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Effect of zinc on germination and early development of lettuce CV. Mimosa meadow

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ABSTRACT

The Micronutrients perform numerous metabolic functions, and the deficiency of minerals such as zinc is considered a worldwide public health problem. The evolution of knowledge about the importance of micronutrients has increased the interest in obtaining foods capable of fully meeting human nutritional needs. Agronomic biofortification of food emerges as part of the solution to this problem, contributing to the supply of micronutrients. For this experiment lettuce was chosen because it is one of the most consumed leafy vegetables with high nutritional value. The experiment was conducted at the Capão do Leão Campus of the Federal University of Pelotas, using cv lettuce seeds. mimosa meadow and had the following objectives: to determine the effects of different concentrations of ZnSO4 on germination, in addition to analyzing the potential for biofortification of seeds through soaking in solutions of different concentrations of ZnSO4. Three assays were performed: one with application of zinc solution in the substrate, and two assays of soaking the seeds in solutions containing different concentrations of ZnSO4, for 2 hours or 16 hours. From these tests, the germination percentage, fresh mass of the seedlings and germination speed index (GSI) were analyzed. Data were submitted to statistical analysis using Tukey's test with ANOVA ($p \leq 0.05$). The germination potential of seeds soaked for 2 hours or 16 hours in the different treatments was not affected in the Zn concentrations studied.

Keywords: Lettuce, Biofortification, Zinc sulfate.

1 INTRODUCTION

Nutrients are known to be necessary in maintaining health, however, micronutrient deficiency is common worldwide, both in developed and developing countries, and it is estimated that more than 30% of the world's population is zinc (Zn) deficient (PINTO et al., 2015; Sheoran et al., 2022).

Zn is an essential mineral for the functioning of the human body, acting as a cofactor of at least 300 enzymes, also contributes to the functioning of the immune system, sensory functions, neurobehavioral development, growth and physical development. Zn deficiency affects approximately one third of the world's population, being responsible for a significant part of the cases of respiratory infections, malaria, diarrhea, among others, since it significantly compromises the immune system (RÍOS et al., 2008).

Biofortification is a strategy that has been increasingly applied in order to increase one or more compounds in order to improve the nutritional or functional potential of foods of plant origin (SALTZAN et al., 2013). However, much of the research conducted is for biofortification of grains and cereals, mainly rice, wheat and corn, which are widely consumed by the general population. But, due to the dynamics of the flow of Zn in these crops, the nutrient is concentrated in the leaves and is little available in the grains, for this reason, plants that have the leaves as an edible part become the target of biofortification studies with Zn, thus assisting in the supply of this nutrient to the population.

Lettuce (*Lactuca sativa L.*) besides being one of the most consumed leafy vegetables in the world is considered of high nutritional value, besides being a source of fiber and having a low caloric index (TACO, 2011; USDA, 2019). However, the Zn content present in this vegetable is low in relation to the daily consumption needs, which are 11 mg for adults (NIH, 2022).

This work aims to evaluate the effect of solutions containing different concentrations of zinc sulfate (ZnSO4) on the germination of cv lettuce seeds. Mimosa Meadow.

2 MATERIALS AND METHODS

The work was conducted at the Capão do Leão Campus of the Federal University of Pelotas in Pelotas-RS, at the Laboratory of Plant Cell and Molecular Biology of CDTec and in the plant growth room of the Department of Zoology and Genetics of the Institute of Biology. In the experiment, cv lettuce seeds were used. mimosa meadow, free of pesticides acquired in local commerce, from lot Isla 140128-003, from the 17/17 crop.

Each of the assays was conducted with statistical repetitions, and the analysis of germination percentages and average germination numbers was performed using Tukey's test with ANOVA ($p \leq 0.05$), using the Statistix 8 software, and the graphs were generated from the Graphpad Prism 8 software.

2.1 GERMINATION TEST

In the first assay, together with the germination test, carried out according to the Rules for Seed Analysis (BRASIL, 2009), the effect of different concentrations of Zn on the germination process was evaluated. The test was performed with four replicates of 100 seeds, and the seeds were distributed in gerbox boxes, on 2 sheets of germite paper and moistened with each of the different treatments, in the proportion of 2.5 times the weight of the substrate paper (FRANZIN et al., 2004; KIKUTI and FILHO, 2012; FRANDOLOSO et al., 2017; ROSSETTI et al., 2020). The five treatments applied contained different concentrations of Zn: water (0); Troppa solution (0.0001% or 0.001 g/L of Zn); $ZnSO_4$ (0.1% or 0.006 mol/L); ZnSO4 (1% or 0.06 mol/L); ZnSO4 (10% or 0.6 mol/L). The assay was kept in a growth room at 20ºC, without photoperiod control.

