THE EFFECT OF HOMOEOPATHIC POTENCIES OF A FUNGICIDE, ACROBAT® (Dimethomorph and Mancozeb), ON DOWNY MILDEW (Peronospora parasitica) OF CABBAGE SEEDLINGS (Brassica oleracea)

Dissertation submitted in partial compliance with the requirements for the Master's degree in Technology in the department of Homoeopathy at Natal Technikon.

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ABSTRACT

The purpose of this study was to expand on previous research in Homoeopathy, using the cabbage (*Brassica oleracae*) and crucifer downy mildew (*Peronospora parasitica*) disease system. More specifically, this study aimed to evaluate the biological effects of homoeopathic treatments made from a fungicide, Acrobat®, on the development of downy mildew on cabbage seedlings. The object was to demonstrate the Arndt-Schulz Law or Hormesis (inhibition of growth at high concentrations and growth stimulation at low concentrations) using homoeopathic treatments prepared as per homoeopathic methodology, by dilution and succussion.

The experimental subjects were trays of healthy cabbage seedlings that were inoculated with downy mildew by introducing one infected cabbage seedling per tray. Eleven treatments, including two controls and nine homoeopathic treatments, were applied every seven days. Distilled water (minimum control), and Acrobat® 2g/litre (maximum control), provided a framework against which dose-related effects of the homoeopathic treatments (7XH, 9XH, 12XH, 7CH, 9CH, 12CH, 15CH, 30CH, 200CH) were measured. Disease ratings were performed every two to three days for six ratings in terms of percentage leaf area infected. The results were analysed at a 95% confidence level using Multifactor analysis of variance (MANOVA). The trial was done twice.

Trial One did not yield any statistically significant results. Trial Two showed Acrobat® to be the most effective treatment in terms of disease control. The 30CH was the most effective homoeopathic treatment in terms of disease control, followed by the 200CH. However, neither showed statistical differences from the distilled water control. A few interesting trends emerged. The 7XH, 9XH, 12XH and 9CH tended to stimulate fungal growth, although not statistically significant, whereas the 30CH and 200CH tended to have an inhibitory effect on the disease progress.

Therefore the higher dilutions had a greater effect on inhibiting the disease process. Although this was not statistically significant, the trend was unexpected in terms of the original hypothesis based on the Arndt-Schulz law. A dose-response to the different potency levels was expected in terms of the Arndt-Schulz law whereby fungus growth will increase with increasing dilution.

There is much experimental data supporting the Arndt-Schulz law or hormesis but researchers in this field have not previously performed experiments using diluted and succussed substances. Given the novel nature of this research and the trends that emerged, further investigation is warranted.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF GRAPHS

LIST OF FIGURES

Page

DEFINITIONS OF TERMS

Allopathy

A therapeutic system in which disease is treated by producing a morbid reaction of another kind or in another part, the prime intention of which is to suppress the symptoms. The term is derived from Greek meaning 'other suffering' and coined by Samuel Hahnemann (founder of homoeopathic medicine) as a label for the dominant medical tradition of his time. It is a term applied loosely but not always correctly to the present day practice of mainstream (orthodox) medicine (Vithoulkas 1986, Gaier 1991).

AUDPC

The area under disease progress curve (AUDPC) was determined by plotting the percentage leaf area infected against time and calculating the area under the curve. It is used to evaluate the development of the disease (Berger 1988).

Avogadro's hypothesis (Number)

The number of molecules in one mole of any substance is 6.02554×10^{-23} as demonstrated by Amedeo Avagadro (1776 – 1856). According to laws of chemistry, there is a limit to how many serial dilutions can be made without losing the substance altogether. Solutions diluted beyond Avogadro's number (i.e. homoeopathic potencies equal to and greater than the 12CH and 24XH) have no molecules left in the solution, that can be detected with methods currently available (Kayne 1997).

Dibble

To sow, plant or prepare the soil by making holes with a hand tool (The Pocket Oxford Dictionary 1992).

Foliar disease rating

Disease severity is visually assessed with the aid of logarithmic rating scales of percentage leaf area infected for foliar diseases (Horsfall and Cowling 1978).

Isotherapeutic agents

These are preparations also called 'Homoeopathized Allopathica' (Tautopathics) which are defined as homoeopathic remedies made from toxic medicines to be used to treat an iatrogenic condition or in the standard method according to similarity of the disease and drug pictures (Gaier 1991).

Potency

A state of altered remedial activity to which a drug is taken by means of a measured process of deconcentration and the introduction of kinetic energy through succussion or trituration (see below) (Gaier 1991).

Decimal potency

A homoeopathic potency scale, introduced by Hahnemann, in which one part of mother tincture (see below) is added to nine parts of diluent which is subjected to vigorous shaking or grinding known as succussion or trituration respectively. Each successive decimal potency refers to the number of successive one in ten dilutions (Gaier 1991).

Centesimal potency

A homoeopathic potency scale, introduced by Hahnemann, in which one part of mother tincture (see below) is added to 99 parts of diluent which is subjected to vigorous shaking or grinding known as succussion or trituration respectively. Each successive centesimal potency refers to the number of successive one in 100 dilutions (Gaier 1991).

Pharmacopoeia

An authentic reference work containing monographs of medicines and other therapeutic agents. It also contains specifications of the sources and standards for the strengths and purity of the base substances and mother tinctures, formulae and methods of preparation of these substances as well as descriptions of processes for the testing of starting materials (Frazer, 1992).

Mother tincture

Liquid preparations resulting from the extraction of constituents from suitable source material with alcoholic or hydroalcoholic solutions, which form the starting point for most homoeopathic medicine manufacture (Gaier 1991).

Succussion

The action of shaking up vigorously a liquid dilution of a homoeopathic medicine in its vial or bottle, where each stroke ends with a jolt, usually by pounding the hand engaged in the shaking action against the other palm (Gaier 1991).

Supersuccussion

A method of making homoeopathic medicines where continuous succussion without further dilutions is used. Herr Stallmeister Jenichen (1787-1845), who was an equerry and homoeopath, pursued this concept originally introduced by Hahnemann, and in this way he was said to have

manufactured very high potencies up to and including the 2500th, 8000th and 16 000th dilution levels (Muzumdar 1988).

Trituration

As one of the methods of preparation of homoeopathic drugs, it is the act of prolonged grinding with a mortar and pestle to reduce a homoeopathic drug (usually insoluble) to a fine powder, while at the same time, amalgamating it thoroughly with *Saccharum lactis* (lactose) by rubbing the combination with the pestle in the mortar (Gaier 1991).

CHAPTER ONE – INTRODUCTION

This study was aimed at extending previous research in Homoeopathy and in the cabbage and downy mildew disease system by investigating the response of this biological system, to a chemical, in different doses. Research biologists have rediscovered the Arndt-Schulz law, which states that biological systems respond differently to chemicals in different doses. Much recent experimental evidence has shown biological, physiological or biochemical responses to a drug at a low dose may be completely opposite to that response when a larger dose is administered. Whether or not this supports homoeopathic theory has yet to be thoroughly investigated, especially in terms of the Arndt-Schulz law applying not only to chemicals in dilution, but also to diluted and succussed chemicals (Robinson 1992).

Homoeopathic research using plants as experimental subjects is thought to eliminate the effect of suggestion (also known as the 'placebo' effect) that is normally encountered in human clinical trials (Pelikan and Unger 1971). Downy mildew, caused by *Peronospora parasitica*, is a major plant disease faced by nurseries and farmers in Kwazulu Natal. Cabbage seedlings (*Brassica oleracea*) were used because they are highly susceptible to downy mildew. The cultivar used was Green Coronet. Acrobat® was used because it is presently the most effective fungicide for controlling downy mildew (Laing 1999).

Homoeopathic potencies (7XH, 9XH, 12XH, 7CH, 9CH, 12CH, 15CH, 30CH and 200CH) were made from Acrobat®. This range of potencies were chosen on the basis that they are the most commonly used potency levels in homoeopathic practice, and because similar potencies were applied in trials by Brammer (1994) and Curnow (1997). The decimal potencies were added for further comparison between the controls and the centesimal potencies.

In this study, distilled water and Acrobat® (2g/litre) were used as minimum and maximum controls respectively. They provided the framework against which dose-related effects of the homoeopathic treatments, including decimal (5XH, 7XH, 9XH) and centesimal (5CH, 7CH, 9CH, 15CH, 30CH, 200CH) potencies, were measured. The aim of the trial was therefore to evaluate inhibition and stimulation of downy mildew (*Peronospora parasitica)* on infected cabbage seedlings (*Brassica oleracea*) as a result of these homoeopathic treatments. The effects of the treatments were evaluated using foliar disease rating in terms of percentage area leaf infected. A dose-response to the different potency levels was expected in terms of the Arndt-Schulz law whereby fungus growth would increase with increasing dilution.

1.1 The Aim of the Study

The aim of this investigation was to evaluate the biological effects of homoeopathic medicines made from a fungicide on fungal growth on cabbage seedlings.

1.2 The Statement of the Objectives

1.2.1 The First Objective

The first objective was to determine the effect of a (maximum) control on development of downy mildew on cabbage seedlings by the application of a fungicide, Acrobat®.

1.2.2 The Second Objective

The second objective was to determine the effect of a (minimum) control on development of downy mildew on cabbage seedlings by the application of distilled water.

