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The Effects of a 45x Potency of Arsenicum album on Wheat Seedling Growth – a Reproduction Trial

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Key Words
Homeopathy · Isopathy · Ultra high dilution · UHD · Arsenic trioxide

Summary
Background: Independent replications of preclinical investigations of homeopathic potencies are rare. However, they are a necessary tool to determine the relevant factors modulating the effects of homeopathic potencies in preclinical systems. **Objective:** The goal of the present study was to reproduce a trial published in 1997. An Italian group of researchers investigated the effect of Arsenicum album 45x on the growth of wheat which had been previously poisoned with a material dose of Arsenicum album. The homeopathic treatment was associated with increased wheat shoot growth significantly different from the control group (+24%, $p < 0.001$). **Materials and Methods:** Wheat poisoned with a sublethal dose of Arsenicum album was cultivated in either Arsenicum album 45x, water 45x, or unpotentized water. After 7 days, shoot length was measured. Reproducibility was assessed in eight independent experiments. **Results:** Arsenicum album 45x significantly inhibited wheat shoot growth (–3%) compared to treatment with unpotentized water and water 45x ($p = 0.011$ and $p = 0.037$). Within the experimental series performed in this reproduction trial, the effects of Arsenicum album 45x proofed to be reproducible. The wheat seed species used did not seem to have a significant impact on the experimental outcome. **Conclusion:** The result of this replication trial is a reversal of the original study, since Arsenicum album 45x inhibited wheat shoot growth instead of enhancing it. Nevertheless, high homeopathic potencies may induce statistically significant effects in biological systems. However, the magnitude and direction of these effects seem to depend on yet unknown parameters.

Schlüsselwörter
Homöopathie · Isopathie · Ultrahohe Verdünnung · UHD · Arsentrioxid

Zusammenfassung
Hintergrund: Unabhängige Replikationen präklinischer Untersuchungen homöopathischer Potenzen sind selten, jedoch notwendig zur Bestimmung aller relevanten Faktoren, welche die Wirkung homöopathischer Potenzen in präklinischen Systemen beeinflussen. **Zielsetzung:** Das Ziel der vorliegenden Studie war die Reproduktion eines im Jahre 1997 veröffentlichten Experiments. Eine italienische Forschergruppe hatte den Einfluss von Arsenicum album D45 auf das Wachstum von Weizen untersucht, welcher mit einer stofflichen Dosis von Arsenicum album vorvergiftet worden war. Die homöopathische Behandlung war mit einer signifikanten Stimulierung des Sprosswachstums assoziiert (+24%, $p < 0{,}001$). **Material und Methoden:** Mit einer sublethalen Dosis von Arsenicum album vorbehandelter Weizen wurde entweder in Arsenicum album D45, Wasser D45 oder unpotenziertem Wasser kultiviert. Nach 7 Tagen wurde die Sprosslänge gemessen. Die Reproduzierbarkeit wurde in 8 unabhängigen Experimenten untersucht. **Ergebnisse:** Arsenicum album D45 induzierte eine Hemmung (–3%) des Sprosswachstums, verglichen mit der unpotenzierten bzw. potenzierten (D45) Wasserkontrolle ($p = 0{,}011$ bzw. $p = 0{,}037$). Innerhalb des Reproduktionsversuchs war der Effekt von Arsenicum album D45 reproduzierbar. Die untersuchten Weizensorten schienen keinen relevanten Einfluss auf das experimentelle Ergebnis zu haben. **Schlussfolgerung:** Das Resultat dieser Versuchsreplikation ist eine Umkehr der im Originalversuch beobachteten Effekte, da Arsenicum album D45 das Wachstum der Weizensprosse hemmte statt es zu fördern. Hohe homöopathische Potenzen können demzufolge in biologischen Systemen statistisch signifikante Effekte hervorrufen. Die Grösse und Richtung dieser Effekte scheinen jedoch von noch unbekannten Parametern abhängig zu sein.

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Introduction

One of the major challenges of homeopathic fundamental research today is the reproduction of preclinical studies that have shown a significant effect of highly diluted and potentized substances compared to control groups. Apart from animal intoxication models [1], we do not know any experimental study which could be successfully reproduced by an independent research team. Some studies successfully assessed external reproducibility by means of a multicenter design [2–4]. A number of published replication studies either failed to reproduce the effects seen in the original trial [5–14] or resulted in differing effects [15–19]. There might be general phenomena such as presumed chronobiological effects [20–22] or unidentified cross-contamination [23] that make homeopathic studies hard to reproduce. But many of the researchers trying to repeat a certain experiment were facing much more elementary problems, including documentation deficits of the original trial with regard to procedures, preparation of the potencies, etc.

