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Original research and audit

A pilot statistical study with homoeopathic potencies of Arsenicum album in wheat germination as a simple model

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Abstract
A blind, randomized laboratory trial to study homoeopathic potencies of Arsenicum album on wheat germination is proposed as a simple model which allows a rigorous statistical analysis. The parametric tests show that the differences between the treatment groups cannot be explained as a mere effect of intrinsic seed variability.

KEYWORDS: *Arsenicum album;* Wheat; Seed germination; Poisson distribution

Introduction

Experimental studies testing homoeopathic treatments on biological systems are generally very difficult due to the complexity of the system itself and its interaction with the environment. Moreover, as there is no complete theory to explain the action of homoeopathic potencies on living matter, it is very hard to deal with the contradictory results sometimes obtained. For this reason we sought a simple model in order to relate directly any effect to the corresponding treatment. Experimental work with plants, as with other biological systems, has often yielded unreliable results, due to an insufficient number of replications or inappropriate use of statistical tools.[1] Most of the plant studies have involved seed germination,[2-5] a normal process probably already fully optimized. In our seed germination model, a number of potencies of *Arsenicum album* were used as growing medium and run in parallel. We chose *Arsenicum album* (As_2O_3) because of its highly toxic effect on plants. The compound is well known to have a depressant effect on plant growth and vitality, even in dilutions of $10^{-6.6}$ It is also known that monocotyledons, as compared to dicotyledons, are more sensitive to the action of arsenic compounds,[6] therefore wheat seed (Triticum durum) was used for our study.

The conceptual nucleus of this experiment was limited to testing whether the results can be explained as 'intrinsic variability' of the seed under examination, excluding the action of homoeopathic potencies attenuated beyond the Avogadro limit. To this end particular attention was given to the statistical analysis of our results.

Finally, we would point out that the main aim of this pilot study was to implement a methodology useful in further experiments.

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We therefore decided not to stress the seeds with previous arsenic treatment, so as to avoid introducing new variables.

Materials and methods

Trial design

Two experiments were carried out, the first in 1991/92 and the second in 1992/93. MEC variety wheat seed was selected for integrity and placed median groove upwards in two concentric circles on sterilized sand in 10 cm diameter Petri dishes. These were randomly distributed in a circle in a germination box with a wooden base and glass walls and cover that was mounted on an electrically driven plate rotating at 90 rpm, to obtain maximum homogeneity of experimental conditions, and kept at room temperature (20°C), in daylight and at a high humidity. Decimal potencies (23 to 45) of arsenic trioxide (As_2O_3—BDH Chemicals) were freshly made for each experiment, starting from 0.2 % mother tincture in distilled water; successive 'dilutions' were made with distilled water, with 70 vigorous impacts after each dilution stage. The same method was used to prepare distilled water potentized to 30x (*H_2O* 30x). We also tested arsenic trioxide 'diluted' to 10^{-30}, but non-potentized (As_2O_3 10^{-30}). All these preparations, used as growing media and utilizing the same sample of distilled water, were made at the same time and stored in a refrigerator at 4 °C.

In the first experiment we tested mother tincture (As_2O_3 0.4 %), *As_2O_3* 23x, *As_2O_3* 30x and distilled water (H_2O) as control; 45 ml of medium was pipetted into each dish without disturbing the seeds. Each treatment was in duplicate (2 dishes, 50 seeds each). The experiment consisted of 10 successive tests (4,000 seeds). Germinated seeds were counted every 48 hours using a blind protocol, and the final estimate given after 192 hours (8 days).

In the second experiment we tested 6 potencies of *As_2O_3* (23x, 25x, 30x, 35x, 40x, 45x), potentized distilled water (*H_2O* 30x), 'diluted' As_2O_3 (As_2O_3 10^{-30}) and distilled water (H_2O) as control. 20 ml of medium was pipetted into each dish and 3 dishes (33 seeds each) were prepared per treatment. The experiment consisted of 16 tests (9,504 seeds), alternating two groups of treatment samples, with the following scheme:

A) H_2O; *H_2O* 30x; *As_2O_3* 23x, 30x, 40x.
B) H_2O; *H_2O* 30x, As_2O_3 10^{-30}; *As_2O_3* 25x, 35x and 45x.

