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Analysis of *Macrotyloma uniflorum Lam*. (kulthi) seed germination rate under $ZnCl_2$ stress

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Abstract: *Macrotyloma uniflorum Lam*. (kulthi) highly widespread form of legume crop that is frequently seen in southern India is a member of the Fabaceae family. Carotene, Thiamine, Riboflavin, Niacin, Vitamin C, and minerals are abundantly found in *Macrotyloma uniflorum*. Antibacterial, antimicrobial, and antioxidant capabilities of *Macrotyloma uniflorum* nourish the skin and lessen recurrent skin infections. The current study intends to demonstrate the major impact of $ZnCl_2$ on seed germination of *M. uniflorum*. Morphological parameters, including as shoot length, root length, and fresh weight, were also examined after germination. Different concentrations of $ZnCl_2$ (2%–10%) were applied to healthy, young seeds. The impact of metal stress ($ZnCl_2$) on the germination of *Macrotyloma uniflorum* seeds, outcomes of seeds treated with $ZnCl_2$ and untreated seeds were contrasted. The largest percentage of seed germination 80% is seen at 4% zinc chloride concentration, while the lowest 30% is seen at 10% zinc chloride concentration. While the highest shoot length and root length, respectively, are found at 4% concentration of $ZnCl_2$.

Keywords: *Macrotyloma uniflorum* Lam, seed germination and $ZnCl_2$ stress.

INTRODUCTION

Macrotyloma uniflorum is also known as horse gramme and madras bean is grown exclusively in India in Karnataka, Andhra Pradesh, Odisha, Tamil Nadu, Madhya Pradesh, Chhattisgarh, Bihar, West Bengal, Jharkhand, and in the foothills of Uttaranchal and Himachal Pradesh (Kumar, 2016; Durga, 2012; Krishna, 2006). About 100 gm of dry *M. uniflorum* powder contain minerals

including calcium, zinc, iron, and magnesium as well as carotene, thiamine, riboflavin, niacin, and vitamin C, however the fat content is relatively low (0.58%) in comparison to the protein (25%), carbohydrate (60%) and vitamin C content. Madras bean contributes greatly to human nutrition since it has antibacterial, antimicrobial, and antioxidant characteristics that nourish the skin and decrease recurrent skin infections (Bolbhat and Dhumal 2012).

Heavy metal is defined as metal with a specific density more than 5g/cm3 (Järup, 2003). Heavy metals play a critical role in a number of physiological processes that occur in the living world when present in typical concentration ranges. Numerous environmental factors, including human activity, industrialization, transportation, the use of pesticides in agriculture, and urban waste, have a direct impact on the distribution and concentration of metals (Khlifi and Hamza-Chaffai, 2010). Heavy metal concentrations that are too high can be harmful to living organisms. Due to negative impact over living organism, heavy metals can be conceder as most harmful environmental pollutants (Jaishankar et al., 2014).

Metal ions import phytotoxicity has an effect on many biochemical processes, including photosynthesis, seedling development, and others.Exposure to cadmium, lead, and ecotoxicity has negative effects on seeds, leading them to germinate more slowly, postpone germination times, and grow seedlings more slowly (Nagajyoti et al., 2010). After iron, zinc is the transition metal that exhibits the greatest necessity in basic biochemical physiological reactions.It facilitates the creation of photosynthetic pigments, nucleotide synthesis, chlorophyll synthesis, and enzyme activation. Other advantages of zinc include its capacity to lessen the damaging effects of other heavy metals like boron and the negative impacts of free radical activity in a plant's body (Marschner, 2011; Adiloglu and Adiloglu., 2006; Gunes et al., 2000).

A little amount of zinc is also ingested by plants through organs above ground from the atmosphere. Plants ingest zinc mostly through the roots from groundwater. The key internal and external elements that influence zinc absorption are the concentration of other heavy metals in the soil, its pH value, and zinc intake (Balafrej et al., 2020). The aim of the present study was to scrutinize the effect of different concentration of zinc as $ZnCl_2$ on seed germination in *Macrotyloma uniflorum*.

MATERIAL AND METHODOLOGY

The healthy and fresh seeds of *Macrotyloma uniflorum* were used to examine the effect of zinc ions on seed germination. After being surface sterilized with 0.1% $HgCl_2$, the seeds were repeatedly washed in distil water. Additionally, $ZnCl_2$ solutions in the concentration ranges of 2%, 4%, 6%, 8%, and 10% were created. Over 3ml of $ZnCl_2$ were given on alternate days. For each concentration in comparison to the control seed germination, after shocking, all treated seeds were then spread over cotton and filter paper.

