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Use of plants in basic research in homeopathic potentisation

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Plants are apt test organisms to study biological effects of potentised substances. We provide a short overview of the current state of knowledge and discuss some recent research results of our laboratories, yielding evidence for specific effects of highly diluted homeopathic preparations.

Introduction

Ever since the beginning of basic research into biological effects of highly diluted and succussed (homeopathically potentised) substances, plants have been used as test organisms to investigate and document any such effects.

As early as 1923, Lili Kolisko started a series of publications that documented her extensive work in this area.[1-4] She investigated the biological effects of series of potencies, mostly decimal potencies up to 30x, but sometimes also up to 180x, of a large variety of substances, typically metal salts. She mainly used a wheat seedling germination and growth assay, but also performed experiments with sunflowers, crocus, hyacinths, gladiolus, and other plants. Her main interest in this research area was to determine the so-called potency curve – a graphical representation of the effects measured as a function of potency level – specific for each substance investigated. This was based on the proposition of Rudolf Steiner,[5] that one would observe effective and ineffective potency levels within a given consecutive series of potency levels when performing respective experiments.

Lili Kolisko was confident that she had observed specific effects of highly diluted potentised substances, and that she had also gained evidence for the occurrence of biologically active and inactive potency levels in defined sequences as R. Steiner had predicted. Later, her work was criticised for lacking a statistical evaluation; however, at that time, statistics were either not developed in the present form or not easily available.

Use of plants in homeopathic basic research

Amongst the more than 1000 experimental studies that were published in the realm of basic research in homeopathy until today,[6] three recent reviews of a multinational collaboration identified 157 publications that reported on the use of plants to study biological effects of homeopathically potentised substances.[7-9] Three main types of bioassays could be distinguished: healthy plants, abiotically stressed plants, and plant-pathogen systems (phytopathological assays).

The 157 publications described a total of 167 experimental studies. 84 studies included statistics and 48 had a Manuscript Information Score (MIS) > 5 allowing proper and detailed interpretation. 29 studies had adequate controls to identify specific effects of homeopathic preparations, reporting significant effects of decimal and centesimal homeopathic potencies, including dilution levels beyond Avogadro's number. There were many individual studies with diverse methods and only a few replication trials. 10 studies reported on the use of systematic negative control (SNC) experiments in addition to experiments with potentised preparations. These SNC experiments increased study quality since they yielded thorough information on the reliability of the experimental set-up.

Evidence for potency curves

The most consistent and intriguing result of these reviews is the confirmation of Lili Kolisko's observation of alternating biologically effective and ineffective potency levels within a given series of potencies. All studies that tested series of consecutive potency levels reported such a non-linear and discontinuous relation between effect and potency level. An example of this phenomenon is given in Figure 1a.

Such non-linear effects may be considered as surprising and unexpected. Therefore, experiments in this area of research have to meet exceptionally high standards in order to exclude false-positive as well as false-negative results. The best way to control and document the stability of the experimental set-

up are systematic negative control experiments.[10] In such experiments, the identical set-up is used as in experiments with homeopathic potencies (the same number of plants, identical cultivation conditions, analogous randomization procedures), but the plants are all identically treated (e.g. with water from the same batch). The data acquired are analysed by identical statistical procedures as in the experiments with homeopathic potencies. In case the statistical analysis does not reveal any significant effects i.e. differences between the samples, the occurrence of systematic errors (e.g. due to spatial temperature gradients or any other inhomogeneities in the growth chamber) can be excluded with very high certainty, and the experimental set-up can be considered as reliable. Furthermore, systematic negative control experiments allow fitting an optimal statistical model that is not too conservative in order also to exclude false-negative results. Figure 1b illustrates the results of such a series of systematic negative control experiments used to control the experimental set-up that was applied in the experiments with homeopathically potentised gibberellic acid (Fig. 1a).

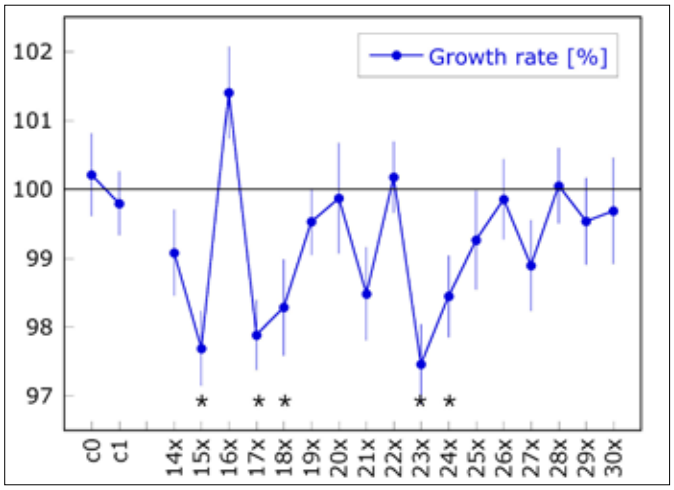

Fig. 1a (above): Growth rate of duckweed (Lemna gibba L.) treated with potencies of gibberellic acid (14x–30x) or controls (unsuccussed water c0 or succussed water c1). Data are from 5 independent experiments with 5 replicate samples each (thus n= 25 for each experimental condition), and are expressed as mean ± standard error relative to the pooled control c (mean of c0 and c1). Potency levels marked with an asterisk were statistically distinguishable from the pooled water control c (p<0.05, protected Fisher's LSD-test). All experimental conditions were blinded with respect to the experimenter and randomly allocated.*

Are these peculiar non-linear effects (Fig. 1a) reproducible? This question must be asked, but care has to be taken not to apply reproducibility as dogma to experimental research, in the sense that research has to be reproducible in order to document real effects.[12] Reproduction trials are powerful and necessary scientific tools, but not only to identify false-positive or false-negative results, but also to reveal possible external or internal conditions, i.e. influencing factors, confounders, that modulate effects of homeopathic preparations.

