FUNDAMENTAL RESEARCH

Germination and vigor of lettuce seeds (Lactuca sativa L.) pelleted with homoeopathic preparations Alumina and Calcarea carbonica subjected to toxic levels of aluminum**

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Background: Aluminum toxicity is the most important factor limiting the growth of plants in acid soils, whereas current treatments are unfeasible. For this reason, alternatives are sought for, among which homoeopathic treatment.

Aims: This study aimed at evaluating the influence of homoeopathic preparations *Alumina* 6cH, *Alumina* 12cH, *Calcarea carbonica* 6cH and *Calcarea carbonica* 12cH on the germination and vigor of lettuce seeds subjected to toxic levels of aluminum in paper-solution. At the same time, it was sought to develop a new procedure to apply homoeopathic preparations in plants (pelleting).

Methods: The statistical design was entirely randomized (CRD) with 6 treatments and 4 repetitions. Treatments included: 1) pelleted seeds/talc + *Alumina* 6 cH; 2) pelleted seeds/talc + Alumina 12cH; 3) pelleted seed/talc + *Calcarea carb* 6cH; 4) pelleted seeds/talc + *Calcarea* carb12cH; 5) pelleted seeds/talc + distilled water; 6) non pelleted seeds (control). Variables evaluated were: germination percentage (GP), germination speed index (GSI) and radicle length (RL).

Results: There was significant difference in GSI and RL – variables that reflect the vigor of seeds - between the samples treated with homoeopathic preparations and the controls

Conclusions: Homoeopathic preparations *Alumina* 6cH and 12cH and *Calcarea carbonica* 6cH and 12cH had significant effect on the vigor of lettuce seeds subjected to stress conditions.

Keywords: aluminum toxicity; homoeopathic preparations; lettuce; germination; vigor; pelleted seeds

Introduction

Aluminum toxicity is the most important limiting factor for the growth of plants in acid soils.[1] It is held that the main effect of toxic levels of aluminum is a reduction in the rate of radicular growth of sensitive plants, since they affect the lengthening and division of cells.[2] This restriction decreases the plant's ability to take water and nutrients from the subsoil due to superficial rooting; in this way, it becomes less productive and more susceptible to drought.

To attempt to decrease this toxic effect in such soils correctives (calcareous compounds) and fertilizers are added in-depth. Nevertheless, the techniques currently available for this goal are unfeasible, partly because there is not yet a method to control the exchangeable aluminum at the sub-superficial level of soils, partly due to the cost of correctives and finally, and partly due to the large areas of soils presenting significant degrees of damaging acidity;[3] furthermore, these resources are not renewable, thus, they can be exhausted. For these reasons, new technologies are sought for.

Homoeopathy is one among such technologies; it was approved in Brazil by the Ministry of Agriculture and Supply in 2008.[4] The use of diluted and agitated substances in agriculture, especially in plants, is spreading fast. Notions and methods proper to

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**The article was originally published in International Journal for High Dilution Research, 2010; 9(33): 138-146. Article is reprinted with consent of the author and the publisher.

homoeopathy are used in several aspects of agriculture, including the quality of seedlings,[5] germination,[6] plagues control,[7] plants' diseases,[8] increase of active principles,[9] metal detoxification,[10] and plant metabolism .[11]

Homoeopathy has potential to contribute to sustainable agriculture, this is the management of resources in order to satisfy the ever-changing human needs and at the same time keep or improve the quality of the environment and conserve the natural resources.[12] Through self-regulation, plants respond with high intensity to homoeopathy when employed in stress conditions.[13]

Despite positive results, both in the academic and practical levels, not too much is known regarding the physiological mechanisms of action of homoeopathic preparations in plants, nor about the methods to employ them.

This study seeks to contribute to the development of a technology to facilitate the germination of plants in poorly favorable environments, as well as to the introduction of an innovative method to employ homoeopathic preparations, such as seeds pelleting. In this context, it was sought to evaluate the influence of homoeopathic preparations *Alumina* and *Calcarea carbonica* as seed pellets on the germination and vigor of seeds of lettuce subjected to toxic levels of aluminum in paper solution.