In the context of the scientific exploration of a presumed mode of action of homeopathic potencies, reproduction of a study primarily serves as a tool for a better understanding of the conditions necessary to observe a certain phenomenon. However, we do not know the precise factors which could be critical for the action of a homeopathic preparation. Taking the protocol of a successful study and applying it to another context of time, space, climate and persons involved in the experiment is one way to learn more about conditions important for a homeopathic remedy's successful effects. In addition, reproduction may serve as a tool to identify false positive results of inappropriate experimental designs. Apart from that, establishing a new biological model appropriate for the investigation of homeopathic effects is a very time, money and energy consuming process. Taking this into consideration, it is highly beneficial to draw on experiences which have been previously gained with an established model.

The aim of the present study was to reproduce a work published in 1997 in the British Homeopathic Journal by an Italian group of researchers [24]. The purpose of this study was to determine the effect of high dilutions of Arsenicum album on wheat seedlings from seeds previously poisoned with the same substance. The authors reported a significant stimulating effect of Arsenicum album 45x on wheat shoot growth compared to a control group (pure water). Other parameters, such as growth of the primary and secondary roots, were not significantly influenced by Arsenicum album 45x treatment. In 2 other publications the group also demonstrated a stimulating effect of several arsenic potencies on wheat germination rates [25, 26] of poisoned as well as unpoisoned seeds. To our knowledge no independent researcher has ever tried to reproduce these remarkable findings. Independently of our own study, Betti et al. reassessed the results of their initial study [24] in another independent trial [27].

Materials and Methods

Design of the Original Trial

In the original setting [24], the seeds had been stressed with a sublethal material dose of Arsenicum album (0.1‰), which decreased shoot growth by approximately 50% compared to non-stressed seeds. Each seed was grown on a filter paper, to which it was fixed by a piece of clay. The paper was inserted in a cellophane envelope (12 × 20 cm) which was then placed into a cardboard envelope. This technique ensured that shoots and roots would grow in natural light and darkness. A total of 360 cardboard envelopes, each containing one seed, were placed on a board where the seedlings were cultivated for 7 days. 60 seeds had not been stressed before and were treated with pure water. 150 seeds were stressed and then treated with pure water. Another 150 seeds were stressed and then treated with Arsenicum album 45x. Applying a blind protocol, the length of the shoots and roots of the seedlings was measured every 24 hours from day 2 until day 7.

Design of the Present (Reproduction) Trial

Given the inevitable changes in the experimental setting already mentioned above (different laboratory, researchers, climate, etc.), it seemed advisable not to introduce any new variables into the study design. Thus, important materials such as wheat seeds (*Triticum aestivum L.*, varieties Pandas and Mec), pure water (p.A., Merck, Darmstadt, Germany) and Arsenicum album (BDH Chemicals, Poole, England) were used from the same source as in the original trial. Nonetheless, some aspects of the experimental set-up had to be changed for technical, statistical or practical reasons (table 1). First of all, the number of completed experiments differed, since we repeated the experiment 8 times modifying several parameters such as the wheat species (Pandas, Mec) and the age of the potencies (see below). As in the original trial, Pandas seeds were pre-treated by 30-min poisoning with a 1‰ As_2O_3 watery solution (p.A.) and subsequent rinsing with tap water for another 60 min. Seeds were allowed to dry in ambient air and stored in darkness until used in the experiments. Mec wheat variety seeds proved to be more resistant to As_2O_3 poisoning (see below), so additional treatment with 1.2‰ As_2O_3 solution was necessary to induce visible effects on growth and germination rate. Furthermore, the 60 non-stressed seeds treated with water were replaced by a control group of 150 plants which had been stressed with material arsenic before being treated with potentized water 45x. For the potentization of water, precisely the same instructions were followed as for the potentization of Arsenicum album. The intention was to rule out purely mechanical effects of potentization as the reason for the observed significant differences between the arsenic- and water-treated groups. Such non-specific effects caused by agitation could be an increase in ion content from the wall of the potentization vessel or higher carbon dioxide solubility with consequential alteration in pH-value. If such effects are relevant for wheat shoot development, they would lead to significant differences between water-treated groups and groups treated with potentized water.

After potentization, the 3 cultivation parameters were blinded and randomly allocated to 3 groups. The cardboard envelopes comprising the differently treated seeds were placed in cardboard boxes in a strict rotation sequence: 10 seeds of group 1 followed by 10 seeds of group 2 followed by 10 seeds of group 3 and so on (corresponding to the order in which they where planted). The open boxes allowed diffuse natural light to enter, which assured a day-night rhythm. The roots were covered by the lower part of the cardboard envelope allowing them to grow in the dark.

