

## EFFECT OF HIGH DILUTIONS OF SODIUM CHLORIDE SOLUTIONS ON WHEAT GERMINATION - PRELIMINARY STUDY

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### Abstract

*The effect on germination and growth of high (homeopathic) dilutions on different plant species was studied since 1923, beginning with the experiments of Kolisko. Few other similar experiments with isopathic models are mentioned in the literature, illustrating the effects of homeopathic treatments on germination and growth of wheat, but they used mainly arsenic oxide (As<sub>2</sub>O<sub>3</sub>) as a stressor and then the same substance as therapy in a diluted form. Other studies were performed with sodium chloride as a stressor, but mainly on cowpea and beans. Our study used wheat in an experiment with sodium chloride as a stressor and various solutions of Natrium muriaticum (high dilutions of sodium chloride) as therapy. Different results were obtained regarding the number of germinated seeds and the length of the coleoptiles, showing that homeopathic dilutions of Natrium muriaticum have a different effect on the wheat germination and its growth, depending on the treatment dilution, opening a new field of research in the domain of abiotic stress in plants treated with high diluted medicines.*

**Key words:** wheat, germination, growth, abiotic stress, Natrium muriaticum.

### INTRODUCTION

High dilutions of medicinal substances have been used with success in the last two centuries, according to the principles of similarity, like cures like, in homeopathic medicine.

Homeopathic preparations are diluted and mechanically agitated (potentized) substances prescribed on the basis of the principle of similitude (like cures like), as stated in the textbooks of homeopathic medicine (Bungetzianu, Chirilă, 1983).

Three main types of scales are used, the decimal, the centennial and the 50-milesimal. The symbols used for these three scales are: D or X for decimal, C for centesimal and LM for 50-milesimal.

In the case of the decimal scale, dilutions are made in successive steps of 1/10 for each new dilution, in the centesimal scale, the proportion being 1/100 for each step and in the 50-milesimal dilution the proportion is 1 / 50000.

The dilution techniques are classified in two major categories, the Hahnemannian (symbol

DH, CH) and the Korsakovian (K) techniques. Sometimes the Hahnemannian dilutions are simply named by letters D or C. For Hahnemannian technique, dilutions are done using a new vial at each new dilution step, and in the case of the Korsakovian method, using a single vial, which is emptied and refilled at each new dilution step, approximating that the liquid remaining on the vial walls would represent 1/100 of the initial volume contained therein, and would always be filled with diluents up to the starting level.

Homeopathic medicines usually have an initial tincture called mother tincture, which is most commonly obtained by maceration, in case of plants. The most widely used solvent is concentrated alcohol of 90 degrees to obtain a mother tincture, and for the subsequent dilutions alcohol of 45 degrees. For solids, deconcentration is initially carried out by trituration of the substance in a mortar with lactose powder. The proportion of mixtures will also be in the 1/10 or 1/100 scale (1 gram of useful substance is mixed with 9 grams of lactose for the decimal scale or 99 grams of

lactose for the centesimal scale). Homeopathic solutions may be used as such or in the form of lactose or sucrose granules impregnated with the initial solution.

Recent studies of electron microscopy HRTEM and FESEM show that all homeopathic dilutions, starting with centesimal 6 (CH 6) or 50-milesimal 1 / LM 1 dilution, contain nanoparticles with elements of the original substance, which place homeopathy in the domain of nanomedicine (Rajendran, 2015).

The effect on germination and growth of high (homeopathic) dilutions on different plant species was studied since 1923, beginning with the experiments of Kolisko.

A number of experiments on wheat are mentioned afterwards in literature, illustrating the effects of homeopathic treatments on germination and growth of wheat impaired by abiotic stress, as isopathic models, where a substance in high concentration is used as a stressor and then, as therapy, in a high homeopathic dilution.

