

## ORIGINAL PAPER

# Use of homeopathic preparations in experimental studies with healthy plants

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**Background:** The last comprehensive review of experimental research on effects of homeopathic treatments on plants was published in 1984, and lacked formal predefined criteria to assess study quality. Since then several new studies with more advanced methods have been published.

**Objectives:** To compile a review of the literature on basic research in homeopathy with healthy plants with particular reference to studies investigating specific effects of homeopathic remedies.

**Methods:** The literature search included English, French, German, Italian, Portuguese and Spanish publications from 1920 to April 2009, using predefined selection criteria. We included experiments with healthy whole plants, seeds, plant parts and cells. The outcomes had to be measured by established procedures and statistically evaluated. We developed a Manuscript Information Score (MIS) and included only publications which provided enough information for proper interpretation ( $MIS \geq 5$ ). A formalised Study Methods Evaluation Procedure (SMEP) was used to evaluate these studies, and the subgroup of studies with adequate controls to identify specific effects.

**Results:** A total of 86 studies in 79 publications was identified, 43 studies included statistics, 29 had  $MIS \geq 5$ , and 15 studies investigated the specificity of homeopathic preparations. Specific effects of decimal, centesimal and fifty millesimal potencies were found including dilution levels far beyond the Avogadro number. In consecutive series of potencies only some of the tested potencies showed effects. There were many individual studies with diverse methods and very few reproduction trials.

**Conclusions:** Healthy plant models seem an useful approach to investigate basic research questions about the specificity of homeopathic preparations. More investigations with more advanced methods are recommended, especially in the sectors of potentiation techniques, effective potency levels and conditions for reproducibility. Systematic negative control experiments should become a routine procedure to control the stability of the experimental systems. *Homeopathy* (2009) 98, 228–243.

**Keywords:** Review; Basic research; Homeopathy; Potentiation; Potentiated dilutions

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## Introduction

The last comprehensive review of experimental research on the effects of homeopathic treatments on plants was authored by Scofield<sup>1</sup> in 1984. Since then several new investigations have been published, and more advanced methods used. In addition, the review by Scofield did not use any formal predefined criteria to assess study quality such as the criteria introduced by Becker-Witt for physico-chemical research<sup>2</sup> and Bluth and Witt for *in vitro* research<sup>3,4</sup> in homeopathy.

It is thus interesting to compile a new review of the literature about the effects of homeopathic treatments of plants. This would make it possible to determine the current state of research and to base future studies on research questions raised by results of prior work. We used predefined criteria to include only the publications which provided enough information to be interpreted properly. Furthermore, we focused on identifying studies which investigated specific effects of homeopathic remedies, i.e. effects related to the substance potentised.

Homeopathic basic research with plants can be divided into three major fields: experimental models with healthy plants, poisoned or impaired plants, and infected plants (phytopathological models). This publication will review the studies which experimented with healthy plants. The review about phytopathological models is published by Betti *et al.* in this issue<sup>5</sup>, the review about studies with impaired plants will be published elsewhere.

## Methods

### Sources for the literature search

We retrieved most of the literature from the authors' personal libraries. These literature collections were compiled by searching and collecting basic research articles for years, mostly by checking bibliographies of reviews and articles, by manually scanning scientific journals, and by information from colleagues. Additionally, the HomBRex Database<sup>6</sup> (maintained by the Karl und Veronica Carstens-Stiftung, Essen, Germany) was used. Searching with the help of standard online literature databases (e.g. MEDLINE<sup>®</sup>) was not very successful, because most studies are not indexed there.

### Literature selection

This review covers publications that reported on experiments with homeopathy in healthy plants. This includes experiments with whole plants, parts of plants, plant cells and plant seeds. Studies were excluded if they featured with plants which were poisoned, infected, or stressed on purpose, e.g. by special experimental conditions such as the absence of light for seeds that require light for germination. Outcome parameters had to be measured by established procedures, e.g. length, weight, leaf area or secondary metabolites. Studies using unconventional methods such as Kirlian photography or bio photon emission as measurement techniques were not included in this review. Publications in German, English, French, Italian, Spanish or

Portuguese were reviewed. In order to gather a comprehensive literature collection, we included all relevant publications from January 1920 to April 2009. Any earlier and later publications were excluded.

In some publications, multiple experiments or studies with differences in methods, set-up or results were described in one paper. If they were described separately, we decided to subdivide the publications into 'studies'.

The studies identified by this selection procedure showed marked differences in methodology and manuscript information content. To compile a comparable and informative publication pool, we developed three further selection criteria.

*Statistics:* We excluded all publications which did not use a statistical evaluation of the results (minimum: mean/median, number *n* and standard deviation or standard error).

*Manuscript information score (MIS):* The MIS was developed to include only publications with sufficient information to be interpreted properly (see Table 1). In the MIS, a maximum of 10 points were given for 5 category groups. A minimum of 5 points was necessary for the study to be included in the review. All publications were independently evaluated by two reviewers. Any differences in rating were resolved by discussion.

*Study Methods Evaluation Procedure (SMEP):* The SMEP evaluates important features of the study set-up. Our focus was on the control samples, because adequate controls are of particular importance investigating specific effects of homeopathic preparations. We distinguished eight different types of control. In addition, four methodological categories (blinding, randomisation, number of independent experiments, systematic negative control experiments) were reviewed.

Studies that provide evidence of specific effects of homeopathic remedies, i.e. effects related to the mother tincture diluted and therefore implying some sort of "memory" of the potentisation medium, have to be well designed to distinguish results from artefacts. Some authors suggested that silicates, other molecules and various ions are dissolved from the potentisation vessel during the succussion process<sup>7</sup>. Unsuccessful potentisation medium contains fewer of these contaminants<sup>8</sup>. Using an unsuccessful potentisation medium as sole control may generate false-positive results when it comes to identifying treatment effects that are specific to homeopathic remedies.

Essential controls to avoid these kind of artefacts consist of working with a succussed potentisation medium or with a potentised potentisation medium (diluted and succussed in the same way as the potentised test substances)<sup>7</sup>. If the test substance first has to be dissolved or triturated in a medium other than the final potentisation medium, the most adequate control to identify specific effects is potentised solvent (e.g. potentised lactose or acetone without any other primary substance), provided that the solvent is potentised in the final potentisation medium. Further valid controls for identifying specific effects are other potentised test substances, if they follow analogous methods of production.

In addition to the controls just discussed, four further controls are itemised in the SMEP to depict the complete

**Table 1** Assessment of the manuscript information content by the MIS. A maximum of 10 points were given for 5 category groups and a minimum of 5 points was necessary for the study to be included in the review

<i>MIS</i>	<i>Fully described</i>	<i>Partly described</i>	<i>Not mentioned</i>
<i>Score</i>	<i>2 points</i>	<i>1 point</i>	<i>0 points</i>
Experimental setup	Detailed information is given: way of treatment of plants, growth period, time of measurements, etc.	Only some details are described or few information about the set-up is given	No information is given about the experimental set-up
Materials	All materials used in the experiments are described with trade name, etc.	Some materials used in the experiments are described or mentioned	No information is given about the materials used
Measuring instruments	Measuring instruments are described in detail, operation mode, trade name, type, etc.	Measuring instruments are only mentioned	There is no information about measuring instruments in the paper
Potentisation	Potentisation technique, date and time of potentisation and potentisation medium are described in detail	Some information about potentisation technique is given	No information about potentisation, only the potentised test substance is mentioned
Controls	Detailed information eg: sterile distilled water from the same batch of distilled water...	Some information about the sort of control is given: e.g.: water control	Controls are not mentioned or not done

list of controls chosen in the studies. The unsuccessful potentisation medium is a common control in basic research in homeopathy. In combination with a succussed or with a potentised potentisation medium, it makes it possible to investigate if there is a succussion effect on the potentisation medium. Dilutions of the test substance (diluted in the same dilution steps as for potentisation but without succussion) can be regarded as a control to identify the importance of succussion in the potentisation process. In the diluting process, however, properly stirring the dilution is necessary to yield a homogeneous distribution of the molecules in the dilution medium. It seems difficult to us to create a precise distinction between stirring and potentisation. The positive control is a material dose of a test substance with a well-known effect. In comparison to the potencies, it can be used to investigate the modification of the specific substantial effect through potentisation. Few studies with adult plants used a control group with no treatment.

