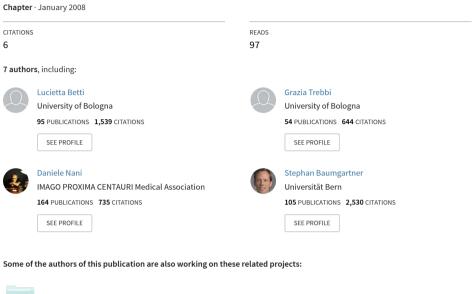
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Models with Plants, Microorganisms and Viruses for Basic Research in Homeopathy





Herb drug interactions View project



Possible effects of Visum album Resina 10% on gynecologic vaginal/zervical neoplastic diseases and their preliminary stages, lichen sclerosus et atrophicus, HPV View project

L. V. Bonamin *Editor*

Signals and Images





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Contributions and Contradictions about High Dilution Research



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Chapter 7 Models with Plants, Microorganisms and Viruses for Basic Research in Homeopathy

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Introduction

Most criticism about homeopathy concerns the lack of a scientific basis and theoretical models. In order to be accepted as a valid part of medical practice, a well-structured research strategy for homeopathy is needed. This is often hampered by methodological problems as well as by gross underinvestment in the required academic resources. Fundamental research could make important contributions to our understanding of the homeopathic and high dilutions mechanisms of action.

Plant- and microorganism-based experimentation appears suitable to this goal, making it possible to overcome some of the disadvantages of clinical trials: botanical and microbial trials do not present neither placebo effect nor ethical problems, and rely on a very cheap and almost inexhaustible source of biological material (Betti et al., 2003a). Moreover, relatively simple model systems can be adopted so that a more direct treatment/effect relationship and large data samples for structured statistical analyses can be obtained. This is a very important feature because it allows a large number of experimental repetitions and external replications to be performed, useful for studying the problem of irreproducibility so often reported in homeopathic literature (Steffen, 1984; Baumgartner et al., 1998; Binder et al., 2005). In fact, one of the major challenges of homeopathic fundamental research today is the reproduction of preclinical studies that have shown significant effects of ultra highly diluted substances compared to control groups. The lack of reproducibility represents a crucial difficulty in testing homeopathy and has stimulated explanations of homeopathic

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treatment effects using complexity theory (Hyland and Lewith, 2002). On the basis of the experimental evidences in wheat and tobacco models (Betti et al., 2003b; Brizzi et al., 2005), a recent proposed hypothesis is that the systematic reduction of variability might be one of the peculiar actions of ultra high dilutions (Nani et al., 2007). Furthermore, since the main cell structures and functions are common in the majority of eukaryotes (Lam et al., 2001; Carrington and Ambros, 2003), plant and eukaryotic microbial bio-assays could be of interest also from a medical point of view, at least as complementary to clinical studies.

Since the pioneering works of Kolisko on wheat germination (Kolisko, 1923) and Junker on growth of microorganisms (paramecium, yeast, fungi) (Junker, 1928), a number of experiments have been performed either with healthy organisms (various physiological aspects of growth) or with artificially diseased organisms, which may react more markedly to homeopathic treatments than healthy ones. In the latter case, the preliminary stress may be either abiotic, e.g. heavy metals, or biotic, e.g. fungal and viral pathogens or nematode infection. Research has also been carried out into the applicability of homeopathic principles to crop growth and disease control (agrohomeopathy): because of the extreme dilutions used, the environmental impact is low and such treatments are well suited to the holistic approach of sustainable agriculture (Betti et al., 2006). Unfortunately, as Scofield reported in an extensive critical review (Scofield, 1984), there is little firm evidence to support the reliability of the reported results, due to poor experimental methodology and inadequate statistical analysis. Moreover, since there is no agricultural homeopathic pharmacopoeia, much work is required to find suitable remedies, potencies and dose levels.

Recently, high methodological standards have been applied to basic research into homeopathy with different plant and microbial model systems: external influences (such as light, temperature, humidity, soil and seed quality) have been considered; most handling steps and instrumental measurements have been carried out blind in order to exclude unconscious influences by the researcher; experimental designs have been guided by adequate statistical standards.