Materials and Methods

Choice of the homoeopathic preparations

Homoeopathic preparations Alumina 6cH, *Alumina* 12cH, *Calcarea carb* 6cH and 12cH were selected according their indications in plants.[14] *Alumina* is indicated in slow-growing plants; growth interrupted due to excessive absorption of aluminum; culture in soils rich in aluminum leading to the accumulation of toxic levels; culture in acid soils. *Calcarea carbonica*: indicated in plants sensitive to adverse conditions; slow production of roots; slow growth with late sprouting, slow germination, smaller seeds and higher rate of sterility.

Preparation of the homeopathic dilutions

Agitated dilutions of *Alumina* and *Calcarea carbonica* were prepared from homoeopathic matrixes in dilution 5cH (Schraiber) obtained at a homoeopathic pharmacy in Viçosa, Minas Gerais, Brazil in October, 2009; further dilutions were prepared at the Homoeopathic Laboratory of the Department of Phytotechnics at Federal University of Viçosa (DFT/UFV) according to the guidelines in the Brazilian Homoeopathic Pharmacopoeia.[15] Succussion was carried out through a "mechanic-arm" device model DENISE 10-50 (UTIC). Homoeopathic solutions *Alummina* 6cH and 12cH, and Calc 6cH and 12cH were prepared immediately before the pelleting of seeds.

Seeds

For this study it was used naked lettuce seeds brand Isla ® type "Regina de Verão", batch #22419 proceeding from Chile; germination percentage 91%; purity: 99.4%.

Pelleting of seeds

To pellet the seeds we used our own method of impregnation, observing the weight/volume ratio, as 1 gram of powder per ml of adhesive. Powder was Synth® inert pharmaceutical talc ($Mg_3Si_4O_{10}(OH)$; and adhesive corresponded to homoeopathic preparations Alum 6cH, Alum 12cH, Calc 6cH, Calc 12cH and distilled water. The latter were made impregnate the talc in a Petri dish diving rise to a semi-pasty, integral and white powder, which was later used to coat the seeds.

Determination of the toxic dose of aluminum

A pretest was carried out to establish the concentration of Al^{3+} that best made patent its toxic effect on seeds of lettuce *(Lactuca sativa L.)* through the method of paper-solution.[16] In this sense, the best behavior was observed with a concentration twice the tolerable levels of lettuce cultures, which bears about 3.0 cmmolc/dm^3 of Al^{3+}.[17] Consequently, it was used a solution corresponding to 540 mg/ l^{-1} of $AlCl_3.6H_2O$.

Experimental Design

The study was carried out at the Ultradilutions Laboratory of DFT/UFV, geographic coordinates 42° 52'W and 42° 50'W (longitude), and 20° 44'S and 20° 47'S (latitude). The statistical designed was entirely randomized (CRD), with 6 treatments and 4 repetitions; each experimental unit comprised 25 seeds. Treatments were: 1) pelleted seeds/talc + Alum 6cH; 2) pelleted seeds/talc + Alum 12cH; 3) pelleted seeds/talc + Calc 6cH; pelleted seeds/talc + Calc 12cH; pelleted seeds/talc + distilled water (control1); non-pelleted seeds (control 2).

All seeds were placed in a germination chamber Tecnal® model TE-401in gerbox boxes with germitest paper previously moistened by 8 ml of aluminum solution (540 mg/ l^{-1} of $AlCl_3.6H_2O$), kept at 20ºC, photoperiod 16 hours light and 8 hours dark, for 7 days. The tests of germination and vigor followed the guidelines and criteria established by the Ministry of Agriculture.[18] The

experiment was carried out in a double-blind design, using symbols to name each treatment in order to avoid potential interferences.

Evaluated variables

Variables assessed included: germination percentage (GP), radical length (RL); germination speed index (GSI). Data were subjected to variance analyses and means were compared through Tukey's test with 5% probability with software SAEG. GP was established on the 7th day after seeding, counting the total number of seeds in each lot; GP data were transformed into $\sin^{-1}\sqrt{x/100}$ RL was measured at the end of the study with a digital paquimeter, and the results were expressed in millimeters (mm). GSI was carried out following Maguire[19] as the addition of the ratio between the number of germinated seeds each day and the day of evaluation: IVG = G1/N1 + G2/N2 + G3/N3 + ... + Gn/Nn, where G1, G2, G3, ... , Gn = number of germinated seeds in the day of observation, and N1, N2, N3, ... , Nn = the number of days after seeding.