1.2.3 The Third Objective

The third objective was to determine the effect of homoeopathic medicines on development of downy mildew on cabbage seedlings by the application of a range of potencies of homoeopathically prepared Acrobat®.

1.3 The Hypotheses

1.3.1 Hypothesis One

It was hypothesized that Acrobat® has a biological effect on development of downy mildew on cabbage seedlings.

1.3.2 Hypothesis Two

It was hypothesized that distilled water has no biological effect on development of downy mildew on cabbage seedlings.

1.3.3 Hypothesis Three

It was hypothesized that a range of potencies of homoeopathically prepared Acrobat® would have biological effects on development of downy mildew on cabbage seedlings.

1.4 Delimitations

1.4.1 Delimitation One

The emphasis of this study was on the observed biological activity of the homoeopathic treatments in terms of percentage fungal growth and not on the mechanisms of action of the medicine, or on the disease process.

1.4.2 Delimitation Two

This study was limited to the treatment of plant fungal disease, and did not include treatment of animal or human subjects. Only cabbage seedlings infected with downy mildew were utilized.

1.4.3 Delimitation Three

This study did not attempt to investigate any other homoeopathic medicines and potencies other than those stipulated.

1.5 The Assumptions

1.5.1 Assumption One

It is assumed that the homoeopathic medicines have been prepared according to the method as provided in the Homoeopathic Pharmacopoeia unless otherwise stipulated.

1.5.2 Assumption Two

It was assumed that the homoeopathic medicines were functionally active at the time of utilization.

1.5.3 Assumption Three

It was assumed that the trays in which the seedlings were grown, chemical sterilant used on the trays and the sprayers that harbour the treatments, were homoeopathically inert.

1.5.4 Assumption Four

It was assumed that the viability of the batch of seed used was uniform for each sample obtained from the batch.

1.5.5 Assumption Five

It was assumed that the homoeopathic medicines obtain specific effects and that their effects were not attributed to 'suggestion', commonly known as the placebo effect.

CHAPTER TWO - LITERATURE REVIEW

2.1 Cabbage - an introduction

Cabbage is included in the family Cruciferae, belonging to the Genus *Brassica* of the plant kingdom. The family name, Cruciferae, is derived from Latin meaning 'cross-bearer' and was so named because of the simple cross formed by the four petals of all cruciferous flowers (Laing 1996).

It is a biennial plant native to the rocky shores of Europe and can be considered one of the most ancient of the common and currently eaten vegetables (Bensen 1993). There has been some question as to whether cabbage is also native to England or whether it was introduced by the Romans or by the Saxons, both of whom where great lovers of cabbage and kale (Philips and Rix 1993). In addition, cabbage holds an historical place in South Africa. Under the orders of the Dutch East India Company, Jan Van Riebeeck established a victualling station at the Cape in 1652. Thus colonization of this region was initiated by the need for fresh supplies. These included vegetables, water and meat for the passing Dutch ships on their way to the East in order to combat scurvy in the sailing crew (de Kock 1924 as cited by Laing 1996). Cabbages were an important vegetable grown for the ships and the origin of *Cape spitz* cabbage, which is unique to South Africa, can be traced to this early period (Anonymous 1989a as cited by Laing 1996).

In the 3000 or so years during which it has been cultivated as a food crop, the wild cabbage has been developed into several distinct vegetables (Philips and Rix 1993). Broccoli, Brussels sprouts, cauliflower, collards, kale and kohlrabi are all plants belonging to the same species, *Brassica oleracea*. These different plants came about by encouraging the development of one element already present in the original plant. For example, concentration on flower development resulted in both cauliflower (centripetal florescence) and broccoli (centrifugal florescence). So besides being one of the oldest cultivated vegetables, cabbage is also one of the most versatile (Root 1980 as cited by Laing 1996).

The earliest records of cultivated cabbages date from around 600BC when kale was mentioned in early Greek literature (Philips and Rix 1993). The Greeks ate a good deal of it not because it was a delicacy, but because it was widely available and nutritious (Root 1980 as cited by Laing 1996). The Romans ate raw cabbage to eliminate 'hangovers' (Nonnecke 1992 as cited by Laing 1996).

Even today the humble cabbage is known for its many health-promoting properties. With both red and green types, it is one of the most nutritious and yet least expensive vegetables. Raw

cabbage is an excellent source of vitamin C; it is alkali forming in the gut, high in cellulose and has a very low calorie content. Nutrients as found in one kilogram are as follows: calories 43, protein 2.1g, fat 0.3g, carbohydrates 7.9g, calcium 68.9g, phosphorus 46.7g, iron 0.7mg, vitamin A 24 I.U., thiamine 0.1mg, riboflavin 0.1mg, niacin 0.04mg and ascorbic acid 78.4g (Bensen 1993).

2.2 Fungal disease in plants

2.2.1 The downy mildews

The downy mildews are fungal pathogens that cause severe epidemics on many crops and are difficult to control by either management or fungicide practices. They can cause heavy or total loss of crops especially when young plants or shoots become infected in the field (Agrios 1997). *Peronospora parasitica* has a global distribution and attacks all Brassicas. Epidemics are very rapid and extremely damaging to nurseries. There is no good genetic host resistance to the disease and few effective fungicides are available for combating infections owing to fungicide resistance (Laing 1999). Cabbage seedlings (*Brassica oleracea*) were selected for use in this study because they are highly susceptible to downy mildew.

Peronospora parasitica is an Oomycetous fungus from the Peronosporacea family. It is an obligate parasite of the higher plants. Infection occurs via the airborne transmission of sporangia (McMeekin 1960). The latent period of infection (length of time between infection and first visible signs of disease) for *Peronospora parasitica* is two days, which means that the disease progresses very rapidly (Laing 1999). First, long white sporangiophores emerge through the stomata. Later, the affected surface appears light brown to gray and may then turn yellow, shrivel up and die (Agrios 1997). The fungus thrives in conditions of high humidity, leaf wetness being the critical factor for infection (Spencer 1981, Laing 1999). Refer to Figure 1 for its disease cycle.

2.2.2 Disease rating

Disease assessment in plants is a process of measuring disease intensity quantitatively. This is most commonly done in terms of disease incidence and disease severity. By definition, disease incidence is the number of diseased sampling units expressed as a proportion or percentage of the total number of sampling units assessed. Disease severity (which was assessed in this research) is defined as the area of a sampling unit affected by disease and expressed as a proportion or percentage of the total area of the sampling unit (Nutter and Schultz 1995). The Brophy/Laing rating scale (Refer to Figure 2) was used to assess the disease progress in this

research (Brophy and Laing 1992). To increase the accuracy of the ratings, a computer program (Disease Pro) was used by the researcher to practice disease rating in an artificial situation and develop the skill before having to do the actual trial ratings and also in between ratings, to maintain rating accuracy.

2.2.3 Chemical control

In South Africa, control of this pathogen is heavily dependent on the use of chemicals (Brophy and Laing 1992). New methods of disease control need to be sought due to the rising costs of chemicals, associated ecological problems but most importantly, the development of resistant *Peronospora parasitica* strains, (Nui et al. 1983, Brophy and Laing 1992). Since the 1980's, several systemic fungicides, such as metalaxyl, propamocarb and fosetyl Al have considerably improved the ability of nursery-workers to control this disease (Agrios 1997). In a study by Brophy and Laing (1992), 11 fungicides were screened for efficacy against crucifer downy mildew. Metalaxyl-based fungicides were found to be ineffective owing to fungicide resistance. A synergistic effect was found between mixtures of systemic and protectant fungicides. Acrobat® is a combination of Dimethomorph (systemic) and Mancozeb (protectant), which is at present the best fungicide for Brassica downy mildew and has been proven to be highly effective in controlling *Peronospora parasitica* (Curnow 1997, Laing 1999).

2.3 Homoeopathic preparations

The homoeopathic preparations used in this trial fall into the category of Isotherapeutic agents. The potencies of Acrobat® are 'Homoeopathized Allopathica' (Tautopathics) which are defined as homoeopathic remedies made from toxic medicines to be used to treat an iatrogenic condition or in the standard method according to similarity of the disease and drug pictures. The following are examples of recently proven 'homoeopathized allopathica' with full, established drug pictures: Chloramphenicol, Cortisonum and Penicillinum (Gaier 1991). Acrobat®, however, has not been homoeopathically proven and in this trial, was used not on the basis explained above, but according to allopathic principles. This was done in order to investigate dose-related effects on fungal growth and compare them with two controls, namely distilled water (minimum control) and Acrobat® 2g/litre (maximum control).

