

## ORIGINAL PAPER

# The use of plant-based bioassays in homeopathic basic research

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**Objectives:** The objective was to evaluate homeopathic basic research studies that use plant-based bioassays. With this in view, a compilation was made of the findings of three systematic literature reviews covering plant-based bioassays in the three fields of healthy, abiotically, or biotically stressed plants. This compilation focused on investigations using advanced experimental methods and detailed descriptions, also with the aim of supporting the design of future experiments.

**Methods:** Publications included had to report on studies into the effects of homeopathic preparations on whole plants, seeds, plant parts and cells. Outcomes had to be measured by established procedures and statistically evaluated. A Manuscript Information Score (MIS) was applied using predefined criteria to identify publications with sufficient information for adequate interpretation (MIS  $\geq 5$ ). Additional evaluation focused on the use of adequate controls to investigate specific effects of homeopathic preparations, and on the use of systematic negative control (SNC) experiments to ensure the stability of the bioassay. Only a fraction of the studies reported here were performed with 'ultra high' dilutions, whereas other studies were performed with moderate or high dilutions.

**Results:** A total of 157 publications were identified, describing a total of 167 experimental studies. 84 studies included statistics and 48 had a MIS  $\geq 5$ , thus allowing adequate interpretation. 29 studies had adequate controls to identify specific effects of homeopathic preparations, and reported significant effects of decimal and centesimal homeopathic potencies, including dilution levels beyond Avogadro's number. 10 studies reported use of SNC experiments, yielding evidence for the stability of the experimental set-up.

**Conclusion:** Plant models appear to be a useful approach for investigating basic research questions relating to homeopathic preparations, but more independent replication trials are needed in order to verify the results found in single experiments. Adequate controls and SNC experiments should be implemented on a routine basis to exclude false-positive results. *Homeopathy* (2015) ■, 1–6.

**Keywords:** Review; Basic research; Homeopathy; Potentisation; Agriculture; Phytopathological models; Field trials; Impaired plants; Noxa

## Introduction

Over 1000 experimental studies have previously been published in the field of basic homeopathic research.<sup>1</sup> The three major areas of homeopathic basic research

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**Table 1** Assessment of the manuscript information content by a 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

MIS Score	Fully described 2 points	Partly described 1 points	Not mentioned 0 points
Experimental setup	Detailed information is given: mode of treatment of plants, growth period, time of measurements, etc.	Only some details are described or only a little information about the set-up is given	No information is given about the experimental set-up
Materials	All materials used in the experiment 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
Potentiation	Potentiation technique, date and time of potentiation and potentiation medium are described in detail	Some information about potentiation technique is given	No information about potentiation; only the potentiated test substance is mentioned
Controls	Detailed information, e.g.: 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

with plants have recently been reviewed. The first review of experimental studies on healthy plants was published by Majewsky *et al.*<sup>2</sup> Betti *et al.*<sup>3</sup> published a review of plants infected by viruses or bacteria (phytopathological models) and Jäger *et al.*<sup>4</sup> published a review of studies on abiotically stressed plants. In this publication we have compiled the three Reviews to give an overview of the complete field of homeopathic Basic Research with plants up to 2010. For information on further studies up to 2015, see the contribution on repetitions of fundamental research models in ultra high dilutions by Endler *et al.* in this issue.

## Methods

In the three reviews,<sup>2–4</sup> a search of the literature considered publications that reported homeopathy experiments using healthy plants, plant pathological models (*in vitro* and *in planta*), plant field trials and abiotically stressed plants, and involved whole plants, seeds, plant parts and cells. Outcomes had to be measured by established procedures and statistically evaluated. Using a Manuscript Information Score (MIS), publications were identified that provided sufficient information for proper interpretation (MIS  $\geq$  5, Table 1). A further evaluation was based on the use of adequate controls to investigate specific effects of homeopathic preparations and on the use of systematic negative control (SNC) experiments.

Only a fraction of the studies reported here were performed with ‘ultra high’ dilutions, whereas other studies were performed with moderate or high dilutions.

## Results

The studies included were conducted from 1920 (healthy plants), respectively 1965 (abiotically stressed plants) and 1969 (plant pathological models) to 2009, respectively 2010 (abiotically stressed plants). In 157 publications a total of 167 experimental studies were described. 84 studies (50% of all studies) included statistics and 48 studies (29% of all studies) had a MIS  $\geq$  5 allowing adequate and

detailed interpretation. 29 studies (17% of all studies) had adequate controls to identify specific effects of homeopathic preparations, and reported significant effects of homeopathic potencies, including dilution levels beyond Avogadro’s number. 10 studies (6% of all studies) involved the use of SNC experiments (Table 2).