Some older studies compared different potency levels of the same test substance, without factoring another control into the analysis.

We checked four further methodological key factors. We determined whether the described experiments were carried out under blind conditions. We also evaluated whether artefacts that might be produced by differing local growing conditions like temperature and light were avoided by randomising the samples in time and space (e.g. pots with plants or the germination vessels). Additionally we checked whether the stability of the chosen experimental set-up was demonstrated by systematic negative control experiments. These are experiments with the same set-up as potency experiments using only one control substance for all samples (e.g. distilled water). With successfully standardised laboratory conditions and homogeneous quality of seeds or plants, no significant differences between the samples may be observed.

We also assessed the number of independent experiments: does the study consist of only one experiment, or were there multiple independent experiments being carried out?

All publications were independently evaluated by two reviewers. Any differences in assessment were resolved by discussion.

*Extraction of information:* The final step was thoroughly extracting the information from the study reports. At this point, studies that investigated specific effects were finally selected and sorted.

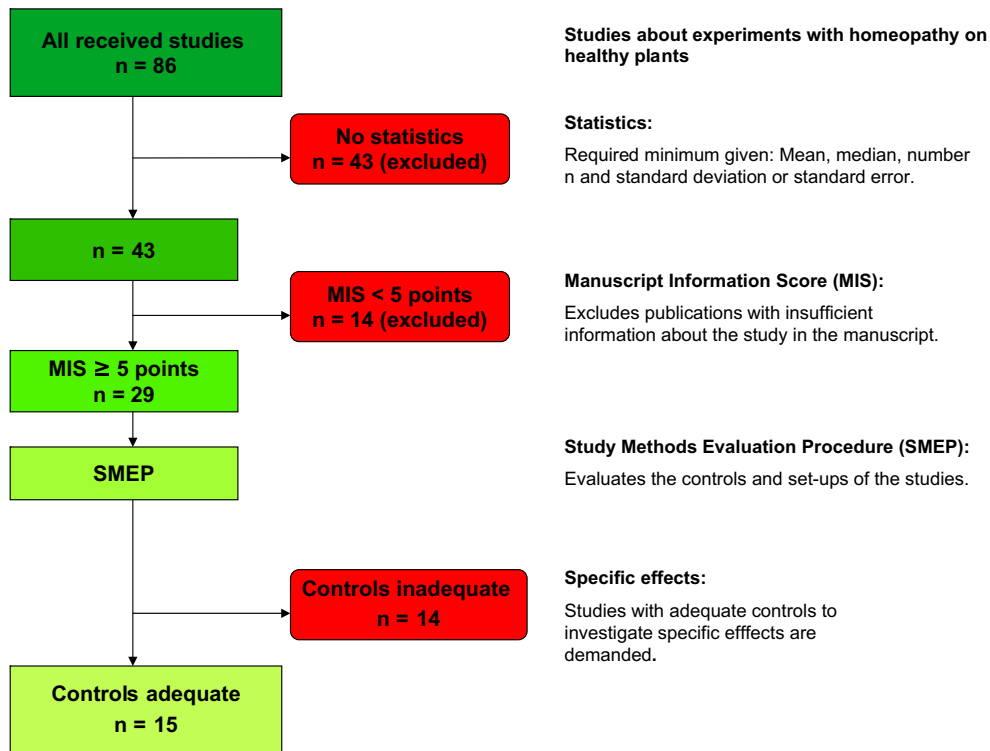
## Results

In some publications, multiple experiments or studies with differences in methods, set-up or results were described in one paper. If they were described separately, we decided to subdivide the publications into 'studies'. A total of 79 publications, including 86 studies, were identified<sup>9–87</sup>. The first publication was by Kolisko<sup>26</sup> in 1923, whilst the newest by Scherr<sup>85</sup> in 2009. Of these 79 publications (86 studies), 43 either did not use a statistical analysis to evaluate the results, or did not mention the statistics in the publication<sup>9–51</sup>. 36 publications (43 studies) with statistics (published from 1962 to 2009) remained within the reviewing procedure<sup>52–87</sup>.

In the following we will always refer to the number of studies (instead of the number of publications). We thus have 43 studies from 36 papers which had some sort of statistical evaluation and which were included in the further reviewing process (for an overview, see [Figure 1](#)).

## MIS

29 of 43 studies achieved 5 or more points in the MIS. They thus contained sufficient information for more detailed interpretation. [Table 2](#) gives an overview of the



**Figure 1** Overview of the review process. A total of 79 publications was subdivided in 86 experimental studies (see text).

studies, methods used, plant models, tested substances and potency levels, controls, and results the authors reported.

### SMEP

By applying the SMEP to the 29 studies with  $MIS \geq 5$  we identified the controls applied as well as the following key methodological factors: blinding, randomisation number of independent experiments and systematic negative control experiments. Due to the heterogeneity of the items checked, rating of the studies with a quantitative sum score did not seem adequate to us. Depending on the objectives and set-ups of the studies, different preparations may be regarded as adequate controls. Our main focus was to identify the studies which used adequate controls to investigate specific effects of homeopathic preparations.

**Controls and specific effects:** Eighteen studies used only one control, while 11 studies included two or three controls. Of the 18 studies with one control, twelve studies used unsuccessful potentisation medium as the control and six studies one of the other types of control. Three of these 12 studies mentioned unsuccessful potentisation medium as control, but evaluated the results by comparing with other potency levels of the same test substance (see Table 2, column controls). Fifteen of the 29 studies included adequate controls to identify specific effects of the tested homeopathic remedies (see discussion below).

**Blinding and randomisation:** In the group of 29 studies with five or more points in the MIS, 11 studies were carried out under blind and randomised conditions. Four studies were blinded only. In six studies, the samples of the experiments were distributed at random, but the researchers were

not blinded to the treatments. Eight of the 29 studies do not mention blinding or randomisation (see Table 2).

**Systematic negative control experiments:** Five studies described systematic negative control experiments to control any disruptive influences of inhomogeneous laboratory conditions (see Table 2, column Methods).

**Number of independent experiments:** Ten of 29 studies comprised only one experiment. Fourteen studies carried out two to eleven experiments included. Three studies consisted of 20–40 experiments. In two studies the number of independent experiments was not clearly identifiable in the manuscript (see Table 2).

**Combination of quality criteria for studies of specific effects:** Four of 15 studies with controls to identify specific effects used all quality criteria (blinding, randomisation, systematic negative control experiments, several independent experiments).<sup>55,56,85</sup>

### Extraction of information

This part includes only the 29 studies with sufficient information available ( $MIS \geq 5$ ), including the studies, which investigated the specificity of homeopathic preparations (Table 2).

