

**A STUDY OF THE EFFECTIVENESS OF HOMOEOPATHICALLY
PREPARED DILUTIONS OF ABSCISIC ACID, MOLYBDENUM AND
ALLOPURINOL IN INHIBITING OR PROMOTING THE GERMINATION
OF BARLEY SEEDS (*HORDEUM VULGARE*).**

BY

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Master's Degree in Technology: Homoeopathy in the Faculty of Health
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**I, Nicole Evans, do hereby declare that this dissertation represents my own work
both in concept and execution.**

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ABSTRACT

Introduction

This study investigated the effectiveness of homoeopathic dilutions of abscisic acid (ABA), molybdenum and allopurinol on inhibiting or promoting the germination of barley seeds (*Hordeum vulgare* cv. Stirling, ex Caledon, Western Cape, South Africa, 1998 harvest). Recent research involving ABA and seed germination has shown mixed results, with Bruni (2001), finding there to be statistically significant biological effects, but Couchman (2001) not.

Objective/Aim/Purpose

The purpose of this study was to evaluate the effectiveness of homoeopathic dilutions of ABA, molybdenum and allopurinol (two substances which have an effect on ABA metabolism), especially those above the 10^{-23} level (Avogadro's dilution limit), on germination, in light of recent findings.

Abscisic acid, a plant hormone and molybdenum, a trace element, both play an essential role in inducing dormancy of the seed. Allopurinol, a therapeutic drug, has also been shown to affect ABA metabolism and therefore seed germination. The study used all three substances individually and in combination, in homoeopathic dilutions ranging from 4CH to 200CH potency.

Methodology

There were 7 treatments with 5 potencies per treatment (4CH, 9CH, 15CH, 30CH and 200CH). Each potency level for each treatment had a control, which meant there were 5 controls per treatment.

The seeds (distally cut) were placed in 9cm Petri dishes (20 seeds in each), with 5 repetitions, 100 seeds per dilution level with one control of 20 seeds. There were thus 600 (120 x 5) seeds per treatment and 4200 seeds in total (600 x 7 treatments). Seeds were germinated in the dark at a constant temperature. Counts were done every 24 hours for 3 days and the data recorded. The criterion for germination was radical emergence.

Results

The data was analysed statistically using Univariate Analysis of Variance (STATISTICA version 6). The results showed statistically significant interaction between treatments and potencies and a One-Way Anova was then used to analyse each treatment to determine the effectiveness of each potency. Statistically significant differences were noted between potencies for each treatment.

From the results it was clear that the most effective treatment for stimulating germination was the treatment utilizing homeopathic dilutions of allopurinol.

The most effective treatment for inhibiting germination was the treatment utilizing ABA in homeopathic dilutions.

The 30CH (10^{-60}) showed a statistically significant effect on the stimulation of germination across almost all treatments, whereas the 15CH (10^{-30}) showed a statistically significant effect in inhibiting germination in most treatments.

Conclusion

It is evident from the results of this study that all the treatments produced distinct biological effects, whether it be stimulating germination or inhibiting germination in homeopathic dilution.

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CHAPTER ONE

INTRODUCTION

Civilization began its development when man started to cultivate plants for the food that their "seeds" provided, especially the cereals - wheat and barley in the near East and Europe, rice in Asia and maize in the Americas.

Virtually all of man's exploitation of plants in agriculture depends upon seeds - that they can be stored, transported, multiplied and most important of all germinated (Bewley and Black, 1978:2).

Barley, a major world crop, ranks among the top 10 crops and is fourth among the cereals. Barley contributes significantly to the world's food supply as human food, malt products and livestock feed. It also serves as an important experimental or model plant species for numerous studies in malting and brewing chemistry, plant breeding methodology, genetics, cytogenetics, pathology, virology, physiology and biotechnology (Nilan and Ullrich, 1993:1).

Barley is a grass belonging to the family Poaceae, the tribe Triticeae and the genus *Hordeum*. The chief taxonomic characteristic of *Hordeum* is its one-flowered spikelet. Three spikelets alternate on opposite sides at each node of the flat rachis of the spike or head. Thus is formed a triplet of spikelets at each node - the central and the two laterals. Each spikelet is subtended by two glumes.

When all three spikelets are fertile, the spike is described as six-rowed. When only the central spikelet is fertile, the spike is two-rowed (Nilan and Ullrich 1993:2).

Cultivated barley is self-pollinated with an extremely low occurrence of cross-pollination. Cultivated barley (*H. vulgare*) is one of 31 *Hordeum* species. *H. vulgare* comprises two subspecies - both cultivated and wild forms. All are closely related in terms of a number of biological factors and are interfertile. The cultivated forms are now considered the subspecies *vulgare* of *H. vulgare*, whereas the wild forms are described as the subspecies *spontaneum* of *H. vulgare* (Nilan and Ullrich, 1993:3).

Barley is among the most ancient of the cereal crops. Archeological studies have revealed two-rowed barley cultivation by about 8000 B.C. However, other evidence indicates that barley in essentially the form we know it today existed and was used at least 17 000 years ago in the Nile River Valley of Egypt (Nilan and Ullrich, 1993:3).

Cultivated barley is adapted to and produced over a wider range of environmental conditions than any other cereal. It is grown farther toward the poles, into deserts and at higher elevations than any other cereal and practically any other crop species (Nilan and Ullrich, 1993:4).

It is estimated that the annual world production of barley exceeds 180 million metric tons, of which about 35% is destined for malting. This industry then depends on germination under controlled conditions on a massive scale (Bewley and Black,1994:378).

The viability and dormancy of barley selected for malting are of critical importance. Viabilities lower than 98% are unacceptable, as is dormancy of more than 3-4% of the grain. Dormancy can be a serious problem in freshly harvested grain, especially in some seasons and geographical locations. Such grain has to be stored, sometimes at elevated temperatures to accelerate afterripening, which increases the costs incurred in malting. However, some dormancy is required, especially while the grain is still on the mother plant, to prevent preharvest sprouting, which barley is prone to do (Bewley and Black, 1994:382). Large losses to the malting industry have been experienced over the years in the Western Cape due to preharvest sprouting (Cairns, 1998).

There is a need to try to control the germination process, because of the economic implications of lack of germination or preharvest germination and two hormones are of special interest with regards to barley seed germination. They are abscisic acid (ABA) and gibberellic acid (GA). Studies done on the effects of plant hormones on germination are numerous. Until recently little research however, had focused on the effect of using these plant hormones in homoeopathic dilutions, despite the following observation by Black (1991:99): An area of concern in research with abscisic acid (ABA), is that most of the work

uses high concentrations of ABA. Millimolar concentrations are often applied exogenously in the course of research, even though, typical endogenous concentrations are effective at micromolar levels.

Homoeopathic research into the germination process has shown homoeopathic dilutions to have a significant effect on the process.

Homoeopathic experiments on wheat seeds were conducted from 1923 - 1959 and the results showed that growth was promoted by lower dilutions, then inhibited with higher dilutions and finally stimulated at even higher dilutions (Kayne,1991).

Betti, Brizzi, Nani and Peruzzi (1994) carried out a study on the effect of homoeopathic potencies of *Arsenicum album* in wheat germination. Their results show that the differences between treatment groups cannot be explained as a mere effect of intrinsic seed variability. In a follow-up study in 1997, the effect of *Arsenicum album* 45x on wheat seed germination and seedling growth again showed the homoeopathic treatments to have had a significant effect.

Couchman (2001) and Bruni (2001) both conducted research using ABA and barley seed as their experimental model in testing the effect of homoeopathic dilutions on α -amylase production in barley seeds. Couchman (2001), found there to be no effect on production of α -amylase, but suggests that more research needs to be undertaken. Bruni (2001), however, in his study found there to be a significant difference between control groups and treatment groups. His

study compared the effectiveness of high and ultra high dilutions of ABA prepared by serial dilution and succussion as opposed to dilutions prepared by serial dilution alone.

Neither study tested the effect of ABA in homoeopathic dilution on germination. Several studies have however tested the effect of another plant hormone, gibberellic acid, on germination and α -amylase production, Pieterse (2002), Balding (2002), Stubbs (2002), Him Lok (2001). Him Lok (2001) found that the barley seed germination plant model successfully demonstrated biological effects of homoeopathically prepared GA in various potencies. This model also demonstrated that certain effects not produced by a substance in its crude form (unpotentised) state are only able to manifest when the substance is used in its homoeopathically-potentised state.

Molybdenum, an essential trace element found in the soil and allopurinol, a therapeutic drug used in the prevention of hyperuricaemia, have both been shown to affect levels of ABA (Modi and Cairns, 1994 and Cairns *et al.* 1998). This study will therefore investigate the effect of these two substances in homoeopathic dilution, individually, and in combination with ABA on barley seed germination.

Plant studies offer an effective means to test the effectiveness of homoeopathic dilutions on living tissue as the "placebo effect" can be discounted.

Homoeopathic treatments are also very cost effective and in an industry such as agriculture the need to cut costs are enormous.

1.1 The aim of the study

The aim of this investigation was to evaluate the biological effects of homoeopathic treatments on barley seed germination.

1.2 The statement of the objectives

1.2.1 The first objective

The first objective was to determine the efficacy of homoeopathic dilutions on the germination of barley seeds by the application of a range of different potencies of homoeopathically prepared ABA.

1.2.2 The second objective

The second objective was to determine the efficacy of homoeopathic dilutions on germination of barley seeds by the application of a range of different potencies of homoeopathically prepared molybdenum.

1.2.3 The third objective

The third objective was to determine the efficacy of homoeopathic dilutions on the germination of barley seeds by the application of a range of different potencies of homoeopathically prepared allopurinol.

1.2.4 The fourth objective

The fourth objective was to determine the efficacy of homoeopathic dilutions on the germination of barley seeds by the application of a range of different potencies of homoeopathically prepared combination of ABA and molybdenum.

1.2.5 The fifth objective

The fifth objective was to determine the efficacy of homoeopathic dilutions on the germination of barley seeds by the application of a range of different potencies of homoeopathically prepared combination of ABA and allopurinol.

1.2.6 The sixth objective

The sixth objective was to determine the efficacy of homoeopathic dilutions on the germination of barley seeds by the application of a range of different potencies of homoeopathically prepared combination of molybdenum and

allopurinol.

1.2.7 The seventh objective

The seventh objective was to determine the efficacy of homoeopathic dilutions on the germination of barley seeds by the application of a range of different potencies of homoeopathically prepared combination of ABA, molybdenum and allopurinol.

1.3 The Hypotheses

1.3.1 Hypothesis one

It is hypothesized that homoeopathically prepared ABA, prepared in different potencies, has a biological effect on barley seed germination.

1.3.2 Hypothesis two

It is hypothesized that homoeopathically prepared molybdenum, prepared in different potencies, has a biological effect on barley seed germination.

1.3.3 Hypothesis three

It is hypothesized that homoeopathically prepared allopurinol, prepared in different potencies, has a biological effect on barley seed germination.

1.3.4 Hypothesis four

It is hypothesized that the homoeopathically prepared combination of ABA and molybdenum, prepared in potencies, has a biological effect on barley seed germination.

1.3.5 Hypothesis five

It is hypothesized that the homoeopathically prepared combination of ABA and allopurinol, prepared in potencies, has a biological effect on barley seed germination.

1.3.6 Hypothesis six

It is hypothesized that the homoeopathically prepared combination of molybdenum and allopurinol, prepared in potencies, has a biological effect on barley seed germination.