Only distal water can given to seed as a control. Now, smeared germination conditions are applied to all Petri dishes. The period of seed coat rupture, just after the radical and plumule formed, was used to compute germination.The percentage of seed germination and new generated root and shoot length were observed in each alternative day. All work was performed in Basic Biotechnology Lab ofSchool of applied and life Sciences, Uttaranchal University Dehradun.

RESULT AND DISCUSSION

The examination of the germination rate revealed that the quantity of seeds that germinated was influenced by the amount of $ZnCl_2$ present in the medium. With the increase in $ZnCl_2$ concentration, the number of seeds that germinated was significantly more than in the control sample without $ZnCl_2$.After each alternative days, the control sample's shows higher percentage of germination in initial period then after 3 day, $ZnCl_2$ treated seeds shows increase rate of germination till 6% after that germination percentage goes down. It was discovered that samples with 10% $ZnCl_2$ concentrations had the least germination (Table 1). Golak and Taranath, 2015 observed the insignificant impact of zinc on seed germination.

Khot et al. (2012) stated that the improved germination may be attributable to photo-sterilization and photo-generation of active oxygen anions such as superoxide and hydroxide anions, which improve seed stress tolerance and stimulate capsule penetrating for water and oxygen consumption required for rapid germination. In the present study, it was observed that initially there was no significant effect on germination till the $ZnCl_2$ concentration was 6% but the germination reached to 50% at 3rd day and then germination was progressed at a slower pace. More concentration had visibly reduced and significant effect on germination (Figure 1). Auwal

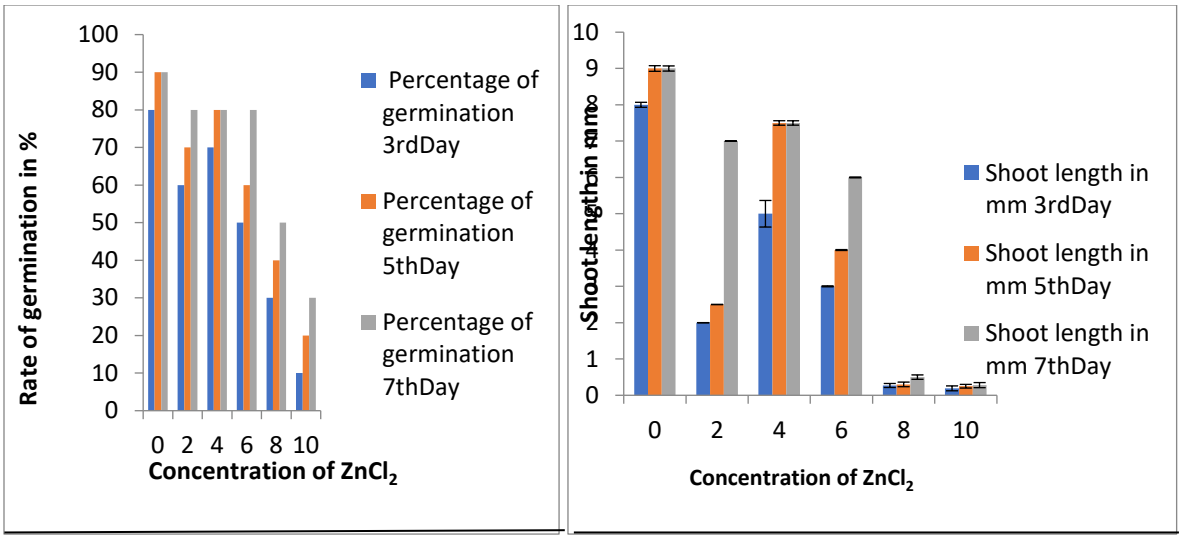
et al. (2016), reported that zinc stress or any other heavy metal stress has proven to be very dangerous for the human health, plant growth and other microbes and organisms.

Similarly, the shoot length was also affected but unlike during germination, the effect on shoot length was visible in slower concentration prominently. The difference in control and the sample shoot length grown in the presence of 2% $ZnCl_2$ has quite difference in the data collected by observing the samples and as concertation of $ZnCl_2$ kept on increasing, the data showed lower growth observed in the shoot length of the studied plant species (Figure 2). Moghaddamet al., 2011 studied that seed germination rapidly reduced, owing to $ZnCl_2$ toxicity on root development and shoot length.