There was a week's break between two consecutive experiments. The count was done as in the first experiment, but every 12 hours, starting from the 36th hour and with the final estimate made at the 96th hour.

Statistical analysis

The percentage of germinated seed and the average germination time were assessed separately. We decided not to do a standard bioassay because of the particular nature of the homoeopathic potencies, in which the effect is not proportional to the potency, but should rather follow an oscillatory trend;[2,3] in fact, the different potencies could be regarded as different qualities of treatment.

Percentage of germinated seeds. We fitted a Poisson model to the number of non-germinated seeds, stopping our observations after four days. Taking the Poisson distribution of H_2O as the null-hypothesis, we studied the significance of the different treatment groups. Moreover, we tried to assess the possible seasonal effects on germination.

Average germination time. For both years we recorded the average and the standard deviation within each treatment and control group. Since the standard deviations were not so different, we did a parametric one-factor ANOVA to compare the different treatment effects on average germination time.

Results

Percentage of germinated seeds

In the first experiment the base solution of As_2O_3 (0.2%) completely inhibited seed germination. This treatment was therefore not repeated in the second experiment. We also

Treatment group	Germination after 4 days (%)	Germination after 8 days (%)	Difference
H_2O	96.5	98.4	1.9
As_2O_3 0.2%	0.0	0.0	0.0
As_2O_3 23x	96.6	97.4	0.8
As_2O_3 30x	97.6	98.7	1.1

TABLE 1. Percentage of germinated seeds in the first experiment (1991/1992)

found that germination was almost complete after 4 days (Table 1). Hence in the second experiment we planned to stop each test after 96 hours, shortening the interval between two subsequent observations.

Fig. 1 shows the global percentage trend of germinated seeds as a function of time. Combining all the data of the treatment groups and control, we distinguished 2 seasonal periods: the final percentage after 4 days observation was almost the same for the 2 periods, but in the late winter period (February to March) the germination time was less than in early winter (November to January).

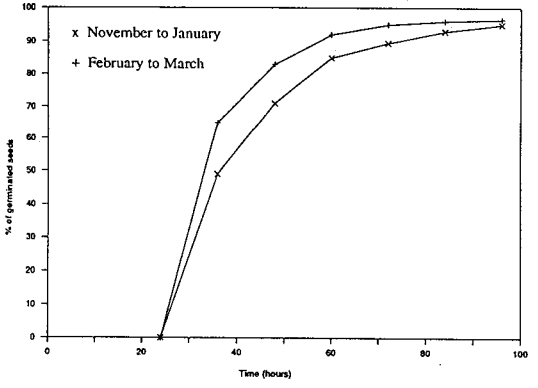

FIGURE 1. Global percentage trend of germinated seeds as a function of time

Table 2 shows the final percentages of germinated seeds for both experiments. In the 2nd and 4th columns the control group percentages are given with a base of 100, and the other data with respect to this new scale. It can be seen that the figures referred to As_2O_3 23x and 30x, the only repeated treatment groups, are almost the same in both experiments with respect to the latter scale.

In order to make a parametric statistical analysis of our data, we fitted the number of non-germinated seeds of each treatment group with a Poisson distribution, which assigns to any non-negative integer number x the following probability:

$$P(x) = \frac{e^{-\lambda}\lambda^{x}}{x!}$$

where λ denotes a parameter which is, at the same time, the distribution mean and variance. This was done only for the second experiment, in which we had more data to compare (Table 3). We first of all considered the distilled water group. The Poisson parameter λ is well estimated by the sample mean, the average number of non-germinated seeds. Referred to 33 seed groups, the control group mean was 1.79.

Table 4 shows the experimental results and the theoretical frequencies, together with the corresponding cumulative frequencies, which give the distribution function. The values observed are very close to the theoretical ones. Kolmogorov distance, which gives the greatest differences between two distribution functions (here, observed and theoretical dfs.), is 3.5 per cent, corresponding to less than 2 observations (the total sample size was 48). Fig. 2 indicates the theoretical and observed cumulative frequencies.