1.3.7 Hypothesis seven

It is hypothesized that the homoeopathically prepared combination of ABA, molybdenum and allopurinol, prepared in potencies, has a biological effect on barley seed germination.

1.4 Delimitations

1.4.1 Delimitation one

The emphasis of this study is on observed biological activity in terms of barley seed germination and not on the mechanism of action of the medicine or of the germination process.

1.4.2 Delimitation two

This study is limited to observation of biological activity with respect to barley seed germination and not the effects on growth.

1.4.3 Delimitation three

This study is limited to the treatment of plants, only the barley seed is utilized, and does not include treatment of animal or human subjects.

1.4.4 Delimitation four

This study will not attempt to investigate any other substances and potencies other than those stipulated.

1.5 The Assumptions

1.5.1 The first assumption

It is assumed that the homoeopathic medicines provided are prepared according to the monographs as provided in the Homoeopathic Pharmacopoeia unless otherwise stipulated.

1.5.2 The second assumption

It is assumed that the homoeopathic medicines are functionally active at the time of utilization.

1.5.3 The third assumption

It is assumed that the controlled environmental conditions stipulated in the experiment are effective and efficient over the entire duration of the study.

1.5.4 The fourth assumption

It is assumed that the material upon which and in which the experimental samples are germinated (i.e. filter paper and Petri dishes) are homoeopathically inert.

CHAPTER TWO

REVIEW OF THE RELATED LITERATURE

2.1 Introduction

The fundamental elements to this research are barley, barley seed germination, the plant hormone - ABA, the substances molybdenum and allopurinol, and homoeopathy in agriculture.

The history, nature, and importance of barley, was covered in Chapter One.

This literature review is focused on barley seed germination and the role of the above mentioned substances on the process.

2.2 Seed - the reproductive germ of flowering plants

Seeds form by the combination of mature male and female gametes, coming from the stamen and pistil of the flower, respectively, in a process known as fertilization or syngamy. In addition to the formation of a seed, the process of fertilization is responsible for the level of genetic variation present in the zygote. Fertilization in angiosperms typically occurs by either self- or cross-fertilization (Desai, Kotecha, and Salunkhe, 1997:7).

Botanically seeds are essentially young plants whose life activities are going on at a minimum rate. They represent the most critical phase of a plant's life cycle and are responsible for the evolutionary continuum of plant species (Desai, Kotecha and Salunkhe, 1997:7).

The seed is derived from the fertilized ovule. In almost all cases the following can be recognised as the fertilized ovule develops: (1) the testa - the product of one or both integuments of the ovule; (2) the perisperm - derived from the nucellus; (3) the endosperm - produced as a result of fusion between one male generative nucleus and the two polar nuclei to form the triploid endosperm nucleus; (4) the embryo - the result of fertilization of the oosphere (ovum) by a male nucleus (Bewley and Black, 1994:5).

2.3 Barley seed structure

A typical hulled barley kernel from the outside inward is composed of lemma and palea enclosing and cemented to the caryopsis. The rachilla lies within the crease of the kernel near the base and on the ventral or palea side. The caryopsis is composed of pericarp, integuments, starchy endosperm and germ. The outer layer of the endosperm is made up of the aleurone cells. This structure is the major enzyme synthesizing area of the kernel. The germ is partly imbedded in the endosperm at the base of the kernel on the lemma or dorsal side and is held at an oblique angle to the axis of the kernel. The germ is

composed of the embryonic axis, which develops into the seedling at germination, and the adjacent scutellum. The latter structure secretes the hormones that stimulate enzyme release and synthesis. The enzymes hydrolyse constituents of the endosperm to products that nourish the growing seedling (Dickson, 1969:108).

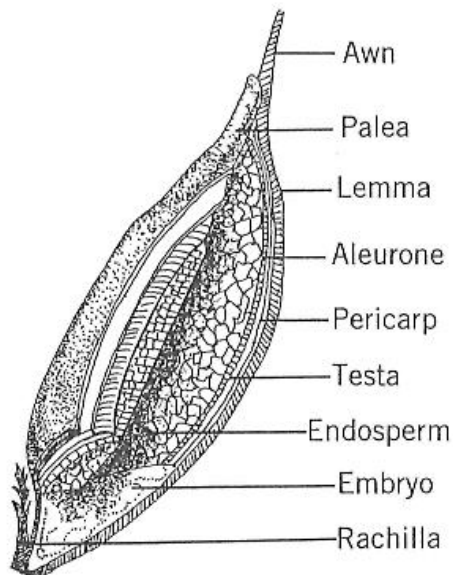


Figure 2.1 Barley kernel (Smith, W. 1995)

2.4 Germination

Germination begins with water uptake by the seed and ends with the start of elongation by the embryonic axis, usually the radicle (Bewley and Black, 1994:1).

The process of germination is subdivided into three phases. During phase I, rapid hydration (imbibition) of the seed occurs and metabolism begins. During phase II, a lag follows while major metabolic events take place in preparation for radicle emergence. The final event phase III, is characterised by radicle elongation and marks the end of germination and the beginning of seedling growth (Nykiforuk and Johnson-Flanagan, 1998:254).

Germination of viable barley grain is initiated by the uptake of water. Cuticularized layers in the husk, the pericarp and the testa-nucellus act as barriers to the penetration of both water and solutes and most of the water that penetrates undamaged grain does so near the embryo, probably through the micropylar region. Water subsequently spreads through the grain, but at different rates in the various regions. Not unexpectedly, hydration of the central endosperm occurs relatively late in the uptake process and mechanical damage to the grain can greatly accelerate the rate of water penetration (Fincher and Stone, 1993:247-248).

Following the initiation of germination, hydrolytic enzymes, which are synthesized mainly in the aleurone and scutellum, are secreted into the nonliving starchy endosperm, where they catalyze the depolymerization of storage polymers. The degradation products move along a diffusion gradient generated by their active uptake into the epithelial layer of the scutellum. After absorption into the scutellum, they are translocated via a developing vascular system to the

seedling, where they serve the immediate nutrient and energy needs of the enlarging and differentiating embryo in the period before the establishment of the photosynthetic leaf and absorptive root systems. Based on in vitro evidence, the synthesis and secretion of the hydrolases from these tissues appear to be controlled principally by the hormones GA and ABA (Fincher and Stone, 1993:248).

2.4.1. Measurement of germination

The extent to which germination has progressed can be determined roughly, by measuring water uptake or respiration, but these measurements give only a very broad indication of what stage of the germination process has been reached. No universally useful biochemical marker of the progress of germination has been found. Therefore, state Bewley and Black (1994), the only stage of germination that we can time fairly precisely, is its termination! Emergence of the axis (usually the radicle) from the seed normally enables one to recognise when germination has gone to completion, though in some cases where the axis may grow before it penetrates through the surrounding tissues, the completion of germination can be determined as the time when a sustained rise in fresh weight begins. The degree to which germination has been completed in a population is usually expressed as a percentage, normally determined at time intervals over the course of the germination period. Germination curves are usually sigmoidal - a minority of seeds in the population germinates early, then the germination

percentage increases more or less rapidly, and finally the relatively few late germinators emerge. The curves are often positively skewed because a greater percentage germinates in the first half of the germination period than in the second. But, although the curves have the same general shape, important differences in behaviour between populations are evident. Some curves flatten off when only a low percentage of the seeds have germinated, showing that this population has a low *germination capacity*, that is, the proportion of seeds capable of germinating is low.

The shape of the curve also depends on the *uniformity* of the population, that is, the degree of simultaneity or synchrony of germination.

Although seed samples may be alike as far as both germination capacity and uniformity are concerned, they may be very different in their *rate* of germination. The rate of germination can be defined as the reciprocal of the time taken for the process to be completed, starting from the time of sowing. This can be determined for an individual seed, but it is generally expressed for a population (Bewley and Black, 1994).

2.4.2 Internal conditions

2.4.2.1 Dormancy

After a seed matures, germination follows, but these two processes are normally separated both by time and space. The interval between the two events may

vary from a couple of hours to many years and from a few centimetres to thousands of kilometres. Delayed germination is not accidental - it represents the physiological mechanisms that keep the seed in a nongerminating state. Since young plants are vulnerable to the hazards of drought and extremes of heat and cold, it is advantageous for the seed to remain in an inactive condition until it reaches a time and place for germination (Desai, Kotecha and Salunkhe, 1997:29).

Bewley and Black, 1982, (as cited by Desai, Kotecha and Salunkhe, 1997:30), distinguished the term quiescence from dormancy; the former refers to nongermination of seed due to either lack of water or unsuitable environmental conditions, whereas the response of the seed to fail to germinate under favourable conditions points to dormancy of the seed, due to its internal germination blocks.

When quiescence is extended for longer periods, the seed may enter a new state of dormancy, called secondary dormancy, which can be broken under suitable temperature conditions (Desai, Kotecha and Salunkhe, 1997:30).

This review will focus solely on primary dormancy and its development in the seed.

Primary dormancy develops in seeds during their maturation on the mother plant (Le Page-Degivry, 1998:203). ABA is the most essential factor for dormancy induction during seed development (Karssen, 1995:345).

In many seeds, levels of endogenous ABA peak around mid-maturation but decline sharply thereafter; levels at maturity are (generally) extremely low. This decline in ABA during the later stages of maturation may release the seed from the constraints of development and allow germination to occur. In dormant seeds, the germination process is blocked and radicle emergence is prevented in a seed that is fully imbibed and metabolising.

In a study done by Karssen *et al*, 1983, on the induction of dormancy during seed development by endogenous ABA, the use of mutants with an ABA deficiency allowed the authors to investigate the role of ABA in seed physiology. Using mutants of *Arabidopsis thaliana* they showed that the ABA-deficient developing seeds (*aba*) do not become dormant; ABA-insensitive (*abi*) mutants have normal or increased levels of endogenous ABA but resemble phenotypically the ABA-deficient mutants, exhibiting reduced seed dormancy.

That dormancy induction depends not only on ABA synthesis, but also partially or wholly on seed sensitivity to ABA, is clearly demonstrated by Walker-Simmons, 1987, (as cited by Karssen,1995:338) in a comparison of a sprouting-resistant and a sprouting-susceptible wheat cultivar. Though dormancy varies, the ABA concentrations are closely similar. Isolated embryos of both cultivars differ, however, in their reaction to ABA. Embryo germination of the high-dormancy (nonsprouting) cultivar is inhibited by ABA concentrations that do not affect embryos from the low-dormancy grains.

A role of ABA in dormancy is also indicated by the observation that exogenous ABA prevents germination and induces dormancy. For example, lettuce seeds, which require red light to germinate, do not germinate when illuminated in the presence of ABA (Taiz and Ziger, 1991:485).

ABA is one of the most important naturally occurring inhibitors inducing dormancy and because of this, it is the most important factor in inhibiting precocious germination or pre-harvest sprouting.

2.4.2.2 Pre-harvest sprouting

Cereal grains, especially wheat, maize, rice and barley, are prone to germinate while still on the ear of the parent plant in the field. This is known as preharvest sprouting. Preharvest sprouting (unlike vivipary) generally occurs in mature, dried seed still held on the ear of the mother plant when they become wetted by rain or very high humidities (Bewley and Black, 1994:382).

Preharvest sprouting occurs because grain is too germinable. During the life of a seed, a period of rest (quiescence, sometimes also dormancy) normally intervenes between embryo development and germination itself. The controlling factors enforcing rest involve constraints imposed by parental tissues and effects of inhibitors such as ABA (Bewley and Black, 1994:385).