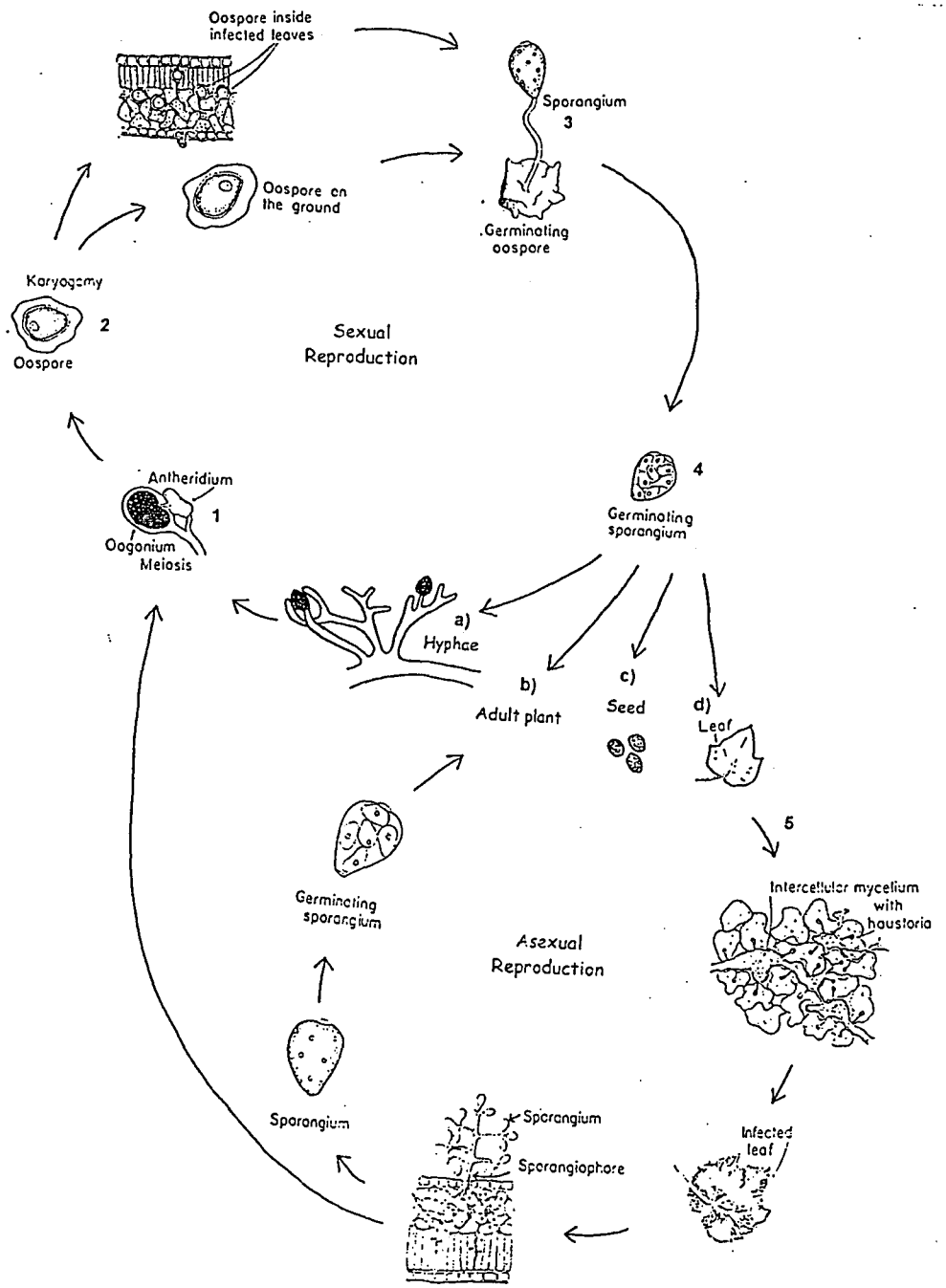


Figure 1. Disease cycle of downy mildew of cabbages caused by *Peronospora parasitica* (adapted from Agrios 1997).
1.Dry weather at the end of season triggers sexual reproductive cycle. 2. Formation of oospore. 3. Growth of sporangia. 4. Germinating sporangia act like spores to: a) produce hyphae which traverse plant structures as infection advances. b) (re)infect adult plant → localized → spread to young tissues & seeds. c) infect seeds → systemic growth. d) infect leaves & cotyledons of seedlings → localized becoming systemic. 5. Asexual reproduction cycle initiated by return of humid season.

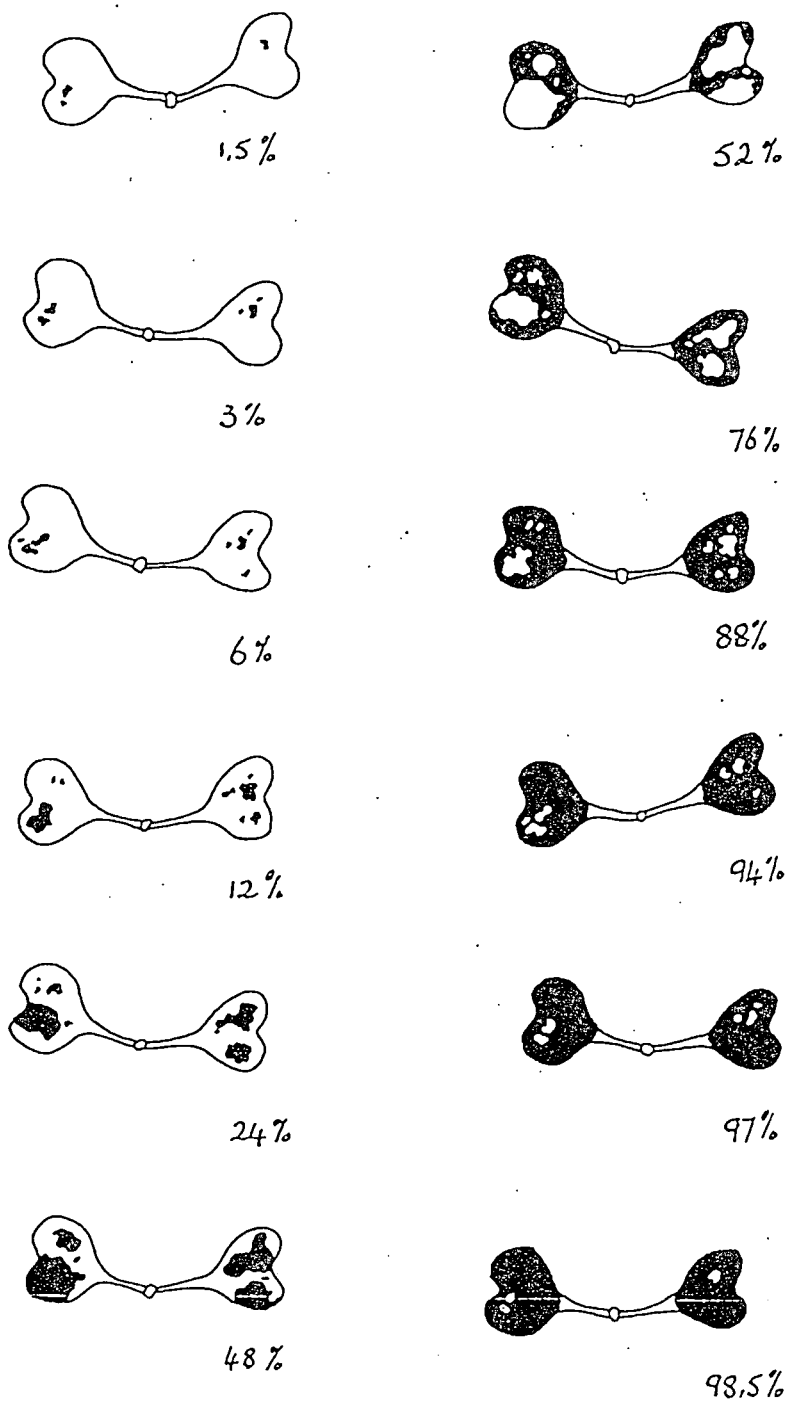


Figure 2. Brophy / Laing rating scale in terms of percentage leaf area infected by *Peronospora parasitica* on the cotyledons of brassica seedlings (Brophy and Laing 1992).

2.4 Homoeopathic agricultural research

Various studies have been carried out in order to test the efficacy of homoeopathic medicine in the agricultural context. Scofield (1984) and Hopkins (1998) have reviewed many of these studies. Research studies directly relevant to homoeopathy and plant fungal disease will now be discussed.

Khanna and Chandra conducted four trials in which they investigated the effects of selected homoeopathic remedies in a range of potencies, on four different plant fungal diseases, namely tomato fruit rot (1976a), leaf blight of wheat (1976b), guava fruit rot (1977) and mango fruit rot (1978). The remedies chosen were those normally used to treat human fungal diseases (Scofield 1984a). Up to 10 homoeopathic remedies, each in potencies from 1-200 (centesimal), were used as both pre- and post-inoculation treatments and the inhibition of spore germination by each treatment was studied. The homoeopathic remedies, which completely inhibited spore germination in vitro, were screened further for their efficacy by checking the disease in vivo. The most effective remedies and potencies were thus identified. For example, Kali iodatum 149C proved to be the most effective treatment in checking and preventing tomato fruit rot caused by *Fusarium roseum*. Similarly, Lycopodium clavatum 190C was shown to be the most effective both in reducing percentage fruit infection as well as percentage rot on mango fruit rot caused by *Pestalotia mangiferae* (Khanna and Chandra 1976a, 1976b, 1977, 1978).

In a study by Brammer (1994) on the control of downy mildew (*Peronospora parasitica)* of cabbage seedlings (*Brassica oleracea*), three fungicides (S151, Optimo® and Phyton27®), a water control and an isopathic preparation of *Peronospora parasitica* were tested. It was demonstrated that the isopathic preparation of *Peronospora parasitica* in homoeopathic dilutions had a statistically measurable effect on fungal growth in terms of foliar disease rating. Out of four potencies tested (5CH, 9CH, 15CH, and 30CH), the 5CH and 9CH consistently stimulated growth of the fungus, thus causing a higher level of disease in those seedlings. However, one of the limits of this trial is that higher potencies were not used.