Results and Discussion

There were significant differences among treatments regarding the variables related to the vigor of seeds (GSI and RL), whereas there was no difference regarding GP, as shown in Table 1. Int J High Dilution Res 2010; 9(33): 138-146 141

Table 1. Table 1. Summary of variance analysis of germination percentage (GP), germination speed index (GSI) and radicle length (RL) in seeds of lettuce non-pelleted (control) and pelleted (with homeopathic preparations Alumina and Calcarea carbonica) subjected to toxic levels of aluminum

Variation source	Degree of freedom	Mean squares		
		GP	GSI	RL
Treatments	5	65,86ns	17,01**	445,32**
Residues	18	88	0,83	4,69
Total	23			
Variation coefficient (%)		10,99	8,29	11,65

(**) Significant at 1% probability respectively with f-test.
(ns) Non significant at 5% probability with f-test.

Treatment 5 (control 1) did not differ from non-pelleted Treatment 6 (control 2) in all 3 evaluated variables (Tables 2, 3) showing thus that the impregnating substance (pharmaceutical talc) had no interference effects between both controls and, thus, that the pelleting of seeds by itself, without homoeopathic preparations, produces no benefit.

Figures 1 and 2 illustrate the germination and vigor of lettuce seeds on the fourth day and seven

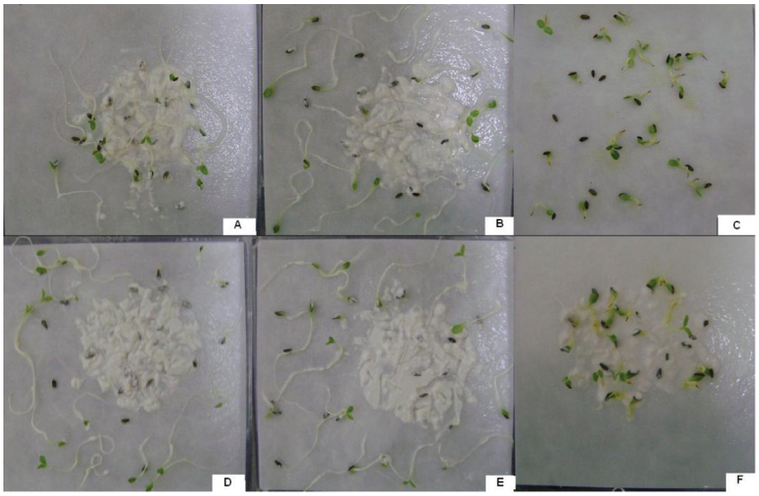

Figure 1 – Lettuce seeds, paper solution with toxic levels of aluminum (4th day): A) Treatment 3 (pelleted seeds/talc+Calc 6cH); B) Treatment 4 (pelleted seeds/talc+Calc 12cH); 6) Treatment 6 – control 2 (non-pelleted seeds); D) Treatment 1 (pelleted seeds/talc+Alum 6cH); E) Treatment 2 (pelleted seeds/talc+Alum 12cH); F) Treatment 5- control 1 (pelleted seeds/talc+distilled water).

days respectively after the mounting of the experiment. Regarding RL, Table 2 shows the significantly higher means of Treatments 1, 2, 3 and 4 by comparison with both control treatments, therefore, the effectiveness of low dilutions of Alumina and Calcarea carbonica on the physiological aspects of the seeds of lettuce. These results corroborate previously obtained ones,[20] where it was observed an increase in the weight of the dry mass of lettuce treated with homoeopathic preparation *Arnica montana* in dilutions 6cH and 12cH, which was