Herein, only subsequent literature published after Scofield's critical review (Scofield, 1984) will be considered. Therefore, the present overview is divided in 4 sections:

- 1. Models based on healthy plants, microorganisms, and viruses
- 2. Models with impaired plants and microorganisms (abiotic stress)
- 3. Phytopathological models using infected plants (biotic stress) and
- 4. Field trials

Models Based on Healthy Plants, Microorganisms and Viruses

Among the numerous plant model systems studied, the classical test of wheat germination and growth (Kolisko, 1923) has been repeatedly used as a basic model for research in homeopathic potencies. But also many other organisms were introduced. Table 7.1 gives an overview about the publications released after 1984. Species, treatment, working variable and effect are reported.

Table 7.1 Overview about of bioassays with healthy plants, microorganisms, or viruses: references, experimental main features and observed effect are reported

Reference	Species	Treatment	Working variable	Effect
Andrade, 2001	Chambà•	Homeopathic treatments	Coumarins content	+
Baker and Smith, 1985	Yeast	Reanalysis of data from Steffen, 1984	Periodicity with potencies	+/-/0
Baumgartner et al., 2004	Dwarf pea	DK, <i>DH</i> plant hormones, PÇ	Shoot growth	+/0
Betti et al., 1994	Wheat	$DH \text{ As}_2 0_3$	Germination	+
Bonato and da Silva, 2003	Radish	CH, MCH Sulfur	Shoot and root growth	+
Bornoroni, 1991	Oat	IAA, CH CaCO, PC	Shoot growth	+/0
Brack et al., 2003	Bacterium	DH 3,5- dichlorophenol	Luminescence	-/0
Endler and Pongratz, 1991	African violet	DH IBA, PC	Root and leaf growth	+
Engstler, 2004	Yeast	CH homeopathic treatments***	In vitro growth (optical density)	+/-/0
Glatthaar- Saalmüller et al., 2001	Viruses	D Euphorbium resinifera, Pulsatilla pratensis, Luffa operculata; Euphorbium compositum SN	Growth of virus plaques	-/0
Grange et al., 1987	Bacteria	CH homeopathic treatments*; mother tinctures**	In vitro growth	-/0
Hagelberg, 1987	Yeast	CH homeopathic treatments**, PC	In vitro growth (optical density)	0
Hamman et al., 2003	Barley	CH gibberellic acid	Germination, root and shoot growth	+/0
Khanna et al., 1992	Fungi	DH and CH homeopathic treatments****	Respiration of germinating spores	-/0
KoCH et al., 1995	Yeast	DH salicylic acid, CuSO ₄ , NaNO ₅	In vitro growth (no. of cells)	0
Malarczyk et al., 2003	Fungi	CH guaiacol, ethanol	Enzymatic activity	+/-/0
Pongratz and Endler, 1994	Wheat	DH AgNO ₃ , PC	Germination, shoot growth	+
Pongratz et al., 1998	Wheat	DH AgNO ₃	Shoot growth	+
Scherr et al., 2007	Duckweed	DH homeopathic treatments*****, PÇ	Frond (leaf) growth	+/-/0

(continued)

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Table 7.1 (continued)

Reference	Species	Treatment	Working variable	Effect
Scherr et al., 2006	Yeasts	DH homeopathic treatments****, PÇ	Growth kinetics	-/0
Steffen, 1984	Yeast	CH Pulsatilla	In vitro growth (no. of cells)	0
Steffen, 1985	Yeast	DH AgNO ₃ , CuSO ₄ , HgCl ₂ , NaCl	In vitro growth (no. of cells)	0
Tschulakow et al., 2005	Dinoflagellate	Succussed and unsuccussed medium	Bioluminescence	+

