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Communications in Soil Science and Plant Analysis

ISSN: 0010-3624 (Print) 1532-2416 (Online) Journal homepage: https://www.tandfonline.com/loi/lcss20

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To cite this article: Adão Felipe dos Santos, Otoniel Magalhães Morais, Renato de Mello Prado, Aguinaldo José Freitas Leal & Rouverson Pereira da Silva (2017) Relation of Toxicity in Corn Seeds Treated with Zinc and Salicylic Acid, Communications in Soil Science and Plant Analysis, 48:10, 1123-1131, DOI: 10.1080/00103624.2017.1323097

To link to this article: https://doi.org/10.1080/00103624.2017.1323097

Accepted author version posted online: 28 Jun 2017.
Published online: 28 Jul 2017.

COMMUNICATIONS IN SOIL SCIENCE AND PLANT ANALYSIS
2017, VOL. 48, NO. 10, 1123–1131
https://doi.org/10.1080/00103624.2017.1323097

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Relation of Toxicity in Corn Seeds Treated with Zinc and Salicylic Acid

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ABSTRACT

The beneficial effect of corn seed treatment with zinc (Zn) is directly related to the source used. The excess of this micronutrient causes seedling stress and reduces growth. Thus, assuming that the use of exogenous phytohormones can minimize such effects, we evaluated different doses and sources of Zn for the treatment of maize seeds with or without salicylic acid. The experiment took place in the laboratory, and two factorial experiments, 2 × 4 + 1, were performed in a randomized design. The seeds were treated with either ZnO or $ZnSO_4$ at doses of 0.5, 1, 2, and 3 g.kg^{-1} seed with four replications, differing only by the addition of 4.14 mg L^{-1} salicylic acid. Treating seeds with Zn and salicylic acid did not affect germination. ZnO led to a greater increase in dry mass in corn seedlings as compared with zinc sulfate, especially at higher doses (2 and 3 g kg^{-1} seed). Seed treatment with sulfate reduces root and shoot length, and salicylic acid did not attenuate this toxic effect. Dry mass is not affected when oxide is used. Salicylic acid reduces the accumulation of zinc in the treatment of corn seeds, regardless of the source used.

ARTICLE HISTORY
Received 10 February 2016
Accepted 14 February 2017

KEYWORDS
Germination seedling; micronutrient; oxidative stress; phytohormone; zea mays

Introduction

Zinc (Zn) can be applied to the soil (broadcast application or the seeding furrows) and foliar. It is distributed by broadcasting, and much of the incorporated fertilizer is adsorbed by the soil. When Zn is applied by seeding furrow, adsorption is reduced; however, a small part of the root system is in contact with Zn from the fertilizer. When foliar application is performed, Zn is unable to reach all parts of the plant due to the low mobility in the phloem (Malavolta 1980).

Faced with the difficulties of applying and distributing Zn in plants, an alternative method used to meet the needs of the seedlings and to ensure their first roots are in contact with the nutrient is seed treatment. This technique allows a more uniform distribution of nutrient per plant area (Slaton et al. 2001). According to Ribeiro and Santos (1996), applying Zn to seeds also promotes the accumulation of nutrients in the plant, especially in the aerial part due to the increased Zn content in that area.

The use of Zn at concentrations up to 6–10 mL kg^{-1} seed has generated significant results in the development of corn, wheat, soybean, sunflower, peanut, and mustard (Singh 2007). When studying the effects of Zn from different sources, Prado, Natale, and Mouro (2007) found lower concentrations of zinc in the tissues of maize seedlings treated with oxide zinc (ZnO, insoluble in water) than in those treated with sulfate of zinc ($ZnSO_4$, soluble in water). Those authors also report beneficial

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effects of using ZnO during the initial development of sorghum seedlings. However, reports suggest that Zn has a depressant effect when applied as zinc sulphate (Yagi et al. 2006).

The results of these studies show that there are differences between the sources of Zn used to treat seeds. This micronutrient can be toxic to the plant, especially to the root zone, suggesting that there is a need for a better understanding of the effects of different sources of zinc on the growth of corn plants.