The durum wheat (*Triticum durum* L.) seedling model stressed with arsenic trioxide and treated with the homeopathic preparation arsenic trioxide 45 X, is the most frequently investigated model in plants. This isopathic approach was introduced by an Italian team of researchers (Betti et al., 1997). Other studies were performed with sodium chloride as stressor, but mainly on other species of plants. Different researchers concluded that salinity has a negative impact from the initial stages of seed germination and seedling growth, it affects the physiological and biochemical processes in mature plants (Nawaz et al., 2012) and among abiotic stress factors, it is one of the most important in many arid and semiarid regions (Ibrahim, 2016).

In 2005, Tighe studied the effect of *Natrium muriaticum* 12 CH, 18 CH and 24 CH on Cress (*Lepidium sativum*) germination and growth, after the plant was stressed with a sodium chloride solution 1%, by watering in the solution for 16 hours. This type of experiment is known as the sodium chloride cress seedling model. After 96 hours of incubation, germination decreased with potency levels 12 CH and 24 CH, while the 18 CH potency had no significant effect. Seedling growth showed a

trend towards inhibition also with 12 CH and 24 CH.

*Natrium muriaticum* in 6 CH and 30 CH dilutions, compared with NaCl 5% was also studied on *Phaseolus vulgaris* and it was concluded that *Natrium muriaticum* had a significant effect of stimulating the growth, especially in 6 CH dilution (Lensi et al., 2010). Another model consisted of seeds of *Vigna unguiculata* pre-treated with *Natrium muriaticum* and then stressed with NaCl. In the pretreated group the germination was increased, when compared with the control group, stressed with the same substance (Mondal et al., 2012). Sukul et al., in 2012, showed that another remedy, Sepia 200 CH in 1:1000 dilution counteracts the effect of salt stress in cowpea seedlings. In 2016, a study performed by Mondal also on cowpea seeds pointed out that potentized *Natrum muriaticum* 200 CH can be used with positive results on plants grown on brackish soils.

In 2015, Delian and Lagunovsci-Luchian studied the effects of saline stress on germination and vigour of primed *Daucus carota* L. seeds and concluded that potassium phosphate and ascorbic acid can be used as an alternative priming treatment to obtain higher percentage of germination and early vigorous seedling growth under saline stress conditions. Other interesting studies concerning priming and salinity are quoted by Delian et al., in 2017, and describe the germination rate of tomato seeds after priming with sodium chloride and gibberellic acid (GA), in a study performed by Nakaune et al. (2012) who report values of 4.9 and 4.6 times higher at 36 hours after sowing compared to hydro-primed seeds, with endogenous abscisic acid levels being similar after sowing. The results suggest that the effect of sodium chloride is produced by an increase of the GA4 content via GA biosynthetic genes activation, with increase in the expression of genes related to endosperm cap weakening.

Recently, in September 2017, Sarkar et al. demonstrated that *Natrum muriaticum* 30 CH and 200 CH, as well as the high concentration of sodium chloride solutions produce biological effects, initiating their action on specific binding sites of a protein, like bovine serum albumin (BSA). Repetition of the same dose of

the drug produces an increasing saturation of the binding sites of the protein.

Agrohomeopathy is a novelty in Romania both for plant protection, growth and plant immunity. Our study is the first experiment of this kind, exposing a variety of wheat to a moderately high salinity level and observing the effects of different treatments with *Natrium muriaticum* on germination and growth, comparatively with the control samples.

## MATERIALS AND METHODS

The wheat Glossa variety (*Triticum aestivum* L.) was used in the experiment. The wheat is the most frequently used model in agrohomeopathic research, due to the easy possibility to observe its germination and growth in a relatively short time, in normal or controlled modified conditions.

The experiment took place in a natural environment (in the laboratory) and the growth chamber of USAMV Bucharest, for two different variants of study, at 23°C, 60% relative humidity each and a 12/12 h day/night regime in the growth chamber and 10/14 h day/night regime for the natural conditions, in November, 2017.