The parameters evaluated in this assay were: the percentage of germination, through the average amount of normal seedlings and the fresh mass of normal seedlings. In the final count, at 7 days only the normal seedlings of each repetition were considered, and the data were expressed as average percentage of germination for each treatment (BRASIL, 2009). It was also made analysis of the fresh mass of normal seedlings, from the random collection of 10 seedlings per plot, which were weighed on an analytical balance with precision of 0.001 g (RODRIGUES et al., 2013).

2.2 2-HOUR SOAKING TEST

The assay sought to determine the germination potential of seeds soaked in solutions containing different concentrations of ZnSO4, after the pre-soaking period of 2 hours (GARBIM et al., 2014). For each treatment, 200 seeds were soaked for 2 hours using 15 ml Falcon tubes, each containing different treatments: water (0); Troppa solution (0.0001% or 0.001 g/L of Zn); $ZnSO_4$ (0.1% or 0.006 mol/L); ZnSO4 (1% or 0.06 mol/L); ZnSO4 (10% or 0.6 mol/L) and then distributed in 4 Petri dishes of 9 cm in diameter, containing two sheets of germite paper, soaked in water in the proportion of 2.5 times the weight of the paper used as substrate. The assay was kept in a growth room at 20ºC, without photoperiod control.

The effect of the solutions at 7 days was analyzed considering the average number of normal seedlings and fresh mass, from the random collection of 10 seedlings per plot weighed on an analytical balance with an accuracy of 0.001 g.

The parameters evaluated in this assay were: average number of normal seedlings 7 days after the installation of the tests and the fresh mass of the normal seedlings.

2.3 16-HOUR SOAKING TEST

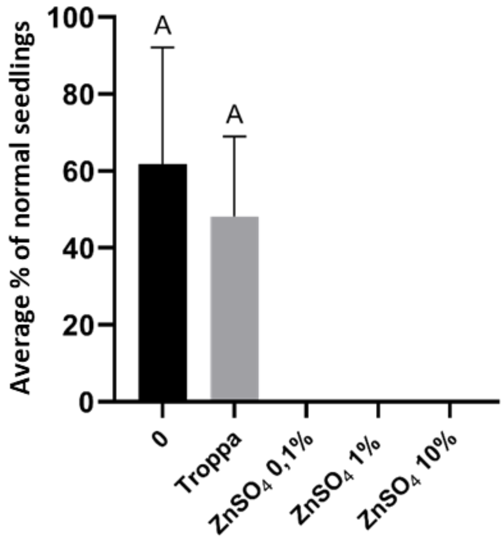
The assay sought to determine the germination potential of seeds soaked in solutions containing different concentrations of ZnSO4, after the pre-soaking period of 16 hours (SOARES et al., 2012). For each treatment, 200 seeds were soaked for 16 hours with the different treatments: water (0); Troppa solution (0.0001% or 0.001 g/L of Zn); $ZnSO_4$ (0.1% or 0.006 mol/L); ZnSO4 (1% or 0.06 mol/L); ZnSO4 (10% or 0.6 mol/L) and then distributed in 4 Petri dishes of 9 cm in diameter, containing two sheets of germite paper, soaked in water in the proportion of 2.5 times the weight of the paper used as substrate. The effect of the solutions was analyzed at 4, 7, 10 and 14 days after sowing, considering the average number of normal seedlings and the fresh mass of 10 seedlings at 14 days collected randomly and weighed on an analytical balance with an accuracy of 0.001 g.

The parameters evaluated in this assay were: average number of normal seedlings, fresh mass of normal seedlings and germination speed index. The average number of normal seedlings was calculated according to the number of normal seedlings obtained at 4, 7, 10 and 14 days after the installation of the assay. The germination speed index (GSI) was calculated by the formula IVG = (G1/N1) + (G2/N2) + ... + (Gn/Nn), where: G = number of normal seedlings computed in the counts and N = number of days from sowing to counting (KIKUTI and FILHO, 2012; SOARES et al., 2012).