**Plant models:** The most frequently used experimental plant model was the seedling model. The influence of homeopathic potencies on the germination and the growth of the seedlings was investigated in 13 studies with wheat seeds,<sup>53,54,57,60,73,75–78</sup> in three studies with dwarf peas,<sup>55,56</sup> in one study with *Sida rhombifolia*<sup>67</sup> and in one study with mung seed.<sup>62</sup> A second experimental plant model investigated the influence of homeopathic potencies

**Table 2** This table includes all studies with MIS  $\geq 5$ . Publications were subdivided in studies when multiple experiments were described in one publication. The table lists plant species, experimental methods, number *n* of independent experiments, potentised test substances, tested potency levels, controls, and effective potency levels–control<sup>3</sup>

<i>Author &amp; Year</i>	<i>Reference</i>	<i>Plant</i>	<i>Methods</i> <sup>1</sup>	<i>Independent experiments</i>	<i>Tested substances</i>	<i>Tested potency levels</i>	<i>Controls</i> <sup>2</sup>	<i>Effective potency levels–control</i> <sup>3</sup>
Scherr 2009	85	Duckweed ( <i>Lemna gibba</i> L.)	b; r; s	Gibberellic acid, Kinetin, Lemna m.: 5; Silver n.: 6	Gibberellic acid, Kinetin, Silver nitrate, Lemna minor	14x–30x	U + S	Gibberellic acid: 15x, 17x, 18x, 23x, 24x
Sukul 2009	87	Lady's finger ( <i>Abelmoschus excelentus</i> L. Moench)		1	CCC, CCC (nano), MH	30c, 200c	P	30c, 200c
Baumgartner 2008	56	Dwarf peas ( <i>Pisum sativum</i> L.), cv. 'Früher Zwerg'; harvests 1997–2000	b; r; s	8	Gibberellic acid	17x, 18x	U + S	17x
Marques 2008	67	<i>Sida rhombifolia</i>	r	5	Cymbopogon winterianus Jowitt (Citronella) CCC, CCC (nano), MH	3c, 6c, 12c, 24c, 30c	U	3c, 6c, 12c, 24c, 30c
Sukul 2008	86	Pigeon pea ( <i>Cajanus cajan</i> L. Millsp.)		1		30c, 200c	P	30c, 200c
Scherr 2007	84	Duckweed ( <i>Lemna gibba</i> L.)	b; r; s	1	Silver nitrate, Copper sulphate, Gibberellic acid, Auxin, Kinetin, Lactose, Lemna minor, Methyl jasmonate, Metoxuron, Phosphorus, Potassium nitrate, Sulphur	14x–30x	U + S	Silver nitr: 24x, 28x, 29x; Kinetin: 14x, 16x, 20x, 23x, 26x, 27x, 30x; Phos: 21x, 25x, 29x
Rossi 2006	79	Lettuce ( <i>Lactuca sativa</i> ), cv. 'Veronica'	r	1	Carbo vegetabilis	6c, 12c, 30c, 100c, 200c	U, (N)	6c, 12c, 30c, 200c
Baumgartner 2004-I	55	Dwarf peas ( <i>Pisum sativum</i> L.), cv. 'Früher Zwerg'	b; r; s	Series 1: 4	Gibberellic acid, Kinetin, Auxin, Abscisic acid	I: 12x–30x	U + S; (P-C)	Gibberellic acid: 13x, 15x, 17x, 23x; Kinetin: 19x
Baumgartner 2004-II	55	Dwarf peas ( <i>Pisum sativum</i> L.), cv. 'Früher Zwerg'	b; r; s	Series 2: 6; Series 3: 4	Gibberellic acid	II: 17x; III: 17x	U + S	17x
Chapman 2004	61	Lettuce ( <i>Lactuca sativa</i> ), cv. 'Tom Thumb'	b; r	1	Sulphur, Silicea	LM1	S	Silicea, Sulphur: 1LM

Andrade 2001	52	<i>Justicia pectoralis</i> L. (Jacqu.)	b; r	1	Justicia; Acanthaceae; Cumarina P.A.; Guaco; Phos; Sulphur; Arnica montana; Humic acid	3c	(U) <b>P</b>	Justicia; Phos; Sulphur; Arnica montana; Humic acid: 3c
Brizzi 2000	60	Wheat ( <i>Triticum durum</i> L.), cv. 'MEC'	b; r	unclear	Arsenicum album	23x, 27x, 25x, 30x, 35x, 40x, 42x, 45x	U; (D; <b>P</b> )	27x, 25x, 30x, 35x, 40x, 42x, 45x
Betti 1994-I	57	Wheat ( <i>Triticum durum</i> L.), cv. 'MEC'	b; r	10	Arsenicum album	23x, 30x	U	No effects
Betti 1994-II	57	Wheat ( <i>Triticum durum</i> L.), cv. 'MEC'	b; r	8	Arsenicum album	23x, 25x, 30x, 35x, 40x, 45x	(U; D) <b>P</b>	40x + 45x
Pongratz 1994a-I	78	Wheat, cv. 'Mephisto'	b	unclear	Silver nitrate	24x–26x	U	24x, 26x
Pongratz 1994a-II	78	Wheat, cv. 'Mephisto'	b	2	Silver nitrate	24x	<b>P</b>	24x
Pongratz 1994a-III	78	Wheat, cv. 'Mephisto'	b	1	Silver nitrate	24x–26x	U	24x, 26x
Endler 1991	63	African violet ( <i>Saintpaulia</i> sp.)	b; r	3	Indole butric acid	33x	<b>S</b>	33x
Pongratz 1990	77	Wheat, cv. 'Mephisto'	b	10	Silver nitrate	24x	<b>P</b>	24x
Chou 1986	62	Mung seed		1	Fertilizer <sup>4</sup>	1x–24x	U; D; (P-C)	Potency levels not mentioned
Noiret 1979b	73	Wheat, cv. 'Hardi'		4	Copper sulphate	5c, 7c, 9c	U; <b>S</b>	5c, 7c, 9c
Pelikan 1971	76	Wheat, own cultivation	r	40	Silver nitrate	8x–19x	U	13x, 14x
Basold 1968-I	54	Wheat, cv. 'Künzel'	r	24	Silver nitrate	8x–19x	(U)	not investigated
Basold 1968-II	54	Wheat, cv. 'Künzel'	r	20	Silver nitrate	6x–30x	(U)	not investigated
Pelikan 1968	75	Wheat, own cultivation	r	2 × 6	Lead nitrate	8x–19x	U	8x, 10x–12x, 16x
Basold 1967	53	Wheat		4	Ferrosulphat	3x–32x	(U)	not investigated
Boiron 1965a	59	Wheat, cv. 'Vilmorin n° 27'		1	Mercury chloride	3x–18x	U	9x, 10x
Boiron 1963	58	Wheat, cv. 'Vilmorin n° 27'		8x: 11; 16x: 8; 18x: 6	As <sub>4</sub> Na <sub>2</sub> H	3x–18x	U	8x, 16x, 18x
Netien 1962	69	Wheat, cv. 'Maitre Pierre'		1	Cobalt chloride	2x–18x	U	8x, 12x, 15x, 18x

<sup>1</sup> Methods: b = Blinding; r = Randomisation; s = Systematic negative control experiments, as identified from the publication.

<sup>2</sup> Controls: U = Unsuccussed potentiation medium; **S** = Succussed potentiation medium; **P** = Potentiated potentiation medium; D = Diluted test substance, P-C = Positive control; N = No treatment group. Adequate controls to identify specific treatment effects (S, P) are printed in bold. Treatment effects were compared to the controls without brackets.

<sup>3</sup> Effective potency levels: List of all potency levels which were significantly effective in any of the measured parameters, compared to the control without brackets.