An alternative method to minimize stress associated with excess Zn in treatment seeds is the addition of salicylic acid (SA), which has many beneficial effects including the ability to minimize stress. This compound helps plants adapt to different environmental conditions, including low (Ahmad et al. 2015) and high temperatures (Takaki and Rosim 2000; Sun et al. 2009), drought, and excess metals (Tamás et al. 2015). Furthermore, SA is associated with the formation of adventitious roots and stems (Hinojosa 2005).

The addition of SA may increase the viability of Zn-treated corn seeds, and its application at low concentrations may induce resistance to stress generated by Zn (Horvath, Szalai, and Janda 2007), when treatment is carried out via seeds. SA might be considered a key compound of the plant defense mechanism, which can inhibit the activity of specific peroxidases responsible for producing large amounts of Reactive Oxygen Species (ROS) (Greggains et al. 2000).

These findings suggest that the concentration and toxicity of Zn in maize seedlings may depend on the source of the micronutrient used. Furthermore, it is important to study the effects of Zn in the presence and absence of SA as this may mitigate the stress caused by excess Zn. Therefore, we aimed to evaluate the doses and sources of Zn used to treat maize seeds with and without the use of SA.

Material and methods

Two experiments were performed at the Seed Analysis Laboratory of State University of Southwest Bahia—UESB, Vitória da Conquista, Bahia-Brazil, using a completely randomized design.

Experiment I

A factorial 4 × 2 + 1 design was employed using maize seeds of cultivar BRS-4051. The treatments consisted of doses of 0.5, 1.0, 2.0, and 3.0 g kg^{-1} seeds with four replications. Two sources of Zn were used: $ZnSO_4$ powder (35% Zn) and ZnO (80% Zn), with four replicates of each condition. ZnO solution was prepared by adding 92.75 g of ZnO and 10 mg lignosulfonate to 1 L, which was then used to treat seeds with different concentrations of Zn. $ZnSO_4$ was applied after moistening at a concentration of 15 mL/100 g seeds to facilitate fertilizer adherence to the seed. This wetting was also adopted for the control treatment.

The characteristics examined were germination, shoot length, root length, dry mass of the aerial part (MSPA), root dry mass (MSR), dry plant matter (MSP), and Zn content in the shoot.

The experiment was conducted on germitest paper previously moistened with distilled water, equivalent to 2.5 times the dry weight of the paper. To position the seeds, a 2 cm line was drawn in the upper-third of the paper in a longitudinal direction so that the radicles were toward the bottom of the paper, thereby facilitating their root growth. The rolls were packed in plastic bags and placed vertically in the germination chamber at 25 °C for 7 days in Brazil (2009). After this period, the normal emerged seedlings (roots and aerial part) were withdrawn and measured, and the average earnings per seedling as expressed in centimeters.

Next, the two sides were evaluated, eliminating the remains of seeds and placing the shoots and roots in paper bags. These were then placed in a forced air circulation oven at 65 °C until a constant mass was obtained, which was subsequently used to determine the dry mass of the samples. The Zn content of the samples was determined according to the method described by Bataglia et al. (1983). Then, we estimated the quantity of accumulated Zn in the shoot and calculated the accumulated Zn use efficiency in the shoot and efficiency of utilization given by the dry mass of the whole plant over the of the plant Zn accumulation whole (g /mg).

Experiment II

The second experiment was performed shortly after the completion of the first, using seeds from the same batch and evaluating the same variables as previously described. The second experiment differed only by the addition of SA to all treatments, with the exception of the control. In seeds treated with ZnO, SA was dissolved in the previously prepared solution of ZnO (92.75 g ZnO lignosulfonate + 10 mg L^{-1}) at a concentration of 4.14 mg L^{-1}. When ZnSO4 was used, this was applied after wetting seeds with an aqueous solution of 4.14 mg L^{-1} SA at a concentration of 15 mL/100 g seed; the $ZnSO_4$ was added later in salt form.

The data obtained in both experiments were subjected to analysis of variance using the test F (>0.05) to differentiate between the effects of different Zn sources. When significant dose effects were found, a regression analysis was performed, opting for a higher degree of significance.

