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Wheat and ultra high diluted silver nitrate – further experiments and re-analysis of data

Waltraud Scherer-Pongratz, Peter Christian Endler*, Harald Lothaller and Saundra Stephen

Interuniversity College for Health and Development Graz/Castle of Seggau, Austria

Background: **Since 1926, an influence of a dilution of silver nitrate (24x) on the growth of coleoptiles of wheat seedlings was described. The aim of the study discussed here is the critical proof of the reliability of a test system which has been quoted as a basic model for the research on homoeopathy for decades.**
Methods: **Grains of winter wheat (*Triticum aestivum*) were observed under the influence of extremely diluted silver nitrate (10^{-23}) prepared by stepwise dilution and agitation according to a protocol derived from homeopathy ('24x'). Analogously prepared water and/or inert water was used for control. Thirty experiments including 5000 + 5000 grains were performed by 5 researchers.**
Results: **Stalk lengths clearly indicate that development is enhanced by the probe silver nitrate 24x as compared to control. When the experiments 1989–1995 were pooled, means and SD for silver nitrate 24x-groups were 42.3 ± 26.9 mm and for water control groups 34.7 ± 22.2 mm. Verum stalk length was 21.9% bigger than control (100%) ($p < 0.01$; $d = 0.31$, i.e. small). For the experiments 1998–2014, means and SD were 73.7 ± 21.7 mm and 60.5 ± 20.9 mm. Verum stalk length was 21.7% bigger than control (100%) ($p < 0.01$; $d = 0.62$, i.e. medium). From the results one may hypothesize that the result is more marked in experiments showing an average mean of stalk length between ca. 50 and 90 mm in contrast to smaller or bigger mean lengths.**
Conclusion: **The previous findings were confirmed by the results.** *Homeopathy* (2015) **104**, 246–249.

Keywords: Wheat; Silver nitrate; Ultra high dilution; Homeopathy

Introduction

Studies involving plants have turned out to be an interesting field to investigate 'homeopathically' prepared dilutions and ultra high dilutions (UHDs).[1,2] In UHD 1994,[3] experiments on the effect of a dilution of silver nitrate 24x, specially prepared according to the instructions for the preparation of homoeopathic remedies on the growth of coleoptiles of wheat seedlings were described. The team followed a historical protocol (Kolisko 1926,[4] see also the work by Pelikan and Unger[5]) and own preliminary experiments.[6] When the seedlings were watered with the dilution, a typical effect was found, silver nitrate 24x significantly enhancing development. This is opposite to the effect of silver nitrate in molecular dose, which inhibits life processes. Between 1994 and 2014, the study on silver nitrate 24x was repeated (for experiments 1998, see Pongratz *et al.*[7]).

*Correspondence: Peter Christian Endler, Interuniversity College for Health and Development Graz/Castle of Seggau, Austria.
E-mail: college@inter-uni.net
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Methods

Plants

Experiments were performed on wheat (*Triticum aestivum*, 1989–1998: Mephisto, 2003 and 2014: Capo variety) grain grown without herbicides or pesticides. Around 10% of the grains were ruptured, and these were removed prior to the experiment.

Researchers

Experiments were performed between 1989 and 2014 by different researchers (Table 1), further details have been published.[3,6,7]

Table 1 Experiment = experiment number referring to the sequence between 1989 and 2014; researcher = EB, E Bermadinger, Institute of Plant Physiology, Graz University; FV, F Varga, Institute of Plant Physiology, Vienna University; WS, W Scherer-Pongratz, Boltzmann Institute Graz and Interuniversity College Graz; CE, PC Endler, Boltzmann Institute Graz and Interuniversity College Graz; AN, A Nograsek, Department of Nature Preservation, County of Styria; year = year the experiment was performed in; N = number of grains per group