For the publications with MIS  $\geq$  5 (48 studies), further detailed information was extracted. The plant primarily used in these 48 experiments was wheat (23 studies). Dwarf peas and duckweed were used in 3 studies each. Other plant organisms were used in no more than one or two studies. The most widely administered homeopathic preparation was silver nitrate (9 studies), followed by arsenic (8 studies), gibberellic acid (6 studies) and cina (4 studies). Other preparations were utilized in one, two or three studies at most. The most applied stressor was arsenic (6 studies). Other stressors were used in one, two or no more than three studies. Widely varying measurement parameters were used, but in all three fields (healthy, abiotically, or biotically stressed plants), number and size of plants, parts of plants or pathogenic organisms were measured. In some studies the concentration of plant secondary metabolites<sup>5</sup> or other biochemical substances<sup>6,7</sup> was used as outcome parameter. Furthermore the idea of using variability instead of mean values to measure the effects of homeopathic preparations has taken place.<sup>8,9</sup> In four model systems a consistent reduction of variability was found when investigating the effects of Arsenicum

**Table 2** A total of 167 experimental studies were included in the review process

Studies	Healthy plants	Phytopathological models	Abiotically stressed plants	Total
Identified	86	44	37	167
With statistics	43	19	22	84
MIS $\geq$ 5	29	6	13	48
With adequate controls	15	6	8	29
With SNC experiments	5	1	4	10

album 45x. Moreover a range of different potentiation techniques was used in the studies. Intensity, duration and movement of succussion differed considerably for example. There were plant studies, which compared the succussed and unsuccussed potentiation medium statistically and found no significant difference between these controls.<sup>9–12</sup> None of the studies identified any linear relationship between potency level and effect size. Within series of potency levels, alternating active and inactive potency levels were observed. With healthy plants, some of the tested potency levels were found to stimulate germination, but other potency levels inhibited it.<sup>13</sup> With stressed plants, likewise, no linear relationship between potency level and effect size was observed, but in one study with stressed plants no inhibiting potency levels at all were found.<sup>12</sup>

## Discussion

Using plant models for basic homeopathic research in offers several advantages. Due to the short experimental running time and space saving conditions it is usually possible to test many plants simultaneously. Thus, within an experimental set-up a large data-set can easily be generated and provides an opportunity to test several potency levels within the same experiment, to achieve a high degree of standardisation, and to observe and analyse individual living entities at different points in time during the experiment. The avoidance of the placebo effect as well as the ethical problems of studies on animals or humans are additional advantages. The disadvantages of plant-based bioassays are the lack of a *Materia Medica* and the absence of differentiated (e.g. also mental) symptoms, with corresponding difficulties in applying the Law of Similars and selecting the most adequate homeopathic remedy. These drawbacks do not occur in an isopathic approach with stressed plants.

In general the reviews show empirical evidence for specific effects of highly diluted, potentised substances, but the many separate studies using several methods meant there were few replicated trials.

### Reproducibility

With the emphasis on the issue of reproducibility, Endler *et al.*<sup>14</sup> identified four plant models replicated in homeopathic basic research with significant effects: Kolisko's results from potentised silver nitrate on wheat<sup>15</sup> were partly confirmed by Pongratz *et al.*<sup>16,17</sup> The outcomes of an isopathic approach by Betti *et al.* on potentised arsenic<sup>18</sup> were confirmed by the same working group.<sup>8,13,19</sup> Findings on potentised gibberellic acid and dwarf peas by Baumgartner<sup>9</sup> *et al.* were confirmed by the same working group<sup>10</sup> for one specific seed batch. The results concerning potentised gibberellic acid and wheat<sup>20</sup> by Pflieger were also partly verified by the same working group.<sup>21,22</sup>

However, there are also difficulties with reproducibility, within as well as between laboratories. Results with potentised arsenic on wheat carried out by Binder *et al.*<sup>23</sup> and Lahnstein *et al.*<sup>24</sup> were different from those found by Betti *et al.*<sup>18</sup> in a meta-analysis of all experiments. In 2008

Baumgartner *et al.*<sup>10</sup> found a different result with potentised gibberellic acid for one specific seed batch of dwarf peas compared to 2004.<sup>9</sup> A different outcome using potentised gibberellic acid for wheat growth at different times of the year was found by Thieves *et al.*<sup>25</sup> and Reischl *et al.*<sup>26</sup> compared to Pflieger.<sup>20</sup>