The connection between endogenous ABA and the prevention of precocious germination is especially evident in those ABA-deficient mutants that germinate while still on the mother plant. Another example is that the addition of ABA to the incubation medium holds the isolated embryo in the embryogenetic mode and therefore somehow mimics the natural constraints that suppress precocious germination *in planta* (Karssen, 1995:338).

Of relevance to this study was the finding that immature embryos of wheat are prevented from germinating precociously when ABA is supplied in the culture medium (Kermode, 1995:282).

2.4.3. External conditions

2.4.3.1 Light

The effect of light and of photoperiod on germination of seeds of some species may involve the pigment phytochrome. If phytochrome is not present in the active form on seed drying, then germination will be light dependent. For cereal seed there appears to be no light requirement (Kruger and LaBerge, 1983).

Dunwell (1981) incubated embryos of *Hordeum vulgare* in the dark in his study of dormancy and germination.

In a study by Van Beckum, Libbenga and Wang (1993), on the responses of barley grains to ABA and GA, the incubation was done in the dark.

This study will therefore follow the trend of most experiments and the barley seeds will be germinated in the dark.

2.4.3.2. Temperature

In a study done by Dunwell (1981), it was shown that confusion may arise by quoting optimum germination temperatures, as he found that at each time between 1-8 days different temperatures gave maximum germination. He found that embryo germination of barley seed occurs at all temperatures above 5°C up to 25°C. For the purpose of this study 15°C was chosen.

2.5 Barley seed germination studies

Experiments on barley seed germination are usually from the viewpoint of a seedsman who needs grain capable of generating new seedlings in the field, or the malster who routinely needs to produce malts of specified qualities, in the shortest possible times, and in high yield (Briggs,1992:369).

The central importance of barley grain germination in the plant's life cycle and in the production of beer and whisky has attracted intense research interest into the physiology and biochemistry of the process (Fincher and Stone, 1993:247).

Studies using barley seeds are numerous due to a simple procedure that can isolate the viable aleurone layers that retain hormone sensitivity and an ability to secrete active enzymes. This has resulted in the widespread adoption of barley aleurone layers as a model experimental system for in vitro investigations of plant hormone action, the synthesis and secretion of hydrolytic enzymes and the

regulation of plant gene expression role of GA and ABA in the induction of hydrolytic enzymes in germinating barley (Fincher and Stone, 1993:248-249).

For the purpose of this study, the attention is focused solely on barley grain germination, with radical emergence being the measurement of germination having taken place, and the effects of ABA, molybdenum and allopurinol in homeopathic dilution on that process.

Previous studies (not in homeopathic dilutions) done on the effect of ABA on the germination of barley seed, have shown that the addition of ABA delayed and inhibited germination (Van Beckum, K.R. Libbenga and Wang, 1993; Dunwell, 1981; Wang, Heimovaara-Dijkstra and Van Duijn 1995).

Wang et al (1995) achieved results that suggest that ABA is a diffusable factor that can inhibit barley grain germination. According to their hypothesis, isolated dormant embryos germinate because ABA diffused out and nondormant embryos germinate because the amount of ABA is already low in the embryo. Therefore, it should be possible to manipulate germination of both dormant and nondormant embryos by addition of ABA to the medium.

2.6 Abscisic acid

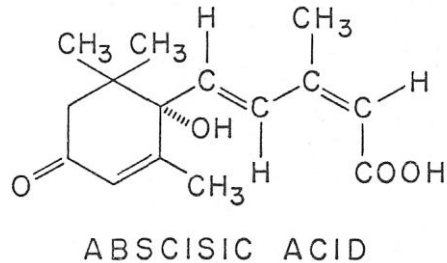


Figure 2.2 (Davies, 1991).

In 1963 ABA was first identified and chemically characterized in California by Frederick T. Addicott and his coworkers, who were studying compounds responsible for abscission of cotton fruits. They named one active compound *abscisin I* and called a second (much more active) compound *abscisin II*. *Abscisin II* proved to be ABA. In the same year, two other research groups had very likely discovered ABA as well. One group was led by Philip F. Wareing in Wales; they were studying compounds that caused dormancy of woody plants, particularly *Acer pseudoplatanus*. They named their most active compound *dormin*. The other group was led by R. F. M. Van Steveninck, first in New Zealand and then in England; they were studying a compound or compounds that accelerated abscission of flowers and fruits of the yellow lupine. Because it became evident (in 1964) that *dormin* and the lupine compound were identical to *abscisin II*, physiologists agreed in 1967 to call the compound *abscisic acid* (Salisbury and Ross, 1992; Taiz and Zeiger, 1991).

ABA has been found to be a ubiquitous hormone in vascular plants. It has been detected in mosses, but appears to be absent in liverworts. Several genera of fungi make ABA as a secondary metabolite. Within the plant, ABA has been detected in every major organ or living tissue from the root cap to the apical bud. ABA is synthesized in almost all cells containing chloroplasts or amyloplasts. Surprisingly, ABA was found in the mammalian brain, but it is unclear whether it originates from ingested food or is a regular brain metabolite (Taiz and Zeiger, 1991:482-483).

ABA is a 15 carbon sesquiterpenoid composed of three isoprene residues. In addition, the carbon skeleton of the molecule bears a close similarity to the terminal rings of carotenoids such as violaxanthin. It can exist in either the *trans* or *cis* configuration, and the latter, being optically active, has a (+) and (-) enantiomer. Both the *cis* and *trans* isomers of ABA may be extracted from plant tissues, although only the former isomer exhibits biological activity (Roberts and Hooley, 1988:21).

This study is not concerned with the biosynthetic pathway through which ABA is produced, although what is important, is the enzymatic role molybdenum and allopurinol play in the metabolism of ABA as will later be discussed.

ABA is also a crucial factor during seed development, being involved in storage protein synthesis, desiccation tolerance and prevention of precocious germination (Wang, 1996:68).

ABA counteracts the effect of GA on α -amylase synthesis in germinating cereal grains and may be involved in defense against insect attack by inducing gene transcription, notably for proteinase inhibitors, in response to wounding (Davies, 1995:10).

In many seeds, levels of endogenous ABA peak around mid-maturation, but decline sharply thereafter; levels at maturity are (generally) extremely low. This decline in ABA during the later stages of maturation, release the seed from the constraints of development and allows germination to occur (Kermode, 1992:297).

ABA inhibits germination in the phase that the seeds are highly sensitive to external stimuli. Due to inhibited germination, induction of secondary dormancy can proceed. In this respect, the action of ABA is comparable to other germination inhibiting conditions such as osmotic stress, darkness (when seeds are light requiring) and supra-optimal temperatures (Hilhorst and Karssen, 1992:232).

ABA plays a pivotal role in dormancy regulation during seed development. During seed development, ABA is involved in the regulation of a number of processes, such as suppression of precocious germination, induction of LEA proteins, development of desiccation tolerance and induction of dormancy. Application of

exogenous ABA inhibits seed germination of many species (Hilhorst and Karssen,1992:235).

The connection between endogenous ABA and the prevention of precocious germination is especially evident in those ABA-deficient mutants that germinate while still on the mother plant (vivipary) (Karssen, 1995:338).

It seems that even extremely low endogenous ABA levels are sufficient to suppress precocious germination (Hilhorst and Karssen, 1992:228).

In a study done by Wang, Heimovaara-Dijkstra and Duijn (1995:586) the endogenous ABA in isolated embryos from both dormant and non-dormant grains during germination was analyzed. The inhibitory effect on germination of a higher number per well of isolated dormant embryos was due to diffusion of endogenous ABA out of the embryos and accumulation of ABA in the incubation medium. Moreover, there was de-novo synthesis of ABA in embryos isolated from dormant grains during incubation but not in embryos isolated from non-dormant grains. The inhibitory effect of ABA on germination of embryos isolated from dormant grains could be mimicked by addition of ABA to the medium in which dormant embryos had been placed. Embryos isolated from nondormant grains were insensitive to addition of ABA and medium from dormant embryos.

Investigation of the role of ABA in dormancy in cereal grains has mainly been focused on developing grains.

The study done by Wang et al (1995) suggests that ABA is a diffusible factor that can inhibit barley grain germination. According to this hypothesis, isolated dormant embryos germinate because ABA diffuses out and nondormant embryos germinate because the amount of ABA is already low in the embryo. Therefore, it should be possible to manipulate germination of both dormant and nondormant embryos by addition of ABA to the medium.

2.7 Molybdenum

Molybdenum is an essential trace element for most living systems, including microorganisms, plants and animals. Molybdenum and tungsten are the only second and third row transition metals that are required for the growth of at least some organisms. These metals are found associated with a diverse range of redox active enzymes that catalyze basic reactions in the metabolism of nitrogen, sulphur and carbon. Molybdenum is incorporated into proteins as the molybdenum-cofactor (Mo-co), which contains a mononuclear Mo atom coordinated to an organic cofactor named molybdopterin. Mo-co-containing enzymes catalyze the transfer of an oxygen atom, ultimately derived from or incorporated into water, to or from a substrate in a two-electron redox reaction. (Kisker, Schindelin and Rees, 1997:234).

The molybdenum cofactor is shared by nitrate reductase, xanthine dehydrogenase and ABA aldehyde oxidase. (Walker-Simmons, Kudrna and Warner 1989).

In a study done by Cairns and Kritzing (1992), molybdenum-treated wheat, produced seed which was significantly more dormant than that harvested from the molybdenum-deficient plants. Molybdenum treatment also resulted in a higher nitrate, protein and ABA content of the seed. Their findings would seem to indicate that molybdenum has the potential to restrict pre-harvest sprouting in wheat growing in soils deficient in molybdenum.

A barley mutant (*Hordeum vulgare* L) was identified with low basal levels of ABA and with reduced capacity for producing ABA in response to water stress. The mutation is in a gene controlling the molybdenum cofactor. This study done by Walker-Simmons *et al.* (1989), indicated that ABA biosynthesis at some developmental stages is dependent upon a molybdoenzyme.

A study done by Cairns, Cowan and Smit (unpublished paper) on the effect of molybdenum (in the form of potassium molybdate) on ABA and its precursor xanthoxal (XAN), showed that molybdate had little or no effect on the formation of XAN, but stimulated ABA production. Also, of relevance was the use of the other substance utilized in this study, allopurinol (review to follow), in combination with molybdenum on seed germination. The combination of molybdenum and

allopurinol showed an increased germination rate and an inhibition of ABA synthesis or an increased catabolism of ABA.

No known study has examined the effects of molybdenum in homoeopathic dilution on its own or in combination with ABA and allopurinol using a barley seed germination model. This study aims to show that molybdenum in homoeopathic dilution can have a biological effect on germination.

2.8 Allopurinol

Allopurinol [4-hydroxypyrazolo-(3,4-*d*)pyrimidine], an isomer of hypoxanthine, is a substrate and potent inhibitor of the metalloflavoprotein, xanthine oxidoreductase, because it binds tightly to the reduced molybdenum component of the enzyme (Hille and Massey, 1981 as cited by Montalbini and Della Torre, 1995). For this reason it has been extensively used in the medical field to relieve hyperuricemia, because the major metabolic fate of allopurinol in humans is oxidation by xanthine oxidase to the corresponding xanthine analogue, oxipurinol (Rundles et al., 1969 as cited by Montalbini and Della Torre, 1995).