Curnow (1997) also investigated the effect of an isopathic preparation of *Peronospora parasitica* in four potencies (9CH, 30CH, 200CH, 1M) as compared to two fungicides, Acrobat® and Bravo® on cabbage seedlings infected with downy mildew. Preventative applications were found to be more effective for all treatments. Acrobat® was proven to be the most effective in the control of the disease. In contrast, none of the homoeopathic treatments provided adequate disease control. There were no significant differences between homoeopathic potencies except for the

30CH, which faired significantly better in disease control than the 200CH. The 200CH caused a much higher level of disease relative to all the other treatments.

In a synthesis of Brammer (1994) and Curnow's (1997) results, the following is apparent: the 5 and 9CH (from Brammer's trial) caused stimulation of fungal growth, the 30CH (from Curnow's trial) caused high levels of fungal growth (but less, relative to the 200CH) and the 200CH (from Curnow's trial) caused even higher levels of fungal growth. Neither Brammer (1994) nor Curnow (1997) used a distilled water control so it is not absolutely clear whether the raised disease levels were as a result of the stimulatory actions of the different potencies or of the natural progress of the disease. How these potencies would have faired against a distilled water control would have provided a more accurate account in terms of evaluating for hormetic effects.

2.5 The Arndt-Schulz law and hormesis

The Arndt-Schulz law is a theory that has been used in support of homoeopathic medicine, one of its basic tenets being that the (healing) power of a drug increases with dilution (Scofield 1984b, Hahnemann 1997). The law, also known as Hueppe's Rule, states that substances, which inhibit biological processes at sublethal levels, may be expected to stimulate them at dilute concentrations. Schulz first described this phenomenon in 1888, when he found that low concentrations of many chemical agents had a stimulatory effect on the growth and respiration of yeasts. Later, in 1896, Hueppe demonstrated that bacteria also behaved in this way (Scofield 1984b). When the Arndt- Schulz law was first disclosed to the scientific community, it was considered to be universally applicable. Since it provided no inherent explanation for how and why the phenomenon occurs, and when too many exceptions were found, the law fell into disrepute (Stebbing 1982, Furst 1987). However, there is a reawakening of interest in this phenomenon (Robinson 1992).

The Arndt-Schulz law forms the basis of the phenomenon of *hormesis*, a term first proposed by Southam and Erhlich in 1943 to describe the stimulatory effects on any organism caused by low levels of potentially toxic agents (Scofield 1984b). In his view of *hormesis*, Stebbing (1982) quotes many examples of growth stimulation in a range of taxa, by substances that are toxic to the organisms in higher levels. Although he concentrated on growth hormesis, the term is not restricted to growth stimulation and may well be applied to stimulatory effects of any kind due to potentially toxic substances. It can be applied to physiological stimulation by low doses of toxic chemicals such as was originally reported by Schulz when he discovered increased CO_2 production, an index of both yeast respiration and growth. More recently, radiation hormesis as a result of low levels of radiation exposure has been documented to cause: increased longevity,

increased growth and fertility in plants and animals and a decrease in frequency of cancer. One possible mechanism of radiation hormesis is the stimulation of the immune system as the immune system itself is already recognized as being particularly radiosensitive (Sagan 1987).

Apart from what is implied by the definition, growth hormesis is constituted by the formation of a β concentration-response curve (Refer to Figure 3). The curve consists of a single stimulatory peak at concentrations immediately below those that cause inhibition. In contrast, the familiar α concentration-response curve usually found in growth experiments with toxic substances, shows no departure from zero at normal and low concentrations followed by progressive inhibition above a threshold level. Most examples of growth hormesis conform to the β curve. In the studies reviewed by Stebbing (1982) where research data has been precise and comprehensive and where many concentrations of the toxicant were used, the graphs plotted conform closely to a β curve. Therefore, evidence seems to justify the original assumption implicit in the Arndt-Schulz law: that hormesis is a phenomenon that occurs in the same way in different organisms as a result of exposure to different kinds of toxic agents (Stebbing 1982).

He suggests that if different examples of hormesis share a common explanation, it may lie in regulatory overcorrections by the organisms biosynthetic control mechanisms to low levels of inhibitory challenge. This results in growth that is greater than normal. More specifically, homeostatic (control of a steady state) and homeorhetic (control of growth as a rate process) feedback mechanisms in an organism respond to perturbation non-specifically and may overcorrect for adaptive reasons to low levels of inhibitory challenge. Because growth hormesis has been shown to be a widely distributed phenomenon both in the taxonomic and toxicological senses, any control mechanism whose behaviour might account for hormesis therefore must operate at the lowest levels of organization and be common to all forms of life (Stebbing 1987).

Stebbing (1982) quoted the lowest concentration of any substance that demonstrated growth hormesis was equivalent to a homoeopathic dilution of 4CH. This is a very 'low potency' by homoeopathic standards and much higher (more dilute) potencies are more frequently used in homoeopathic practice. Therefore, the question is whether hormesis is applicable to homoeopathic high potencies? Scofield (1984b) was doubtful whether it operates at these levels and as a consequence, whether hormesis could provide an explanation for the action of homoeopathic medicines.

However, Brammer (1994) showed growth stimulation caused by her 5CH and 9CH potencies. Although Curnow (1997) was not able to reproduce the findings, she found that the 30CH and 200CH potencies caused the highest fungal growth. In light of these findings, whether or not the

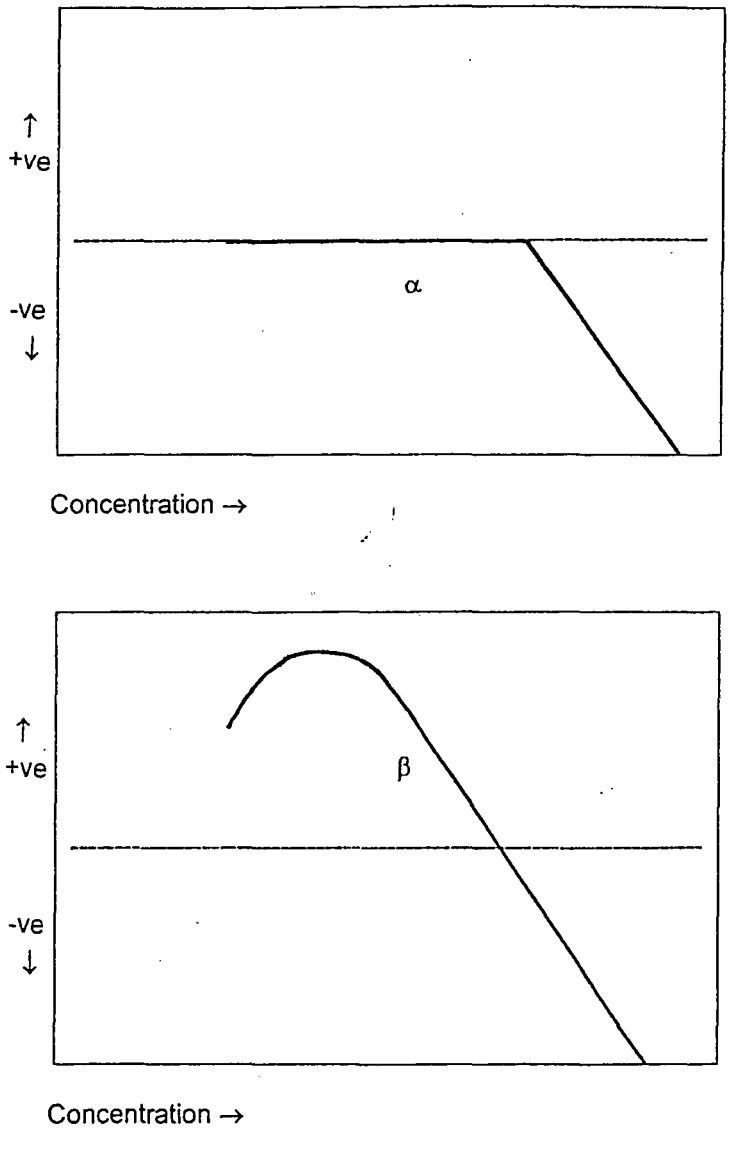


Figure 3. α and β Concentration - response curves identified by Townsend and Luckey (1960) The β curve was redrawn by Stebbing (1982).

Arndt-Schulz law applies to homoeopathic potencies is still open to further research. Furthermore, a homoeopathic potency is made up according to a process of serial dilution and succussion called potentization (Gaier 1991). In Stebbing's (1982) examples, only diluted substances were used. Therefore, for the purpose of scientific research, the action of a diluted substance cannot be equated with one that is the same concentration but also succussed. The effect of succussion also needs to be taken into account. Theories abound but no conclusion has been reached in modern physics to explain the process and effects of potentization (Hopkins 1998). Studies need to be done comparing the action of diluted and potentized substances.