3 RESULTS AND DISCUSSION

In the first assay, the results obtained allowed us to observe the effect of the different concentrations of ZnSO4 on the germination of cv lettuce seeds. Mimosa Meadow. The treatments whose solutions present concentrations from 0.1% affected the germination process and seedling growth (Figure 1).

Figure 1 – Effect of different concentrations of $ZnSO_4$ on the germination of lettuce seeds cv. mimosa meadow



Germination is directly influenced by water whose absorption by the seed triggers metabolic processes that lead to seedling development (ASAHIDE et al., 2012). Toxic substances can directly influence these processes, which is confirmed by the reduction or inhibition of germination, which occurs due to the low amount of endosperm in lettuce seeds. Seeds poor in endosperm become more susceptible to the conditions of the environment, especially in the early stages of development (RODRIGUES et al., 2013).

The Zn concentrations studied negatively affected the germination of cv lettuce. mimosa meadow, when kept in contact with the seeds throughout the period of the experiment. Asahide et al. (2012) suggest that Zn concentrations of 0.25 mmol/L already demonstrate a deleterious effect, negatively affecting the germination process of lettuce seeds. According to Peduto, Jesus, Kohatsu (2019) the impairment of root structures is expected when the seeds are in prolonged contact with toxic substances. In addition, excess Zn is known to hinder the growth of the main roots and cause the shortening and yellowing of the lateral roots (REICHMAN, 2002).

Hydration stimulates the seed to initiate the respiratory processes essential to germination (BISOGNIN et al., 2016). The water is absorbed by the seed in three phases, in phase I there is rapid absorption, in phase II the absorption is slower and in phase III, when the emergence of the radicle occurs, the absorption intensifies again (RODRIGUES et al., 2012). Thus, the pre-soaking period should contemplate the phases I and II of germination, which are preparatory phases, without advancing to phase III, which is characterized by cellular elongation and emission of the radicle (SANTOS et al., 2008).

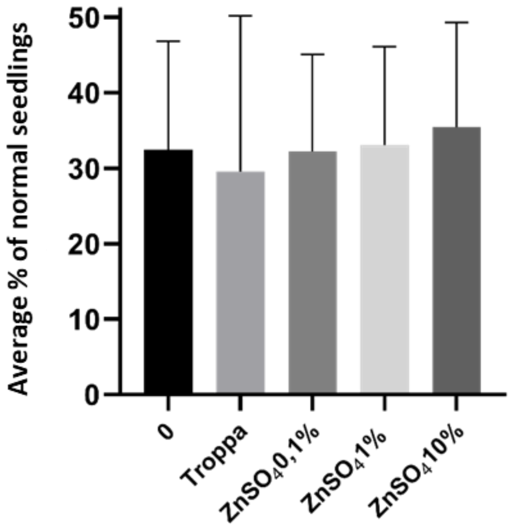
The soaking period of the seeds favors the beginning of the germinative pre-metabolism, reducing the germination period, and accelerating the emergence of seedlings. There are many factors that influence the success of the pre-soaking process, such as the forms of water supply, use of saline or osmotic solutions, number of hydration and dehydration cycles, in addition to the duration of the hydration period (SANTOS et al., 2008).

With the monitoring of the physiological performance of seeds at different times of imbibition it was found that tomato and cabbage seeds respond better to periods of 20 and 72 hours of soaking (BISOGNIN et al., 2016). For lettuce seeds these times are not yet well determined, but Sousa et al. (2020), describe that the soaking times for kale-butter, which are 6, 12 and 18 hours, can also be applied to lettuce. Other studies on lettuce seeds show that pre-soaking lettuce for 2 hours with eucalyptus essential oils showed good results (GARBIM et al., 2014) and pre-soaking in biostimulant solution for 16 hours did not alter their germination capacity, but improved their germination speed and plant vigor (SOARES et al., 2012; OLIVE TREE; TAKAHASHI; MIGLIORANZA, 2014).

In an assay that evaluated the imbibition and the effects of hydroconditioning by immersion of lettuce cv. Regina in distilled water, Rodrigues et al. (2012) identified that phase I occurred until 2 hours after soaking and phase II until 6 hours. Menezes et al. (2006), describe that the osmoconditioning for 96 hours added to the use of 200 mg/L of gibberellic acid, increase the speed of germination and the length of the seedlings, but the intensity of these effects varies with lettuce cultivars. Bisognin et al. (2016), testing periods of hydroconditioning of lettuce seeds of 0, 5, 10, 15, 20, 25, 30 and 35 hours, described that for these times the responses were not positive, and suggests that for lettuce hydroconditioning during shorter periods is more appropriate.