The same fitting procedure was used for the other control groups. Table 5 shows the estimated value of λ and the Kolmogorov distance for each control group. It can be seen that the other 2 control group data fit a Poisson model even better than the distilled water group data.

At this point we applied the global test for several Poisson distributions,[7] in order to compare the different treatment and control groups simultaneously. We decided to reduce the number of classes by considering only H_2O as a control group and merging As_2O_3 40x and 45x, which are similar and show the same average, as can be seen in Table 3. Such classes are well-distinguished and internally homogeneous. The null hypothesis is that all the Poisson distributions involved have the same λ parameter. Denoting by k the number of classes (here $k = 6$), we can compute the $\hat{\chi}^2$ statistics, as shown in Sachs,[7] that—if the null hypothesis holds—follows a chi-squared distribution with $k - 1$ (in this case, 5) degrees of freedom (d.o.f.). Since the observed value $\hat{\chi}^2 = 11.88$ is greater than the 95th percentile of the distribution with 5 d.o.f., say 11.07, we can reject the null hypothesis with a significance level less than 5%. Once proved that there is a significant effect of the prefixed potencies on the number of non-germinated seeds, we can reasonably make paired comparisons between con-

Treatment group or control	1st experiment		2nd experiment	
	germ. seeds (%)	control = 100	germ. seeds (%)	control = 100
H_2O (control)	96.5	100.0	94.6	100.0
H_2O 30x	—	—	96.0	101.5
As_2O_3 10^{-30}	—	—	95.5	101.0
As_2O_3 23x	96.6	100.1	95.1	100.5
As_2O_3 25x	—	—	96.7	102.2
As_2O_3 30x	97.6	101.1	96.0	101.5
As_2O_3 35x	—	—	95.2	100.6
As_2O_3 40x	—	—	96.8	102.4
As_2O_3 45x	—	—	96.8	102.4

TABLE 2. Percentage of germinated seeds after 4 days (in both experiments) and comparison with H_2O control group, supposed equal to 100

Treatment group or control	No. of tests	No. of non-germinated seeds		Significance vs H_2O
		total	mean	
H_2O (control)	48	86	1.79	—
H_2O 30x	48	63	1.31	< 1%
As_2O_3 10^{-30}	48	71	1.48	< 5%
As_2O_3 23x	24	39	1.62	NS
As_2O_3 25x	24	26	1.08	< 1%
As_2O_3 30x	24	32	1.33	< 5%
As_2O_3 35x	24	38	1.58	NS
As_2O_3 40x	24	25	1.04	< 1%
As_2O_3 45x	24	25	1.04	< 1%

TABLE 3. Number of non-germinated seeds in each treatment group

Value of x	Observed frequencies		Poisson frequencies		Distance
	$f(x)$	$F(x)$	$f^*(x)$	$F^*(x)$	$\lvert Fx) - F^*(x)\rvert$
0					
0	0.146	0.146	0.167	0.167	0.021
1	0.354	0.500	0.298	0.465	0.035
2	0.229	0.729	0.266	0.731	0.002
3	0.167	0.896	0.161	0.892	0.004
4	0.062	0.958	0.070	0.962	0.004
5 or more	0.042	1.000	0.038	1.000	0.000

TABLE 4. Observed and theoretical Poisson frequencies for the number x of non-germinated seeds., where $f(x)$ = single point frequency and $F(x)$ = cumulative frequency

Control	Est. of (i.e. x)	Kolmogorov distance d_K	N of observations corresp. to d_K $n\,d_K$ (here, $n = 48$)
H_2O	1.79	0.035	1.68
H_2O 30x	1.31	0.019	0.91
As_2O_3 10^{-30}	1.48	0.018	0.86

TABLE 5. Fitting observed frequencies with a Poisson distribution: comparison between control groups

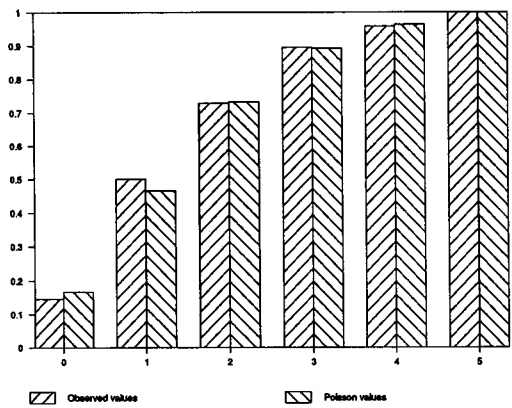

FIGURE 2. Observed cumulative frequencies of the control group (H2O) and corresponding Poisson distribution frequencies (x-axis: no. of non-germinated seeds in a single experiment; y-axis - frequency)