Due to the observation in the original trial that the growth of the roots was apparently not influenced by Arsenicum album 45x treatment, the present study focused on shoot growth. In the original trial the differences in shoot length between the groups tended to increase over time. The highest significance level was reached on day 7 of the growth period. A single measurement on day 7 allowed us to adopt a computerized measurement of the shoot length, as the plants could easily be photocopied

Table 1. Comparison between the experimental set-up of the original trial and the reproduction trial

Parameters	Original trial	Reproduction trial
Number of experiments	1	8 (plus 2 experiments with only pure water to monitor the stability of the system)
Poison	Arsenicum album (As_2O_3)	batch of original trial
Potentization medium	Water p.A., Merck	Water p.A., Merck
Intoxication of seeds	1‰ Arsenicum album, 30-min exposure, 60 min rinsing with tap water	1st series: compare original trial 2nd series: 2 consecutive intoxications with 1,2‰ and 1‰ Arsenicum album; exposure and rinsing according to original protocol
Number of seeds per experiment	360	450
Species of wheat	Mec	Pandas, Mec (5 experiments each)
Treatment groups	60 seeds, non-stressed, treated with water 150 seeds, stressed, treated with water 150 seeds, stressed, treated with Ars. alb. 45x	150 seeds, stressed, treated with water 45x 150 seeds, stressed, treated with water 150 seeds, stressed, treated with Ars. alb. 45x
Randomization	unknown	yes
Cultivation conditions	shoot: diffuse natural light root: darkness	shoot: diffuse natural light root: darkness
Arrangement of cardboard envelopes	on a board	in five boxes each containing 90 envelopes
Measured parameters	length of shoot, primary and secondary roots	length of shoot
Time of measurement	2nd, 3rd, 4th, 5th, 6th, 7th day of cultivation	7th day of cultivation
Blinding	yes	yes
Mode of measurement	manual	computerized by scanning photocopies of the plants
Potentization of As_2O_3 / water	single-glass-method, 100 ml polyethylene vessels, 70 beats against a firm base	multiple-glass method, 100ml polyethylene vessels, 70 beats against a firm base
Amount of potency per seed	3.2 ml	3.3 ml

Table 2. Schedule of the performed experiments

	E11	E12	E13	E14	E15	E21	E22	E23	E24	E25
Date	July 2001	Feb 2001	Oct 2001	Nov 2001	Dec 2001	Dec 2002	Dec 2002	Jan 2003	Jan 2003	Feb 2003
Type of experiment	Water	Pot	Pot	Pot	Pot	Water	Pot	Pot	Pot	Pot
Wheat species	Pandas	Pandas	Pandas	Pandas	Pandas	Mec	Mec	Mec	Mec	Mec
Starting point potentization	–	MT	40x E12	40x E12	40x E12	–	MT	40x E22	40x E22	MT

Type of experiment: Pot = potency treatment; Water = water-control experiment testing the stability of the experimental set-up. Starting point of fresh potentization: MT = mother tincture, 40x = 40x Arsenicum album and water, resp.

and subsequently scanned by means of the software Tracking (A. Fritschy, Informatik-Lösungen, Zürich, Switzerland).

As regards the method of potentization, the only difference to the original experiments consisted in the use of the (less controversial) multiple-glass method. The exact procedure (type of movement, number of beats, etc.) was adopted during a visit to the laboratory of M. Perruzzi (Milan, Italy) where the original trial was performed. Succussion was accomplished through 70 strong beats directed downwards against a firm base.

Experiments Performed

In some preliminary experiments, the effects of various material doses of Arsenicum album on germination rate and seedling growth were investigated. After that, 2 series of main experiments were carried out, both of them in Arlesheim near Basle, Switzerland (table 2). Two different species of wheat seeds were used – Pandas for the first series of 5 experiments (February 2001 to December 2001) and Mec for the second series of 5 experiments (December 2002 to February 2003). Mec wheat was used in the original trial, whereas the Italian group for germination rate experiments are currently using Pandas wheat since the Mec variety is no longer available. Thus, the importance of the wheat species for the experimental outcome could be estimated. For each experiment, aqueous potencies were freshly prepared on the day of the experiment, starting either from the mother tincture (1‰ As_2O_3 solution) or from a As_2O_3 40x potency produced for an earlier experiment, and intermediately stored in a refrigerator at 5–8 °C. Exactly the same procedure was adopted for the potentized water control (water 45x). The impact of an intermediate storage of homeopathic potencies was examined in this process. Furthermore, the stability of the experimental setting was tested. Sometimes experiments provide false positive results due to a systematic error associated with the experimental set-up. To rule out such phenomena, 2 experiments (E11, E21) were carried out using only pure water as treatment for all 3 groups. If the statistical analysis of these two experiments showed no significant differences between the 3 groups treated with pure water, it can be concluded that the experimental set-up is stable. That is to say that differ-