<sup>4</sup> Fertilipzer content: 10% Nitrogen, 1% Ammoniacal Nitrate, 0.6% Nitrate Nitrogen, 8.4% Urea Nitrogen, 15% Phosphoric acid, 10% Potash.

on the growth of adult plants: there were two studies with duckweed,<sup>84,85</sup> two studies with lettuce,<sup>61,79</sup> and one study with *Justicia pectoralis*,<sup>52</sup> pigeon pea<sup>86</sup> and lady's finger,<sup>87</sup> respectively. The third experimental plant model used plant slips: three studies investigated the modification of the O<sub>2</sub> consumption of wheat seedling slips<sup>58,59,69</sup> and another study investigated the growth of slips of african violet.<sup>63</sup>

*Measured parameters:* The most commonly used experimental outcome (26 of 29 studies) was plant growth. Growth was measured by three categories: size (including shoot and root length, leaf area, leaf number or stem diameter), weight (including fresh and dry weight) and germination rate (and speed). Twelve of these 26 studies measured the effect on the plant growth by size only.<sup>53–56,62,63,75,76,84,85</sup> Ten of the 26 studies measured further parameters: three of the ten studies<sup>52,86,87</sup> also measured weight and secondary metabolites or biochemical parameters; two studies<sup>67,77</sup> additionally reported weight and germination rate; three studies<sup>78</sup> measured size and germination rate, and two studies<sup>61,79</sup> measured size and weight. Three studies<sup>57,60</sup> measured growth by germination rate and germination time, whereas one study<sup>73</sup> used only weight as the measuring parameter. Besides plant growth, three of 29 studies measured the O<sub>2</sub> consumption of wheat seedlings<sup>58,59,69</sup>.

*Experimental plant systems and treatment:* We distinguished between timing and the route of application of the treatment, to identify how the contact between plant and homeopathic remedy was effected.

*Timing:* In six studies<sup>55,56,58,59,69</sup> the treatment of the plants was carried out before cultivation, 22 studies<sup>52–54,57,60–63,67,75–79,84–87</sup> seedlings or plants were treated over a longer period during the cultivation, and one study<sup>73</sup> had pre-treatment combined with treatment during cultivation.

*Route of application:* Most of the germination as well as plant slip experiments (20 studies<sup>54–60,62,63,69,73,75–78</sup>) assured the plants contact with the homeopathic potencies by immersing them into the potencies for a certain period, mostly during the whole time of experiment. One study<sup>53</sup> watered the plant substrate in the pots with potencies or control at the beginning of the experiments. Two other ways of treatment were the application with a spray in four studies<sup>52,79,86,87</sup> and the daily application of one drop potency or control on the soil in one study.<sup>61</sup> One study<sup>67</sup> covered the seed with filter paper, soaked in potency or control, respectively. Two studies<sup>84,85</sup> used waterplants, continuously floating in potency or control solutions.

*Potentiation:* All 29 studies with MIS ≥ 5 were checked for potentiation technique. Eleven studies<sup>54–56,62,78,84,85</sup> used the multiple-glass method for potentiation. Two of them<sup>55</sup> used the single- and the multiple-glass method. In 17 studies no detailed information about the use of the method was given.<sup>52,53,57–61,63,67,69,73,75–77,86,87</sup> In 15 studies<sup>53–60,62,69,84,85</sup> the test substances were potentiated by hand. About 12 different methods of hand succussion were used. Most differences were found in the number of succussion beats; beyond that, there were also differences in whether the potencies were succussed with or without hitting, or in a vertical or in horizontal movement.

Four studies<sup>67,73,75,76</sup> used a succussion machine for potentiation. For machine succussion, essentially three different techniques were employed: in two studies<sup>75,76</sup> potencies were succussed for 4.5 min, in the third study<sup>73</sup> they were succussed with 200 beats in 20 seconds, the fourth study<sup>67</sup> potentiated with 100 beats. In 10 studies<sup>52,61,63,77–79,86,87</sup> no information was given about whether succussion was done by hand or by machine.

*Plants and potentiated substances:* The germination experiment with wheat is the most commonly used experimental model: more than half of the papers with statistics (23 of 43 studies) used wheat. In the following, we have summarised all plant models that were described in the 29 studies with MIS ≥ 5, including the studies, which investigated the specificity of homeopathic preparations (see Table 2).

#### a) Wheat

With 16 of 29 studies, wheat is the most frequently investigated plant model.

Seven studies<sup>54,76–78</sup> with wheat investigated the effect of Silver nitrate. With only unsuccessful potentiation medium as control, the potency levels 8x–13x (2 studies), 6x–30x, 24x and 24x–26x (2 studies) were tested. The potency levels 13x, 14x, 24x (2 studies) and 26x influenced growth of the wheat seedlings. Two of the 7 studies evaluated the effects by comparison of potency level to potency level, respectively, without factoring the control into the analysis of the results.<sup>54</sup> Two studies<sup>77,78</sup> compared silver nitrate 24x with potentiated potentiation medium, and found it to be specifically effective (increase of the germination rate).

The influence of Arsenicum album potencies on the germination rate and speed of wheat seeds was investigated in three studies<sup>57,60</sup> with a huge dataset. The first, a pilot study, tested the potency levels 23x and 30x compared to unsuccessful potentiation medium. The other two investigated the effect of 23x, 30x, 35x, 40x and 45x, and the third study additionally 27x and 42x. The second study compared the potencies to unsuccessful potentiation medium, potentiated potentiation medium (H<sub>2</sub>O 30x) and diluted test substance (Arsenicum album 10<sup>-30</sup>) as control. The third study compared the potencies to unsuccessful potentiation medium. This study additionally investigated the potentiated potentiation medium and the diluted test substance, both in the same potentiation levels or dilution steps as potencies used, in comparison to unsuccessful potentiation medium. The first study did not find significant potency effects. In the second study, significant differences were found compared to three sorts of control: between 25x, 30x, 40x, 45x and the unsuccessful control; between a pool of the 40x and 45x data and the potentiated control (increase of the germination rate); between 25x, the 40x–45x-pool and the diluted test substance. In the third study all tested potency levels showed significant effects compared to unsuccessful control. Some of the potency levels stimulated wheat germination whereas others induced inhibition of the germination process.

A multi-centre study with wheat<sup>73</sup> investigated the specific effect of Copper sulphate 5c, 7c and 9c compared to the

unsucceded and the succeded potentisation medium. They tested four different ethanol-water mixture ratios of the potentisation medium and found the strongest effects (for all potency levels) with a potentisation medium with 20% ethanol.

Ferrous sulphate (3x–32x)<sup>53</sup> and lead nitrate (8x–19x)<sup>75</sup> were also tested with the wheat germination model. The first study evaluated potency levels only against each other. Potency levels 8x, 10x–12x and 16x of lead nitrate modified wheat growth significantly compared to unsucceded potentisation medium.

Three studies<sup>58,59,69</sup> investigated the effect of homeopathic potencies on the O<sub>2</sub> consumption of wheat seedling slips. The substances Cobalt chloride (2x–18x), Mercury chloride (3x–18x) and As<sub>4</sub>Na<sub>2</sub>H (3x–18x) were investigated, compared to unsucceded potentisation medium. The O<sub>2</sub> consumption of the seedling slips was significantly increased by the potency levels of 8x, 12x, 15x and 18x of Cobalt chloride, as well as the potency levels 9x and 10x of Mercury chloride, and the potency levels 8x, 16x and 18x of As<sub>4</sub>Na<sub>2</sub>H.

#### b) Dwarf peas

Two publications<sup>55,56</sup>, reporting 3 studies, investigated the specific effects of potentised plant hormones (compared to succeded and unsucceded potentisation medium) on the growth of dwarf peas. Gibberellic acid was tested in 12x–30x, 17x, as well as 17x and 18x. Specific effects of Gibberellic acid 13x, 15x, 17x (3 studies) and 23x were found (increase of shoot length). Kinetin, Auxin and Abscisic acid were tested in the potency levels 12x–30x. Only Kinetin 19x showed a specific effect on the growth of dwarf peas (enhancing shoot length). These effects were verified by systematic negative control experiments.

#### c) Duckweed

Two studies<sup>84,85</sup> investigated specific effects of homeopathic potencies on the growth of duckweed (*Lemna gibba* L.). The first study was a screening of twelve test substances in the potency levels 14x–30x, compared with succeded and unsucceded potentisation medium. In the second study experiments with four test substances used in the first study were repeated several times. Gibberellic acid, Kinetin, Silver nitrate and Lemna minor were investigated in both studies. Auxin, Copper sulphate, Methyl jasmonate, Metoxuron, Phosphorus, Potassium nitrate, Sulphur and Lactose were only investigated in the first study. Silver nitrate 24x, 28x and 29x; Kinetin 14x, 16x, 20x, 23x, 26x, 27x and 30x (decrease of the growth rate) as well as Phosphorus 21x, 25x and 29x (increase of growth) showed specific effects in the screening. In the second study only Gibberellic acid 15x, 17x, 18x, 23x and 24x showed specific effects (decrease of growth). These effects were verified by systematic negative control experiments.