Results and discussion

Zn applied in the form of ZnSO4 or ZnO did not influence corn germination (Table 1) in experiment I. However, when added to SA (experiment II), there was a higher percentage of germination when ZnO was used (Table 1). There was an interaction between the source and dose of Zn on root length (CR), shoot length (CPA), root dry mass (MSR), dry mass of the aerial part (MSPA), and dry plant matter (MSPLA) in both experiments (Table 1).

Table 1. Germination (Germ), root length (CR), shoot length (CPA), root dry mass (MSR), dry mass of the aerial part (MSPA), and dry mass of the whole seedling (MSPLA) of corn in response to different concentrations of Zn with and without SA.

	Germ	CR	CPA	MSR	MSPA	MSPLA
Doses of Zn (D)	(%)	(cm)			(mg/g)	
			Without SA—Experiment I			
0	99.15	25.28	19.64	1.00	1.32	2.32
0,5	98.73	27.79	20.33	1.04	1.40	2.45
1	97.88	25.31	18.99	1.20	1.35	2.56
2	98.31	23.80	16.90	0.88	1.17	2.05
3	96.65	20.59	12.93	0.73	0.82	1.56
Test F	1.12^{ns}	11.78**	46.23**	87.78**	35.45**	52.27**
Sulfate	97.47 a	25.23 a	17.65 a	0.86 b	1.12 b	1.98 b
Oxide	98.82 a	23.88 a	17.86 a	1.08 a	1.31 a	2.39 a
Test F	2.76^{ns}	3.81^{ns}	0.28^{ns}	87.78**	30.17**	70.45**
Interaction (FxD)	2.63^{ns}	7.15**	8.06**	53.25**	19.47**	43.30**
CV (%)	2,61	8,85	7,00	7,6	9,14	7,09
			With SA—Experiment II			
0	99,15	25.28	19.64	0.97	1.32	2.29
0,5	99.15	25.01	18.39	1.02	1.56	2.59
1	99.57	25.96	18.13	1.00	1.59	2.60
2	99.57	23.84	15.52	0.94	1.47	2.42
3	97.48	19.94	14.45	0.67	1.09	1.75
Test F	1.24^{ns}	18.49**	22.50**	52.89**	81.02**	80.86**
Sulfate	98.14b	22.29 b	16.17 b	0.80 b	1.26 b	2.07 b
Oxide	99.83 a	25.72 a	18.28 a	1.04 a	1.54 a	2.59 a
Test F	5.85*	47.35**	26.75**	177.20**	177.63**	223.39**
Interaction (FxD)	1.59^{ns}	19.36**	19.58**	58.47**	126.32**	114.31**
CV (%)	2.2	6.6	7.5	6.1	4.7	4.7

Means followed by the same letter in the column do not differ by Tukey's test at 5% probability. ns, not significant. * and ** indicate significance at 5% and 1% probability, respectively.

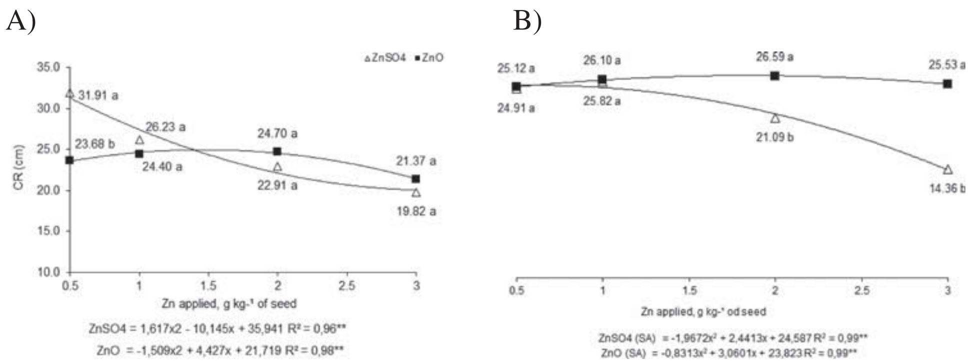

Figure 1. Root length without (A) and with salicylic acid (B).

Corn seeds treated with Zn at a concentration of 0.5 g kg^{-1} using ZnSO4 had longer seedling roots as compared with those grown in the presence of ZnO (Figure 1). The root length decreased with increasing doses of Zn, indicating possible toxicity in the root zone (Marschner 1995). The application of high Zn concentrations close to the corn roots affected the growth of the root system causing phytotoxicity, a fact that was also noted by Rosolem and Ferrari (1998).