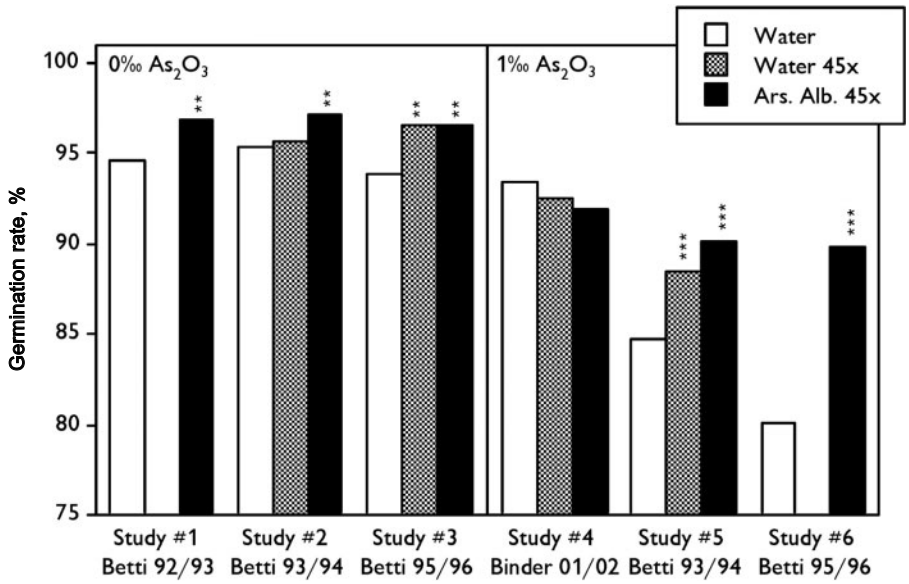

Fig. 1. Germination rate of wheat seedlings, treated with either unpotentized water, water 45x or Arsenicum album 45x in 6 different trials. Study 1: Betti et al., 1994 [25]; study 2–3: Brizzi et al., 2000 [26]; study 4: present study; study 5–6: Brizzi et al., 2000 [26]. Wheat seedlings were not pretreated in studies 1–3 and poisoned with 1‰ As_2O_3 in studies 4–6. Statistically significant differences to the unpotentized water control are indicated by *(1% < p < 5%), **(0.1% < p < 1%), ***(p < 0.1%). The number of seedlings per study was comparable.

ences occurring in experiments with homeopathic potencies are more likely to be attributable to the different treatments than to intrinsic features of the experimental set-up.

Statistics

With respect to wheat shoot length of germinated seedlings, statistical analysis was based upon parametrical analysis of variance (ANOVA). The two-step procedure of a preceding F-test for global significance (at the level $p < 0.05$) and following planned pairwise comparisons by the LSD-test (least significant difference test) yields optimal protection against type 1 and type 2 errors [28] and was therefore adopted in our study. Furthermore, this type of analysis is empirically robust against minor violations of its theoretical assumptions (normality, homogeneity of variances). Homogeneity of variances was assessed by the Levene test and normality of data distribution by the Kolmogorov-Smirnov test. As regards the germination rate, differences were assessed with the chi square test. For the wheat shoot length of all seedlings (germinated and non-germinated), the Kruskal-Wallis-ANOVA test and succeeding Mann-Whitney-U test for planned comparisons were used since these data sets showed strong non-normal distributions. All calculations were carried out with the software Statistica 4.1 (Statsoft Inc., Tulsa, OK, USA).

Results

Effects of Material Doses of Arsenicum album on Wheat Germination and Growth

Similar to the results of Betti et al. [24] with Mec seed variety, poisoning with 1‰ As_2O_3 proved to be the maximum dose that could be applied to Pandas wheat seedlings. Any higher concentrations of As_2O_3 led to a drastic decrease in the germination rate (<40%) rendering experiments virtually impossible (data not shown). Our treatment of Pandas wheat with 1‰ As_2O_3 led to a reduction in shoot length of –14.0% (mean I, n = 867). This is considerably less than was observed in the original trial (–58%). The effects on the germination rate were also less pronounced (fig. 1). Since any higher concentration of As_2O_3 for pre-treatment was impossible, the main experiments with homeopathic potencies were conducted with Pandas seeds poisoned with 1‰ As_2O_3. This procedure is also formally identical with the original trial.