#### d) *Justicia pectoralis* Jacqu. (L.)

One outdoor study<sup>52</sup> investigated the specific effects of 8 test substances on the growth and the production of secondary metabolites (coumarine) of *Justicia pectoralis* Jacqu. (L.). 3c potencies of Arnica montana, Justicia, Phosphorus, Sulphur, Humic acid, Acanthaceae, Cumarina P.A. and Guaco were compared with succeded and

unsucceded potentisation medium; the first five preparations increased the coumarine content significantly.

#### e) African violet

One study<sup>63</sup> investigated the specific effect of the plant hormone Indole butric acid 33x on the rooting and the development of new leaves of african violet slips. Enhancing root growth, the 33x showed a specific effect, compared to the succeded potentisation medium.

#### f) Lettuce (*Lactuca sativa*)

One outdoor study<sup>61</sup> investigated the specific effects of Silicea 1LM and Sulphur 1LM on the growth of lettuce. For both test substances specific effects were found (decrease of weight and breadth), in comparison to the succeded potentisation medium. Another outdoor study<sup>79</sup> investigated the effect of Carbo vegetabilis (6c, 12c, 30c, 100c, 200c) on the growth of lettuce, compared with unsucceded potentisation medium and an untreated control group. All potency levels were found effective.

#### g) Mung seed

One publication<sup>62</sup> investigated the effects of the potency levels 1x–24x of a fertilizer on the growth of mung seedlings. Regrettably, the author only reported that the potencies were effective (compared to unsucceded potentisation medium, the diluted test substance and a positive control), but no detailed information about the potency levels was given.

#### h) *Sida rhombifolia*

One study<sup>67</sup> investigated the effect of *Cymbopogon winterianus* Jowitt (Citronella) in the potency levels 3c, 6c, 12c, 24c and 30c on the germination and growth of *Sida rhombifolia*. The potency levels 6c, 12c and 30c showed significant effects (in comparison to the unsucceded control) on the parameter root and shoot length, fresh weight, germination rate, germination time and speed. The 3c was found effective on the root and shoot length, and the 24c influenced the root length and the fresh weight significantly.

#### i) Pigeon pea and

#### j) Lady's finger

Two outdoor studies<sup>86,87</sup> investigated the specific effects of potencies of two growth retardants normally used in agriculture on multiple parameters of growth and plant biochemistry. The potency levels 30c and 200c of CCC ((2-chloroethyl) trimethyl ammonium chloride), CCC (nano) and MH (Maleic hydrazide) were tested in pigeon pea and lady's finger. Growth and biochemical parameters of both plants were significantly influenced (increase) by the potencies of CCC and CCC (nano) and MH.

*Tested substances:* In the 29 studies with MIS ≥ 5 the effect of 32 different test substances was investigated. Twenty-two studies investigated only one test substance. Five studies examined two to four test substances. Moreover, there were two screenings, one study with eight different test substances and another study with twelve.

The most frequently used test substances were Silver nitrate (nine studies), Gibberellic acid (five studies), and Kinetin and Sulphur in three studies each (See also Table 2, column Tested substances).



**Table 3a** This table includes all 15 studies (with MIS  $\geq$  5) using adequate controls to investigate specific effects of homeopathic preparations. Sub-table 3a lists the 6 studies consisting of only one independent experiment, sub-table 3b all 9 studies with multiple experiments. The table lists plant species, experimental methods, number n of plants per treatment and experiment, number n of independent experiments, measured parameters, way of treatment, potentised substances, potentisation method, tested potency levels, controls, statistics and effective potency levels

Author & year	Reference	Plant	Methods <sup>1</sup>	Number n (per treatment and experiment)	Number of independent experiments	Measured parameters	Treatment <sup>2</sup>	Tested substances	Potentisation <sup>3</sup>	Tested potency levels	Controls <sup>4</sup>	Statistical tests	Effective potency levels <sup>5</sup>
Sukul 2009	87	Lady's finger ( <i>Abelmoschus excelentus</i> L. Moench)		10 plants	1	Length, girth and weight of shoots; length and weight of roots; number of leaves/plant; weight, area and water content of leaves; chlorophyll and protein content	D: 1:500 in water diluted potencies, with a spray, 2 times daily, only the first 2 days of exp.	CCC, CCC (nano), MH	H: Mother tinctures: Triturations of CCC, MH, CCC+Copper nano particles with lactose; Potentisation: 1:100 dilution steps in Alcohol 90%, Succussion: 10 beats	30c, 200c	P	One way ANOVA, t-test	30c, 200c (all test substances)
Sukul 2008	86	Pigeon pea ( <i>Cajanus cajan</i> L. Millsp.)		20 plants	1	Leaves- and branches number, shoot girth at day 75; shoot weight, root length, number of flowers/plant at day 150; chlorophyll-, carbohydrates- and protein-content of leaves	D: with a spray on several days of exp., 1:500 in water diluted potencies	CCC ((2-chloroethyl) trimethyl ammonium chloride), CCC (nano), MH (Maleic hydrazide)	H: Mother tinctures: 1g CCC/MH per 1 ml Alcohol 90%, 1 g CCC+Copper nano particles triturated with lactose per 1 ml Alcohol 90%; Potentisation: 1:100 dilution steps in Alcohol 90%, Succussion: 10 beats	30c, 200c	P	One way ANOVA, t-test	30c, 200c (all test substances)
Scherr 2007	84	Duckweed ( <i>Lemna gibba</i> L.)	b; r; s	5 beakers per parameter	1	Fronde area and frond number related growth rate, day 0–7, 0–3, 3–7	D: The waterplants grew in potency or control	Silver nitrate, Copper sulphate, Gibberellic acid, Auxin, Kinetin, Lactose, Lemna minor, Methyl jasmonate, Metoxuron, Phosphorus, Potassium nitrate, Sulphur	H: horizontal, no hitting; multiple-glass method	14x—30x	U + S	With F-test protected Fisher's LSD	Arg nitr: 24x, 28x, 29x; Kinetin: 14x, 16x, 20x, 23x, 26x, 27x, 30x; Phos: 21x, 25x, 29x;
Chapman 2004	61	Lettuce ( <i>Lactuca sativa</i> ), cv. 'Tom Thumb'	b; r	17–25 plants	1	Plant height, weight, breadth, 55 days after germination	D: 1 drop on the soil, daily	Sulphur; Silicea	Globuli (Helios Homeopathic Pharmacy) solved in dist. water	LM1	S	Student's t-test	LM1
Andrade 2001	52	<i>Justicia pectoralis</i> L. (Jacqu.)	b; r	16 plants	1	Fresh and dry weight, leaf area, coumarine content	D: weekly application with a spray	Justicia; Acanthaceae; Coumarine P.A.; Guaco; Phosphorus; Sulphur; Arnica montana; Humic acid	Hahnemannian, no detailed information	3c	(U); P	Scott-Knott-test, t-test	Justicia; Phos; Sulphur; Arnica montana; Humic acid: 3c
Pongratz 1994a-II	78	Wheat, cv. 'Mephisto'	b	200 seeds	1	Stalk length, germination rate, after 5 days	D: Immersion of the seed	Silver nitrate	H: acc. to HAB; multiple-glass method	24x	P	Chi-square test; one way ANOVA	24x
Scherr 2009	85	Duckweed ( <i>Lemna gibba</i> L.)	b; r; s	5 beakers		Giberellic acid, Kinetin, Lemna minor: 5; Arg nitr: 6 Fronde area related growth rate, day 0–7, 0–3, 3–7	D: The waterplants grew in potency or control	Gibberellic acid, Kinetin, Silver nitrate, Lemna minor	H: horizontal, no hitting; multiple-glass method	14x—30x	U + S	With F-test protected Fisher's LSD	Gibberellic acid: 15x, 17x, 18x, 23x, 24x