However, when ZnO was used, there was an increase in root growth up to a dose of 2 g Zn per kg seed, and a 13.5% decrease relative to the maximum and minimum was obtained when 3 g Zn per kg seed was used. Therefore, treating seeds with Zn reduces the length of maize seedling roots, especially when the source is $ZnSO_4$ at doses greater than 0.5 Zn per kg^{-1} of seed. Excess levels of Zn following application of $ZnSO_4$ to maize seeds can promote hormonal changes in seedlings, with decreases in concentrations of gibberellic acid, zeatin, and indole acetic acid, and increase in concentrations of abscisic acid (Erturk et al. 2015), which compromises metabolic activities, such as cell division.

However, the addition of SA to corn seeds (Experiment II—Figure 1B) minimized toxicity. This can be evidenced by the longer root length when compared to experiment I (without SA). However, this result was observed only when the oxide was used. Thus, it is possible to affirm that the use of higher doses of Zn oxide in the seed treatment associated with SA does not compromise the root system of the corn seedlings.

In addition, when compared to experiment I (Figure 1A), it is possible to note that the association of SA with sulphate provided plants with lower root system. This indicates that high levels of Zn inhibit many metabolic processes in plants, which can result in limited growth root and development, in addition to inducing senescence of the plant (Wang et al. 2009; Parlak Yilmaz 2012).

The maximum shoot length (Figure 2) was obtained at a dose of 0.5 g Zn per kg of seed (Experiment I), reaching values of 21.6 and 19.1 cm, respectively, in response to zinc sulphate and ZnO. Increasing doses of zinc from both sources were observed to decrease shoot length, with decreases of 39 and 34%, in the presence of $ZnSO_4$ and ZnO at the highest dose (3 g Zn per kg seed), respectively. These results are similar to those obtained by Funguetto et al. (2010), who reported that the maximum length of the aerial parts of rice seedlings was obtained with the addition of 0.57 g kg^{-1} Zn to seeds and that higher doses of ZnSO4 promoted the reduction of shoot length of seedlings. The detrimental effect of corn seed zinc treatment on the seedling shoot length was less pronounced following the addition of salicylic acid (Experiment II—Figure 2B), particularly at the highest dose evaluated, which was 3 g Zn kg^{-1} seed. However, when $ZnSO_4$ was used, salicylic acid did not minimize the toxicity of seed treatment. In the present work, the reduction of shoot length occurred regardless of the source used.

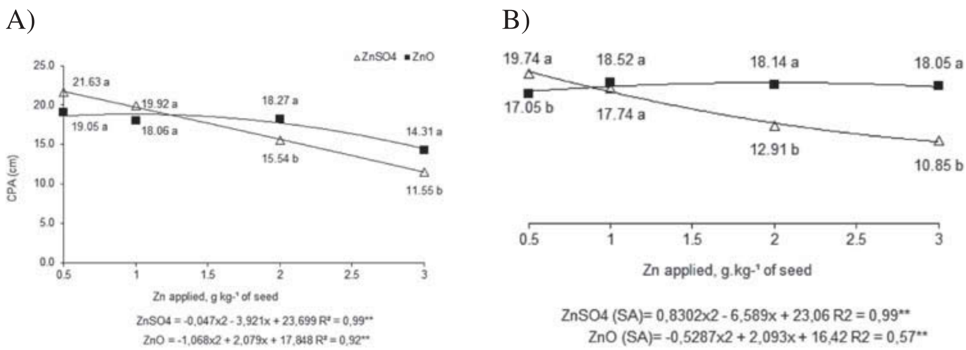

Figure 2. Length of aerial part without (A) and with salicylic acid (B).

In the experiment II, the addition of SA in seed treatment promoted an opposite response. The use of sulfate with SA resulted in the reduction of shoot length of the seedlings (Figure 2B). The exogenous application of SA has as main function to reduce the abiotic stress in order to give conditions for the development of the plants. When compared to Experiment I, these results are positive, which shows effectiveness in the use of SA together with the source of oxide, especially in high doses.