In a study by Cairns, Cowan and Smit (unpublished paper) on the effect of allopurinol and molybdenum on seed dormancy and ABA metabolism it was shown that allopurinol plus potassium molybdate altered the ABA metabolism in the seed produced. It was found that there was either an inhibition of ABA

synthesis or an increased catabolism of ABA. They suggested that the use of allopurinol and molybdenum may be useful tools in the study of ABA metabolism, seed dormancy and germination.

No known homoeopathic research has studied the effect of allopurinol in homoeopathic dilutions on germination on its own or in combination with ABA and molybdenum.

2.9 Homoeopathic potency and potentisation

Homoeopathy is a therapeutic method which clinically applies the law of similars and which uses medicinal substances in weak or infinitesimal doses (Jouanny,1993:11).

The law of similars is the formulation of a physiological state of things, which had already been observed twenty-five centuries ago by Hippocrates and his school. Even at that time, the existence of a similarity between the toxicological action of a substance and its therapeutic action had been observed. "The same things which cause the disease cure it."

In the centuries that followed, other doctors made similar observations but did not come to any practical conclusions. It was not until the end of the 18th century that a German physician, chemist and toxicologist, Christian Samuel Hahnemann further studied the question. He observed that cinchona, a remedy, which at that time was used to treat certain types of malarial fever, toxicologically caused attacks of fever similar to those against which it was therapeutically used. He set

to work testing out his hypothesis by experimenting on himself and others with every medicinal substance known at that time, in order to familiarize himself with the pharmacodynamic action these substances had when administered to healthy people. Having determined what these actions were, he then tried these substances out as therapeutic agents on ill patients who displayed symptoms similar to those induced in healthy people undergoing experimentation.

He observed that his hypothesis was correct, but only when very weak or even infinitesimal doses were used (Jouanny,1993:13).

Hahnemann experimented with various strengths of the medicines because many of the substances used were highly toxic in their crude state and found that although diluting them reduced their side effects it also correspondingly decreased their curative powers. This is when Hahnemann discovered the power of succussion. He started by diluting the medicinal substance in water or alcohol and then vigorously shook the bottle containing the resulting dilution. He did this between each dilution and called the process potentisation or dynamisation. The resulting remedy was not only freed from toxicity, but its curative powers were increased (Garion-Hutchings,1995:10).

Medicines are prepared for homoeopathic use by diluting one part of the original substance (if a solid) or tincture (if a liquid) in nine parts of milk sugar or of a solution of alcohol and distilled water. The mixture is triturated in a mortar or succussed in a bottle for some time until the medicinal substance is uniformly

distributed throughout the diluent, and it is then known as the 1 X dilution. The mixture can also be made in the proportion 1 to 99 and is then known as the 1 C dilution. The process can be repeated as many times as is desired, and the remedies are prepared and used in all dilutions from 1 X or 1 C up to the 200C and beyond, the former being known as "low" and the latter as "high" dilutions (Coulter, 1972:34-35).

According to Avogadro's Law, however, the number of molecules in 1 gram/mole of any substance is approximately 1×10^{24} . Therefore when substances are diluted beyond the 12 C or 24 X levels, it is improbable that a single molecule of the original medicinal substance will remain in the milk-sugar or alcohol used as the diluent (assuming that a homogenous solution has been achieved at each stage). Since homoeopathy makes frequent use of substances diluted well beyond the Avagadro limit, there is much controversy surrounding the effectiveness of these medicines. This study therefore aims to prove the effectiveness of these dilutions, by utilizing an objective model such as seed germination in an attempt to prove biological action still exists at such dilution levels.

2.10 Isopathy

Isopathy refers to the use of the "same" (iso-) instead of the "similar" (homoeo-) as medicines for curing disease (Gaier, 1991: 290). The relevance of isopathy to this study is that this study is using homoeopathically diluted ABA to see if it has

an effect on ABA "metabolism" which then affects seed germination. The use of ABA in homoeopathic dilution falls under the category sarcodes, which is a remedy, homoeopathically made from preparations, or derivatives, of healthy plant, animal or human secretions, excretions or special tissue products (Gaier, 1991: 294).

2.11 Homoeopathic agricultural research

In "An agricultural application of homoeopathy" Kayne (1991), reiterates Scofield's 1984 criticism that despite a great deal of investigation there is only little firm evidence to support the efficacy of homoeopathic potencies, due to poor experimental methodology.

In 1971, Pelikan and Unger pointed out that there has been violent controversy as to whether homoeopathic potencies are therapeutically active or not. The effects observed in patients, and healthy subjects, are often put down to suggestion. However, homoeopathic potencies are also used in the veterinary field and here effects, due to suggestion are not very likely. Unfortunately, the case material available in this field has not been systematically assessed to give evidence of efficacy. The situation is different when it comes to demonstrating the effects of potencies on plants. In this case, there is no possibility of suggestion affecting the results.

This review will focus mainly on studies using germination as a means of verifying the efficacy of homoeopathic treatments. A brief review of previous studies utilizing plant material, will also be covered.

In 1923 L. Kolisko carried out experiments on wheat, sunflowers, gladioli and crocuses (as cited by Pelikan and Unger, 1971), and was able to show that the action of potentized substances can be demonstrated on plant growth.

In 1971 Pelikan and Unger reproduced a small part of Kolisko's work, but with experimental methods more adapted to laboratory conditions. They chose wheat seeds to test the substance silver nitrate on and the potencies ranged from the 8th to the 19th decimal. According to them the results obtained showed that there was a statistically significant effect on plant growth.

In 1932 J. Roy (as cited by Stephenson, 1973:7) made dilutions of germinating barley ranging from 10^{-3} to 10^{-120} . He saturated barley grains with these varying dilutions, planted them, and weighed the amount of barley produced. Of 69 experimental groups, only two used dilutions above 10^{-26} . Each of these two dilutions (10^{-60} and 10^{-120}) differed from the control groups by 8 per cent. However, Stephenson states that because Roy did not describe the range of variation in his control groups, the significance of an 8 per cent variation from the control could not be evaluated.

In 1966, Netien, Boiron and Marin (as cited by Boyd, 1973:258), experimented with seeds obtained from dwarf pea plants sprayed with copper sulphate solution. Half of these were soaked in double distilled water and half in a 15c (10^{-30}) potentized dilution of copper sulphate. The germination of those soaked in the potentized dilution was much increased over those in the distilled water.

In addition, the excretion of copper from treated seeds grown on this 15c dilution was much more rapid than those grown in potentised distilled water.

For a complete review of homoeopathic research involving plants up to 1984, refer to Scofield's (1984) "Experimental research in Homoeopathy - a critical review".

Saxena, Pandey and Gupta (1987:191), studied the effect of the homoeopathic remedies on the incidence of seed-borne fungi and germination of *Abelmoschus esculentus*. A total of 22 fungal species were isolated from the seeds of *A. esculentus*. The remedies Thuja, Nitric Acid and Sulphur in the 200th potency completely checked the growth of all the fungal species in blotter method.

Their results also showed that the percentage of seed germination and root-shoot lengths were increased in all treatments in comparison to controls.

Work done by Bornoroni (1991:275) on the synergism of action between the plant hormone Indoleacetic acid (IAA) and highly diluted solutions of CaCO_3 on the growth of oat coleoptiles, found that the 5CH potency resulted in a

statistically significant increase in growth, as compared with coleoptiles treated with indoleacetic acid alone.

In an article entitled "Characteristics and selected results of research on Homoeopathy", Righetti (1994) states in his reference to plant studies, that it has become increasingly obvious that the use of remedies enhances the growth of plant seeds.

Another important study, which used wheat germination as a model to test the efficacy of homoeopathic potencies, was conducted in 1994 by Betti, Brizzi, Nani and Peruzzi.

This blind randomized laboratory trial studied the homoeopathic potencies of Arsenicum album on wheat germination using a simple model, which allows for a rigorous statistical analysis. The parametric tests showed that the differences between the treatment groups cannot be explained as an effect of mere intrinsic seed variability. The test revealed that the 40x and 45x potencies seemed to have had the most relevant effect on wheat germination.

In 1997, the same team of Betti *et al.* conducted a more detailed experiment using only the 45x potency with the aim of verifying whether homoeopathic treatment has a statistically significant effect, not only on seed germination, but also on seedling growth. They also wanted to add to the quantitative results of the previous study, a qualitative assessment of the effects of homoeopathic treatment on the morphological features of the seedlings.

In the study the treated seedlings were poisoned with sublethal quantities of Arsenic and then treated with the homoeopathic dilution of the same substance, to establish if there was amplification of homoeopathic treatment according to Hahnemann's law of similars which states that "like cures like".

They found that the most marked effect was on stem length, which showed significant recovery increasing with treatment time, whereas root length was not influenced by the homoeopathic treatment.

In 2000 Brizzi, Nani, Peruzzi and Betti wrote a paper on the statistical analysis of the effect of high dilutions of arsenic in a large dataset from a wheat germination model. Their aim was to test the reproducibility of their above mentioned results and to make a comparison between all the experiments they had performed. They found that the global results were consistent, in spite of the fact that the two experiments were performed two years apart. The interaction of succussion and high dilution gave the most relevant results, which seems to put in evidence the existence of a real efficacy of homoeopathic treatments.

Studies done that have important relevance to this study have been carried out by Couchman (2001), Bruni (2001), Pieterse (2002), Balding (2002), Stubbs (2002), Him Lok (2001). The first two studies are of particular relevance as they both involve the use of the plant hormone ABA, which was utilized in this study.

Bruni (2001), did a comparison of the relative effectiveness of high and ultra high dilutions of ABA prepared by serial dilution and succussion as opposed to dilutions prepared by serial dilution alone, on the synthesis of α -amylase in barley endosperm half-seeds. The results showed that unsuccussed ultra high dilutions of ABA are biologically active. Furthermore, a significant difference was noted between the effects of succussed and unsuccussed dilutions of ABA. The unsuccussed dilutions produced less α -amylase than the control dilutions, which indicates that the inhibitory effects of ABA were still present in these dilutions. He found that statistically significant differences were noted between treatment groups and control groups, although not between the group of succussed dilutions when compared with the control. There were also significant differences between the two methodologies employed, but not between the different dilution levels tested.

The conclusion he drew was that ultra high dilutions can have biological effects and that succussion does alter the action of homoeopathically prepared medicines.

Couchman (2001) also investigated the effects of homoeopathic potencies of ABA on the production of α -amylase in de-embryonated endosperm half seeds of barley, but in the presence of gibberellic acid.

She found however that there was no significant difference between the control and the various potencies of ABA. As the two studies have conflicting results

with regards to the effect of homoeopathic dilutions of ABA, there is a definite need for more research to be carried out using a barley seed model.

Other research which has utilized the barley seed germination model includes a study by Him Lok (2001) which looked at the effect of homoeopathically prepared dilutions of GA on germination. She found that biological activity of homoeopathically prepared GA₃ at 15CH was evident in medium vigour seeds, since this treatment resulted in significantly long root development. High-vigour seeds imbibed in homoeopathically prepared GA₃ at 4CH, 30CH and 200CH, germinated faster than the control. Also, among high-vigour seeds, prior imbibition with homoeopathically prepared GA₃ at 4CH, 15CH, 30CH and 200CH solutions stimulated the subsequent development of larger seedlings than those imbibed in GA₃. This study therefore successfully showed that the barley seed germination plant model demonstrated biological effects of homoeopathically prepared GA in various potencies. It also demonstrated that certain effects not produced by a substance in its unpotentised state are only able to manifest when the substance is used in its homoeopathically-potentised state.