Reasons why hormesis has not been recorded more frequently all seem as a result of poor experimental design. For example, most studies dealing with toxic substances are interested in lethal doses rather than organism growth. Concentrations of the substances tested do not usually extend below inhibitory concentrations and into the realm of hormetic concentrations. Unexpected or unwanted (hormetic) results may be dismissed or regarded as aberrant. Lastly, the toxicologist may be unwilling to write about a phenomenon that is not understood. Therefore, failure to observe hormesis is not evidence that it does not occur (Stebbing 1982). It is also postulated that hormesis has previously received little attention because it conflicts with the conventional science paradigm which states that biological observations at high doses can be used to predict effects at low doses. In other words, if high doses overwhelm and poison an organism, then low doses have less of this tendency the more dilute the doses, a relationship where dose is directly proportional to action (Sagan 1987).

2.6 Conclusion

Experimental data supporting hormesis are many but researchers in the field of hormesis have not yet performed experiments using diluted and succussed substances (Robinson 1992). In this study, the object was to see if the phenomenon of hormesis could be demonstrated by the homoeopathic treatments, prepared as per homoeopathic methodology (that is, by dilution and succussion). A dose-response to the different potency levels was expected in terms of the Arndt-Schulz law whereby fungus growth will increase with increasing dilution.

CHAPTER THREE - MATERIALS AND METHODS

3.1 Study design

3.1.1 Research location

The trial took place in the controlled environment of the Plant Pathology greenhouse, School of Applied Environmental Sciences, University of Natal, Pietermaritzburg.

3.1.2 Study population

The study population consisted of cabbage seedlings *(Brassica oleracea* cultivar Green Coronet*)* grown from seed and later inoculated by seedlings infected with downy mildew *(Peronospora parasitica).*

3.1.2 Treatments

A total of 11 treatments were applied. Each treatment was replicated 4 times in each trial.
The treatments were as follows:

- Control One = Distilled water (zero)
- Control Two = Acrobat® 2 gram/litre (maximum)
- Homoeopathic preparations = 7XH, 9XH, 12XH, 7CH, 9CH, 12CH, 15CH, 30CH, 200CH

3.1.3 Repetitions

The trial was done twice for comparison.

3.1.4 Randomization

Randomization was achieved by using a computer program (RBC) to generate the block design for a randomized position of the seedling trays for each treatment group. The experimental design used in this trial was the Randomized Complete Blocks (RCB) design. Each treatment appears in each replication, but each replication is kept in a complete block. This method allows the researcher to detect block effects, due to the position of the different replications.

3.2 Materials

3.2.1 Seeds

Source: McDonald's Seeds, Pietermaritzburg
Cultivar: Green Coronet

3.2.2 Growing medium

Seedling mix of composted pine bark (CPB) obtained from Gromed, Crammond.

3.2.3 Inoculum

Infected seedlings were obtained from the commercial nursery,
Sunshine Seedlings Nursery, Pietermaritzburg.

3.2.4 Greenhouse chemicals

- Plasdip (Copper oxychloride in PVA paint) used for sterilizing trays.
- Fertilizer: 3.1.3(38) @1gram/litre (100ppmN)* which is injected into the irrigation system.

3.2.5 Greenhouse equipment

- 44 x 24-celled Speedling trays
- 11 x high-pressure 'Efekto Polyspray 2' (One per treatment was used with no interchange of spraying units between treatments).
- An automatically operated irrigation system irrigates seedlings daily at 09h00, 12h00 and 16h15. Spraying occurred in the afternoon after the final irrigation on the designated days.

3.2.6 Distilled water

Millipore MilliQ distilled water from the Department of Biochemistry, Pietermaritzburg University was used for Control One and making up the homoeopathic treatments on site.

3.3 Methods

3.3.1 Preparation of trays

Speedling trays were first sterilized using Plasdip, ensuring that the entire surface area of the trays was covered. The trays were then left for approximately 24 hours or until they were dry. Complete drying of the trays is essential to prevent burning of the seedlings by the sterilant.

3.3.2 Seed planting and germination

The trays were filled with composted pine bark (CPB) and then thoroughly moistened with water. A pencil was used to dibble into the CPB to a depth (approximately 5 mm) uniform throughout all the trays. 1056 seeds were selected, excluding damaged or deformed seeds and planted, 1 seed per cavity. This was followed by watering until drip through (entire growing medium saturated with water) using a hand-sprayer. Then the trays were stacked in the medium room and left for two

* Nitrogen: Phosphorus: Potassium (38% active ingredient) at 1 gram per litre (100 parts per million of Nitrogen)

days or until the plumules emerged, at which point they were moved to the tunnel and arranged into groups according to the computer-generated randomized block design. The trays were labeled from 1 – 44 in soft lead pencil to prevent fading and smudging.

3.3.3 Preparation of potencies of Acrobat®

Acrobat® is the combination of a systemic fungicide, Dimethomorph (morpholine) and a protectant, Mancozeb (dithiocarbamate). As a control, it was applied according to the recommendation in a 2 gram/litre ratio (Nel et al 1996).

Both decimal (7, 9,12) and centesimal (7, 9,12, 15, 30, 200) potencies of Acrobat® were selected. This range of dilutions traverses both sides of Avogadro's number, thus allowing for the demonstration of biochemical and biophysical effects. These dilution levels are also chosen because they are similar to those used by Khanna and Chandra (1976a, 1976b, 1977, 1978), Brammer (1994) and Curnow (1997). The decimal potencies were added for further comparison between the controls and centesimal potencies.

The homoeopathic dilutions of Acrobat® were prepared by the researcher according to Method Six for insoluble substances (refer to Appendix A), as specified in the German Homoeopathic Pharmacopoeia (GHP) (British Homoeopathic Association 1985). This is the pharmacopoeia most commonly used by the homoeopathic pharmaceutical manufacturers in South Africa. The researcher prepared the first four potencies by trituration in the fume cupboard of the Department of Chemistry, Technikon Natal. Thereafter, potencies were made up in distilled water, according to Method 8b (refer to Appendix A) in the GHP, due to the possible intrinsic effect alcohol may have on the plant physiology. The fourth and fifth (decimal and centesimal) potencies were not used as final potencies to exclude the effect of lactose powder still present in these dilutions, on the disease system (Hopkins 1999). All the liquid potencies were manufactured in the laminar flow room of the Department of Homoeopathy, Technikon Natal, up to the dilution level one below that required for the study; i.e., 6XH, 8XH, 11XH, 6CH, 8CH, 11CH, 14CH, 29CH and 199CH. The required final potencies were manufactured on site by the researcher immediately before spraying.

3.3.4 Spraying

In each case, the sprayers were filled with 500ml of treatment. The treatments were prepared immediately before spraying in order to ensure optimum activity. The excess was discarded at the end of each spraying. The seedlings were sprayed to leaf wetness just before point of run off (droplets present on the leaves but they don't coalesce and run off). Spraying was performed at

the end of the day, after final irrigation. Spraying with all 11 treatments commenced on Day 7 of the trial. Frequency of spraying for all 11 treatments was every 7 days.

3.3.4.1 Preparation of controls

Control One was prepared by filling the designated sprayer with 500ml of Millipore MilliQ distilled water. Control Two was made up by adding one gram Acrobat® to 500ml tap water (according to a 2 gram/litre ratio), directly into a sprayer and shaking vigorously.

3.3.4.2 Preparation of homoeopathic treatments

The homoeopathic preparations were each made up by adding one part of the previous potency to nine parts distilled water (for the decimal potencies) and one part of the previous potency to 99 parts distilled water (for the centesimal potencies). This was placed into a 500ml clear glass bottle (making up 300ml) and succussed according to the method described in the GHP. This was repeated using a new bottle (to prevent supersuccussion) thus making 600ml per treatment. The final potencies were then transferred into the sprayers ready for use. After each spraying, the clear glass bottles were autoclaved. Spraying units were used for the same treatment throughout the trial and not interchanged.

3.3.5 Inoculation with infected seedlings

On Day 14, one infected seedling was inoculated into a central position in each tray by removing a healthy seedling and replacing it with one infected with downy mildew. This type of inoculation, by artificial introduction of infected seedlings, allows the natural spread of the pathogen and provides levels of disease similar to those occurring in a typical nursery situation (Brophy and Laing 1992).

3.3.6 Disease rating

The Brophy/Laing rating scale was used to assess disease progress (see Figure 2). The parameter assessed was percentage cotyledon leaf / primary leaf area infected of the middle 8 seedlings per tray. The cotyledon leaves were assessed first, by applying an appropriate percentage. If the disease was advanced, the primary leaves were also assessed and rated 100% + percentage of infection on the cotyledon leaves. Therefore, a total of 16 cotyledon leaves per tray were rated.

Frequency of rating was twice per week (Mondays and Thursdays) commencing on Day 16 for a minimum of six ratings. However, once ratings reached five percent, they were done every second day since thereafter the disease progresses at a very rapid rate (Laing 1999).

3.3.7 Statistical analysis

The rating results (percentage leaf area infected) were transformed into area under disease progress curve (AUDPC) values for the two trials. Multifactor analysis of variance (MANOVA) was the method used to analyse the data. Statsgraphics program (Version 7.0) was used to process the data.

CHAPTER FOUR - RESULTS OF THE STUDY

4.1 Results

The rating results were transformed into area under disease progress curve (AUDPC) values for the two trials. Multifactor analysis of variance (MANOVA) was used to analyze the data.

Table One: Summary of results of Trial One.