We used ten sterile Petri dishes with sterile sand, 10 seeds of wheat in each Petri dish. The seeds and the sand were wetted with 10 ml of a sodium chloride solution 4g/l and immediately afterwards we treated them with 10 different solutions, which were numbered from 1 to 10 (so that until the end of the experiment nobody knew which solution was used for the dishes). Each Petri dish was wetted with 5 ml of a different solution (*Natrium muriaticum* D6, D12, C5, C7, C9, C15, C30, C200 and two variants of spring water, one of them being mechanically agitated before the use, corresponding with the succusions made for the CH 7 potencies). The homeopathic dilutions of *Natrium muriaticum* were obtained from the pharmacy, being produced by Plantextrakt, Romania. The salt for the sodium chloride solution was taken from Naturalia, Bucharest (salt without Iodum).

One variant consisting in 10 dishes was placed in the growth chamber (Figure 1) and the other one in a natural environment (Figure 2). Each day afterwards we observed the rate of

germination and the length of the shoots, on a period of 9 days.



Figure 1. Petri dishes in the growth chamber



Figure 2. Petri dishes in a natural environment

The correspondence between the numbers and the different solutions applied was written and kept for the evaluation of results, being the following:

1. *Natrium muriaticum* CH 30;
2. Spring water (Borsec) as control;
3. *Natrium muriaticum* CH 7;
4. *Natrium muriaticum* CH 9;
5. *Natrium muriaticum* CH 15;
6. *Natrium muriaticum* CH 5;
7. *Natrium muriaticum* CH 200;
8. *Natrium muriaticum* D12;
9. *Natrium muriaticum* D6;
10. Potentized (mechanically agitated) spring water CH 7 as additional control.

## RESULTS AND DISCUSSIONS

### Synthesis of previous agrohomeopathic experiments

Till present time, other researchers used mainly arsenic oxide ( $As_2O_3$ ) as a stressor for the germination and growth of wheat and then the

same substance as therapy in a diluted form (Table 1).

Table 1. Overview of bioassays of wheat with abiotic stress (after Jager et al., 2011)

Author	Stress	Treatment	Work variable
Auquierie et al., 1988	NaCl, CuCl, K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	DH NaCl, DH CuCl, DH K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	Shoot growth; fresh and dry weight of shoots, grains and roots
Kovac et al., 1991	As <sub>2</sub> O <sub>3</sub> 0.1%	DH As <sub>2</sub> O <sub>3</sub> , PC	Shoot growth
Betti et al., 1997	As <sub>2</sub> O <sub>3</sub> 0.1%	DH As <sub>2</sub> O <sub>3</sub> , PC	Germination
Brizzi et al., 2000	As <sub>2</sub> O <sub>3</sub> 0.1%	DH As <sub>2</sub> O <sub>3</sub> , PC	Germination
Brizzi et al., 2002	As <sub>2</sub> O <sub>3</sub> 0.1% 0.12%	DH As <sub>2</sub> O <sub>3</sub> , PC	Shoot growth
Binder et al., 2005	As <sub>2</sub> O <sub>3</sub> 0.1%	DH As <sub>2</sub> O <sub>3</sub> , PC	Shoot growth
Brizzi et al., 2005	As <sub>2</sub> O <sub>3</sub> 0.1% 0.16%	DH As <sub>2</sub> O <sub>3</sub>	Germination, shoot growth
Lahnstein et al., 2009	NaCl, CuCl, K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	DH NaCl, DH CuCl, DH K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	Shoot growth; fresh and dry weight of shoots, grains and roots

D, C = decimal, centesimal potency; H = Hahnemannian potency; PC = potentized control (as additional control).

### The agrohomoepathic effect on wheat

The germination rate and the length of shoots responded differently to the treatments made with the different agrohomoepathic solutions applied, the highest growths in day 9 being observed for CH7 and CH15 as illustrated in Figure 3 and Figure 5.



Figure 3. Length of wheat shoots treated with CH7, CH15 and D6 in growth chamber and natural environment

Examining the evolution of germination, the D6 dilutions led to no germinated wheat seed in the growth chamber and 70% germination in natural environment, still, all these with the lowest growth from all samples afterwards (Figure 4).