Table-1 Depicting the percentage of germination, root and shoot length after each alternative day after incubation

S. No	**Conc. of $ZnCl_2$ (%)**	**Percentage of germination**			**Shoot length in mm**			**Root length in mm**		
		3rdDay	**5thDay**	**7thDay**	**3rdDay**	**5thDay**	**7thDay**	**3rdDay**	**5thDay**	**7thDay**
1.	Control	80	90	90	8±0.06	9±0.07	9±0.07	20.35±0.18	22.05±0.20	22.25±0.21
2.	2	60	70	80	2±0.01	2.5±0.01	7.0±0.00	10.65±0.08	12.5±0.09	12.9±0.01
3.	4	70	80	80	5±0.36	7.5±0.06	7.5±0.06	15.5±0.14	18.25±0.16	18.55±0.16
4.	6	50	60	80	3±0.01262	4±0.0126	6±0.0118	5±0.02	6±0.02	8±0.02
5.	8	30	40	50	0.27±0.05	0.3±0.06	0.5±0.06	1.1±0.26	1.5±0.07	2.5±0.01
6.	10	10	20	30	0.19±0.07	0.25±0.05	0.28±0.1	0.05±0.37	0.07±0.35	0.08±0.26

Values expressed as mean±standard deviation (n=3).



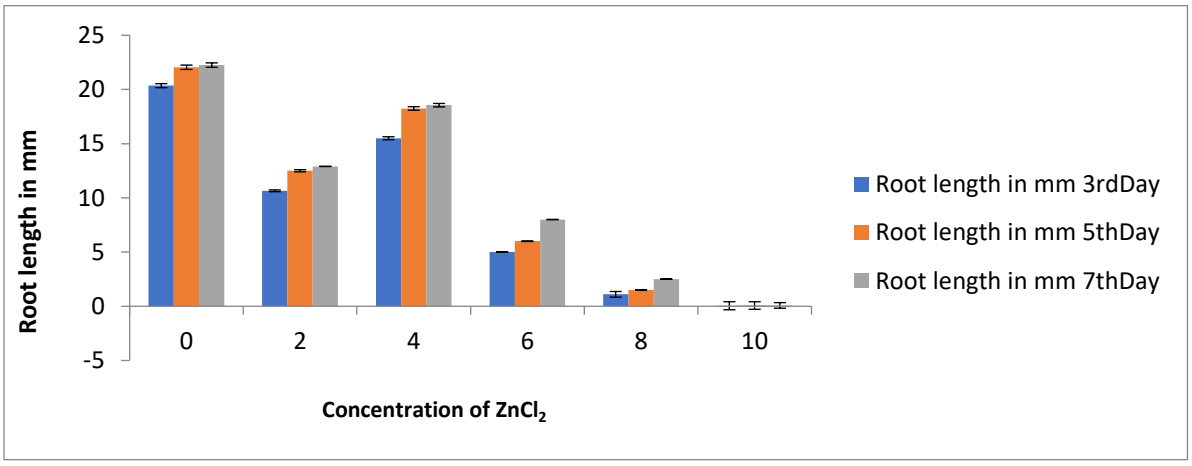


Figure 1: Rate of germination in the presence of different concentration of $ZnCl_2$.

Figure 2: Rate of Shoot length growth in the presence of different concentration of $ZnCl_2$.

Figure 3: Rate of Root length growth in the presence of different concentration of $ZnCl_2$**.**

When the root length was observed in the control plant sample and the plant sample grown in 2% $ZnCl_2$ concentration, the length of root was reduced to half at 3rd day when both the samples were observed but the significant reduce in root length was observed at the 6% $ZnCl_2$ concentration. At the 8% $ZnCl_2$ concentration and 10% $ZnCl_2$ concentration, root length was meaningfully diminished (Figure 3).

From the observations in this study and data collected form the present investigation evidently prove that plant species perform critically in the process of germination and growth when $ZnCl_2$stress is introduced in them. Auwal et al. (2016) suggested that stress is minimal at low and control concentrations, but rises as concentrations rise.

Nevertheless, a few mechanisms must be developed to withstand the harmful effects induced by ion accumulation in plants, heavy metal stresses are massive issues affecting agricultural production globally, causing osmotic stress, ionic toxicity, and disrupting mineral nutrient uptake and translocation, and are expected to evolve into greater issue in the upcoming years.

CONCLUSION

The present study reported the germination rate of horse gram seed under the zinc chloride salt. Bell shape graph is generated; after the from 2 to 4 % rate of seedling is increase and after that it is continuously decreases. This outcome clear that low concentration of Zn is work like a positive regulator and after moderate amount (4%) it decreases the seed germination.

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