Treatment	Disease level on 6th rating	AUDPC
Distilled water	12.766	44.8
Acrobat®	12.866	52.9
7X	12.866	49.725
9X	20.649	92.225
12X	12.233	43.05
7C	17.532	50.25
9C	20.166	76.5
12C	20.799	92.6
15C	15.883	63.2
30C	19.816	66.225
200C	15.299	67.45

ANOVA results:

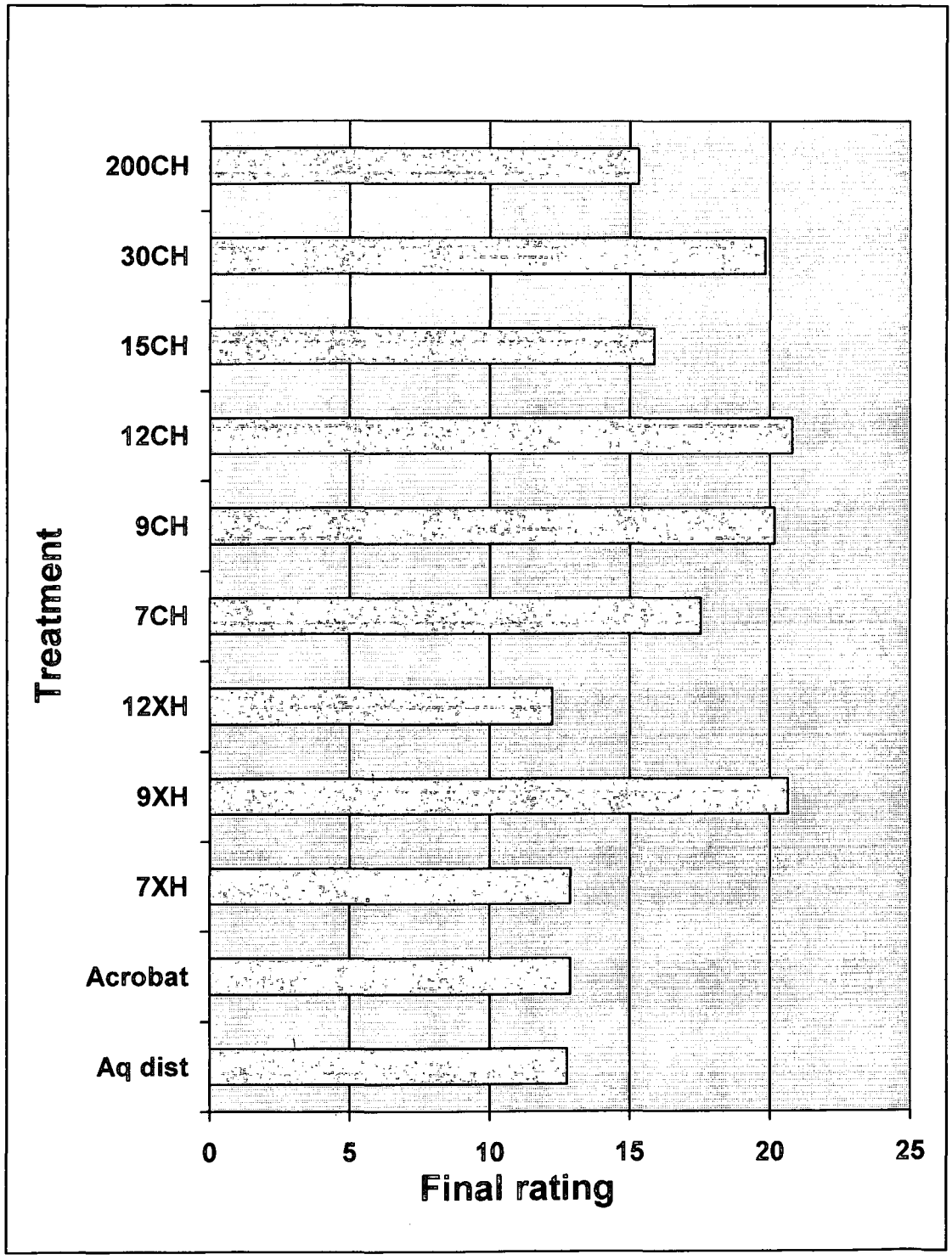
Disease level on 6th rating	F ratio = 0.955	P = 0.5002	Not significant
AUDPC	F ratio = 1.06	P = 0.4216	Not significant

Table Two: Summary of results of Trial Two.

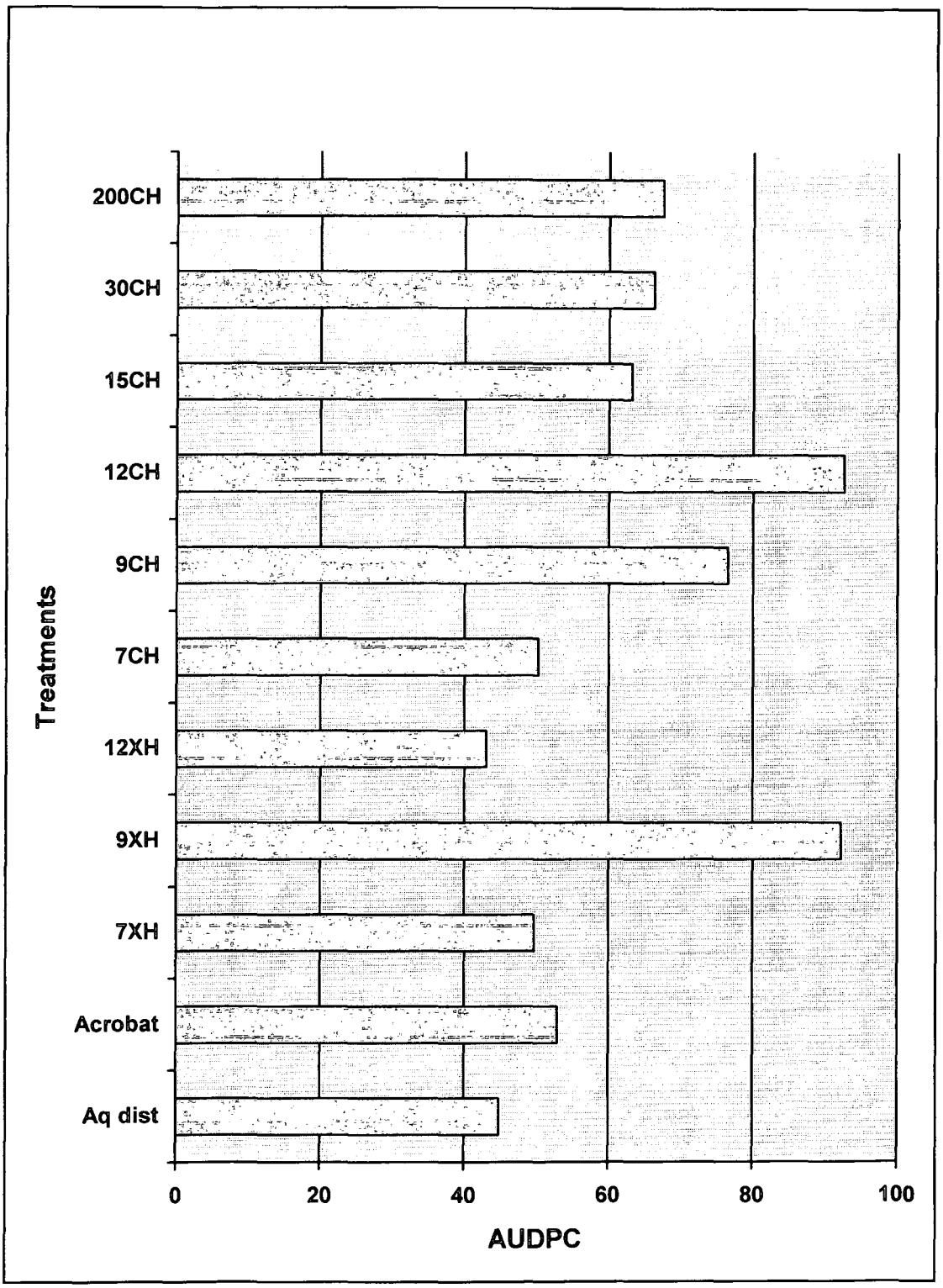
Treatment	Disease level on 6th rating	Mean separation	AUDPC	Mean separation
Distilled water	20.766	BC	59.8	ABC
Acrobat®	5.133	A	18.725	A
7X	25.049	BC	79.3	BC
9X	28.933	C	87.925	C
12X	31.099	C	96.25	C
7C	22.216	BC	71.425	BC
9C	26.849	C	90.675	C
12C	23.741	BC	68.225	BC
15C	24.799	BC	80.525	BC
30C	14.083	AB	40.325	AB
200C	21.049	BC	62.925	ABC

ANOVA results:

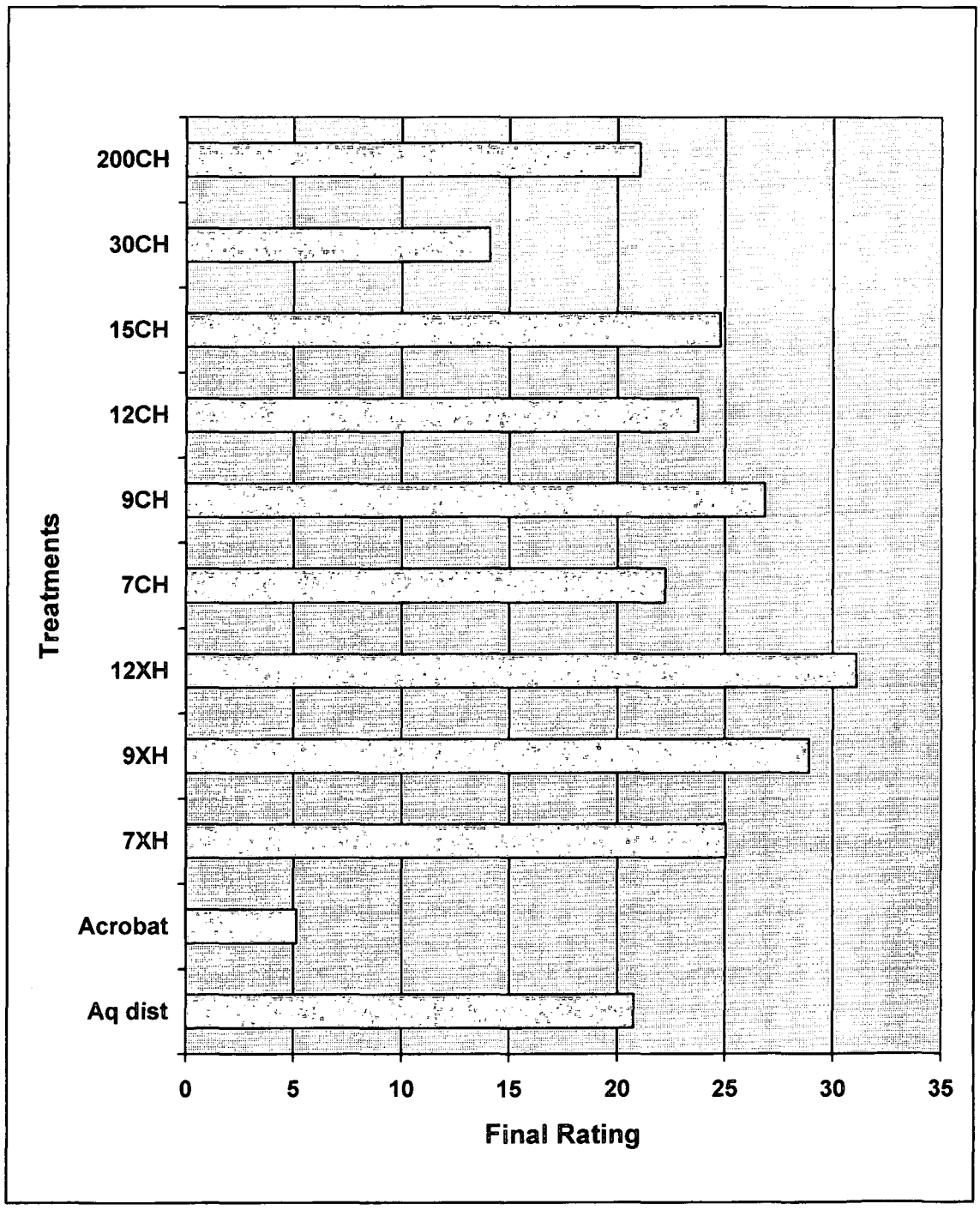
Disease level on 6th rating	F ratio = 2.977	P = 0.01	Highly significant
AUDPC	F ratio = 2.144	P = 0.052	Significant at 5.2% level



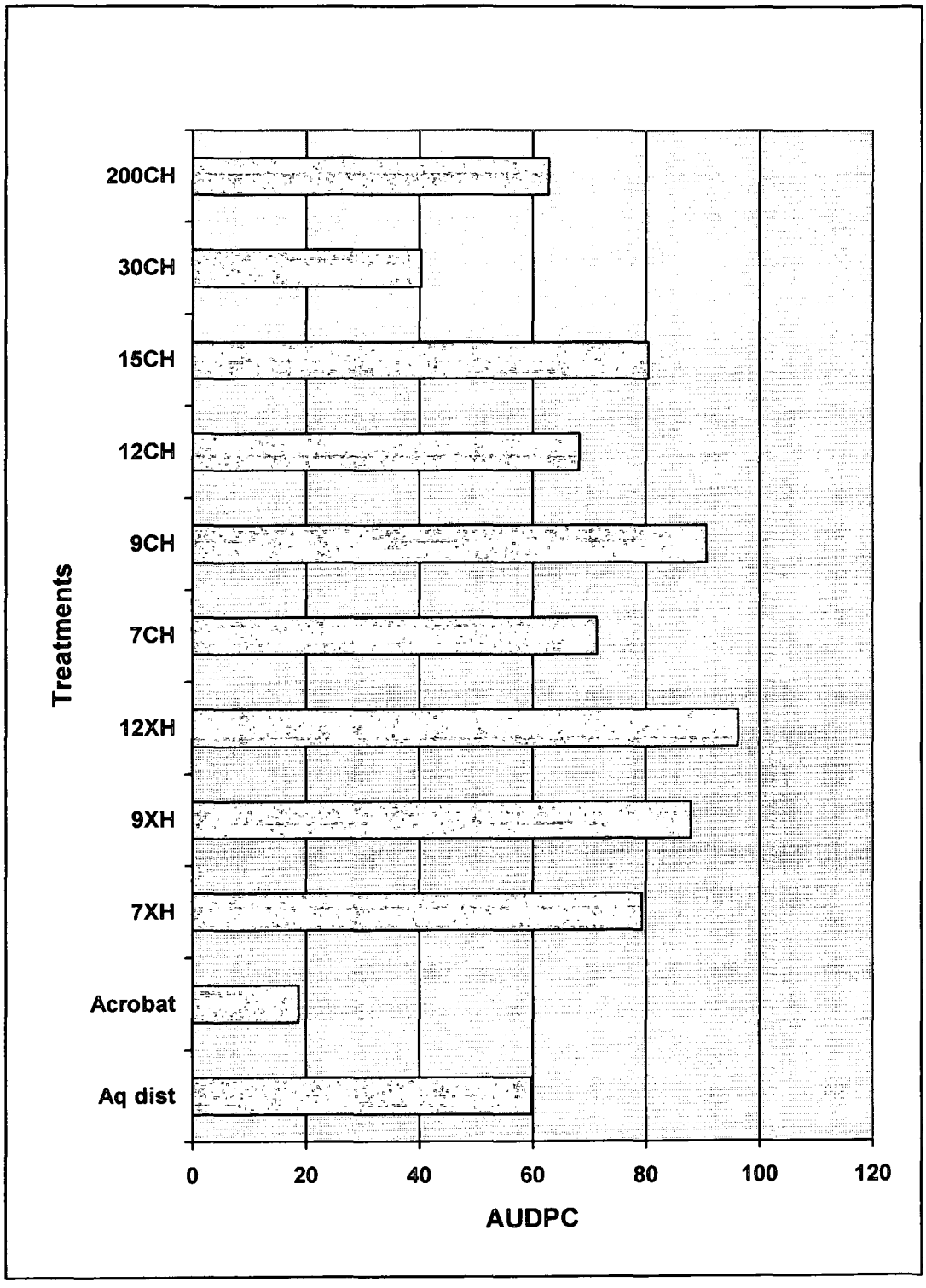
Graph One: Histogram of Final Rating for Trial One



Graph Two: Histogram of AUDPC for Trial One



Graph Three: Histogram of Final Rating for Trial Two



Graph Four: Histogram of AUDPC for Trial Two

CHAPTER FIVE - DISCUSSION

5.1 Trial One

The first trial did not yield any significant results and therefore no conclusions have been drawn. A few problems have been identified that may have contributed to the lack of statistically significant data:

- Presence of a high level environmental error. Downy mildew as a disease is directly dependent the presence of environmental humidity. Trial One was done in April-May where the environmental humidity was higher than for Trial Two in June. As a result, the disease may have progressed too rapidly and thus ratings were unable to reflect accurately, the effect of treatments.
- Rater inexperience resulting in inconsistent ratings.
- Fungicide resistance to inoculum used in Trial One. The inoculum used in Trial One was different to that used in Trial Two although they were obtained from the same source. Therefore, it is possible that the downy mildew from the first inoculum may have been resistant to Acrobat® and for that reason, there was no statistical difference noted between the minimum (distilled water) and maximum (Acrobat®) controls. This was contrary to what was expected based on the results obtained by Curnow (1997) and therefore the researcher has disregarded these results.

5.2 Trial Two

In terms of disease control, Acrobat® was the most effective treatment. The 30CH was the most effective homoeopathic treatment followed by the 200CH in terms of disease control. However, both the 30CH and 200CH were not significantly different from the distilled water control which may suggest possible contamination of the distilled water.

Otherwise, some trends emerged which are worth a mention: the 7XH, 9XH, 12XH and 9CH tended to stimulate fungal growth whereas the 30CH and 200CH tended to have an inhibitory effect on the disease progress. In terms of dilution, the results should be ascending (the higher the potency, the greater the stimulatory effect) but are not, which shows a disparity with regards to dilution levels. The high dilutions had a greater effect on inhibiting the disease process, which shows an inverted trend. Although this was not borne out in Trial One and is not statistically significant, these trends are interesting and warrant further investigation.