The Mec seed batch we used proved to be even more resistant to As_2O_3 poisoning, since treatment with 1‰ As_2O_3 did not lead to a clear reduction of shoot length growth (97.5% compared to the unpoisoned control, n = 400). We thus decided to apply a second subsequent poisoning with 1.2‰ As_2O_3, leading to a visible reduction in shoot length (84.4% compared to the unpoisoned control) with the germination rate still being acceptable (95%). Equivalent to the results obtained with Pandas seeds, any higher As_2O_3 concentration led to a sharp decrease in the germination rate (<30% with 1.5–2.0‰ As_2O_3) making experiments practically impossible.

Water Control Experiments

Descriptive statistics of the results of the 2 water control experiments E11 and E21 are given in table 3. A diagrammatic representation of the results can be found on the left side of figure 2 (water control run). The F-test of ANOVA yields no significant global differences for the 2 experiments, either separately (E11: $p = 0.787$; E21: $p = 0.257$) or pooled ($p = 0.786$). The data distribution does not show deviations from normality for any of the experiments ($p > 0.05$, Kolmogorov-Smirnov test). Thus, the water control experiments revealed no significant differences between the 3 identically treated groups. The system was presumed to be stable.

Main Experiments

Descriptive statistics of all 8 experiments with homeopathic potencies are given in table 4. Non-normality was not observed in any of the experiments (Kolmogorov-Smirnov test). On average, skewness was –0.48 and kurtosis was 0.45. Thus,

Table 3. Descriptive statistics of the water-control experiments E11 and E21 testing the stability of the experimental set-up (mean, standard deviation, number of seedlings germinated)

Exp. No.	Parameter	Mean, mm	Std. Dev., mm	n
E11	water 1	85.15	25.86	131
E11	water 2	87.27	26.77	140
E11	water 3	86.81	26.52	137
E21	water 1	44.67	11.19	142
E21	water 2	44.34	10.79	141
E21	water 3	42.54	12.83	140

Table 4. Descriptive statistics of the potency experiments E12–E15 and E22–E25 (mean, standard deviation, number of seedlings germinated)

Exp. Nr.	Parameter	Mean, mm	Std. Dev., mm	n
E12	Ars. alb. 45x	51.52	15.48	133
E13	Ars. alb. 45x	76.66	21.23	135
E14	Ars. alb. 45x	48.04	16.56	141
E15	Ars. alb. 45x	48.94	15.83	133
E22	Ars. alb. 45x	43.21	13.31	141
E23	Ars. alb. 45x	43.08	11.82	139
E24	Ars. alb. 45x	44.81	12.82	136
E25	Ars. alb. 45x	48.50	13.24	140
E12	water	54.82	15.95	145
E13	water	79.34	19.36	131
E14	water	52.06	15.86	136
E15	water	48.33	17.30	139
E22	water	48.02	12.29	143
E23	water	41.63	13.79	142
E24	water	45.70	12.48	142
E25	water	48.20	14.38	140
E12	water 45x	57.91	16.37	138
E13	water 45x	74.17	23.58	139
E14	water 45x	48.44	14.95	137
E15	water 45x	50.93	16.81	140
E22	water 45x	47.70	11.73	138
E23	water 45x	45.26	11.52	139
E24	water 45x	43.49	11.98	134
E25	water 45x	47.84	15.04	144

deviations from normality were insignificant and were ignored for the subsequent parametrical ANOVA.

In order to calculate a global analysis of variance, data of all experiments performed in a comparable manner (E12–E15, E22–E25) were pooled. The following analysis was based on the shoot length measurements of all germinated seeds. A two-way ANOVA with the independent variables treatment (water, water 45x, Arsenicum album 45x) and experiment number (E12–E15, E22–E25) and the dependent variable shoot length was calculated. The F-test for the factor treatment was significant ($p = 0.026$, fig. 3a). Arsenicum album 45x treatment inhibited shoot growth significantly compared to water treatment ($p = 0.011$, LSD test) and compared to water 45x treatment ($p = 0.037$, LSD test). There was no significant difference between the treatment with water and potentized water 45x ($p = 0.650$, LSD test). This means a complete reversal of the effect observed in the original trial.

In figure 2, potency effects are diagrammed relative to the water control as a function of the number of the experiment. Since the interaction between the independent factors treatment and experiment number was also significant ($p = 0.0023$), some treatment effects were dependent on some factors associated with single experiments. If the parameter water 45x was omitted from the ANOVA – thus, comparing only the effect of Arsenicum album 45x with the unpotentized water control – the treatment effect still yielded significant results ($p = 0.010$), but the interaction did not ($p = 0.12$). Correspondingly, omission of Arsenicum album 45x from the analysis – thus, comparing only the effect of water 45x to the unpotentized water control – yielded no significant main effect for the treatment ($p = 0.65$), but a significant interaction ($p = 0.0037$). This means that the action of Arsenicum album 45x manifests itself in significant and reproducible reduction of shoot length by –3% relative to the unpotentized water control. The action of water 45x is, on average, equivalent to the unpotentized water control, but may result in significant effects ($p < 0.05$) for single experiments with opposite directions, such as shoot growth stimulation (E23) or depression (E13).