D, C, Mc = decimal, centesimal, hundred thousand potency; H = hahnemannian potency; K = korsakovian potency; PC = potentized control (as additional control); IAA = indole acetic acid; IBA = indole butric acid. • = Justitia pectoralis;*= Belladonna, Drosera, Lycopodium, Plumbago, Prunus spinosa, Pulsatilla nigricans, Pyrethrum parthenium, Raphanus, Rhus toxicodendron, Usnea barbata; **= 31 different mother tinctures; ***= Sulphur, Arnica montana, Chamomilla, Bryonia alba, Euphrasia officinalis, Pulsatilla; ***= screening of 49 substances, replications of Arnica montana, Aurum metallicum, Berberis vulgaris, Colchicum autumnale, Conium maculatum, Corallum rubrum, Cyclamen purpurascens, Lycopodium clavatum, Medorrhinum, Mercurius solubilis Hahnemanni, Natrium chloratum, Pulsatilla pratensis, Solanum dulcamara, Stannum metallicum, Strychnos nux-vomica; ****= screening of 14 substances, replications of Azoxystrobin, Phosphorus; *****=Arsenicum album, AsvaganDH, Blatta orientalis, Filix mas, Kali iodatum, Kali muraticum, Lycopodium clavatum, Phosphorus, Thuja occidentalis, Zincum sulphuricum; ******= screening of 12 substances. + = stimulating or increasing effect; - = inhibiting or decreasing effect; +/- = different effects according to the potency used or physiological conditions; 0 = not significant, i.e. effect below natural variability of the experimental system used.

In particular, Pongratz's results (Pongratz and Endler, 1994; Pongratz et al., 1998) confirmed previous data showing that three consecutive potencies of silver nitrate, a substance which in high concentration inhibits germination, induced a typical 'V'-form effect pattern: 24 and 26 *DH* stimulated and 25 *DH* weakened stalk growth. The simplicity of the model made it possible to repeat the experiment in a multi-centre trial, a very important requirement for the validation of high-potency studies.

In experiments on other plant species growth parameters and biochemical responses were evaluated. Between the most recent papers, three studies have examined the effects of homeopathically prepared plant hormones (e.g. gibberellic acid or kinetin) on the germination performance of barley seeds (Hamman et al., 2003), length growth of dwarf peas (Baumgartner et al., 2004) and frond (leaf-like structures) area growth rate of duckweed (Scherr et al., 2007). In all cases, significant effects have been observed, supporting the hypothesis that homeopathic potencies of plant growth substances may be effective.

Also microorganisms and viruses were used as model systems; several studies have been published in the last years. These included different strains of viruses and species of bacteria, yeasts, fungi and one species of dinoflagellates (see Table 7.1). Though it seems to be easier to use microorganisms (compared to plants) with respect to technical handling, it is nevertheless important to exactly control and document the methodological details of all experimental conditions in order to

allow replication experiments. The latter are necessary to verify effects found and to determine all important parameters necessary for successful replication.

Among the studies with microorganisms most often yeasts have been used as model organisms (Steffen, 1984, 1985; Baker and Smith, 1985; Hagelberg, 1987; Koch and Partilla, 1995; Engstler, 2004; Scherr et al., 2006). Some of these works were inspired by the positive results of Jones et al. (Jones and Jenkins, 1983a,b) indicating sensitive reactions of Saccharomyces cerevisiae to potencies of Pulsatilla. However, also with microorganisms there seem to be some difficulties when external replication of reported results was intended (Steffen, 1984). A re-analysis of the data by Baker (Baker et al., 1985) showed similar periodicities with potencies in the work of Steffen et al. (Steffen, 1984) and Jones et al. (Jones and Jenkins, 1983a, b). In general, the yeast model system was reported to be stable and reliable, however varying sensitivity to homeopathic potencies has been found, depending on the measured parameter and on the substances and potency levels tested (Baker et al., 1985; Engstler, 2004; Scherr et al., 2006). It can be hypothesized that different strains as well as different physiological conditions of the cells at the beginning of the experiment may be important when using microorganisms for investigating effects of potentized substances. In the studies named two different yeast species have been used, either Saccharomyces cerevisiae (Steffen, 1984; Koch et al., 1995; Engstler, 2004) or Schizosaccharomyces pombe (Steffen, 1985; Hagelberg, 1987). In one study both species were tested and showed differential reactions in their growth kinetics when treated with homeopathic potencies (Scherr et al., 2006). Engstler (Engstler, 2004) did not come to consistent conclusions because of problems with internal repeatability and sterility.

Substance specifity, i.e. the effect that some potentized substances did affect the measured parameters, whilst others did not when tested in the same experimental set-up, was observed for viruses (Glatthaar-Saalmüller et al., 2001), yeast (Scherr et al., 2006) and fungi (Khanna and Chandra, 1992). Another phenomenon was demonstrated in those studies in which several potency levels were tested simultaneously: there were active and inactive potency levels. This phenomenon has also been demonstrated with bacteria (Brack et al., 2003), yeast (Scherr et al., 2006) and fungi (Malarczyk et al., 2003).