CHAPTER THREE

METHODS AND MATERIALS

3.1 Experimental procedure (study design)

Seven treatments of homoeopathic dilutions on *Hordeum vulgare* (Barley seed) with respect to germination were conducted.

Germination trials were conducted under controlled laboratory conditions, in growth chambers. The growth chambers were set at a temperature of 15°C with 1 degree temperature fluctuations. Each treatment consisted of 5 different dilutions of 120 seeds of which 20 seeds made up the control. The seeds were placed in 9cm petri dishes lined with two Whatmann No. 1 filter papers. Five ml of the relevant potency was dispensed into each petri dish, prior to incubation. Five ml of distilled water was placed in each control dish. All the petri dishes of each treatment and the control (30 dishes) were placed in a separate plastic bag, with an extra 2ml of distilled water in each bag to avoid evaporation.

3.2 Preparation of materials

3.2.1 Setting up of the field trial (technical details)

This trial was run at the Seed Physiology Laboratory of the Faculty of Agriculture at the University of Natal, Pietermaritzburg. The controlled environment under which barley seeds were germinated, consisted of growth chambers set a 15°C (< 1°fluctuation) in the dark. Seeds were germinated in 9cm Petri dishes on two Whatmann No.1 filter papers moistened with 5ml distilled water or test solution. Germination was recorded in 24 hour intervals for 3 days and results were observational. Once the radicle had emerged (first visible sign of germination) the seed(s) was removed from the dish and the number germinated recorded.

3.2.2 Apparatus

Growth chamber/Germination chamber - Labcon (Labex), with identification symbols (labeled) University 1 & 2.

Dispensing pipette (with disposable tip unit) - Socorex (Swiss) 0.5 - 5ml. Utilized to administer above mentioned distilled water and test solutions.

Petri dishes - New 9cm (diameter) petri dishes were utilized. All petri dishes were labeled on the exterior of the lid and base of each petri dish.

3.2.3 Materials

3.2.3.1 Seeds

All treatments utilized variety: *Hordeum vulgare* cv. Stirling, 1998 harvest from the Caledon Farmers Cooperative, (Western Cape, South Africa), provided by the University of Natal, Pietermaritzburg. The seed was not treated with any chemicals.

3.2.3.2 Distilled water

All samples of distilled water were obtained from the same source. Samples were taken from a Milli-Q plus Water Purification System (0.22µm filter), within the Department of Biochemistry, University of Natal, Pietermaritzburg.

3.2.3.3 Plastic bags

Transparent 280 x 330mm bags were utilized in all treatments.

3.2.4 Procedures

- 1 Seed counts were conducted by hand and each seed was distally cut with a scalpel to ensure effective absorption of treatments. (This was decided after doing a pilot study first). All batches of counted seed were refrigerated at 4°C to prevent any possibility of premature germination prior to the commencement of the experiments.
- 2 210 Petri dishes were each lined with two Whatmann No. 1 filter papers and labeled.
- 3 Twenty seeds were placed into each petri dish upon the filter paper.
- 4 There were five repetitions for each dilution level and 1 control. This amounted to 120 seeds per dilution level. There were 5 dilutions per treatment, which meant there were 600 seeds per treatment. With 7 treatments, this amounted to 4200 seeds being used in the study.
- 5 Each test solution and distilled water was added by means of a disposable tip-dispensing pipette (to prevent 'contamination' between test solutions).
- 5 Petri dishes were sealed randomly, collected and placed one upon the other

in 'piles' of 6 (with 30 in one bag), and then placed into plastic transparent bags.

- 6 Two millilitres of distilled water was placed into each plastic bag (preventing any possibility of desiccation), sealed and placed in the growth chamber.
- 7 Recording of measurements was performed every 24 hours which involved physical counts of the number of seeds germinated, that is the first visible protrusion of the radicle through the seed coat. After which the petri dishes were immediately returned to their respective germination chambers.

3.2.5 Potencies utilized

Treatment 1

Absciscic acid: 4CH, 9CH, 15CH, 30CH & 200CH.

Treatment 2

Molybdenum: 4CH, 9CH, 15CH, 30CH & 200CH.

Treatment 3

Allopurinol: 4CH, 9CH, 15CH, 30CH & 200CH.

Treatment 4

Abscisic acid/Molybdenum: 4CH, 9CH, 15CH, 30CH & 200CH.

Treatment 5

Abscisic acid/Allopurinol: 4CH, 9CH, 15CH, 30CH & 200CH.

Treatment 6

Molybdenum/Allopurinol: 4CH, 9CH, 15CH, 30CH & 200CH.

Treatment 7

Abscisic acid/Molybdenum and Allopurinol: 4CH, 9CH, 15CH, 30CH & 200CH.

3.2.6 Dosage levels

All "doses" constituted 5ml test solution or distilled water, directly applied to each sample batch of twenty barley seeds (one petri dish). Dosage combinations (e.g. ABA/Molybdenum) constituted an equivalent total dose quantity, but not of each

combined treatment but rather as a summation, using the equivalent potency of each treatment (substance) combined, that is, 2,5ml of ABA was added to 2,5ml of molybdenum of the equivocal potency, totaling a 5ml dose. In the case of the combination of ABA, molybdenum and allopurinol 1.6ml of each was added.

3.3 Preparation of the potencies

3.3.1 Selection of substances utilized

3.3.1.1 Abscisic acid

- 1 The law of similars states that "that effect which a substance can cause in an organism it can cure in homoeopathic dose", therefore, because ABA inhibits seed germination, in a homoeopathic dose it should have the opposite effect.
- 2 Bruni (2001) found that ultra high dilutions of ABA did have biological effects on barley seed.
3. ABA utilized was obtained from the Sigma Chemical Company and was ABA cis-trans. Its molecular weight is 264.3. The reference numbers for the ABA were: No. A-1012/41 F-3810.

3.3.1.2 Molybdenum

- 1 Molybdenum is an essential trace element required by most microorganisms, plants and animals (Kisker, Schindelin and Rees, 1997:234).
- 2 Molybdenum is found associated with a diverse range of redox active enzymes that catalyze basic reactions in the metabolism of nitrogen, sulphur and carbon. It is incorporated into proteins as the molybdenum-cofactor (Mo-co) (Kisker et al. 1997). One of these Mo-co enzymes is aldehyde oxidase(s), which has been shown to catalyze the last step in the biosynthesis of the phytohormones indolyl acetic acid and ABA (Mendel, 1997:399).
- 3 Cairns *et al.* (1998) suggested that the use of molybdenum may be a useful tool in the study of ABA metabolism, seed dormancy and germination.

3.3.1.3 Allopurinol

- 1 Cairns and Cowan (unpublished paper) found that a combination of allopurinol and molybdenum stimulates overall ABA metabolism.
- 2 Cairns *et al.* (1998) showed in a study that increasing concentrations of allopurinol caused a concomitant increase in ABA.

3.3.2 Selection of potencies utilized

All treatments utilized the 4CH, 9CH, 15CH, 30CH and 200CH.

- 1 There is provision for the investigation of two potencies below (i.e. 4CH and 9CH), and three potencies above (i.e. 15CH, 30CH and 200CH), Avogadro's number with respect to dilution.
- 2 Other recent homoeopathic research by Bruni (2001), Him Lok (2001), Couchman (2001) and Balding (2002) involving plant hormones, utilized the above-mentioned potencies.

3.3.3 Methodology of preparation

- 1 All potencies prepared were done so using a solvent of double distilled water so as to avoid any degree of inhibited germination due to the "carry-over" effect of the alcohol from the Mother tincture as has been reported by Jones and Jenkins (1983).
- 2 ABA and molybdenum were both made up individually using purified water as the liquid vehicle. The centesimal scale of dilution was used with 1 part of the 'Mother tincture' to 99 parts of the vehicle/solvent (purified water). This was then succussed 100 times. This procedure continued until all the

required potencies were made as provided by the German Homoeopathic Pharmacopoeia, Method 5a (GHP, 1978, 20-22).

- 3 Allopurinol in homoeopathic dilution was made up individually using a trituration of one part of allopurinol (in tablet form) to 99 parts of pure lactose powder to produce the 1CH trituration (Hahnemann, 1989). Having produced the 1CH, an identical procedure was used to prepare the 2CH and 3CH. Subsequent liquid potencies were manufactured using distilled water with one part (by weight) being added to 99 parts purified water. Subsequent potencies utilized 1 part (by volume) of the 4CH added to 99 parts of distilled water (by volume), succussed 100 times, forming the 5CH. This procedure continued until all the required potencies were made.

Note:

- 1 All glassware was autoclaved at 121°C for 15 minutes before use.
- 2 All potencies were manufactured under laminar flow (Labair unit with air velocity of 150 Pascals) without the use of fluorescent or ultraviolet lighting.
- 3 One hundred succussions were used between each dilution of subsequent potencies.
- 4 All homoeopathic treatment dilutions were made by the Natura Laboratory in Pretoria, South Africa.

3.4 Frequency of application

Test solution and distilled water (control) treatments constitute a single application (i.e. 5 ml for each petri dish) at the commencement of the trial. No treatments were repeated during the course of the trial.

3.5 The recording of the data/ measurements

Recording measurements were performed every 24 hours for 3 days. This involved physical counts of the number of seeds germinated. Those seeds that had germinated were removed and the petri dish was immediately returned to the germination chamber.

3.6 Statistical analysis

3.6.1 Two-Way ANOVA (analysis of variance)

All treatments and potencies were analysed using the Two-Way ANOVA once a ratio had been found for each potency of each treatment, whereby the number of seeds germinated in the control dish (for each potency in each treatment) was divided by the number of seeds germinated in each treatment dish. This was done on a spreadsheet and the data then analysed using Two-Way ANOVA by

the STATISTICA program (Version 6), to determine the significance for effectiveness of each treatment and potency in relation to the controls.

This also enabled one to see if there was significant interaction between the treatments and potencies. If there had been no interaction, no further analysis would be required.

The significance of an effect was tested as follows:

The null hypothesis states that the effect in charge is insignificant at the given level of significance,

The alternative hypothesis states the effect in charge is significant at the given level of significance.

α is the level of significance of the test.

At the α level of significance,

- 1 The null hypothesis is rejected if the observed significance level (the p value) is less than α .
- 2 The null hypothesis is accepted if the observed significance level (the p value) is greater than or equal to α .

Once it had been established that there was significant interaction between treatments and potencies, the next analysis required was the comparison of

potencies for each treatment with separate One-Way ANOVA's for each treatment.

In other words, the following null hypothesis had to be tested using the one-way analysis of variance method:

$H_0: \mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5$ against the alternative hypothesis,

H_1 : At least 2 of the 5 means (dilutions) differ significantly from each other.

3.6.2 Bonferroni test for variable effectiveness

This test compared potencies within each treatment, to test for significant differences between the potencies within each treatment.

The results will be presented in a table with a corresponding graph clearly demonstrating difference in effectiveness between potencies.

CHAPTER FOUR

RESULTS OF THE STUDY

4.1 The criteria governing the admissibility of the data

The data utilised was observational. Radicle protrusion was the criterion for germination occurring.