Baumgartner 2008	56	Dwarf peas ( <i>Pisum sativum</i> L.), cv. 'Früher Zwerg'	b; r; s	50 seedlings	8	Shoot length after 14 days	B: Immersion of the seed	Gibberellic acid	H: vertical, no hitting; multiple-glass method	17x, 18x	U + S	With F-test protected Fisher's LSD	17x, 18x
Baumgartner 2004-I	55	Dwarf peas ( <i>Pisum sativum</i> L.), cv. 'Früher Zwerg'	b; r; s	23 seedlings	Series 1: 4	Shoot length after 14 days	B: Immersion of the seed	Gibberellic acid; Kinetin; Auxin; Abscisic acid	H: vertical, no hitting; multiple and single glass method	12x–30x	U; S; P-C	With F-test protected Fisher's LSD	Gibberellic acid: 13x, 15x, 17x, 23x Kinetin: 19x
Baumgartner 2004-II	55	Dwarf peas ( <i>Pisum sativum</i> L.), cv. 'Früher Zwerg'	b; r; s	21–60 seedlings	Series 2: 6; Series 3: 4	Shoot length after 14 days	B: Immersion of the seed	Gibberellic acid	H: vertical, no hitting; multiple and single glass method	17x	U + S	With F-test protected Fisher's LSD	17x
Brizzi 2000	60	Wheat ( <i>Tritium durum</i> L.), cv. 'MEC'	b; r	33 seeds per treatment	unclear	Germination rate, germination speed	D: Watering at the beginning of the experiment	Arsenicum album	H: vigorous hitting, 70 impacts	23x, 25x, 27x, 30x, 35x, 40x, 42x, 45x	U; (P; D)	Poisson test, global poisson comparison test, odds ratio	25x, 27x, 30x, 35x, 40x, 42x, 45x
Betti 1994-II	57	Wheat ( <i>Tritium durum</i> L.), cv. 'MEC'	b; r	99 seeds	8	Germination rate, germination speed	D: Watering at the beginning of the experiment	Arsenicum album	H: vigorous hitting, 70 impacts	23x, 25x, 30x, 35x, 40x, 45x	(U; D) P	Poisson test; parametric one-factor ANOVA	40x + 45x
Endler 1991	63	African violet ( <i>Saintpaulia</i> sp.)	b; r	75/74 slips per experiment	3	Rooting, development of new leaves after several weeks	D: Immersion of the plant slips	Indole butric acid	Serial 1:10 dilution, succussion after each dilution step	33x	S	Chi-square test,	33x
Pongratz 1990	77	Wheat, cv. 'Mephisto'	b	unclear	10	Stalk length, dry weight of shoots and roots, after 5 days; germination rate	D: Immersion of the seed	Silver nitrate	Exp. 1–2: 4 min succussion; Exp. 2–6, 8–10: succussion acc. HAB; Exp. 7: 30 impacts per 1 min	24x	P	Not parametric U-tests (Mann-Withney)	24x
Noiret 1979b	73	Wheat, cv. 'Hardi'		10 seeds	unclear; multi-centre study (4 laboratories)	Fresh and dried weight after 4–5 days of germination	B and D: Immersion of the seed	Copper sulphate	M: 200 impacts in 20sec.; alcohol-water ratios: 10:90 up to 40:60	5c, 7c, 9c	U; S	Students and fisher table t-test	5c, 7c, 9c

<sup>1</sup> Methods: b = Blinding; r = Randomisation; s = Systematic negative control experiments, as identified from the publication.

<sup>2</sup> Treatment: B = Before cultivation; D = During cultivation.

<sup>3</sup> Potentisation: H = Hand succussion; M = Machine succussion.

<sup>4</sup> Controls: U = Unsuccussed potentisation medium; S = Succussed potentisation medium (adequate control); P = Potentised potentisation medium (adequate control); D = Diluted test substance, P-C = Positive control. Treatment effects were compared to the controls without brackets.

<sup>5</sup> Effective potency levels: List of all potency levels which were significantly effective in any of the measured parameters, compared to the control without brackets.

**Table 4** This table gives an overview of the 15 studies investigating specific effects, focussing on the tested substances. It shows how many investigations were done with which potentised substance, which plant models were used, which potency levels were investigated and which potency levels showed specific effects

Potentised substances	N of studies	Plants and potencies	Tested potency levels	Effects reported by author	Effect <sup>1</sup>	Ref. nr.
Gibberellic acid	5	Dwarf peas	12x–30x; 17x	13x; 15x; 17x; 23x	I	55
		Dwarf peas	17x	17x	I	55
		Dwarf peas	17x, 18x	17x	I	56
		Duckweed	14x–30x	No effect		84
		Duckweed	14x–30x	15x; 17x; 18x; 23x; 24x	D	85
Silver nitrate	4	Wheat	24x	24x	I	77
		Wheat	24x	24x	I	78
		Duckweed	14x–30x	24x; 28x; 29x	D	84
		Duckweed	14x–30x	Sign. Interaction	D, I	85
Kinetin	3	Dwarf peas	12x–30x	19x	I	55
		Duckweed	14x–30x	14x, 16x, 20x, 23x, 26x, 27x, 30x	D	84
		Duckweed	14x–30x	No effect		85
Sulphur	3	Lettuce	1LM	1LM	D	61
		Duckweed	14x–30x	No effect		84
		<i>Justicia pectoralis</i>	3c	3c	I	52
<i>Arsenicum album</i>	2	Wheat	23x, 30x; 35x, 40x, 45x	40x + 45x	I	57
		Wheat	23x, 27x, 30x; 35x, 40x, 42x, 45x	27x, 30x, 35x, 40x, 42x, 45x	D, I	60
Auxin (IAA)	2	Dwarf peas	12x–30x	No effect		55
		Duckweed	14x–30x	No effect		84
CCC	2	Pigeon pea	30c, 200c	30c, 200c	I	86
CCC (nano)	2	Lady's finger	30c, 200c	30c, 200c	I	87
		Pigeon pea	30c, 200c	30c, 200c	I	86
Copper sulfate	2	Lady's finger	30c, 200c	30c, 200c	I	87
		Wheat	5c, 7c, 9c	5c, 7c, 9c	D	73
Lemna minor	2	Duckweed	14x–30x	No effect		84
		Duckweed	14x–30x	No effect		85
Maleic hydrazide	2	Pigeon pea	30c, 200c	30c, 200c	I	86
		Lady's finger	30c, 200c	30c, 200c	I	87
Phosphorus	2	<i>Justicia pectoralis</i>	3c	3c	I	52
		Duckweed:	14x–30x	21x, 25x, 29x,	D	84
Abscisic acid	1	Dwarf peas	12x–30x	No effect		55
Acanthaceae	1	<i>Justicia pectoralis</i>	3c	No effect		52
<i>Arnica montana</i>	1	<i>Justicia pectoralis</i>	3c	3c	I	52
Coumarine P.A.	1	<i>Justicia pectoralis</i>	3c	No effect		52
Guaco	1	<i>Justicia pectoralis</i>	3c	No effect		52
Indole butric acid	1	African violet	33x	33x	I	63
Humic acid	1	<i>Justicia pectoralis</i>	3c	3c	I	52
<i>Justicia</i>	1	<i>Justicia pectoralis</i>	3c	3c	I	52
Lactose	1	Duckweed	14x–30x	No effect		84
Methyl jasmonate	1	Duckweed	14x–30x	No effect		84
Metoxuron	1	Duckweed	14x–30x	No effect		84
Potassium nitrate	1	Duckweed	14x–30x	No effect		84
Silicea	1	Lettuce	1LM	1LM	D	61

<sup>1</sup> I = Increase; D = Decrease.