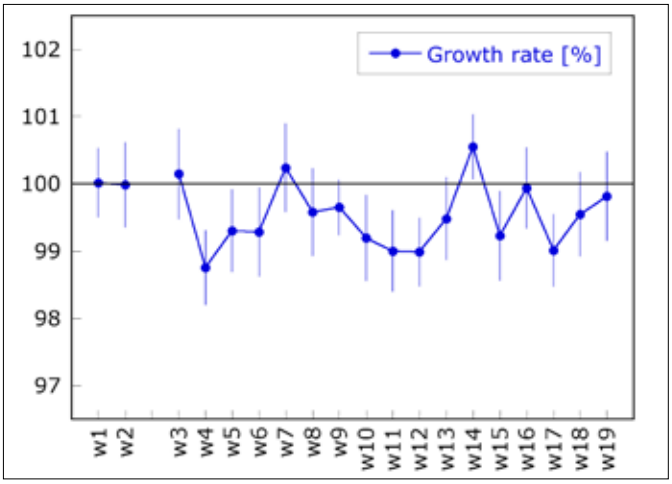

Fig. 1b (above): Growth rate of duckweed (Lemna gibba L.) treated with unsuccussed water only in systematic negative control experiments to investigate the stability of the experimental set-up used. Data are from 5 independent experiments with 5 replicate samples each (thus n= 25 for each experimental condition that was just unsuccussed water) and are expressed as mean ± standard error relative to the mean of water samples w1 and w2. No statistically significant differences between the 19 parameters were observed (ANOVA F-test).

Data for both figures were taken from the investigation of Scherr et al. [11]

Necessary conditions for successful reproducibility

One example for an identification of a probable effect-modulating factor is given in the analysis of a series of experiments with peas (*Pisum sativum* L.). The chosen cultivar "Früher Zwerg" is a gibberellic acid deficient mutant exhibiting dwarf growth. In the first series of experiments, a screening of different substances yielded significant effects for potencies of gibberellic acid and kinetin, two plant growth substances.[13] Repetitive investigations of the effect of gibberellic acid 17x on pea growth revealed seed-lot specific sensitivity of the dwarf peas regarding homeopathic treatment: out of 4 different seed lots (harvests from four different years) only two reacted to the treatment with gibberellic acid 17x, whilst the other two seed lots did not.[14] Chemical analysis of major constituents led to the hypothesis that the chosen pea variety does only react to potentised gibberellic acid when the seeds used in the bioassay form part of a seed lot harvested at a somewhat premature stage. This hypothesis could be confirmed in further experiments (manuscript in preparation).

Another aspect of reproducibility concerns the specificity of the shape of the potency curve. Is the pattern of active and inactive potency levels as visible in Fig. 1a specific for gibberellic acid, i.e. is it the same or at least similar for different organisms? Or is there a specific pattern for each combination of potentised substance and test organism? Or is the pattern specific for the organism used in the bioassay? All these questions are still open for research.

High standardisation possible

Plant-based bioassays can be standardised to a very high degree. The duckweed bioassay discussed above was modified by introducing abiotical stress through application of arsenic. By various measures, e.g. by using a chemically stable arsenic compound and by careful pre-selection of comparably stressed duckweed plants, it was possible to develop a bioassay with a coefficient of variation smaller than 1%.[15] Due to this optimisation, it was possible to obtain a high statistical power that allowed differentiating the effects of different potentised substances. Whilst potentised *Arsenicum album* increased duckweed growth rate, potentised gibberellic acid did not (in contrast to unimpaired duckweed), showing that the introduction of arsenic stress led to a specific sensitization of the duckweed bioassay.[15]

Conclusion

Advantages of using plant models in homeopathic basic research include the potential to generate large datasets with acceptable expenditure, to test several potency levels within the same experiment, to achieve a high degree of standardisation and to observe and analyse individual living entities. Main drawbacks of plant-based bioassays are the lack of an elaborated Materia Medica and the absence of emotional and mental symptoms, and corresponding difficulties in precisely applying the Law of Similars and selecting the most adequate homeopathic remedy.

We are convinced that plant-based bioassays will continue to be a useful approach in basic research into homeopathic potentisation. After further optimisations and laboratory internal and external replication trials, forthcoming applications include possible refinements of production procedures (e.g. method and duration of succussion, stability against external influences such as electromagnetic radiation, suitable sterilisation procedures, etc.) as well as determination of the long sought for mode of action.

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