4.2 Results from the TWO-WAY ANOVA for all treatments and potencies

Univariate Tests of Significance for Effectiveness

Effect	Univariate Tests of Significance for Effectiveness (DATA) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. Of Freedom	MS	F	p
Intercept	221.9373	1	221.9373	1801.659	0.000000
Treatment	11.0680	6	1.8447	14.975	0.000000
Potency	14.2900	4	3.5725	29.001	0.000000
Treatment*Potency	55.4849	24	2.3119	18.767	0.000000
Error	17.2459	140	0.1232		

Table 4.1

The above ANOVA table shows that:

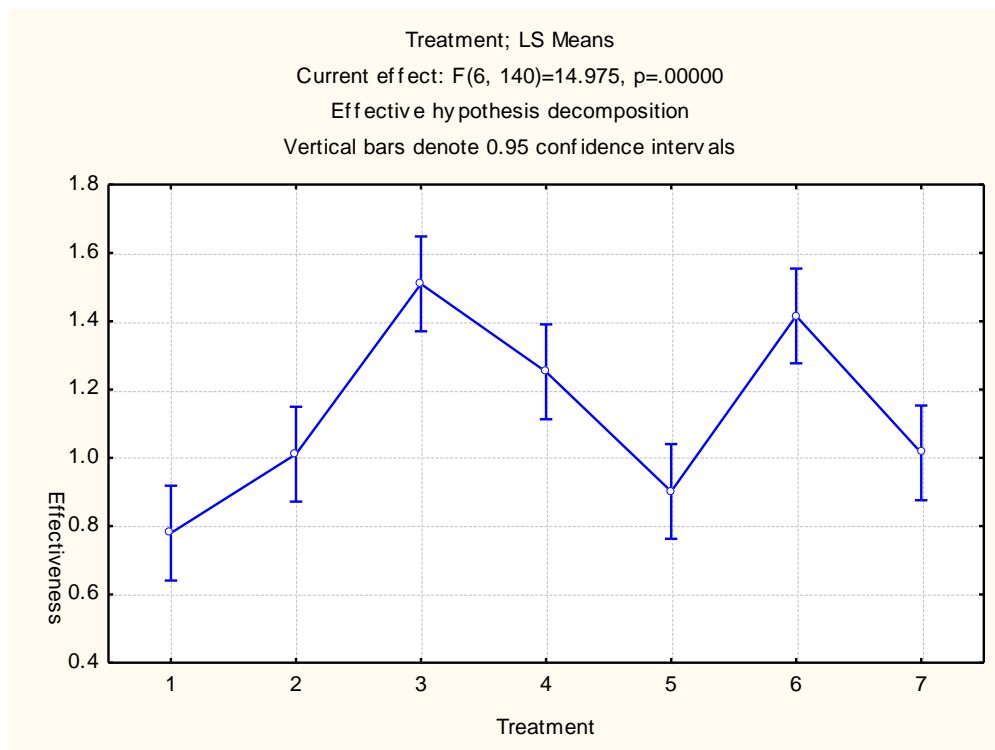
- a) The effects of treatments on germination was highly significant at the $\alpha = 1\%$ level.

b) The effect of potency on germination was highly significant at the $\alpha = 1\%$ level.

c) The effect of potency and treatments was highly significant at the $\alpha = 1\%$ level.

Effectiveness rating corresponds to ability of treatment or potency to stimulate germination. Where a treatment or potency had a high effectiveness, it means that particular treatment or potency caused more seeds to germinate than the control. Where the effectiveness was low, this corresponds to that treatment or potency possibly inhibiting germination.

4.2.1 Results for Treatment; LS Means



Graph 4.1

Treatment 1: ABA alone

Treatment 2: Molybdenum alone

Treatment 3: Allopurinol alone

Treatment 4: ABA + Molybdenum

Treatment 5: ABA + Allopurinol

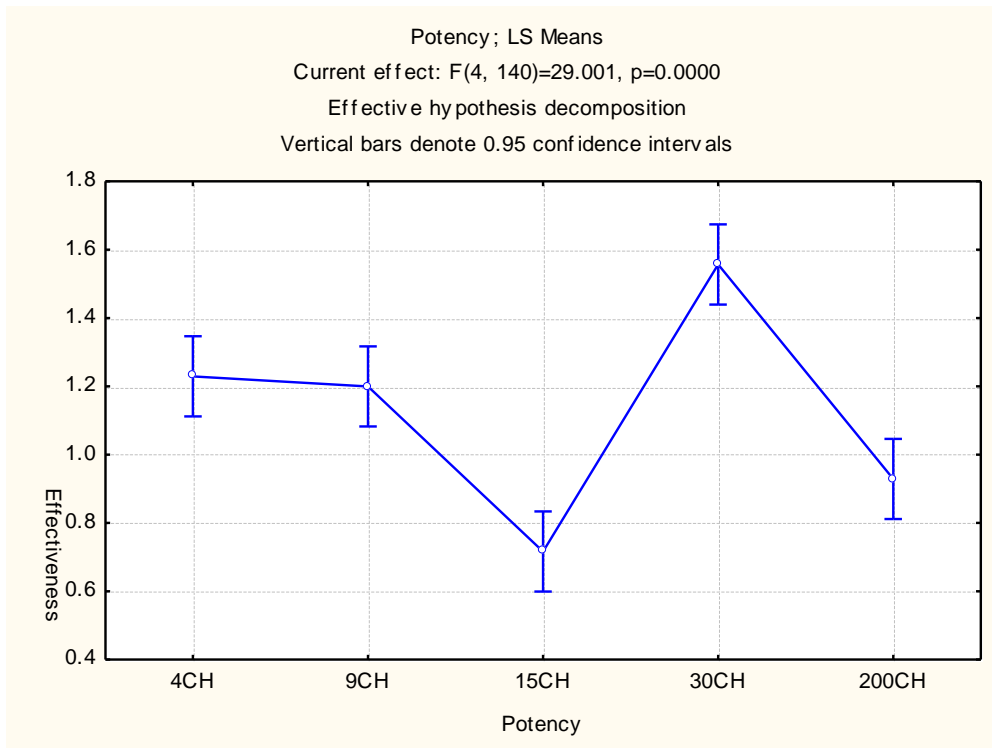
Treatment 6: Mo + Allopurinol

Treatment 7: ABA + Molybdenum + Allopurinol

From the graph it is clear to see that the order of effectiveness is as follows:

- 1) Treatment 3 (allopurinol).
- 2) Treatment 6 (allopurinol + molybdenum)
- 3) Treatment 4 (ABA + molybdenum)
- 4) Treatment 2 (molybdenum) and Treatment 7 (ABA + molybdenum + allopurinol) being of the same effectiveness.
- 5) Treatment 1 (ABA), being the least effective or most inhibitory.

4.2.2 Results for Potencies; LS Means



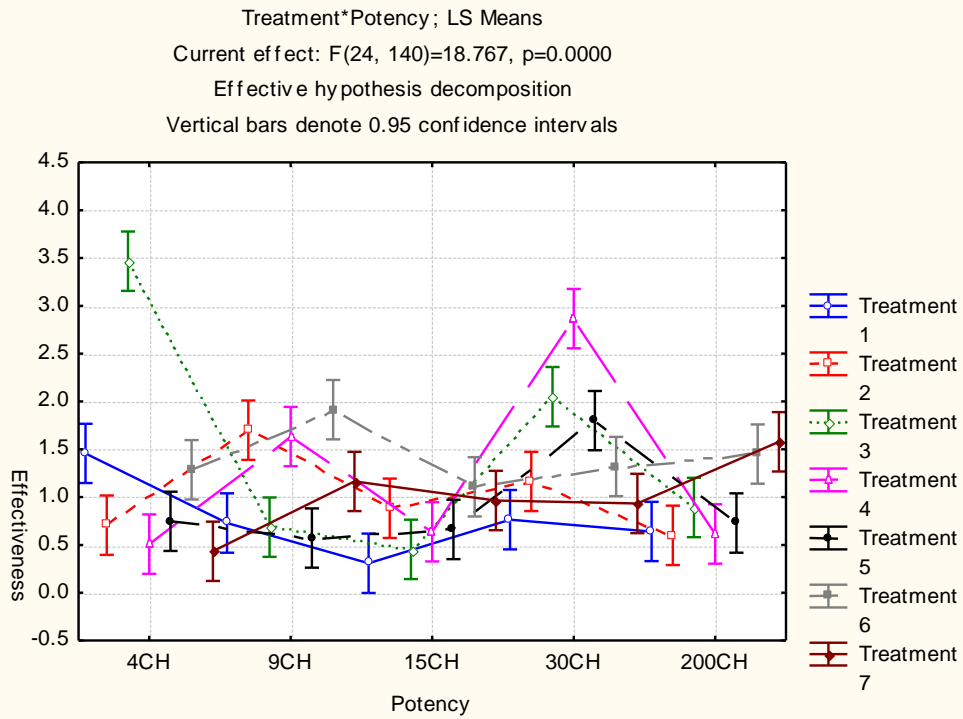
Graph 4.2

From the graph above the results appear as follows:

In order of effectiveness:

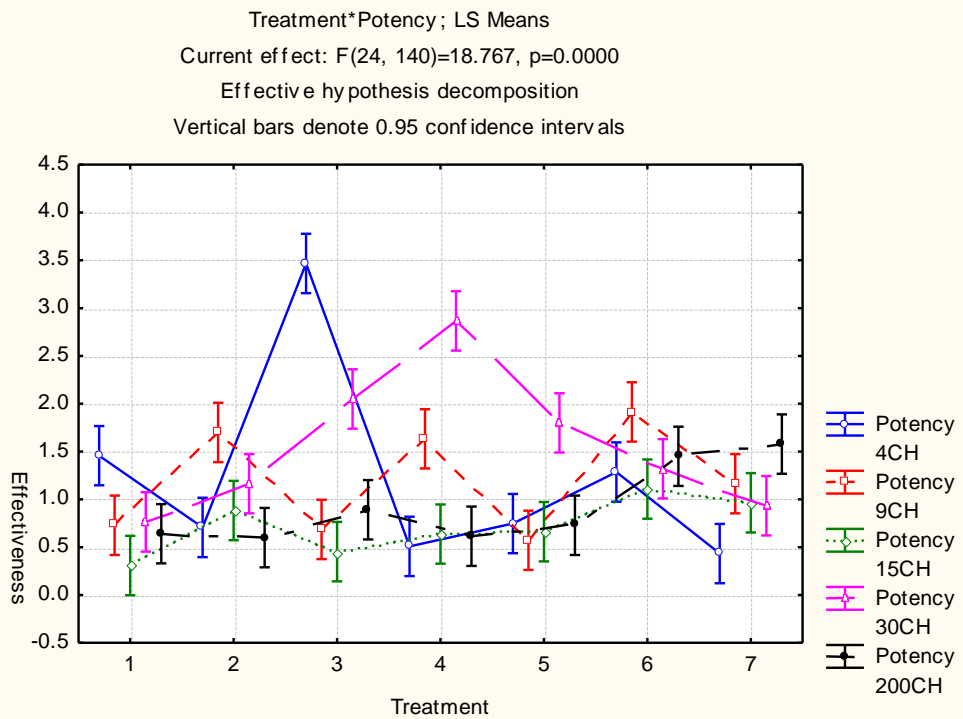
- 1) 30CH potency
- 2) 4CH potency
- 3) 9CH potency
- 4) 200CH potency
- 5) 15CH potency.

Results for Potency*Treatment LS Means



Graph 4.3

Treatment*Potency; LS Means



Graph 4.4

From the previous graphs it is clear that there is significant interaction between treatments and potencies, therefore it was necessary to do a comparison of the potencies for each treatment. A separate ONE-WAY ANOVA was done for each treatment.