*Studies with controls adequate to investigate specific effects of homeopathic preparations:* Fifteen studies included adequate controls to identify specific effects of the tested homeopathic potencies (Table 3). Twenty-five test substances were investigated with eight plants: wheat, dwarf peas, duckweed, lettuce, African violet, *Justicia pectoralis*, pigeon pea and lady's finger (See Table 3, columns Plants, Tested substances and Controls).

The most frequently tested potentised substances were Gibberellic acid (5 studies), Silver nitrate (4 studies), Kinetin and Sulphur (3 studies each), as well as *Arsenicum album*, Auxin, Phosphorus, Copper sulphate, Lemna minor, CCC, CCC(nano) and Maleic hydrazide in 2 studies each. The following test substances were investigated in one study only: Abscisic acid, Acanthaceae, *Arnica montana*, Coumarine P.A., Guaco, Indole butric acid,

Humic acid, *Justicia*, Lactose, Methyl jasmonate, Metoxuron, Potassium nitrate and Silicea (see Table 4).

Four studies<sup>55,63,77,78</sup> investigated the effect of only one potency level (17x; 24x (2 studies); 33x). All four studies found the tested potencies to be significantly effective. Another study<sup>61</sup> investigated one potency level (1LM) of two test substances, and both significantly influenced the growth of the plants. One study<sup>73</sup> tested three potency levels (5c, 7c and 9c) of one substance: all potency levels induced significant effects. Furthermore, two studies<sup>86,87</sup> investigated two potency levels (30c, 200c) of three test substances; significant effects of all potency levels and test substances were observed.

In a screening, one study<sup>52</sup> tested the potency level 3c of eight different test substances. Five of the potentised substances showed specific effects. The phenomenon that

only some test substances influenced plant growth was also found in the second screening, where specific effects were observed only for three of twelve potentised substances.<sup>84</sup>

Some of the experiments,<sup>55,56,77,78</sup> investigating only one or few potency levels were based on preceding studies with potency level series.<sup>27,55,78</sup> In these experiments, only some potency levels seemed to be effective. That only some or few potency levels seem to be biologically active was observed in nearly all studies investigating specific effects of series of potencies.<sup>55,57,60,84,85</sup> Three of these studies verified their results by systematic negative control experiments; therefore this phenomenon does not seem to be an artefact.

When similar potency levels of the same substance were tested with two different plant organisms, some potency levels showed effects for both plants, e.g. Gibberellic acid in dwarf peas<sup>55</sup> and in duckweed.<sup>85</sup> The tested intersecting potency levels were 14x–30x, and concordant effective potency levels were 15x, 17x and 23x, which increased growth for dwarf peas and decreased for duckweed. Another example are potencies of Silver nitrate with wheat (two studies<sup>77,78</sup>) and with duckweed.<sup>84</sup> In all three studies the 24x was found to be effective.

Two studies – one with dwarf peas,<sup>56</sup> the other with duckweed<sup>85</sup> – found significant interactions between the treatment and the experiment number. This means that the effects were not identical for the different experiments. The results of both studies were verified by systematic negative control experiments, so this effect does not seem to be due to instabilities of the experimental systems. A further phenomenon is the opposite effect of several potency levels of the same substance in one study.<sup>60</sup> Two potency levels (27x and 35x) of *Arsenicum album* inhibited wheat germination, whilst 30x, 40x, 42x and 45x stimulated it.

In five studies which used unsuccessful and succeeded potentisation medium as control,<sup>55,56,84,85</sup> no significant difference between succeeded and unsuccessful control was found. In two further studies,<sup>57,60</sup> however, when unsuccessful and potentised potentisation medium were used as control, H<sub>2</sub>O 30x and 45x (i.e. succeeded control) induced a significant modification of wheat germination.

**Reproducibility:** There are only very few investigations of the external reproducibility in the group of studies with  $MIS \geq 5$  and most of them repeated experiments of Kolisko<sup>27</sup> (published 1926), whose results were evaluated without statistical analysis. Two studies<sup>54,76</sup> which investigated the effect of *Argentum nitricum* (8x–19x) on the growth of wheat seedlings both found significant effects, but the effective potency levels were not identical. One study<sup>78</sup> investigated the effects of three potency levels of a series (*Argentum nitricum* 24x–26x), and found the same phenomenon as Kolisko, 24x and 26x were more effective than 25x. Additionally, a multi-centre study<sup>73</sup> with four participating laboratories investigated the effect of Copper sulphate 5c, 7c and 9c, on the germination of wheat seeds. Effects were mostly similar, but there was imprecision in potency production between the laboratories.

Internal reproducibility was investigated by five<sup>55,56,84,85</sup> studies, three with dwarf peas and two with duckweed,

which included all of the quality criteria (see Table 2, column 3). In these studies, a significant interaction between treatment and experiment number was observed for some test substances; the effects of certain potency levels varied between inhibiting, inducing and no modification of plant growth. To determine conditions for successful reproducibility, one study<sup>56</sup> with dwarf peas investigated Gibberellic acid 17x and 18x, using four different harvest lots of the same seed cultivar. Significant effects on plant growth were found only for Gibberellic acid 17x for two of the four seed harvests, and Gibberellic acid 18x did not induce any significant growth modification. Different results in carbohydrate content for the four pea seed batches indicated that differing seed quality could cause problems in the reproducibility of the results in experimental plant systems.

A further study<sup>86,87</sup> repeated the investigation of 3 potentised plant retardants, maintaining the set-up, but replacing the plant model. Here, the test substances were effective in many parameters of the two plant models, but the effects were not always the same.

## Discussion

Almost all the studies observed effects of homeopathic potencies on plants, even in high dilutions far beyond the Avogadro number. It is conspicuous that no linear or monotonous relationship between potency level and effect size was observed in any of the studies that tested a series of potency levels. Consistently across all studies, only some of the potency levels of a series were found to be effective, and not all potency levels were effective in the same way. In three studies<sup>55,84,85</sup> investigating potency series from 12x or 14x to 30x, a minimum of one potency level and a maximum of seven potency levels of the series showed significant effects. Another study found some of the tested potency levels stimulated germination, but other potency levels inhibited it.<sup>60</sup> There was no uniform activity of all potency levels of a continuous series. This observation is not an artefact due to multiple statistical testing, since three studies<sup>55,84,85</sup> used the protected Fisher's LSD-Test with predefined statistical hypotheses. Another study<sup>60</sup> used a corresponding statistical procedure with an exact global Poisson test preceding comparisons of single potency levels to the control. Both procedures minimize type 1 as well as type 2 errors. In addition, stability of the experimental system (including the statistical procedure applied) was ensured by systematic negative control experiments in three studies.<sup>55,84,85</sup>

The observation that only some potency levels of a tested series of potencies were effective was not only observed in plant studies. A multi-centre study on the effect of potentised histamine on human basophil cells<sup>88</sup> also showed differing effective potency levels of potency series. In addition, Linde<sup>89</sup> found in his "Critical review and meta-analysis of serial agitated dilutions in experimental toxicology" that Mercury 15c induced a 40% mortality reduction in mercury-intoxicated mice, whilst Mercury 9c was ineffective. Thus the phenomenon of alternating levels of active and inactive potencies seems to be of a general intrinsic

nature among homeopathic potencies in preclinical investigations. Possible consequences of this observation for clinical application should be studied more closely. For example, in case of lack of success of a well-indicated remedy, it might be worth trying other potencies.