Wheat Species: Pandas versus Mec

A two-way ANOVA with the independent variables wheat species (Pandas, Mec) and treatment (water, water 45x, Arsenicum album 45x) and the dependent variable shoot length, normalized to the corresponding experimental mean, yielded – as was expected – a significant treatment effect, but no significant interaction between the effects of treatment and wheat species ($p = 0.74$). This means that the effect of the homeopathic treatment was comparable for the 2 wheat species, as the diagrammatic representation of the mean effects suggests (fig. 3b,c).

Effect of Intermediate Storage of Homeopathic Potencies

In the experiments E13–E15 and E23–E24, potencies were used which had not been produced freshly from the mother tincture (as was done in E12, E22 and E25) but from 40x potencies made for earlier experiments and intermediatel stored in a refrigerator at 5 °C (table 2). In order to analyze the effect of such storage of homeopathic potencies, we again calculated a two-way ANOVA with the independent variables storage (yes/no) and treatment (water, water 45x and Arsenicum album 45x) and the dependent variable shoot length, normalized to the corresponding experimental mean. Again, we found no significant interaction between storage and treatment effects ($p = 0.080$). There was a tendency, however, for potencies freshly produced from the mother tincture to show greater effects (Arsenicum album vs. unpotentized water: –5.1%) than potencies made from a preserved 40x potency (Arsenicum album vs. unpotentized water: –1.7%).

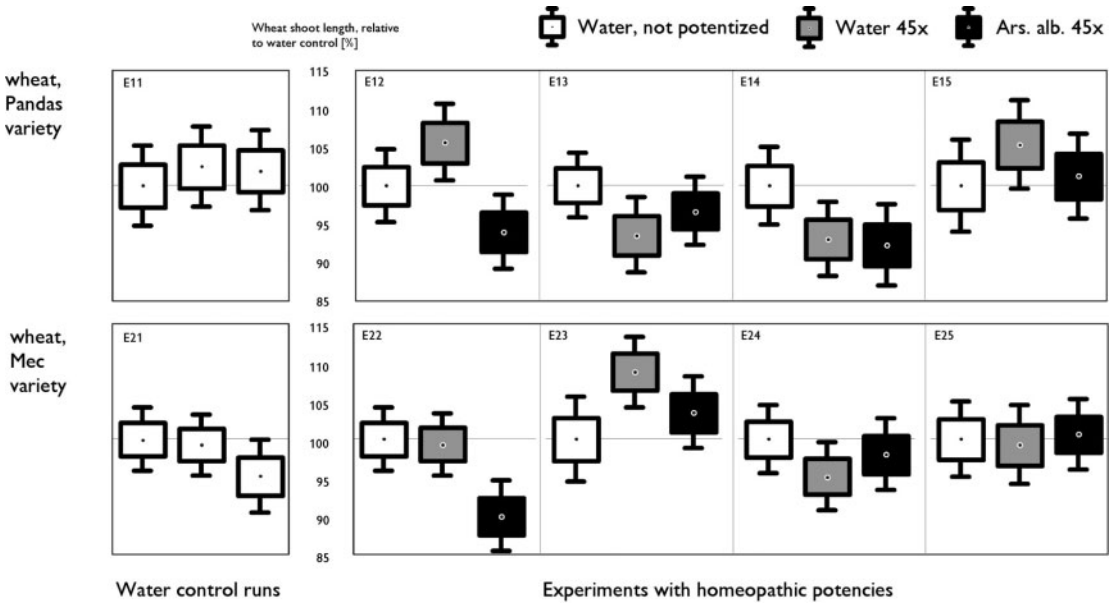

Fig. 2. Wheat shoot length (% relative to the unpotentized water control, mean ± single and double standard error) for all individual experiments. Pure water control runs left of the scale (E11 with Pandas variety, E21 with Mec variety) and experiments with homeopathic potencies run right of the scale (E12–E15 with Pandas variety and E22–E25 with Mec variety).

Germination Rates

Though not the main aim of this study, it is also worth comparing germination rates of the differently treated seeds of our current study with the results of Betti and Brizzi [25, 26]. We did not find significant differences between the 3 treatments water, water 45x and Arsenicum album 45x ($p = 0.396$, chi square test; see also fig. 1, study #4). However the germination rate of Arsenicum album 45x treated seeds tended to be lower than the germination rates of the control groups. This tendency corresponded to the inhibiting effect of Arsenicum album 45x on shoot growth.