A decrease in MSR to 72% was observed following application with sulfate (Figure 3A), which may be due to Zn toxicity, since there was a reduction in root length. Ohse et al. (2012) studied the effect of Zn treatment in watermelon seeds and found similar results when using sulfate, wherein the MSR also decreased when increasing doses of Zn were applied to the seed and when Zn used in the form of oxide does not affect the MSR (Figure 3A). In our work, this fact is related to a small variation in root length when using this source (Figure 1A). However, it is worth mentioning that the treatment of seed with 0.5 g kg^{-1} of seed in the sulfate source provided greater mass of the roots, and thus its use in small quantities proves to be an interesting alternative.

SA application (Experiment II—Figure 3B) reduced the variation in Zn accumulation in response to treatment with different doses and sources of Zn, where it can be observed that the application of 3 g of Zn per kg^{-1} was similar for the doses. It is worth noting that the roots developed more when using this combination ($ZnSO_4$ + SA, Figure 1B). Thus, it is assumed that SA, regardless of the dose

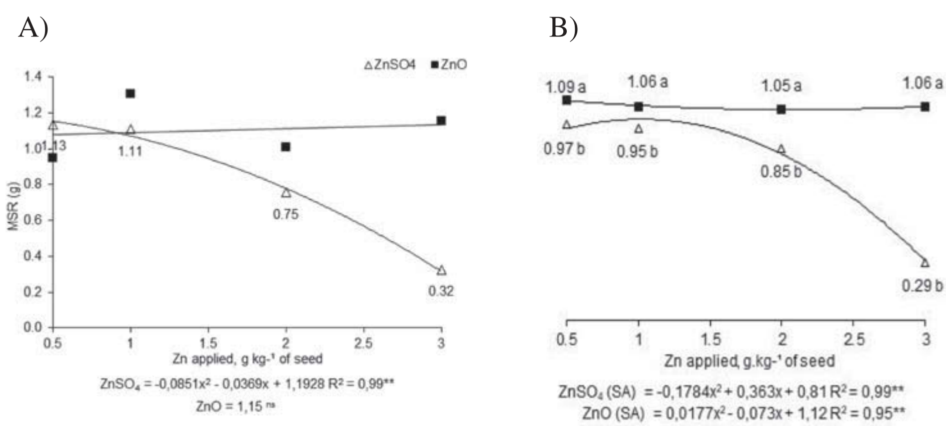

Figure 3. Dry mass of root without (A) and with salicylic acid (B).

and source used, increased the activation of enzymes such as reductase and ascorbate peroxidase, which are responsible for the accumulation of certain osmolytes, such as proline, that reduce the potential for stress (Szepesi et al. 2005; Tari et al. 2002, 2004).

However, when $ZnSO_4$ was used, a decrease in the dry mass of roots was observed depending on the Zn dose used. Silveira, Moraes, and Lopes (2000) analyzed the effect of different SA doses on germination and vigor of rice seeds and also found marked reductions in MSR and MSPA when doses of 10 and 20 μM were used. This decrease can be explained by use of optimal SA dose.

The SA in seed treatment may attenuate possible stress caused by the increase of Zn in the root zone the oxide source; however, the combination with sulfate did not have a positive effect in ithe ncrease/maintenance of MSR, with significant reduction of MSR (Figure 3A).

For the interaction of the doses and sources for shoot dry mass (MSPA), no differences were observed between the sources when applying the doses when they were relatively low (0.5 and 1 g of Zn per kg^{-1}). Increasing doses of Zn induced reduction of MSPA, especially in the sulfate form (Figure 4A). However, with the addition of salicylic acid (Figure 4B), the increase in the doses allowed to differ in the accumulation the oxide source to sulphate in 61% in the highest dose (3 g of Zn per kg^{-1}). The higher accumulation of dry mass of the oxide source can be explained by the fact that the seedlings presented a larger shoot length, indicating the relevant role of Zn in the process of plant metabolism as the main precursor of plant growth promoting hormone biosynthesis.