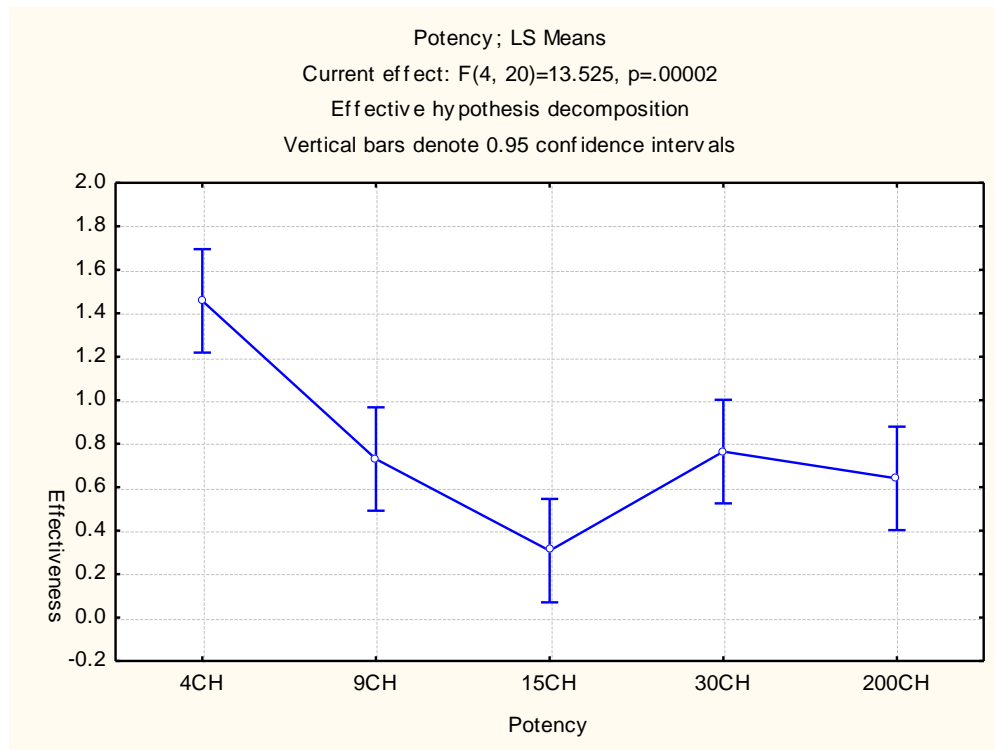
4.3 DATA TREATMENT 1: ABA

ANOVA Results 2: Univariate Tests of Significance for Effectiveness (DATA)

Effect	Univariate Tests of Significance for Effectiveness (DATA) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. Of Freedom	MS	F	p
Intercept	15.19349	1	15.19349	233.5076	0.000000
Potency	3.52012	4	0.88003	13.5251	0.000017
Error	1.30133	20	0.06507		

Table 4.2

Potency; LS Means



Graph 4.5

The graph for Treatment 1 (ABA) shows clearly that the 4CH was the most effective potency for this treatment.

The 9CH, 30CH and 200CH were of roughly the same effectiveness.

The least effective was the 15CH potency.

Bonferroni test; variable Effectiveness (DATA):

Treatment 1: ABA

Bonferroni test; variable Effectiveness (DATA)					
Probabilities for Post Hoc Tests					
Error: Between MS = .06507, df = 20.000					
Potency	{1}	{2}	{3}	{4}	{5}
	1.4571	.72941	.30769	.76364	.64000
4CH		0.002133	0.000007	0.003499	0.000592
9CH	0.002133		0.166136	1.000000	1.000000
15CH	0.000007	0.166136		0.104338	0.526682
30CH	0.003499	1.000000	0.104338		1.000000
200CH	0.000592	1.000000	0.526682	1.000000	

Table 4.3

The Bonferroni test for Treatment 1 (ABA) shows a significant difference

($p < 0.05$) between the 4CH and all the other potencies.

4.4 DATA TREATMENT 2: Molybdenum

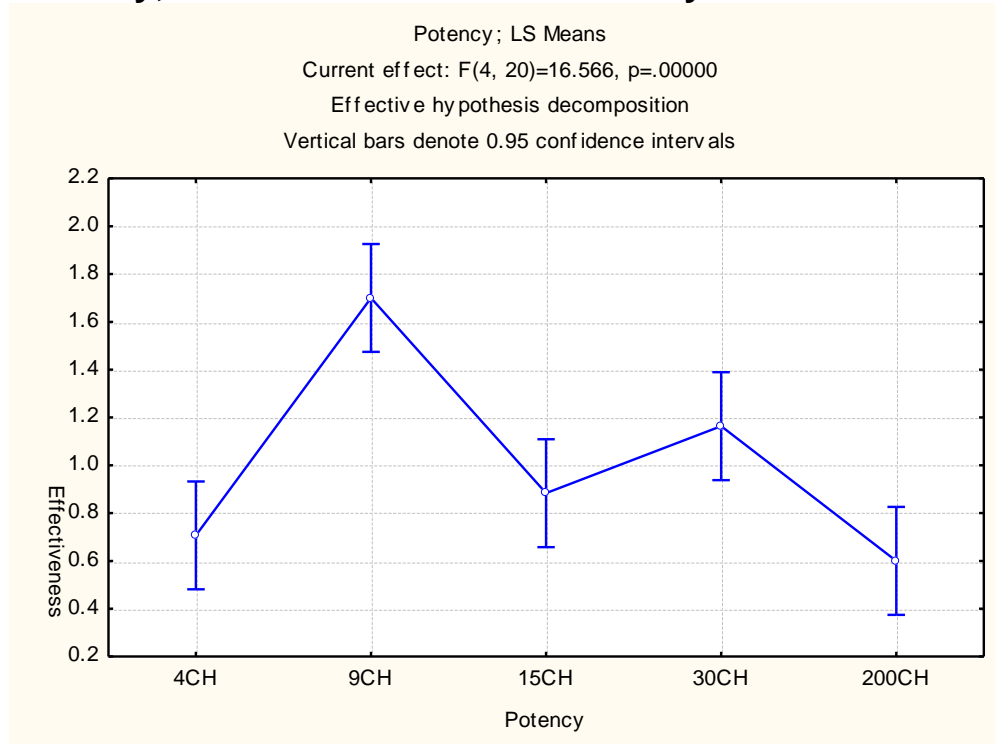
ANOVA Results 2:

Univariate Tests of Significance for Effectiveness (DATA)

Effect	Univariate Tests of Significance for Effectiveness (DATA) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. Of Freedom	MS	F	p
Intercept	25.53924	1	25.53924	436.2376	0.000000
Potency	3.87929	4	0.96982	16.5656	0.000004
Error	1.17089	20	0.05854		

Table 4.4

Potency; LS Means - Treatment 2: Molybdenum



Graph 4.6

Graph 4.6 shows clearly that for Treatment 2 (Molybdenum) the 9CH was the most effective potency.

The second most effective was the 30CH potency.

The third most effective was the 15CH potency.

The fourth most effective was the 4CH potency.

The least effective potency was the 200CH potency.

Bonferroni test; variable Effectiveness (DATA)

Treatment 2: Molybdenum

Bonferroni test; variable Effectiveness (DATA)					
Probabilities for Post Hoc Tests					
Error: Between MS = .05854, df = 20.000					
Potency	{1}	{2}	{3}	{4}	{5}
	.70667	1.7000	.88333	1.1636	.60000
4CH		0.002133	1.000000	0.072995	1.000000
9CH	0.000025		0.000319	0.022292	0.000006
15CH	1.000000	0.000319		0.819323	0.789197
30CH	0.072995	0.022292	0.819323		0.014737
200CH	1.000000	0.000006	0.789197	0.014737	

Table 4.5

The Bonferroni test for Treatment 2 (Molybdenum) shows a significant difference ($p < 0.05$) between the 9CH and all the other potencies.

A significant difference was also noted between the 30CH and the 200CH potency.

4.5 DATA TREATMENT 3: Allopurinol

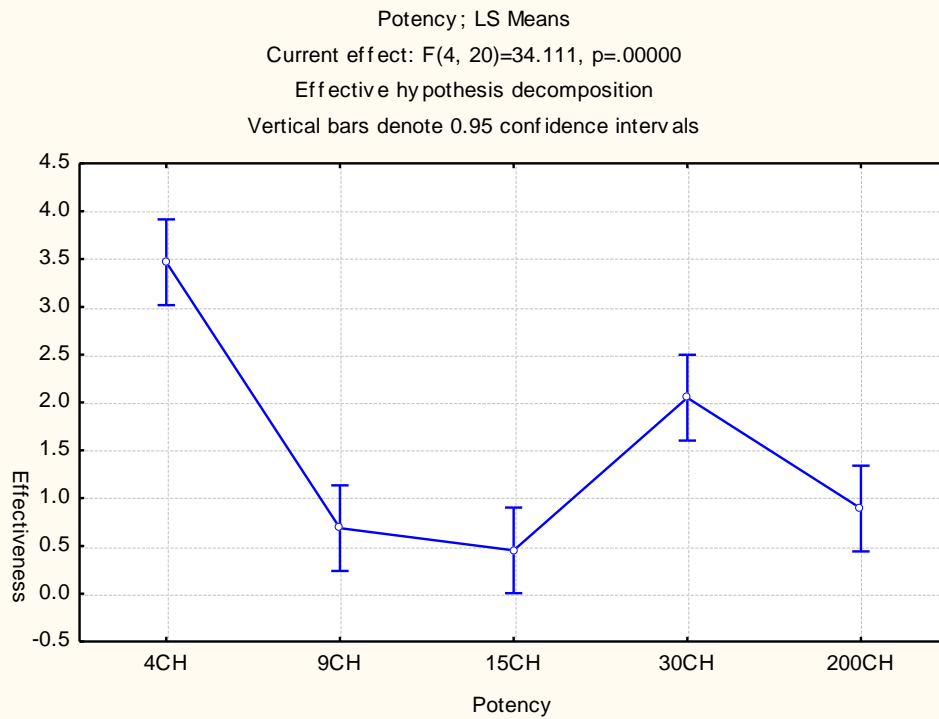
ANOVA Results 2:

Univariate Tests of Significance for Effectiveness (DATA)

Effect	Univariate Tests of Significance for Effectiveness (DATA) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. Of Freedom	MS	F	p
Intercept	56.95152	1	56.95152	246.7085	0.000000
Potency	31.49704	4	7.87426	34.1105	0.000000
Error	4.61691	20	0.23085		

Table 4.6

Potency; LS Means Treatment 3: Allopurinol



Graph 4.7

Graph 4.7 shows clearly that for Treatment 3 (Allopurinol) the 4CH was the most effective potency.

The second most effective was the 30CH potency.

The third most effective was the 200CH potency.

The fourth most effective was the 9CH potency.

The least effective was the 15CH potency.

Bonferroni test; variable Effectiveness (DATA)

Treatment 3: Allopurinol

Bonferroni test; variable Effectiveness (DATA)					
Probabilities for Post Hoc Tests					
Error: Between MS = .23085, df = 20.000					
Potency	{1}	{2}	{3}	{4}	{5}
	3.4667	.68571	.45333	2.0500	.89091
4CH		0.000000	0.0000000	0.001501	0.000000
9CH	0.000000		1.0000000	0.002241	1.000000
15CH	0.000000	1.000000		0.000384	1.000000
30CH	0.001501	0.002241	0.000384		0.010854
200CH	0.000000	1.0000000	1.000000	0.010854	

Table 4.7

The Bonferroni test for Treatment 3 (Allopurinol) shows a significant difference ($p < 0.05$) between the 4CH and all the other potencies.