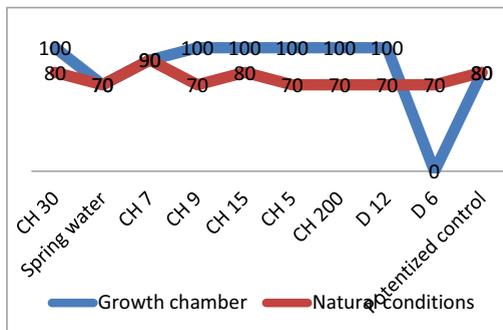


Figure 4. The germination percentage of wheat seeds in growth chamber and natural environment

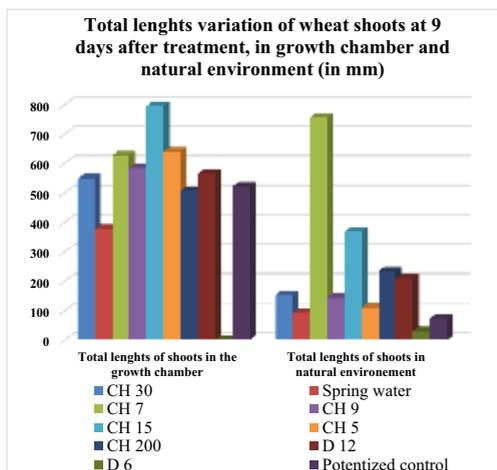


Figure 5. Length of wheat shoots in growth chamber and natural environment (in mm)

As it can be seen in the figure 4, the growth of seeds was also not the same in the different samples and in both variants we noticed an increase in the growth of shoots in samples 3 and 5 (CH 7 and CH 15). The worst results were noticed in the sample 9, in both variants, where *Natrium muriaticum* D6 was used. Seeds from control samples had a modest growth, comparatively with other samples, with the exception of sample 9, where we noticed the smallest growth in the natural environment or no growth present in the growing chamber. The

sample with water CH 7 had a better tendency of germination and growth when compared with sample 2, where simple water was used, in our study, a fact which was also pointed out by Brizzi et al., in 2000 and 2011 (agitated/succused water has better effects on germination when compared with simple water), but is infirmed by other researchers (Baumgartner et al., 2008; Scherr et al., 2009). The D6 dilution is considered a low homeopathic dilution and it is possible that the low dilutions have inhibitory effects when compared with higher ones.

## CONCLUSIONS

The study points out that homeopathic treatments have a certain effect on plants, confirming other studies from literature, depending on the dilution which was used. In our study, low dilutions like D 6 have an inhibitory effect on germination and growth, while higher dilutions stimulate germination and growth. Our present work has to be confirmed by other researches, but it can open a whole domain to be explored, when abiotic stress in plants is concerned. It is also important to take into consideration the possibility to intervene with homeopathic treatments in the case of brackish soils.

Another interesting domain concerns priming solutions tested and used as hydropriming, osmopriming, chemopriming etc. to overcome the action of stressors and we have in view in future to use dilutions of *Natrium muriaticum* as priming solutions to test the effects on germination and growth of wheat, as the next step of our researches.