Difficulties with reproducibility observed in basic research into homeopathic preparations can have several causes: on the one hand, uncontrolled external influences interpreted as treatment effects (false positive results, artefacts), and on the other, unknown and therefore uncontrolled parameters influencing the effects of homeopathic preparations. The latter was observable in the dwarf pea bioassay with potencies of gibberellic acid where seed ripeness was hypothesized to be a crucial parameter.<sup>10</sup> Moreover, intrinsically indeterminate characteristics of dynamised preparations (as also known in chaos theory or quantum physics) and unsuitable measurement parameters, could lead to reproducibility problems. In this respect, reproduction trials are not only necessary scientific tools to identify false-positive or false-negative results, but also to reveal possible conditions modifying the effects of homeopathic preparations. Increased cooperation between laboratories could facilitate identify the crucial parameters for successfully replicating trials.

### Usage of the three fields

Each of the three fields is particularly suited for studying different issues. Healthy plant models are considered as a useful tool for investigating basic research questions about the specificity of homeopathic potency levels, especially the fluctuation between active and inactive potency levels in series of potency levels. For discovering different effects of single potency levels, a model seems to be most suitable in which the effects of single potency levels do not interfere with therapeutic effects. Therefore healthy plants are appropriate to investigate this question. Identifying specific remedy effects requires plant studies of high quality design that include appropriate controls, adequate statistical analysis, and SNC experiments. More trials on the potentiation process itself, and the use of standardised potentiation techniques, would simplify the comparison between different studies.

Of the three research fields in homeopathic basic research with plants, phytopathological models have the largest practical relevance. These models seem to be a useful instrument for investigating the application of homeopathy in agriculture. The support with homeopathic preparations is primarily requested in the field of organic agriculture. However the results obtained must be scrutinised further before any useful effect of the homeopathic treatments can be confirmed and applied. In general, the prospects for agro-homeopathy can be considered promising, but much more experimental work is needed, especially field trials.

Based on the assumption that a characteristic feature of homeopathic preparations is to induce equilibrating, regulating effects, it can be hypothesised that test systems using impaired organisms will yield more stable as well as more pronounced effects as a result of the application of

homeopathic preparations compared to test systems using healthy organisms. This hypothesis was confirmed by Betti *et al.*<sup>27</sup> In consequence of the equilibrating character of homeopathic preparations on test systems with impaired plants, it may be expected that all active potency levels act in the same direction, e.g. promoting growth in a system where the stress factor induces a growth reduction. This opens up the possibility of pooling data from several different potency levels in the statistical evaluation, which in turn might yield more stable effects due to the broader observational basis.

A major concern in test systems with stressed organisms is the increased variance of outcome parameters due to the impairment of the organisms. Hence, when using impaired organisms, a high degree of standardisation is very important for achieving as low a standard deviation as possible. This reduction of variance is in general more easily achieved by using abiotic stressors rather than biotic stressors, because lifeless material causes less variability than live factors. Reduction of the effects' magnitude might be a possible negative aspect of high stabilisation. However, in basic research the absolute magnitude of an effect is less important than in applied science.

### Development of plant-based bioassays

Several experimental parameters in experiments with homeopathic preparations might be relevant for or interact with the effects of the homeopathic treatment. The application of stressors (biotic and abiotic) in test systems with impaired plants leads to additional experimental parameters that require optimization, and, due to their interactions, to even greater scope for variability with a consequent need to tune all parameters so as to maximize the effect size. In relation to homeopathic medicines there are several parameters such as the selection of preparations or the time of application; likewise several parameters exist for the plant organisms, the noxa or pest, and the test system itself (Table 3).

The choice of the homeopathic test substances for plants is a substantial challenge for all models with healthy and impaired plants due to the lack of a *Materia Medica* for plants. But stressing plants leads to the development of new approaches, primarily in isopathic application, which might be a good starting point for tuning the experimental parameters in order to maximize the effect size. After optimization of the experimental parameters, a screening of multiple test substances could be performed to identify homeopathic (rather than isopathic) test substances with stronger effects.