One aim of this review was to identify studies that provide evidence for *specific* effects of homeopathic remedies, i.e. effects implying some sort of “memory” of the carrier substance (e.g. water) for the mother tincture diluted. We identified 15 studies which included adequate (succeeded) controls. In these cases, the results obtained most probably cannot be attributed to non-specific effects, such as molecules and ions dissolved from the potentisation vessel during the succussion process. Five studies<sup>55,56,84,85</sup> compared the succeeded and unsucceeded potentisation medium statistically and found no significant difference between these controls. This may mean that plants in general are not influenced by non-specific succussion effects. If this were true, studies with unsucceeded controls may also be indicative of specific remedy effects. We think, however, that empirical confirmation is needed to prove that this is truly the case for any plant system investigated.

Several studies reported effects of potentised plant growth substances or plant hormones.<sup>55,56,63,84,85</sup> Since even stronger responses were observed in basic research animal models after application of potentised animal hormones,<sup>90–93</sup> one might setup the hypothesis, that human hormones – unknown at Hahnemann’s times – might be promising substances for human homeopathic therapy, corresponding homeopathic drug provings might be very interesting.

In two studies<sup>60,62</sup>, series of potencies and analogous unsucceeded dilutions of the test substances were investigated. One study<sup>62</sup> found significant effects of the potencies in comparison to the diluted controls, and the other study<sup>60</sup> found significant effects for most of the tested potency levels, but no effects of any dilution level of the diluted test substance. If these systems are not sensitive to non-specific succussion effects, these results imply that succussion is a necessary part of the homeopathic remedy production procedure. It thus would be interesting to conduct more investigations involving different sorts of controls to determine the relevance of the non-specific processes during succussion for plant models, to assess the usefulness of the different controls, and determine what intensity or sort of succussion is necessary to yield effective homeopathic preparations.

Some of the studies with specific effects had shortcomings in the quality of the experimental set-up; for example, blinding and randomisation were missing. Moreover, we could not assess the standardisation of the laboratory or ambient conditions during the experiments in most of the publications. Only five studies documented the stability of the experimental system by publishing data about systematic negative control experiments. In our opinion, this is the only way of convincingly demonstrating the stability of the experimental set-up chosen, i.e. that there were no false-positive effects caused by influences of laboratory or ambient conditions.<sup>7</sup> This is a very important point for the quality of a study, but systematic negative control

experiments have not so far been implemented on a routine basis. Especially in research in homeopathy, where in many cases effects are not explicable on molecular level, distinguishing effects from artefacts is essential. One example of the situation, where the instability of the experimental system precluded interpretation of the results, was an extensive investigation about the effect of potentised ambient pollutants (metals) on wheat germination.<sup>94</sup> In this case, the systematic negative control experiments yielded evidence for instability of the experimental set-up. We assume that many of the researchers carried out systematic negative control experiments to prove the stability of their experimental plant system. Without a proper description of the results of these control experiments, however, it is difficult to assess the validity of the study results definitively.

We found only a few published studies<sup>54,76–78</sup> that tried to reproduce earlier findings,<sup>27</sup> and these studies were only partially successful. There may be an unknown number of unpublished experiments reporting unsuccessful attempts to reproduce other trials. The lack of reproduction trials points to problems with reproducibility.<sup>95</sup> One study<sup>56</sup> started to investigate causes for reproduction problems in homeopathic basic research, and hypothesized differing seed quality as a limiting factor. These results have some similarity to the presumption, that basophil cells of different human donors vary in their sensitivity to treatment with potentised histamine.<sup>88,95</sup>

The studies reviewed were conducted for different reasons. Some studies can be interpreted as homeopathic drug provings on plants, corresponding to human homeopathic drug provings as introduced by Hahnemann.<sup>96</sup> The aim of other studies was to look for alternative fertilisation methods for plant production. Further studies strived to develop simple test systems for providing scientific evidence of the specificity of homeopathic remedies, to generate tools to control homeopathic drug production, and to ensure product quality.

Using plant models for research in homeopathy offers several advantages such as studies with large data sets, with a short experimental running time, and avoidance of the placebo effect as well as the ethical problems of trials on animals or humans.<sup>97</sup> The multiplicity of the reviewed studies and the diversity of the results reflect the complexity of the issue, however. In homeopathic drug provings with humans, a large variety of symptoms are depicted to understand the homeopathic effects of a potentised substance. Most homeopathic studies with healthy plants use only one or few measuring parameters, such as growth, length, weight, area or germination rate. This limitation could be one of the reasons why only few of many test substances were found to be effective in screening studies.<sup>84</sup> To deepen the understanding of homeopathic effects on plants, more sophisticated research would be interesting. For example, plant growth could be measured by multiple methods, or parameters mirroring the plant’s physiology and biochemistry could be used, as they are in some newer studies.<sup>52,86,87</sup>

Many different potentisation techniques were used in the studies reviewed: there were considerable variations in the intensity and movement of succussion, for example. Most studies identified effects of the tested potencies, but to

date we have not learned how the different potentiation techniques influence the efficacy of the potentised agents. To establish a closer relationship between homeopathic basic research and human and veterinary homeopathic care, it would make sense to investigate potencies, which were produced by the established procedures also used by homeopathic pharmacies.

Some studies<sup>55,56,77,78,84,85</sup> investigated similar series of potency levels of the same test substance – using different plant models in some cases – but they started potentiation with different concentrations of the mother tinctures. As a result, when we examine the possibility that there could be particular potency levels of one test substance which are effective in multiple plant models, we cannot say whether these potency levels (e.g. the 24x of *Argentum nitricum*, used in two studies) are comparable because of their differing mother tincture concentrations.

One aim of this review was to identify useful models for future studies in homeopathic basic research. The study model should be simple and easily transferable to other laboratories. Moreover, the standard deviation should be as small as possible. The germination model with wheat has been the most frequently used model so far. In spite of a rather high standard deviation it seems to be sensitive enough to identify potency effects. Treating the seeds with liquid immersion is simple. Parameters to measure growth modifications are root and shoot length, fresh and dry weight, and germination rate and speed. Wheat is routinely used for studies with poisoned or stressed plants as well as for studies with healthy plants.<sup>97</sup> Wheat germination studies with information about systematic negative control experiments are still lacking, however.

The study model based on duckweed<sup>84,85</sup> has generated attention. Duckweeds are small monocotyledonous waterplants with predominantly vegetative growth that are used as standard test organisms in ecotoxicology.<sup>98</sup> Due to the uniform plant growth the duckweed studies benefited from a very low standard deviation,<sup>98</sup> though the effects were also quite small. The waterplants grew directly in potency or control, with parameters being the area and number of the fronds, measured using a scanalyzer with an image processing system.<sup>84,85,98</sup> Using systematic negative control experiments, the reliability of the system was assessed over the entire course of investigations. The homogeneity of plant growth, the option of simple treatment and the standardised bioassay all qualify the duckweed model as a very interesting test system. The installation of the measuring technique involves considerable effort and expenditures. Easier and cost-efficient techniques may be developed in future in order to make this bioassay easily suited for multi-centre use.

Other interesting experimental plant models use the concentration of plant secondary metabolites<sup>52</sup> or other biochemical substances<sup>86,87</sup> as outcome parameters. This allows depicting a physiological reaction of the plants to the treatment with homeopathic potencies. These studies demand established techniques for analysis, special laboratory equipment and corresponding experience; this may be challenging for future independent repetitions in other laboratories.

## Conclusions

Healthy plant models seem to be a useful tool to investigate basic research questions about the specificity of homeopathic preparations. Homeopathic basic research could move forward by conducting plant studies of high quality design that includes systematic negative control experiments, blinding, randomisation, adequate statistical analysis, and appropriate controls to identify specific remedy effects. It would be advisable to do more trials on the potentiation process itself, and to use standardised potentiation techniques so that studies can be easily compared. Only few studies attempted independent reproduction trials. Increased cooperation between active laboratories would be advisable for identifying the crucial parameters for successful reproduction trials.

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