## REFERENCES

- Baumgartner S., Shah D., Schaller J., Kämpfer U., Thurneysen A., Heusser P., 2008. Reproducibility of dwarf pea shoot growth stimulation by homeopathic potencies of gibberellic acid. *Complementary Therapies in Medicine*, 16: 183-191, PubMed.
- Betti L., Brizzi M., Nani D., Peruzzi M., 1997. Effect of high dilutions of *Arsenicum album* on wheat seedlings from seed poisoned with the same substance. *Br Hom J*, 86 (April): 86-89.
- Brizzi M., Nani D., Peruzzi M., Betti L., 2000. Statistical analysis of the effect of high dilutions of arsenic in a large dataset from a wheat germination model. *Br Homeopath J*, 89 (2): 63-7.
- Brizzi M., Biondi C., Lazzarato L., Betti L., 2002. Analisi esplorativa del effetto di soluzioni ultramolecolari di triossido di Arsenico sullo sviluppo vegetativo in vitro di plantule di grano. *Statistica*, 62 (3): 515-522.
- Brizzi M., Elia V., Trebbi G., Nani D., Peruzzi M., Betti L., 2011. The efficacy of ultramolecular aqueous dilutions on a wheat germination model as a function of heat and aging time. *Evid based Complement Alternat Med*, 2011 696298, published online 2011 Feb 14. doi: 10.1093/ecam/nep217.
- Bungetzianu Gh., Chirilă P., 1983. Manual de homeopatie. Editura Medicală, București, p. 98-111.
- Delian E., Lagunovschi-Luchian V., 2015. Germination and vigour of primed *Daucus carota* L. seeds under saline stress conditions. *Romanian Biotechnological Letters*, 20 (5): 10833-10840.
- Delian E., Badulescu L., Dobrescu A., Chira L., Lagunovschi-Luchian V., 2017. A brief overview of seed priming benefits in tomato. *Romanian Biotechnological Letters*, 22 (3): 12505-12513.
- Ibrahim E.A., 2016. Seed priming to alleviate salinity stress in germinating seeds. *Journal of Plant Physiology*. In press.
- Jager et al., 2011. Use of homeopathic preparations in experimental studies with abiotically stressed plants. *Homeopathy*, 100: 275-287.
- Kolisko L., 1923. *Physiologischer und physicalischer Nachweis der Wirksamkeit kleinster Entitäten*. Verlag der Kommende Tag AG, Stuttgart.
- Lensi M.M., Siqueira T.J., Silva G.H., 2010. A pilot study of the influence of *Natrum muriaticum* 6 CH and 30 CH in a standardized culture of *Phaseolus vulgaris* L. *Int J High Dilution Res*, 9(30): 43-50. Available from: <http://www.feg.unesp.br/~ojs/index.php/ijhdr/article/view/380/417>.
- Mondal S., 2016. Agrohhomeopathy- new practice in agriculture from seed germination to field trial. *Agrotechnol (Suppl)*, 5: 2.
- Mondal S., Sukul N.C., Sukul S., 2012. *Natrum mur* 200c promotes seed germination and increases total protein, chlorophyll, rubisco and sugar in early seedlings of cowpea under salt stress. *Int J High Dilution Res*, 11: 128.
- Nakaune M., Hanada A., Yin Y.G., Matsukura C., Yamaguchi S., Ezura H., 2012. Molecular and physiological dissection of enhanced seed germination using short-term low-concentration salt seed priming in tomato. *Plant Physiology and Biochemistry*, 52: 28-37.
- Nawaz A., Amjad M., Jahangir M.M., Khan S.M., Cui H., Hu J., 2012. Induction of salt tolerance in tomato (*Lycopersicon esculentum* Mill.) seeds through sand priming. *Australian Journal of Crop Science*, 6 (7): 1199-1203.
- Rajendran E.S., 2015. *Nanodynamics*. Mohna Publications, Kerala, India, 31: 43-242.
- Sarkar T., Konar A., Sukul N.C., Sukul A., 2018. High and Ultra Low Concentrations of Sodium Chloride Initiate their Action on Binding Sites of a Protein. *Environment and Ecology*, 36 (1A): 209-213.

- Scherr C., Simon M., Spranger J., Baumgartner S., 2009. Effects of potentised substances on growth rate of the water plant *Lemna gibba* L. *Complementary Therapies in Medicine*, 17 (2): 63-70, PubMed.
- Sukul S., Mondal S., Sukul N.C., 2012. Sepia 200 CH in 1:1000 dilution counteracts the effect of salt stress in cowpea seedlings but vehicle 90% ethanol proves ineffective in the same dilution. *Int J High Dilution Res*, 11: 237-240.
- Tighe M., 2005. Homeopathy on cress - a preclinical method development using a simple plant growth model. Thesis, University of Westminster, London, UK.