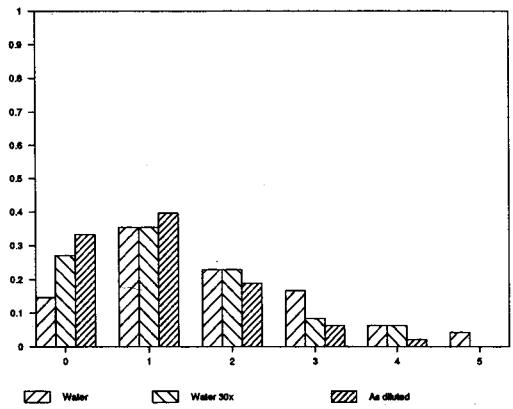

FIGURE 3. Observed frequencies of each number of non-germinated seeds for H_2O, H_2O 30x and As_2O_3 diluted groups (x-axis: no. of non-germinated seeds in a single experiment; y-axis - frequency)

trol and treatment groups.

Let us consider the 6 treatment groups. Under the hypothesis that these groups had the same distribution as the distilled water group, i.e. a Poisson distribution with $\lambda = 1.79$, the total number of non-germinated seeds in 24 independent tests would be a Poisson with parameter $\lambda = 24 \cdot 1.79 = 43$. Therefore, the probability that the number observed is less than 33 would be approximately 5%, whereas the probability of a value lower than 29 would be less than 1%. Comparing these limit values with the results in Table 3, we can state that 3 treatment groups (As_2O_3 25x, 40x, 45x) showed a significance level of less than 1% and another treatment group (As_2O_3 30x) was below 5% significance. The remaining groups (As_2O_3 23x and 35x) were not significant, but showed a smaller average number of non-germinated seeds than the distilled water group.

In order to compare the treatment groups versus H_2O 30x and As_2O_3 10^{-30} we combined As_2O_3 40x and 45x data which had similar potencies and the same average (Table 3), using the same method. When considering H_2O 30x as a control group, the 'double' treatment group (As_2O_3 40x + 45x) was the only one that showed a significance of exactly 5%; when the control reference was As_2O_3 10^{-30}, the double treatment had a significance less than 1%, and for the As_2O_3 25x group significance was less then 5%. The other treatments were not significantly different to the control groups. Fig. 3 and Fig. 4 show a histogram which represents the observed frequencies of each number of non-germinated seeds for the 3 control groups (H_2O, H_2O 30x and As_2O_3 10^{-30}) and for the 3 'best' treatment groups respectively.

Finally, we tried to check the significance

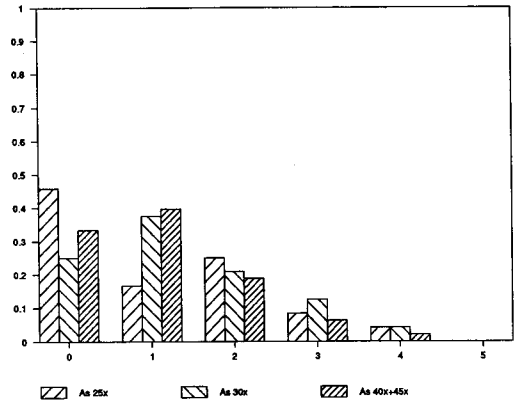

FIGURE 4. Observed frequencies of each number of non-germinated seeds for As_2O_3 25x, 30x and 40x + 45x. (x-axis: no. of non-germinated seeds in a single experiment; y-axis - frequency)

of H_2O 30x and As_2O_3 10^{-30} with respect to distilled water. Using normal approximation (the sample size was large enough to allow it) we obtained a significance of about 5% for As_2O_3 10^{-30} and less than 1% for H_2O 30x.