In the investigations of Betti et al., the arsenic potency enhanced both the shoot growth [24] and the germination rate [25–26]. The germination rate increase after treatment with Arsenicum album 45x was significant in all experiments (fig. 1) with the number of seedlings (n = 792–1782 per parameter) comparable to our set-up (n = 1200 per parameter).

Analysis of Germinated and Non-Germinated Seeds

Another way to analyze the given set of data is to pool germinated and non-germinated seeds, i.e. to attribute a length of 0 mm to all non-germinated seeds and to incorporate these data into the measured shoot length data set analyzed above. These pooled data show a strong non-normal distribution, so only non-parametric methods are appropriate in this case. A Kruskal-Wallis ANOVA of the shoot length data of all 8 experiments, normalized to the corresponding experimental mean, still yielded global significant results ($p = 0.014$). Again, the effect of Arsenicum album 45x is statistically significant – compared to both the unpotentized water control ($p = 0.006$, U-test) and water 45x ($p = 0.024$, U-test) – whilst the parameters water and water 45x cannot be distinguished ($p = 0.638$, U-test).

Analysis of Variability

The variances of the 3 treatment groups (pooled for all experiments) were essentially the same ($p = 0.98$, Levene test). One thus concludes that the treatment with homeopathic potencies did not exert measurable effects on the variability *within* the experiments.

Variability *between* the experiments can be assessed through comparison of the standard deviation of the 8 mean values of all experiments for each treatment parameter (table 4). The corresponding values are 11.6 mm (100%) for the water group, 9.95 mm (85.6%) for the water 45x group and 10.9 mm (94.2%) for the Arsenicum album 45x group. Thus, there was a tendency towards a reduction in inter-experimental variability through treatment with homeopathic potencies.

Discussion

The main and most striking result of this reproduction study is the small (–3%) but statistically significant wheat shoot growth *depression* associated with treatment with Arsenicum album 45x, compared with both the unpotentized and the potentized water control (45x). This depression effect can be regarded as a consistent and internally reproducible phenomenon for the following reasons: 1. The unpotentized and the potentized water control (45x) showed no significant differences. 2. The 2 wheat species (Pandas, Mec) reacted essentially in the same way. 3. There was no significant interaction between Arsenicum album 45x treatment and date of the experiment (i.e. the action of Arsenicum album 45x was reproducible). 4. The shoot growth depression effect is qualitatively in line with the (insignificant) tendency towards an analogous germination rate *reduction* through Arsenicum album 45x.

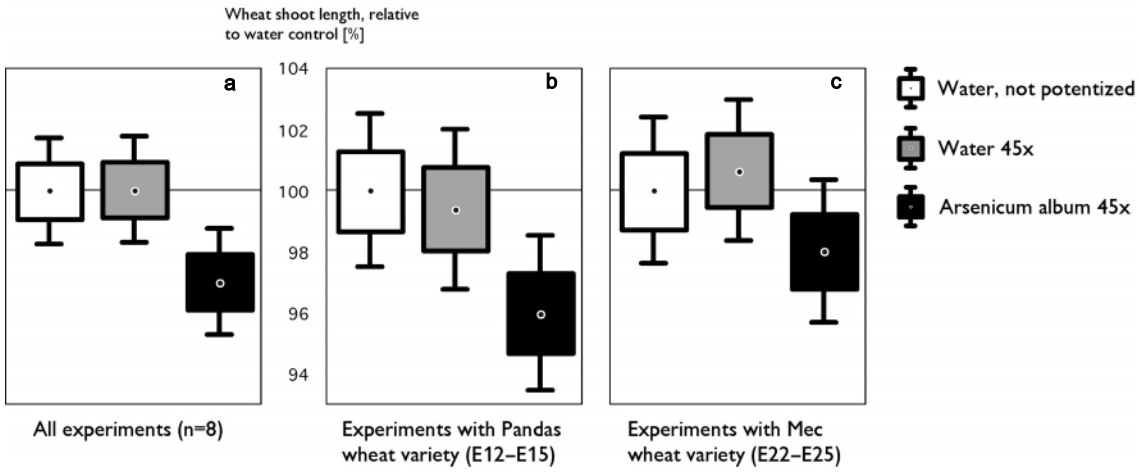

Fig. 3. **a** Wheat shoot length (% relative to the unpotentized water control, mean ± single and double standard error) for all experiments with homeopathic potencies (n = 8). **b** Wheat shoot length for all experiments with Pandas wheat (n = 4). **c** Wheat shoot length for all experiments with Mec wheat species (n = 4).