The importance of the succussion step in the preparation process of homeopathic remedies was investigated by Tschulakow et al. (2005). They studied the effect of succussed and unsuccussed medium when measuring the intensity of bioluminescence in a dinoflagellate. The differences found were highly significant and independent of the number of succussions (in the range between 13 and 64).

Models Based on Impaired Plants and Microorganisms

In Table 7.2 we summarize recent investigations of homeopathic dilutions in systems with abiotic stress. Species, stress factor, treatment, working variable and effect are reported.

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Tab

Auquière et al., 1988 Betti et al., 1997 Binder et al., 2005 Brizzi et al., 2000	Species	00000			١
Auquière et al., 1988 Betti et al., 1997 Binder et al., 2005 Brizzi et al., 2000				of the state of shoots	1
Betti et al., 1997 Binder et al., 2005 Brizzi et al., 2000	Wheat	Ethanol, Lysine	CH Ethanol, Lysine, PC	Shoot growth, weight of shoots	: .
Betti et al., 1997 Binder et al., 2005 Brizzi et al., 2000	Whent	As O	DH As.O., PC	Shoot growth	+
Binder et al., 2005 Brizzi et al., 2000	Wilcar	233	DH As O PC	Shoot growth	1
Brizzi et al., 2000	Wheat	As_2U_3	DH 752 3, 1 C	Germination	-/+
	Wheat	As_2O_3	DH As ₂ O ₃ , FC	Shoot grouth	+
Brizzi et al., 2005	Wheat	As ₂ O ₃	DH As ₂ O ₃ , PC	Silvot growtin	1
Compatible et al. 2003	Feverfew	Adaptation	DH Arnica montana	Shoot growth, partnerionide content	
Carvalho et al., 2004	Feverfew	Water shortage	CH Natrum muriaticum,	Shoot growth, chlorophyll and proline	1,4,0
Cal Valle Co and Tool			nosode	content	0
Carvalho et al., 2005	Feverfew	Adaptation	CH Arnica montana	Shoot growth, partneholide content	ر ان ا
Ecce 1002	Angels's trumpet	Gamma-irradiation	CH Gamma-radiation, PC	Germination	
Egger, 1992	Wheat	NaCl, CuCl, K,Cr,O,	DH NaCl (isopathic), DH	Shoot growth; fresh and dry weight	0/-/+
Novac et al., 1991		7 7	CuCl (isopathic), DH	of shoots, grains and roots	
			K,Cr,O, (isopathic)		9
900	Wheat	Dark germination	DH CuSO.	Shoot growth; fresh of shoots; dry	0/-/+
Lauppert, 1993	11000			weight of shoots, grains and roots	9
1001	Wheat	Dark germination	DH Platinum, Mercurius,	Shoot growth; fresh and dry weight of	0/-/+
Lenner et al., 1991			Cadmium, Plumbum,	shoots, grains and roots	
			Cuprum, Aurum,		
			Argentum nitricum,		
			Sulfur		9
Novic et al., 1990	Wheat	Dark germination	DH Aurum	Shoot growth; fresh and dry weight of	0/-/+
				anonie a	+
Projetti et al., 1985	Lentil	CuSO,	CH CuSO ⁴	Root growin	· ·
Stoffen 1085	Yeast	CuSO.	DH CuSO ₂	In vitro growin	,
Stellen, 1965	Crass	NaCl 4	CH NaCl, PC	Shoot growth, germination	+,

D, C = decimal, centesimal potency; H = hahnemannian potency; K = korsakovian potency; PC = potentized control (as additional control); + = stimulating or increasing effect; - = inhibiting or decreasing effect; +/- = different effects according to the potency used or plant physiological conditions; 0 = not significant, i.e. effect below natural variability of the experimental system used. The work of Bologna University research group (Betti et al., 1994, 1997; Brizzi et al., 2000, 2005) focuses on the statistical analyses of a series of experiments on the same biological model, where a large number of wheat seeds were treated with decimal potencies of arsenic trioxide. The consistency of the different statistical analyses, as well as the reproducibility of most of the experimental results is notable: the As₂O₃ 45 *DH* potency always induces a highly significant stimulating effect compared to control, as well as H₂O at the same potency, whereas As₂O₃ diluted at 10⁻⁴⁵ never show any effect. The reported results confirm that the potentization process is critical to make raise the biological effects secondary to the treatments with respect to control.