In addition to choosing plant species and, where applicable, a stressor, the most appropriate outcome parameter must be found. Closely related to the outcome parameter is the impairment rate. For example *Senapis alba* (mustard) seedlings stressed with copper sulphate showed a 25% reduction in fresh weight and a 88% reduction in chlorophyll content, a parameter also strongly related to light conditions.<sup>28</sup> Stem growth of *Triticum* (wheat) seedlings stressed with arsenic trioxide was inhibited by 60%, whilst roots were inhibited by 30%. Only stem length showed an effect caused by homeopathic preparations.<sup>18</sup> Furthermore,

**Table 3** Experimental parameters, which might be relevant for or interact with the effects of the homeopathic treatment

<i>Homeopathic remedy</i>
– Selection of homeopathic remedy
– Point in time and way of application
– Application rate (dose)
– Mode of production, e.g. trituration or dilution
– Potency level
– Potentisation method
– Quality of prime substance
– Quality of potentisation medium
– Influences during potentisation
<i>Organisms/noxa or pest</i>
– Kind of organism, e.g. r/k-strategist, organisational level
– Kind of noxa, e.g. radiation, inorganic or organic substances
– Kind of impairment, e.g. cytotoxic, genotoxic
– Degree of damage – fitness of organisms
– Mode of impairment, e.g. concentration, point in time
<i>Test system</i>
– Growth conditions, e.g. light, nutrients, temperature
– Point in time of measurement
– Measuring parameter
– Variance
– Sensitivity
– Specificity
<i>Special considerations for experiments with homeopathic remedies</i>
– Crossover effects between potentised preparations and controls, e.g. through agitation, shielding, distance
– Attenuation of efficacy, e.g. through UV-radiation, electromagnetic fields, pressure
– Modulation of efficacy, e.g. constellations, impact of handling material
– Confining factors during experiments, e.g. sterile filtration
– Influence of experimenter

the applicability of outcome parameters depends on their potential to react to external stimuli, their capacity for regulation. For instance, a very low standard deviation of an outcome parameter could be caused by a pre-final state of severely stressed organisms that may prevent a reaction to homeopathic preparations. It might therefore be interesting in future studies to apply various outcome parameters and to compare them, since the response to homeopathic preparations may manifest in various parts or different metabolism pathways of the organism. Measurement parameters such as the concentration of secondary plant metabolites (e.g. metabolomics) make it possible to detect a physiological reaction in plants to treatment with homeopathic potencies, and allow an inference to be made about the mode of action. Using these findings, the plant models could in turn be optimised.

All studies, which compared succeeded and unsucceeded potentisation medium statistically, found no significant difference between these controls,<sup>9–12,29</sup> suggesting that plants are not influenced by non-specific succussion effects and that studies with unsucceeded controls may therefore also be indicative of specific remedy effects. However, this cannot be taken for granted and should be assured for every single model in question.

The production method of potentised preparations should also be considered in the development of test systems. Conducting studies with the same potentisation technique would be useful for ascertaining how different potentisation techniques (e.g. preparation of an aqueous solution, trituration in lactose) impact on the efficacy of the potentised agents in a given test system.

Furthermore, the origin of test substances could also have an impact on the effects of potentised preparations. As long as the mode of action is unexplained, we cannot exclude the possibility that the origin or production of the potentised substance affects the medicinal action of potentised preparations. Thus the origin or production of the substance should be described in detail in future studies.

We are convinced that plant-based bioassays will continue to be a useful approach in basic research into homeopathy. After further optimisations by internal and external replication trials, forthcoming applications include investigation of pharmaceutical factors such as possible improvements to production procedures (e.g. mode and duration of succussion, stability against external influences such as electromagnetic radiation, suitable sterilisation procedures etc.) as well as determination of the mode of action. In organic agriculture, homeopathic preparations may provide an opportunity to strengthen plants against biotic and abiotic environmental influences.

## Conclusion

Homeopathic basic research models using plants are usually short term, allowing large numbers of experimental replications, and they eliminate disadvantages such as the placebo effect or ethical concerns. Results included in three reviews dealing with the three fields of study using plant-based bioassays on healthy, biotically and abiotically stressed plants, support the notion that plant models are a useful approach for researching basic questions in relation to the specificity of homeopathic preparations.

Plant-based studies with healthy plants seem to be better bioassays for studying alternating active and inactive potency levels within series of potency levels. Models with stressed plants particularly when stressed with abiotic stressors yield more stable and more pronounced effects than models with healthy plants and are especially suitable to investigate therapeutic effects of homeopathic preparations. Phytopathological models are most relevant for possible application in agriculture.

There is a need to investigate difficulties arising with independently reproduced trials. Therefore more internal and external replication trials are needed. Further studies should also implement SNC experiments on a routine basis to control system stability and to exclude false-positive results. The use of more complex and multi-variable outcome parameters would also be beneficial.

Plant-based basic research models may be used for investigations of the mode of action of homeopathic substances and may develop into a method for studying the stability of homeopathic preparations against external influences, and comparing different production methods.

## Conflict of interest statement

Neither the authors nor their affiliates have any conflicts of interest to declare. No competing financial interests exist.

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