This result is a complete reversal of the effects observed by Betti and Brizzi. In their experiments, Arsenicum album 45x consistently induced an *increase* in shoot length of arsenic-poisoned seedlings [24, 27], and an *increase* in germination rate for untreated as well as poisoned seedlings [25, 26]. In the following, we discuss the relevance of some factors possibly involved in this effect reversal.

The experiments of Betti et al. on shoot length were conducted in winter (November 1993 to February 1994) at 16–20 °C [24] and in summer (July to August 1999) at 22–25 °C [27]. Thus, seasonality and/or effects of temperature can be excluded as possible reasons for the observed effect inversion (table 2). The experiments of Betti were conducted in Milan/Italy [24] and Bologna/Italy [27], whilst the experiments by Binder (this paper) were undertaken in Arlesheim near Basle/Switzerland. Unidentified factors associated with the geographical position or other peculiarities of the Italian laboratories may influence the experimental system.

Differences due to the quality of the Arsenicum album used can be excluded, since the present study used Arsenicum album from a batch identical with that of the Italian group [24, 27]. The batch of water used for potentization was not identical in the original and the present replication trial. However, we regard this difference to be of minor importance, since the water used was from the same brand (p.A., Merck) guaranteeing a high degree of standardization.

At first glance, the wheat species seems to be of minor importance. In our trial, the reaction of the 2 different species Pandas and Mec was comparable (fig. 3). However, we were not able to induce a growth depression of about 50% as was reported by Betti [24] through poisoning with 1‰ As_2O_3 (Pandas) or 1.2‰ As_2O_3 (Mec) while maintaining a reasonable germination rate (>70%). One might therefore argue that the poisoning effect in our study (–15% compared to –50% in the original trial) was too weak to allow a clear healing effect through Arsenicum album 45x to occur. Therefore, it might be the case that the Mec seed batch used by Betti [24, 27] had another history (harvest quality etc.) and was therefore not comparable with the Mec seed batch available to us. On the other hand, Betti and Brizzi observed in their germination rate experiments [25, 26] statistically significant stimulating effects of Arsenicum album 45x even on unpoisoned seeds (fig. 1).

Another factor differing between the original and replication trials were the persons responsible for potentization and conducting of experiments. Though blinding was part of the protocol in both experiments, one might hypothesize that the personality of the experimenter subconsciously influenced the outcome, i.e. the result of the experiments might depend on the person(s) responsible for the trials. The following parameters will be investigated in additional experiments:

1. Influence of location: M. Binder, who was responsible for the experiments presented in this paper, is to conduct some experimental series at the laboratory of M. Perruzzi in Milan, where the original trial was performed.
2. Influence of the main experimenter: We plan to have a different person repeat the experiments in this study in the same laboratory (at Arlesheim near Basle, Switzerland) with the identical wheat seed and Arsenicum album batch.
3. Influence of harvest quality: We plan to undertake screening with different harvests of different wheat species in order to find a wheat seed batch where arsenic poisoning leads to a more prominent reduction in shoot length (approx. 50%) without large influence on germination rate.

Betti et al. [29] observed that variability within and between experiments was decreased when tobacco plants infected with TMV virus were treated with homeopathic potencies of Arsenicum album. We did not observe any effects of the homeopathic treatment on the variability of the plants' length within the experiments. This may be due to the fact that the distribution of the data set is quite near to normality. Assessment of inter-experimental variability in our data set yielded – analogue to the results of Betti et al. – a tendency for a reduction of variability through treatment with homeopathic potencies.

Finally, the behavior of the 2nd water control (potentized water 45x) needs to be discussed. On average, there was no statistically significant difference between water and water 45x

(fig. 3). One can therefore conclude that the non-specific and purely physicochemical alterations induced through the succussion process such as air suspension and dissolution, vessel-wall ion release etc. did not significantly influence the growth of the wheat shoots. On the other hand, we observed a significant interaction between treatment and experiment number (date) if and only if water 45x is integrated in the statistical analysis. This means that potentized water 45x induces erroneous effects for single experiments and therefore does not seem to be well suitable as an adequate control for experiments on homeopathic potencies. A similar effect was observed by Betti and Brizzi in their germination rate experiments [26]: water 45x sometimes exerted a detoxifying effect and sometimes it did not (fig. 1).

To our knowledge this study is the first independent replication of an experimental model investigating biological effects of homeopathic potencies which showed a statistically significant effect of a high homeopathic dilution most probably not due to experimental artefacts. However, the direction of the effect was inverted compared to the original trial and quantitatively smaller. We agree with Betti et al. [30] that plant models might be an interesting approach to assess the effects of homeopathic potencies in living systems. However, the internal and external factors influencing the reaction of the plants to homeopathic potencies still need to be resolved.

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