The application of Zn induced a decrease in the seed dry mass with and without the addition of SA, especially when $ZnSO_4$ was used (Figure 5A and B). However, it was noted that ZnO had little effect on the accumulation of shoot dry mass with or without SA, indicating that it has no toxicity. It is possible that the addition of oxide to lignin may have had a positive effect, since this will reduce the rate of Zn release from the seedlings, making them able to accumulate a higher amount of dry mass. Similar results were found by Prado, Natale, and Mouro (2007) in experiments conducted in a greenhouse using seed corn, where a higher increase in seedling dry mass was obtained when ZnO was used and a decrease was observed when zinc sulphate was used. Galrão (1996) and Dias and Cicero (2016) also observed a beneficial effect in the treatment of corn seeds using the oxide source.

For the sulfate source it is possible to observe that the doses of the micronutrient Zn induced decrease of dry mass accumulation of the roots reflecting in the dry mass of the plant. Yagi et al. (2006) studying the effect of zinc application on sorghum seeds, and observed that zinc sulfate decreased the accumulation of dry mass of roots and whole plant.

In the Experiment II, of the present study, the addition of SA to treatments and larger doses of Zn in the oxide source (2 and 3 g kg^{-1} of Zn) resulted in the highest total dry mass accumulation (Figure 5B). However, there was no difference when the lowest doses were used. This effect was described by Shakirova et al. (2003) who reported that exogenous application of SA prevents the

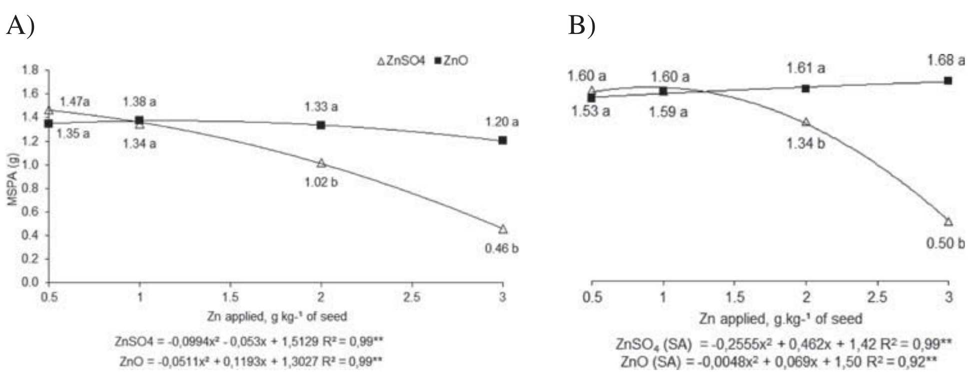

Figure 4. Dry mass of aerial part without (A) and with salicylic acid (B).

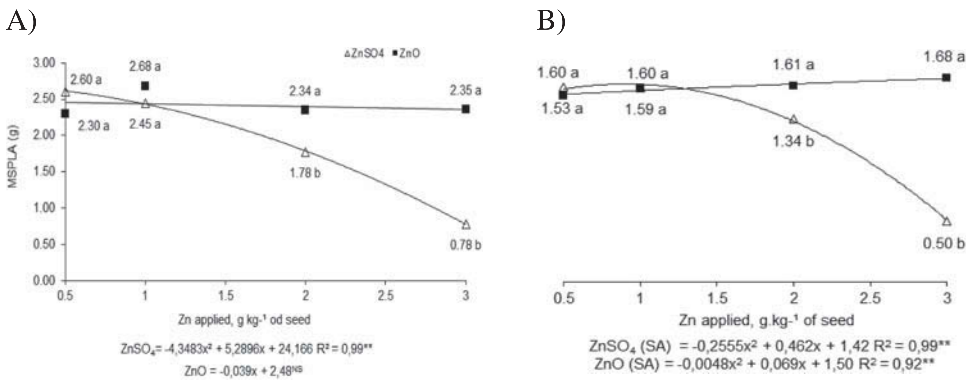

Figure 5. Dry mass of the plant without (A) and with the addition of salicylic acid (B).

reduction of indole acetic acid and cytokinin acid, which would otherwise promote stress, resulting in improved cell division in the root and in the apical meristem, thus, increasing growth and total dry mass accumulation. Moreover, those authors reported that the application of SA to seeds treduces the content of reactive oxygen species and, therefore, the activity of superoxide dismutase (SOD) and peroxidase (POX) enzymes is also reduced in roots of seedlings. In contrast, Krantev et al. (2008) reported that the exogenous application of salicylic acid increased the activities of antioxidant POX and SOD enzymes with a concomitant decline in catalase activity in corn plants.