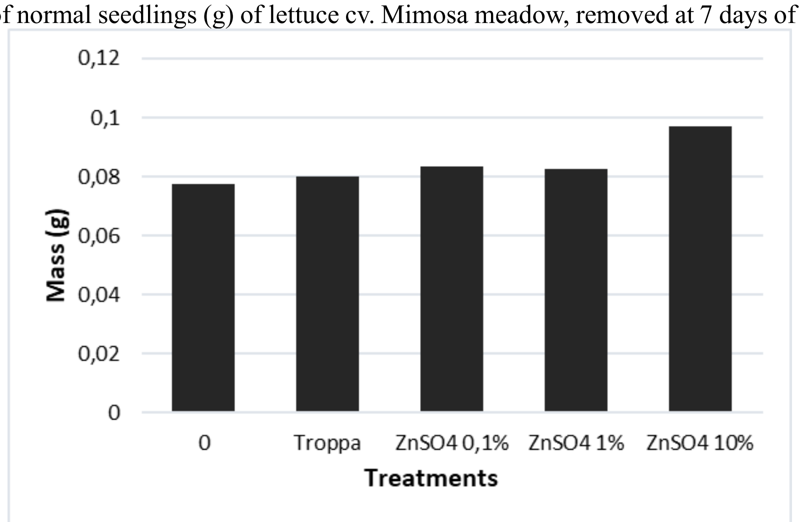
The germination potential of cv. mimosa meadow lettuce seeds soaked for 2 hours in solutions containing different concentrations of ZnSO4 was not affected, allowing the development of seedlings, with no statistical difference in the number of normal seedlings between the treatments studied (Figure 2). At 7 days, it was found that in the treatment with ZnSO 4 10% there was a higher number of seedlings, in relation to the other treatments, the average of normal seedlings for this treatment was 35.42, while for the treatment with Troppa solution the average of normal seedlings was 29.58 (lowest average verified), the treatment with $ZnSO_4$ 0.1% had a mean of 32.25 normal seedlings, the treatment with ZnSO4 1% had a mean of 33.08, and the treatment with water had a mean of 32.50.

Figure 2 – Average number of normal seedlings of lettuce cv. mimosa meadow after pre-soaking the seeds for 2 hours in solutions of different concentrations of Zn.



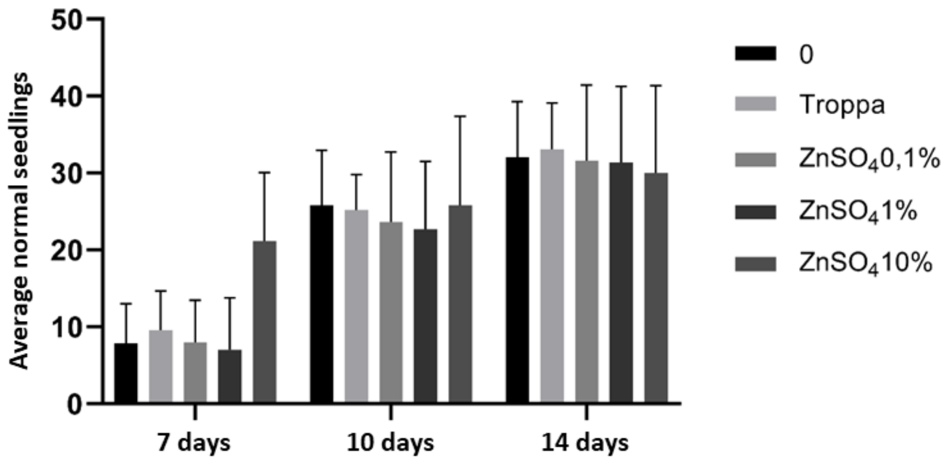
Another factor that also evidences the result is the fresh mass of 10 seedlings collected randomly, the mass in all treatments was quite uniform, but it seems that the increase in the concentration of Zn in the soaking solution slightly favors the growth of the seedlings, presenting more developed seedlings, and therefore with greater mass (Figure 3). The masses ranged from 0.0774 g in the treatment containing water, to 0.0972 g in the treatment with ZnSO4 10%. The other treatments showed little variation, Troppa solution presented mass of 0.0800 g, treatment with $ZnSO_4$ 0.1% presented mass of 0.0834g and treatment with ZnSO4 1% presented mass of 0.0827 g.