CHAPTER SIX - CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

It was expected that Acrobat® would be the most effective control in eradicating the downy mildew. It was expected that the distilled water control would have no effect on the disease process. Finally, it was expected that the homoeopathic preparations would have an effect on the fungal growth as predicted by the Arndt-Schulz Law. That is, the homoeopathic preparations were expected to stimulate the growth of the downy mildew but the higher potencies would cause higher levels of fungal growth than the lower potencies.

However, the results of the trial showed that Acrobat® had the most significant effect on fungal growth causing the lowest final levels of disease on the seedlings. In terms of disease control, Acrobat® was the most effective treatment. The 30CH was the most effective homoeopathic treatment followed by the 200CH in terms of disease control. However, both the 30CH and 200CH were not significantly different from the distilled water control. Some trends emerged: the 7XH, 9XH, 12XH and 9CH tended to stimulate fungal growth whereas the 30CH and 200CH tended to have an inhibitory effect on the disease progress.

6.2 Recommendations

There were a few problems that were encountered during the trial and others that were identified in retrospect:

- The possibility of human error in remedy preparation cannot be discounted as the potencies were made by hand and thus may have invalidated the activity of the potencies.
- The inherent subjective nature of the disease rating coupled with rater inexperience can skew the results of a trial. The recommendation is for rigorous pre-trial training in the field to maximise rating accuracy.
- It may have been beneficial to repeat the trial a third time especially in the light of the two trial's differing results, and the lack of results from Trial One.

6.2.1 Recommendations for further research

Research using plants as experimental subjects, is a very objective way of demonstrating the activity of homoeopathic medicines. There is also much scope for investigation in the field of homoeopathy and plant pathology. The following points are ideas for future researchers who wish to take existing research a step further, in order to replicate, verify and expand on the knowledge already obtained.

6.2.1.1 Alternatives to agrochemical methods

- The first possibility is disease control by treating the plant with homoeopathic remedies selected on the basis of similarity of disease picture to scope of the remedy; and not by directly destroying the pathogen. Research by Khanna and Chandra (1976, 1977and 1978) serves as a good example of this type of investigation.
- The second possibility is disease control using isopathic preparations as attempted by Brammer (1994), Curnow (1997) and Carey (1999) among others. However, this body of research has not produced any spectacular findings. This may be due to either the incorrect basic assumption that isopathic preparations are able to cure the same disease; or more practical problems relating to study design and experimental methods being employed that may not be compatible with homoeopathic preparations in general.

6.2.1.2 Tautopathic preparations to demonstrate Hormesis

- This particular trial is to the researcher's knowledge, novel research. Thus it is only the beginning and rigorous replication of the trial would be needed to try to reproduce and refine the findings.
- Complete range of potencies should be employed for better monitoring of process of hormesis. For example, all the potencies from 1CH to 30CH may be applied, as opposed to only the most commonly used potencies or a random selection of potencies.
- Frequency of dose repetition is another important consideration. The frequency should match the pace of disease and the potency. If the disease is progressing at a rapid rate the homoeopathic treatments should be applied more frequently than once a week (as was done in this trial) because the energy of the medicine is consumed quickly by the disease-ridden organism. Low potencies are generally repeated more frequently than high potencies, which have a deeper action and therefore a more lasting effect.
- Trials comparing diluted and potentized medicines should also be done in order to demonstrate whether succussion has an effect on hormesis.

Although this trial did not produce many statistically significant results, the trends that emerged were interesting. Future research into this area of homoeopathy should concentrate on refining techniques that are compatible with homoeopathic practice and philosophy so that the results obtained can be accurate and meaningful.

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APPENDIX A

Methods Six and Eight (b) from the German Homoeopathic Pharmacopoeia (GHP) (British Homoeopathic Association 1985) used for preparation of homoeopathic potencies of Acrobat®.

Method Six: Triturations

Preparations made according to Method six are triturations of solid basic drug materials with lactose as the vehicle unless otherwise prescribed. Triturations up to and including the fourth dilution are triturated by hand or by machine in a ratio of one to ten (decimal dilution) or one to 100 (centesimal dilution). Unless otherwise stated, the basic drug materials are reduced to the particle size given in the Monograph (mesh aperture). Quantities of more than 1.000g are triturated by mechanical means.

The duration and intensity of trituration should be such that the resulting particle size of the basic drug material in the first decimal or centesimal dilution is below 10μm at the 80 per cent level; no drug particle should be more than 50μm.

Triturations up to and including the fourth decimal or centesimal are produced at the same duration and intensity of trituration.

Trituration by hand

Divide the vehicle into three parts and triturate the first part for a short period in a mortar and pestle. Add the basic drug material and triturate for six minutes, scrape down for four minutes with a porcelain spatula, triturate for a further six minutes, scrape down again for four minutes, add the second part of the vehicle and continue as above. Finally add the third part and proceed as before. The minimum time required for the whole process will thus be one hour. The same method is followed for subsequent dilutions.

For trituration above the 4X or 4C dilute one part of the dilution with nine parts of lactose or 99 parts of lactose as follows: in a mortar combine one third of the required amount of lactose with the whole of the previous dilution and mix until homogeneous. Add the second third of the lactose, mix until homogeneous, and repeat for the last third.

Method Eight (b): Aqueous preparations made from triturations

Preparations made by Method eight (b) are aqueous preparations produced from triturations made by Method six.

To produce a 6X liquid dilution, one part of the 4X trituration is dissolved in nine parts of WATER FOR INJECTIONS and succussed. One part of this dilution is combined with nine parts of WATER FOR INJECTIONS to produce the 6X liquid dilution by succussion. In the same way, the

7X liquid dilution is made from the 5X trituration, and the 8X liquid dilution from the 6X trituration. From the 9X upwards, liquid decimal dilutions are made from the previous decimal dilution with WATER FOR INJECTIONS in a ratio of one is to ten.

6X and 7X made by the above method must not be used to produce further liquid dilutions.

Aqueous preparations made by method eight (b) are normally processed immediately; their use is limited to the manufacture of presentations by methods 11, 13, 14, 15, 39(a) and 39(c), mixtures by method 16, and potentized mixtures by method 40(b).

Aqueous preparations made by method eight (b) must comply with the 'Sterility Test' of the Pharmacopoeia if stored.

Labelling

Preparations made by Method eight (b) carry the designation 'aquos.' after the indication of the potency; the same applies to presentations made from them.

APPENDIX B

Layout of computerised Complete Blocks Randomisation trial design.

Title:	**Trial 1**
Expermental site:	**UNP**
Date:	**18 May 1999**
Treatments:	**11**
Replications:	**4**

TRAY NO.	TREATMENT	NAME
Block 1		
1	10	30C
2	1	distilled water
3	5	12X
4	2	acrobat
5	3	7X
6	6	7C
7	8	12C
8	4	9X
9	9	15C
10	11	200C
11	7	9C
Block 2		
12	1	distilled water
13	6	7C
14	2	acrobat
15	4	9X
16	11	200C
17	5	12X
18	9	15C
19	10	30C
20	7	9C
21	3	7X
22	8	12C

(Appendix B continued)

Block 3

23	10	30C
24	7	9C
25	1	distilled water
26	8	12C
27	3	7X
28	4	9X
29	9	15C
30	11	200C
31	6	7C
32	2	acrobat
33	5	12X

TRAY NO.	TREATMENT	NAME

Block 4

34	4	9X
35	11	200C
36	9	15C
37	8	12C
38	5	12X
39	10	30C
40	3	7X
41	2	acrobat
42	6	7C
43	1	distilled water
44	7	9C

TREATMENTS	NAME	TRAYS			
1	distilled water	2	12	25	43
2	acrobat	4	14	32	41
3	7X	5	21	27	40
4	9X	8	15	28	34
5	12X	3	17	33	38
6	7C	6	13	31	42
7	9C	11	20	24	44
8	12C	7	22	26	37
9	15C	9	18	29	36
10	30C	1	19	23	39
11	200C	10	16	30	35

APPENDIX C

Layout of computerised Complete Blocks Randomisation trial design.

Title: Trial 2
Experimental site: UNP
Date: 17 June 1999
Treatments: 11
Replications: 4

TRAY NO.	TREATMENT	NAME
Block 1		
12	10	30C
13	3	7X
14	7	9C
15	8	12C
16	2	acrobat
17	6	7C
18	4	9X
19	1	distilled water
20	11	200C
21	9	15C
22	5	12X
Block 2		
12	1	distilled water
13	2	acrobat
25	6	7C
26	5	12X
27	10	30C
28	3	7X
29	4	9X
30	9	15C
31	7	9C
32	8	12C
33	11	200C

(Appendix C continued)

Block 3

34	8	12C
35	9	15C
25	3	7X
26	7	9C
27	4	9X
28	5	12X
29	6	7C
30	1	distilled water
31	2	acrobat
32	11	200C
34	10	30C

TRAY NO.	**TREATMENT**	**NAME**

Block 4

34	4	9X
45	6	7C
46	9	15C
47	11	200C
48	1	distilled water
49	10	30C
50	7	9C
51	3	7X
52	8	12C
53	5	12X
54	2	acrobat

TREATMENTS	**NAME**	**TRAYS**			
1	distilled water	8	12	30	38
2	acrobat	5	13	31	44
3	7X	2	17	25	41
4	9X	7	18	27	34
5	12X	11	15	28	43
6	7C	6	14	29	35
7	9C	3	20	26	40
8	12C	4	21	23	42
9	15C	10	19	24	36
10	30C	1	16	33	39
11	200C	9	22	32	37