It should also be noted that the results in the two experiments were similar, with little influence of the treatments when the oxide source was used and significant reduction when sulfate is used. However, the effect of SA was more evident on sulfate, allowing the seedlings to accumulate similar or greater dry mass (2 g Zn per kg^{-1} of seed) under the same conditions.

The application of Zn in the oxide and sulphate forms led to similar dry mass accumulation in maize plants when used on seeds at low doses (0.5–1 g kg^{-1} seed). This is in contrast to the effect of high doses of Zn (2–3 g Zn per kg^{-1} of seed), which results in stress in response to Zn oxide with or without the addition of SA.

Treating corn seeds with ZnO does not result in the loss of plant dry mass accumulation, and toxicity is minimal when varying the doses of Zn from 0.5 to 3.0 g kg^{-1} Zn with or without the addition of SA. However, the application of this micronutrient in the form of sulfate at doses higher than 0.6 and 1.2 g Zn per kg^{-1} of seed in the absence and presence of SA, respectively, induced a loss of dry mass accumulation in corn seedlings (Figure 5).

Treating corn seeds with Zn increased the levels of accumulated Zn in the shoot, especially when applied as sulfate without (Figure 6A) and with the addition of SA (Figure 6B). Zn utilization at doses of 3 g kg^{-1} seed went 62% more efficient when ZnO was used than when zinc sulfate was applied. Prado, Natale, and Mouro (2007) also found that ZnO was used more efficiently. The most likely cause of Zn accumulation following application of $ZnSO_4$ may be related to its greater solubility as compared with ZnO. It is worth remembering that the compounds applied as seed treatment can be absorbed by the plants through the capture of the seed structures, root absorption, or through the coleoptile (Quérou, Euvrard, and Gauvrit 1997).

For the present study, the use of SA little interfered in the accumulation of Zn in the plants, so that it reduced the content of the micronutrient in the organs of the plant. This reduction is more pronounced when the oxide source is analyzed separately, regardless of the dose used. Thus, it is inferred that SA in seed treatment reduces the Zn content in the plant. However, according to Ahmad et al. (2015), use of SA increases the tolerance of the seeds subjected to stress because it

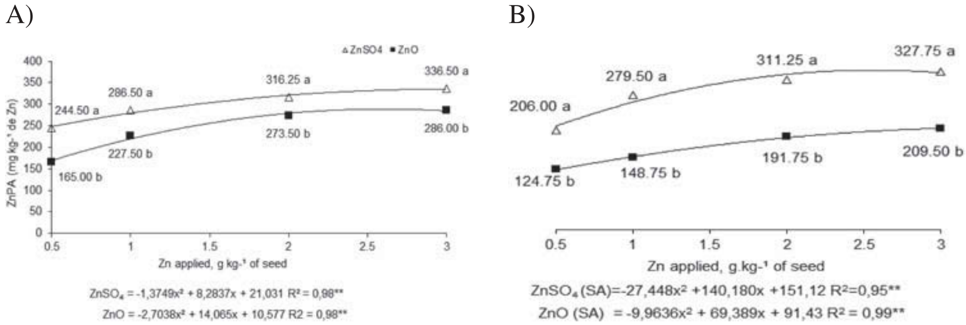

Figure 6. Zinc accumulated in aerial part without (A) and with salicylic acid (B).

induces the antioxidative defenses system, which improves the activity of the enzyme SOD and also reduces the permeability of the seed membranes allowing less loss of ions (Sakr and Arafa 2009).

Conclusions

Treating seeds with Zn and SA does not affect germination of corn.

The toxicity of Zn is more pronounced when the sulfate source is used, so that it reduces root and shoot length.

The source oxide does not affect the dry mass of the plants when corn seeds are treated with this source, especially at higher doses (2 and 3 g de Zn per kg^{-1} of seeds).

Despite the benefits of SA, there was a reduced zinc accumulation in the plant and was not effective in reducing root zone toxicity and shoot development when used in maize seed treatment, regardless of the source and dose used.

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