Figure 3 – Fresh mass of normal seedlings (g) of lettuce cv. Mimosa meadow, removed at 7 days of the 2-hour soaking test.



For the 16-hour soaking assay, the germination potential of seeds soaked for 16 hours in solutions containing different concentrations of ZnSO4 was evaluated from the normal seedlings in the different counting periods.

Figure 4 – Average number of normal seedlings of lettuce cv. mimosa meadow after pre-soaking the seeds for 16 hours in solutions of different concentrations of Zn, in analyses performed at 7, 10 and 14 days after sowing.



During the 14 days, the seedlings developed equally, showing no statistical difference between the treatments used (Figure 4). At 7 days, ZnSO 4 10% the average number of normal seedlings was 21.17, while for the treatment ZnSO4 1% the average of normal seedlings was 7, this was the lowest average verified, the treatment with $ZnSO_4$ 0.1% presented average of normal seedlings 8, the treatment with Troppa solution had an average of 9.58, and the treatment with water had an average of 7.83.

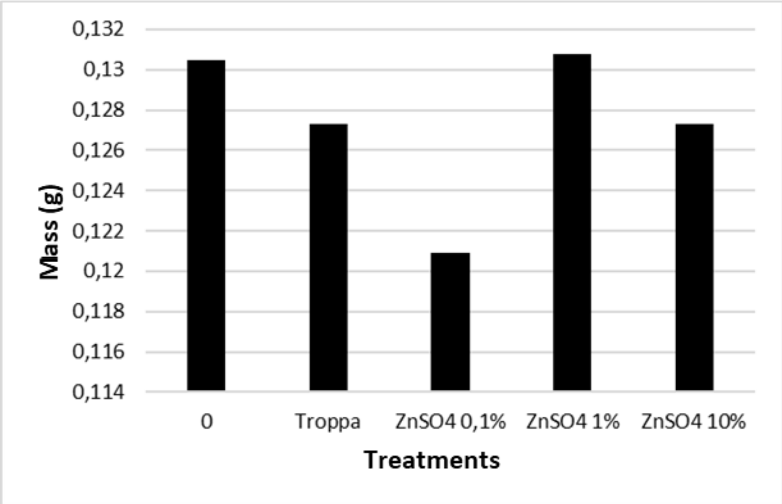
At 10 days, satisfactory growth was observed since all treatments applied promoted uniform germination (Figure 4). The seeds that received the treatment containing water had a mean of normal seedlings of 25.75, those that received the Troppa solution had a mean of 25.17, those that received the solution containing $ZnSO_4$ 0.1% 23.58, those that received the solution containing ZnSO4 1% 22.67 and those that received the solution containing ZnSO4 10% 25.75.

At 14 days, the average number of normal seedlings did not show significant differences, as shown in Figure 4. The seeds that received the treatment containing water had an average of normal seedlings of 32.00, those that received the Troppa solution had an average of 33.10, those that received the solution containing $ZnSO_4$ 0.1% 31.58, those that received the solution containing ZnSO4 1% 31.33 and those that received the solution containing ZnSO4 10% 30.00, which was the lowest average of normal seedlings for this period.

The pre-soaking for 16 hours of cv. lettuce seeds. mimosa meadow in solutions containing different concentrations of Zn did not affect their germination potential or normal morphology. The treatments containing water and Troppa solution presented more developed seedlings, with larger roots, the other treatments present morphology very similar to each other, but smaller in comparison to the treatment containing water and the treatment with Troppa solution.

From the analysis of the fresh mass of 10 normal seedlings (Figure 5) randomly collected, it is observed that the treatment with ZnSO4 1% resulted in more developed seedlings, with a mass of 0.1308 g. However the difference between the masses in all treatments is very small, the treatment containing water presented mass of 0.1305 g, the treatment with Troppa solution presented mass of 0.1273 g, treatment with $ZnSO_4$ 0.1% had a mass of 0.1209 g and treatment with ZnSO4 10% had a mass of 0.1273 g.

Figure 5 – Fresh mass of normal seedlings (g) of lettuce cv. mimosa meadow, removed at 14 days of the 16-hour soaking assay.