Moreover, wheat germination is the theme jointly investigated by Betti and Baumgartner research groups: the result of Baumgartner replication trial (Binder et al., 2005) is a reversal of the original study (Betti et al., 1997), since *Arsenicum album* 45 *DH* inhibited wheat shoot growth instead of enhancing it, whereas Betti replication trial (Brizzi et al., 2005) reassessed the result of its initial study (Betti et al., 1997). Nevertheless, high homeopathic potencies induced statistically significant effects in both experiments, even if the magnitude and direction of these effects seem to depend on yet unknown parameters (Binder et al., 2005). Elucidation of factors responsible for effect size and direction will yield important information, possibly helping to understand the reasons for problems with reproducibility also in other systems.

In some studies it is not easy to decide, if stressed or healthy organisms were used, because in some cases there was no active intervention to cause damage, and in other cases the damages were not exactly defined. For example, Novic et al. investigated the effect of potentized gold on shoot growth of wheat seeds. To increase the sensitivity of the system they aimed to create deficiency conditions through germination in darkness and without addition of nutrients (Novic et al., 1990). Lehner et al. used the same experimental design to test metals like *Platinum*, *Mercurius*, *Cadmium*, *Plumbum*, *Cuprum*, *Aurum* as well as *Argentum nitricum* and *Sulfur* under mentioned deficiency conditions (Lehner et al., 1991). Dark germination is a natural condition for wheat, however. To what extent germinating seeds were stressed by unavailability of nutrients, was not specified. Lauppert used the same experimental design, accounting that germination of seeds in darkness was chosen only to get uniform conditions, but not with the aim of stressing the organisms (Lauppert, 1995).

Carvalho et al. investigated the effect of low potentized *Arnica montana* on stressed feverfew caused by adaptation (Carvalho et al., 2003, 2005). In one study they used *Natrium muriaticum* for the treatment of damages induced by water shortage (Carvalho et al., 2004). Kovac et al. worked with wheat seeds germinated over 6 days in darkness without nutrient and, in addition, supplied with high NaCl-concentrations as abiotic stress factor (Kovac et al., 1991). They emphasized the need of a reduction of variance (see above) to achieve clear results. Tighe also used NaCl for weakening of plants. He chose cress as test organism after comparing wheat, sunflower and cress in preliminary tests (Tighe, 2005). Egger treated *Datura arborea* seeds with gamma radiation and treated the plants with potentized gamma radiation (gamma irradiated lactose; Egger, 1992).

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It is needed to add some methodological considerations on the use of impaired or stressed organisms in preclinical model systems.

All experiments with poisoned plants (as listed in Table 7.2, except one preliminary test from Egger (1992)), used the isopathic approach. Thus, the problem of finding an appropriate remedy according to the *similia principle* could be "avoided". A further advantage of this experimental approach is the possibility to test true *information* effects even in low potencies (low potencies of many substances can influence plants due to molecular – non homeopathic effects; given a pre-existing damage by higher concentrations of the same substance, any effect of a treatment with lower concentration cannot be explained by the material presence of this substance). In addition, low isopathic doses can be a useful tool to validate or not some principles of anthroposophical medicine, in which low potencies might act on a *deeper organizational* level of the organism. Plant models are especially interesting for studying this matter.

In addition, it might be promising in future to try other approaches than the isopathic one in systems with poisoned organisms. Ways to approximate classical homeopathy may be the use of phenomenological or biochemical symptoms on basis of the *similia principle*.

If a systematic reduction of variability is – as aforementioned – a specific effect of homeopathic potencies, i.e. stronger in situations that allow a regulative response of the organism, the homeopathic/isopathic treatment of organisms disturbed by stress should exceed the effect of treatments of healthy organisms in basically equalized conditions. Furthermore, reproducibility might be enhanced if the damaging effect and the corresponding specific organic answer to the homeopathic/isopathic treatment are stronger than the unspecific noise concomitant factors.