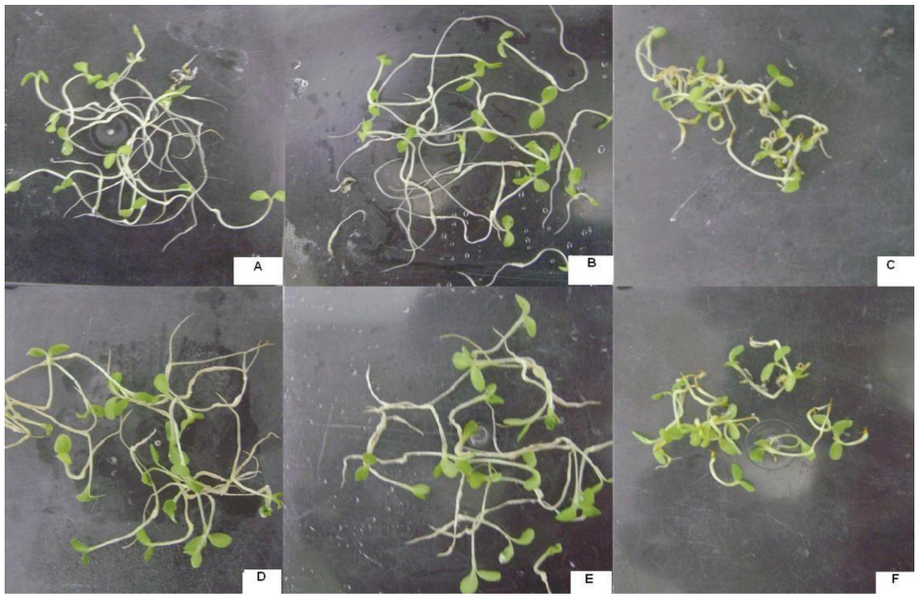

Figure 2 – Small plants resulting from this study, fnal evaluation (7th day). A) Treatment 3 (pelleted seeds/talc+Calc 6cH); B) Treatment 4 (pelleted seeds/talc+Calc 12cH); 6) Treatment 6 – control 2 (non-pelleted seeds); D) Treatment 1 (pelleted seeds/talc+Alum 6cH); E) Treatment 2 (pelleted seeds/talc+Alum 12cH); F) Treatment 5- control 1 (pelleted seeds/talc+distilled water).

not found with dilution 30cH.

Table 2 – Mean values of radicle length (RL) of lettuce seeds both non-pelleted and pelleted with homoeopathic preparations of *Alumina* and *Calcarea carbonica* subjected to toxic levels of aluminum.

Treatments	GSI
1. Pelleted seeds / Talc + Alum 6cH	25,43 a
2. Pelleted seeds / Talc + Alum 12cH	24,34 a
3. Pelleted seeds/ Talc + Calc 6cH	25,14 a
4. Pelleted seeds / Talc + Calc 12cH	24,61 a
5. Pelleted seeds / Talc + distilled water (control 1)	5,62 b
6. Non-pelleted seeds (control 2)	4,40 b

Means followed by at least one small-case letter in the line do not differ significantly between them in Tukey's test at 1% probability.

Regarding the germination speed index (GSI), Table 3 shows that the response of Treatments 1, 2, 3, 4 was similar to RL: all four differed from both controls; Alum 6cH and Calc 6cH obtained the highest means. This shows a positive effect of these homoeopathic preparations on seeds exposed to unfavorable conditions. A similar behavior was seen in plants of *Ocimum basilicum* L. (basil) intoxicated with copper sulfate; following treatment with *Cuprum metallicum* 30cH drastically reduced the effect of the intoxication on the development of the plants.

Table 3 – Mean values of germination speed index (GSI) of lettuce seeds both non-pelleted and pelleted with homoeopathic preparations of *Alumina* and *Calcarea carbonica* subjected to toxic levels of aluminum

Treatments	GSI
1. Pelleted seeds / Talc + Alum 6cH	12.38 a
2. Pelleted seeds / Talc + Alum 12cH	11.82 a
3. Pelleted seeds / Talc + Calc carb 6cH	13.05 a
4. Pelleted seeds / Talc + Calc carb 12cH	11.85 a
5. Pelleted seeds / Talc + distilled water (control 1)	8.17 b
6. Non-pelleted seeds (control 2)	8.60 b

Means followed by at least one small-case letter in the line do not differ significantly between them in Tukey's test at 1% probability.

Conclusions

This study showed that elleting, or the process of coating seeds, with homoeopathic preparations represents an effective alternative means to employ such dilutions in plants.

Homoeopathic preparations *Alumina* 6cH and 12cH, and *Calcarea carbonica* 6cH and 12cH had significant positive effects on the seeds of lettuce exposed to toxic levels of aluminum.

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