Average germination time

Table 6 shows the range of the averages in the different groups to be only 0.038, i.e. about one sixth of the common standard deviation. We performed a parametric 1-factor ANOVA, and the Fisher-test value was very far from the significance threshold.

In the second experiment with a greater number of treatment and control groups, and with a shorter interval between two different recordings, we obtained the scores reported in Table 7. In this experiment the first record was made after 36 hours instead of 48 hours, which might explain the differences between the averages of the two experiments. As can be seen, neither the means nor the standard deviations differ appreciably from group to group. In particular, the maximum range of the group average in Table 7 is equal to 0.079, which is about 40% of the standard deviation. Comparing the averages of the three control groups, they appear almost coincident. Nevertheless, the Fisher test was performed for the data of both tables, and we obtained very low values for the test statistic.

Treatment group or control	No. of tests tests	Mean (days)	Standard deviation
H_2O	20	2.239	0.236
As_2O_3 23x	20	2.222	0.226
As_2O_3 30x	20	2.201	0.238
Overall mean		2.221	0.233

TABLE 6. Statistical analysis of mean germination time in the first experiment

Treatment group or control	No. of tests	Mean (days)	Standard deviation
H_2O	48	1.847	0.221
H_2O 30x	48	1.843	0.207
As_2O_3 10^{-30}	48	1.844	0.219
As_2O_3 23x	24	1.841	0.192
As_2O_3 25x	24	1.906	0.286
As_2O_3 30x	24	1.898	0.184
As_2O_3 35x	24	1.901	0.275
As_2O_3 40x	24	1.827	0.209
As_2O_3 45x	24	1.836	0.240
Overall mean		1.856	0.231

TABLE 7. Statistical analysis of mean germination time in the 2nd experiment

Discussion

Regarding the percentage of germinated seeds, the experimental results show that differences between treatment groups cannot be explained as a mere effect of intrinsic seed variability. This statement was confirmed by parametric statistical methods (Table 3). The parametric approach was justified by the very close approximation of Poisson distribution to the 'number of non-germinated seeds' variable (Fig. 2, Tables 4 and 5). Observing Table 3, an oscillatory trend of the above-mentioned variable can be found as the Arsenicum album potency increases. We also tried to analyse the effect of the potentized distilled water (H_2O 30x) and As_2O_3 10^{-30} separately. It seems that potentized water has

a stronger positive effect (stimulation) on seed germination than the As_2O_3 'dilution'. Moreover, when the solution was potentized and diluted at the same time, this effect seemed stronger, but only for the 'right' potencies (specifically, As_2O_3 25x, 40x and 45x). This might be visualized as a vector composition of the two factors' effect, although these statements need further and more extensive experimental work for confirmation.

Concerning average germination time, we observed very close values of the standard deviation from group to group, and average values could be statistically compared; however, the mean values were not significantly different. This might be attributed to the bias due to difficulties and delay of the first recording (36 or 48 hours), rather than to a lack of effect on average germination time. Research is in progress on this topic.

The simple model we propose here seems to be particularly valid for studying homoeopathic potencies, especially when the conditions for parametric test application hold, i.e. when the experimental variable follows a known distribution law (in our case, the Poisson law) and the number of observations is not too small.

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References

1 Rubik B. Report on the status of research on homoeopathy with recommendations for future research. *Br Hom J* 1989; **78**: 86.

2 Kolisko L. *Physiologischer und physikalischer Nachweis der Wirksamkeit kleinster Entitäten*. Stuttgart: Arbeitsgemeinschaft anthroposophischer Ärzte 1959.

3 Pelikan W, Unger G. Activity of potentized substances. *Br Hom J* 1971; **60**: 233 & 1972; **61**: 86.

4 Jones R, Jenkins M. Comparison of wheat and yeast as in vitro models for investigating homoeopatic medicines. *Br Hom J* 1983; **72**:143.

5 Pongratz W, Bermadinger E, Varga F, Endler PC. Revival of an experiment from 1924. Further botanical studies. *Br Hom J* 1993; **82**:127.

6 Berge H. *Phytotoxische Immissionen*. Berlin: Paul Parey 1963.

7 Sachs L. *Applied statistics*. A handbook of techniques. New York: Springer 1982.

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