The variances of model systems under abiotic stress are highly dependent on the concentration of noxae used. A straight relation has to be found between a measurable damage and a sufficient fitness of the organism that still allows self-healing. The variance increases strongly up to a certain concentration of noxae, thereby causing a very instable system. The further reduction of the organism vital functions leads to a lower system-sensitivity and consequent decreasing of variance, in a second step. Hence, variance and sensitivity correlate. For this reason, a sensitive system with weak organisms has to be highly stabilized to prevent loss of statistical power caused by escalating variance. Anyway, it is important to remember that the equilibrating effect of homeopathic preparations does not necessarily imply a reduction of variance in any case.

Phytopathological Models

In most of the papers available focused on fungal infections (Saxena et al., 1987; Khanna and Chandra, 1989, 1992; Aggarval et al., 1993; Rivas et al., 1996; Rolim et al., 2001) following homeopathic treatments, decrease of disease symptoms, post-harvest losses, fungal germination and respiration rate of germinating spores

were evidenced. Few studies took into account viral infections (Cheema, 1986, 1993; Betti et al., 2003b) and, in this case, a weaker symptomatology was observed. In particular, in blind randomized experiments using tobacco plants carrying tobacco mosaic virus (TMV) resistant gene N, a significant enhancement of plant resistance was obtained following As₂O₃ 5 and 45 DH potencies (Betti et al., 2003b). As far as nematode infection is concerned, a few papers are available as well (Sukul and Sukul, 1999, 2006; Datta, 2006): plants treated with homeopathic preparations showed improved growth (in terms of shoot and root length) and reduced nematode infection (in terms of root gall number and nematode population in root and soil). Root- and leaf-protein content and root-water content were also affected by homeopathic treatments. A brief review about is seen in Table 7.3.

 Table 7.3
 Summary of phytopathological bioassays: references, experimental main features and observed effect are reported

Reference	Species/pathogen	Treatment	Working variable	Effect
Aggarval et al., 1993	Wild taro/ phytophthora colocasiae	Homeopathic treatments**	Disease symptoms, fungal growth and spore germination	, -
Betti et al., 2003b	Tobacco/tobacco mosaic virus	DH As ₂ O ₃ , PC	Virus-induced hypersensitive lesions	-
Cheema et al., 1986	Papaya/papaya mosaic virus	Homeopathic treatments	Disease symptoms	-
Cheema et al., 1993	Tomato/tobacco mosaic virus	Clerodendrum aculeatum, CH Thuja	Disease symptoms	-
Datta, 2006	Mulberry/ m. Incognita	CH Cina	Plant growth, nematode infection	+, -
Khanna and Chandra, 1989	Mango, guava, tomato/ pestalotia spp., fusarium roseum	Homeopathic treat- ments and adju- vants	Post-harvest losses	-
Khanna and Chandra, 1992	Different fungi••	DH treatments	Spore respiration rate, organic acid pool in spores	-
Rivas et al., 1996	Wheat, tomato/ Alternaria solani	CH treatments***	Seed and spore germina- tion	+/-
Rolim et al., 2001	Apple/ podosphaera leucotricha	CH treatments****	Powdery mildew symp- toms	-
Saxena et al., 1987	Reed okra/ seed-borne fungi	CH Thuja, nitric acid, Sulphur, Calcarea carb., Teucrium Q	Fungal spore germination	

(continued)

Table 7.3 (continued)

Reference	Species/pathogen	Treatment	Working variable	Effect
Sukul and Sukul, 1999	Cowpea / Meloidogyne incognita	CH Cina	Plant growth, nematode infection	+
				-
Sukul et al., 2006	Lady's finger/ M. incognita	CH Cina, Santonin, Ethanol	Nematode infection root-protein	-
			and -water content	- "

D, C = decimal, centesimal potency; H = hahnemannian potency; PC = potentized control (as additional control); •• = Alternaria alternata, Colletotrichum gloeosporioides, Fusarium roseum, Gloeosporium psidii, Pestalotia mangiferae, Pestalotia psidii; ** = Kali iodatum, Arsenicum album, Blatta orientalis, Thuja occidentalis; ***= Arsenicum album, Calcarea, Cuprum, Ferrum metallicum, Lycopodium, Natrum, Phosphorus, Selenium, Silicea, Sulphur; **** = Kali iodatum, Lachesis trigonocephalus, Staphysagria, Sulphur, Oidium lycopersici. + = stimulating or increasing effect; - = inhibiting or decreasing effect; +/- = different effects according to the potency used or plant physiological conditions.