Experiment	*Researcher*	*Year*	*N*
1	EB	1989	200
2	EB	1989	200
3	EB	1989	200
4	FV	1989	200
5	WS	1994	180
6	WS	1994	120
7	WS	1994	150
8	WS	1994	140
9	WS/CE	1994	200
10	WS/CE	1994	200
11	WS	1998	120
12	WS	1998	240
13	WS	1998	200
14	WS	1998	200
15	WS	1998	200
16	WS	1998	120
17	WS	1998	120
18	WS	1998	200
19	WS	1998	180
20	WS	1998	120
21	WS	1998	160
22	WS	1998	120
23	WS	1998	160
24	WS	1998	200
25	CE	1998	210
26	AN	1998	100
27	WS	2003	100
28	WS	2003	200
29	WS	2014	200
30	WS	2014	200

Observed development

The initial development of stalks (coleoptiles) was observed after 5 days in experiments 1−10 and 24−30 and after 7 days in experiments 11−23.

Preparation of test solutions

The grains were observed under the influence of an aqueous solution 1:10^{24} part of the weight of the stock solution of silver nitrate (Merck), specially prepared according to 'homoeopathic' instructions. Following the original protocol of 1921,[4] the stock solution contained 10 mg silver nitrate per 1 ml distilled water, and it was diluted in distilled water in steps of 1:10. The solutions, including the stock solution, were agitated according to standardized instructions[3]: at every step a sterile bottle is partly filled with the dilution, and was pushed down at short regular intervals (30 times within 15 s, e.g. against a rubber impediment) to create mechanical shocks. The test dilution prepared in this way (10^{-25} part per weight) was called dilution silver nitrate 24x. Analogously prepared water (water 24x), was used for control, only in some experiments, the control was untreated water. Experiments have shown that differential treatment with water 'x' or with untreated water produces no differences.[8] For the experiments performed in 2014, the control was water 24x.

Independent solution coding

Control and verum were encoded by independent persons. All probes were applied blindly, codes were broken only after the data had been calculated.

Exposition to probes

The grains with the germination furrow downwards were put into glass dishes (diameter 11 cm), each containing 20 ml of the respective probe. Dishes were covered with 1000 ml glass vessels. Stalk lengths were measured after one week. The experiments were performed at room temperature apart from experiments 9−10 that were performed at 15 ± 1°C. Figures illustrating details of the procedure, as well as information on the reliable system homogeneity in negative system performance controls, have been published.[6] Positive control experiments showed inhibition of wheat development at high concentrations of silver nitrate (unpublished data). Most of the experiments were started at noon (8 a.m.−1 p.m.).

Data base

Two sets of dishes for the treatment with dilution silver nitrate 24x and control were always used for the experiments. Dishes were allocated in a stratified randomization process.[6] According to the experiment, 20−30 grains were put into one dish; the numbers of grains per dish were equal in each experiment. Each experiment consisted of two groups of 100−200 seedlings each (see Table 1).

Evaluation of the data

For a description of the data on stalk lengths, at the level of the 30 individual experiments, the statistical mean was used. SD of the mean was calculated at the level of grains, at the level of dishes and at the level of experiments. In order to avoid false positive results, analysis of variance was not calculated for the total of the pooled experiments. Statistical significance (p, analysis of variance) and average effect sizes (Cohen's d, standardized difference of means = absolute difference between means of verum and control group, divided by SD; an effect size >0.2 is regarded as small, >0.5 as medium and >0.8 as large) were calculated for the experiments 1989−1995 and for the experiments 1998−2014 separately.

Results for silver nitrate were also expressed as percent of water control normalized to 100%. At the level of single experiments, results were then represented graphically by zeroing the results of the water control groups and plotting the difference to the silver nitrate groups on the ordinate.

Correlation analysis was performed to investigate a possible correlation between the *average mean of stalk length per experiment and the size of the result (i.e. the difference between groups)*.

Results

More than 5000 grains per group were observed (see Table 1). Means of stalk length per experiment varied