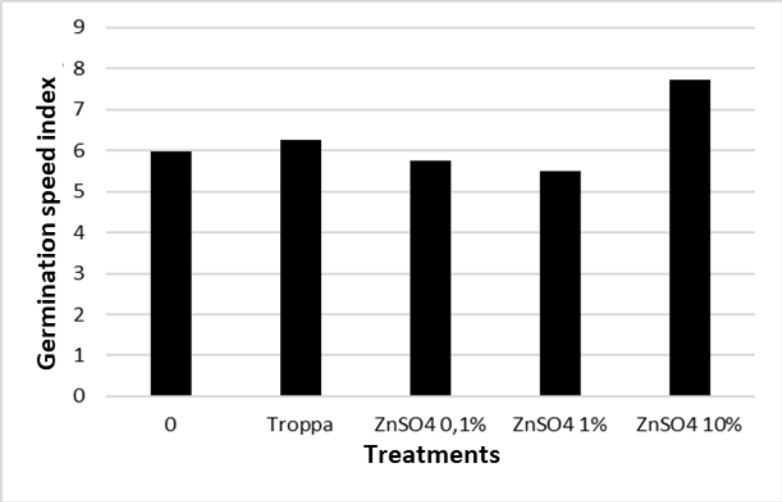


Döor et al. (2017), in a study with bean seeds, observed that the treatment of seeds with different concentrations of Zn did not affect the germination potential, however, it affected the physiological quality of the same interfering in the growth of the roots and the aerial part of the seedlings.

From the number of normal seedlings at 4, 7, 10 and 14 days after the implementation of the experiment contemplating the soaking of cv. mimosa meadow lettuce seeds in solutions containing different concentrations of Zn for 16 hours, the IVG was determined (Figure 6). In the treatment ZnSO4 10% the seedlings developed faster, presenting an IVG of 7.74, while the other treatments had a much lower IVG, the treatment containing water presented 5.98, the treatment with Troppa solution presented 6.25, the treatment with $ZnSO_4$ 0.1% presented 5.76 and the treatment with ZnSO4 1% presented 5.50, which may be a subjective variation, since the analysis is visual.

According to Silva and Cicero (2014), lots that have higher germination speed, usually, have greater agility in the reorganization of cell membranes, which in turn, enables a rapid and uniform growth of seedlings.

Figure 6 - Germination speed index of cv. mimosa meadow lettuce seeds after soaking for 16 hours in solutions of different Zn concentrations.



According to Bisognin et al. (2016), the soaking of lettuce seeds in water, had a negative effect on the IVG, with a drop in this index between the times of 0 to 5 hours of imbibition, between the times of 5 to 25 hours, the index seems constant, but from 25 to 35 hours, there is again a drop in the IVG. Soares et al. (2012), in a study of the soaking of lettuce seeds for 16 hours in solutions containing different concentrations of biostimulants, observed that the optimal concentration differs between 2 different cultivars, and that higher concentrations of biostimulant decrease the IVG of the seeds. This leads us to believe that the soaking periods, in addition to the concentrations of nutrient solutions, are specific to each species and between different cultivars of the same species (OLIVEIRA; TAKAHASHI; MIGLIORANZA, 2014; BISOGNIN et al., 2016).

Comparing the results of the average number of normal seedlings between the 2 seed soaking assays of lettuce cv. mimosa meadow with solutions containing different concentrations of Zn, 2 and 16 hours, at 7 days after the implementation of the experiment, the soaking time of 2 hours seems to be more appropriate, since it generated a greater amount of normal seedlings, and visually, generated morphologically more uniform seedlings, and more developed compared to the same period in the 16-hour soaking. This result is in line with the result obtained by Bisognin et al. (2016), where shorter soaking times seem to be more appropriate.

4 CONCLUSION

ZnSO4 concentrations of 0.1%, 1% and 10% negatively affected the germination process of cv lettuce seeds. Mimosa meadow, also interfering in its development, when exposed continuously to zinc.

The germination potential of cv. lettuce seeds. Mimosa meadow soaked for 2 hours or for 16 hours in solutions containing different concentrations of ZnSO4 was not affected, allowing the development of seedlings.

The fresh mass of normal seedlings soaked for 2 hours in solutions containing different concentrations of ZnSO4 was higher in the treatment containing ZnSO4 10% and in the seeds soaked for 16 hours was higher in the treatment containing $ZnSO_4$ 1%.

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