Field Trials

Scientific literature provides very few and outdated descriptions of field trials (Table 7.4). Aside from two studies on trees affected by virus (Sinha, 1976) or fungus (McIvor, 1980), only two papers are easily available. Kayne reported the results of a field trial on rye grass (Kayne, 1991). The application of homeopathic sprays (CH Sulphur and mixture of CH Sulphur, Silicea and Carbo vegetalis) did not give significant effects on plant growth; however some methodological hints for testing homeopathic treatments emerged: the choice of remedy, potency and frequency of application is crucial and should be made with great care to ensure the best chance of success. In a more recent paper (Diniz, 2006) the efficacy of a homeopathic preparation for tomato late blight control was evaluated. The treatment was prepared from tomato tissue infected by Phytophthora infestans, agent of tomato late blight (isopathic treatment at 30 CH potency). Also in this case, no significant effect has been observed with respect to control.

A three year project (2004–2006) on biological control of dark leaf spot caused by *Alternaria brassicicola* in cauliflower was financed by the Marche region (Italy) to the Betti research group. The field trial results showed that As₂O₃ *DH* 35 could significantly reduce infection level on cauliflower heads with respect to control. A resistance increase in tobacco plants against tobacco mosaic virus following treatments with As₂O₃ 45 *DH* has been already reported (Betti et al., 2003b) and here it was shown the significant effects of As₂O₃ 35 *DH* in the control of dark leaf spot disease. It is noteworthy that in different plant/pathogen interactions different potencies of the same substance have different efficacy. Since As₂O₃ 35 *DH* was diluted above Avogadro's number, there were no arsenic molecules in the treatment, thus, it can be used in agricultural practice without introducing pondered arsenic into the environment. These results need further investigations, but they seem to support the

Table 7.4 Summary of field trials: references, experimental main features and observed effect are reported

Reference	Species/pathogen	Treatment	Working variable	Effect
Diniz et al., 2006	Tomato/ Phytophthora infestans	CH isopathic treatment	Late blight symptoms	0
Kayne, 1991	Rye grass	CH Sulphur, Silicea, Carbo vegetalis	Plant growth	0
Trebbi et al., 2008	Cabbage/ Alternaria brassicicola	DH As ₂ O ₃	Disease severity	-

D, C = decimal, centesimal potency; H = hahnemannian potency; - = inhibiting or decreasing effect; 0 = not significant, i.e. effect below natural variability of the experimental system used.

possibility of an agricultural application of potentized drugs. The privileged target of agro homeopathy could be small farms (and in particular, those of nutraceutical and herbalist sectors) practicing organic and sustainable agriculture that strive to be environmentally responsible, economically viable, and socially just.

However, putative adverse effects for cattle and consumer health must also be considered. Remains of the potentized remedy itself, as well as processes induced in plants, might cause reactions in animals and humans. Given the fact that the exact nature of these preparations is still unknown and that there is not any analytical methods to detect application of homoeopathic/isopathic potencies, it would be advisable to proceed with caution. Application of homoeopathic/isopathic potencies in organic plant production should be tested thoroughly before any products are sold to the public.

Conclusions and Perspectives

The literature on homeopathy or isopathy and plants or microorganisms is limited and not always easily available. Nevertheless, interest in this field appears to be growing in recent years and several projects are in progress, mainly in Central and South America. In general, the potential prospects for such treatments in plant and microbial basic research and agriculture can be considered promising. In particular, much more work is needed, especially at field level, since the influence of environmental and agronomical factors (temperature, drought, humidity, plant cultivars and so on) might significantly change the quality of yields and, thus, the results of successive experimentations. In addition, there is no certain knowledge about the most effective potency level or potency type (d/c), and neither adequate doses nor application frequencies of potentized dilutions for plants are sufficiently known.

The use of plants and microorganisms in homeopathic basic research has a considerable potential. We think that it is possible to use such model systems to elucidate basic nature and working principles of homeopathic/isopathic preparations and to develop quality control instruments (e.g. for optimization of production, storage, and transport procedures).

Finally, it has to be stressed that results of all research and projects, whether successful or not, should be made widely available so that others can learn from these, avoiding duplication and inefficiency. Moreover, replication of results and multicentre trials should be performed, to be published in international journals with an impact factor or wide circulation, to gain credibility and facilitate funding.

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