhance the root uptake of Zn. In pigeon pea, direct root uptake, remobilizations from vegetative tissues or combination of both of these two approaches are the main source of Zn in grains .The increased root uptake of Zn and root to shoot transfer could not proportionately increase the grain Zn concentrations indicating that internal translocation /re-translocation of Zn from vegetative tissues to grains is the major bottleneck for improving grain Zn concentrations. (Impa *et al*., 2013a).

These results show that there is a negative effect towards germination by zinc treatment. Minimum use of Zinc containing compounds in fungicide, pesticide and nematicide is recommended. Special care should be taken to monitor the toxic pollutants available in immediate environment. The accumulation of such type of toxic pollutants in larger concentration by crop can produce harmful effects to crop and ecosystem as well.

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**Conflict of Interest**
The author declares that there is no conflict of interest.

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