Significant difference ($p < 0.05$) was also noted between the 30CH potency and all the other potencies.

4.6 DATA TREATMENT 4: ABA and Molybdenum.

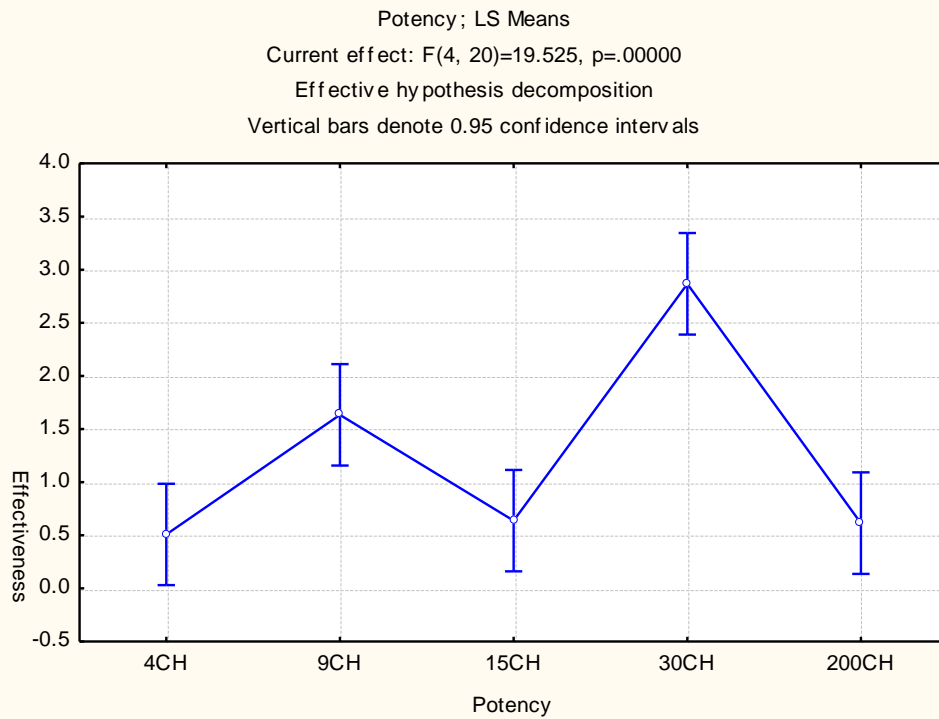
ANOVA Results 2:

Univariate Tests of Significance for Effectiveness (DATA)

Effect	Univariate Tests of Significance for Effectiveness (DATA) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. Of Freedom	MS	F	p
Intercept	39.18107	1	39.18107	149.6036	0.000000
Potency	20.45424	4	5.11356	19.5249	0.000001
Error	5.23799	20	0.26190		

Table 4.8

Potency; LS Means Treatment 4: ABA + Molybdenum



Graph 4.8

Graph 4.8 shows clearly that for Treatment 4 (ABA + Molybdenum) the 30CH is the most effective potency.

The second most effective was the 9CH potency.

The third most effective are the 15CH and the 200CH potencies.

The least effective dilution was the 4CH potency.

Bonferroni test; variable Effectiveness (DATA)

Treatment 4: ABA + Molybdenum

Bonferroni test; variable Effectiveness (DATA)					
Probabilities for Post Hoc Tests					
Error: Between MS = .26190, df = 20.000					
Potency	{1}	{2}	{3}	{4}	{5}
	.50769	1.6333	.63750	2.8667	.61429
4CH		0.023741	1.000000	0.000005	1.000000
9CH	0.023741		0.059505	0.010953	0.050566
15CH	1.000000	0.059505		0.000011	1.000000
30CH	0.000005	0.010953	0.000011		0.000009
200CH	1.000000	0.050566	1.000000	0.000009	

Table 4.9

The Bonferroni test for Treatment 4 (ABA + Molybdenum) shows a significant difference ($p < 0.05$) between the 30CH and all the other potencies.

A significant difference ($p < 0.05$) is also noted between the 4CH and the 9CH potency.

4.7 DATA TREATMENT 5: ABA + Allopurinol.

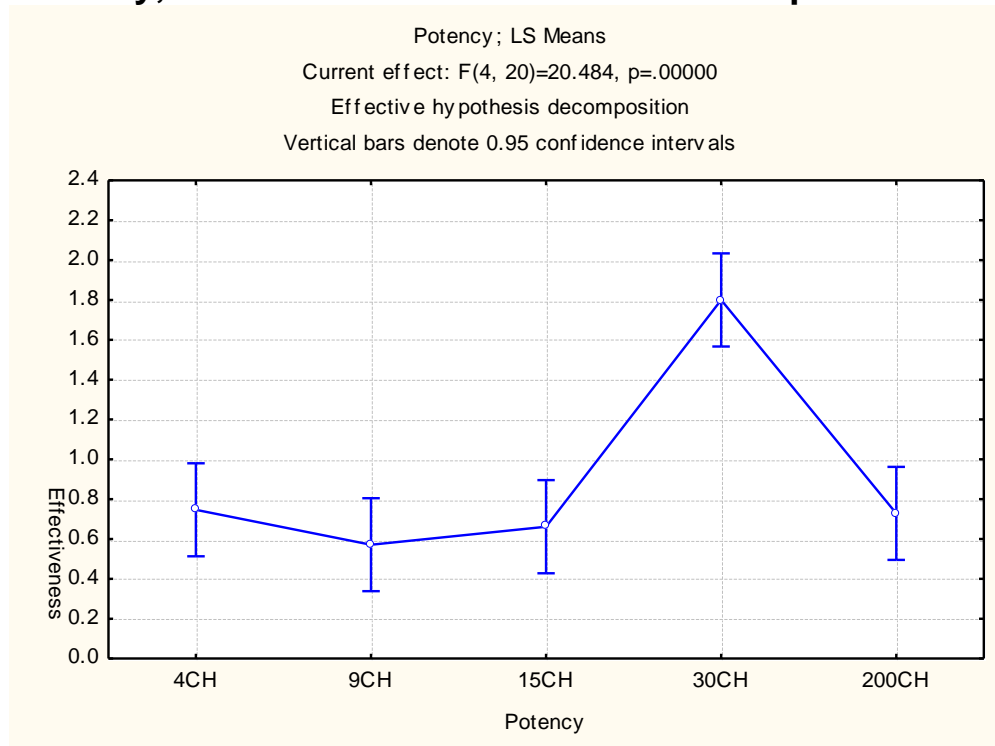
ANOVA Results 2:

Univariate Tests of Significance for Effectiveness (DATA)

Effect	Univariate Tests of Significance for Effectiveness (DATA) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. Of Freedom	MS	F	p
Intercept	20.32391	1	20.32391	324.0677	0.000000
Potency	5.13854	4	1.28464	20.4837	0.000001
Error	1.25430	20	0.06272		

Table 4.10

Potency; LS Means Treatment 5: ABA + Allopurinol



Graph 4.9

Graph 4.9 shows clearly that for Treatment 5 (ABA + Allopurinol) the 30CH was the most effective potency.

The other potencies performed at roughly the same effectiveness.

Bonferroni test; variable Effectiveness (DATA)

Treatment 5: ABA + Allopurinol

Bonferroni test; variable Effectiveness (DATA)					
Probabilities for Post Hoc Tests					
Error: Between MS = 0.06272, df = 20.000					
Potency	{1}	{2}	{3}	{4}	{5}
	.74667	.57143	.66154	1.8000	.72857
4CH		1.000000	1.000000	0.000018	1.000000
9CH	1.000000		1.000000	0.000002	1.000000
15CH	1.000000	1.000000		0.000006	1.000000
30CH	0.000018	0.000002	0.000006		0.000014
200CH	1.000000	1.000000	1.000000	0.000014	

Table 4.11

The Bonferroni test for Treatment 5 (ABA + Allopurinol) shows a significant difference ($p < 0.05$) between the 30CH and all the other potencies.

4.8 DATA TREATMENT 6: Molybdenum + Allopurinol.

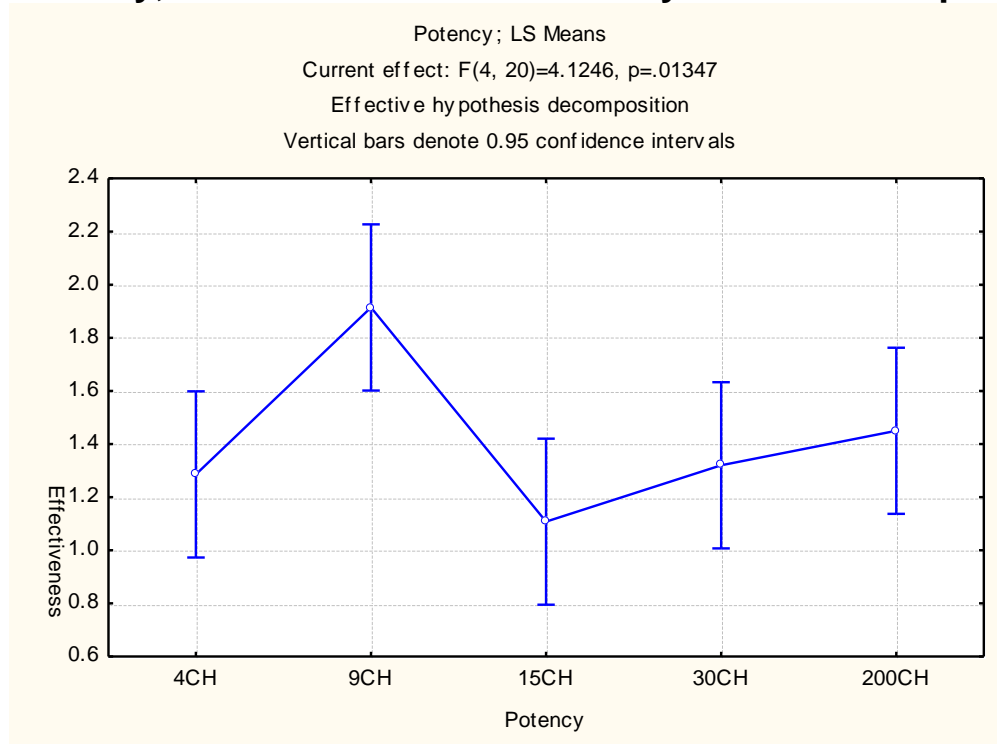
ANOVA Results 2:

Univariate Tests of Significance for Effectiveness (DATA)

Effect	Univariate Tests of Significance for Effectiveness (DATA) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. Of Freedom	MS	F	p
Intercept	50.09373	1	50.09373	445.9131	0.000000
Potency	1.85344	4	0.46336	4.1246	0.013474
Error	2.24679	20	0.11234		

Table 4.12

Potency; LS Means Treatment 6: Molybdenum + Allopurinol



Graph 4.10

Graph 4.10 shows clearly that for Treatment 6 (Molybdenum + Allopurinol) the 9CH was the most effective potency.

The second most effective was the 200CH potency.

The 4CH and the 30CH performed at roughly the same effectiveness.

The 15CH was the least effective potency.

Bonferroni test; variable Effectiveness (DATA)

Treatment 6: Molybdenum + Allopurinol

Bonferroni test; variable Effectiveness (DATA)					
Probabilities for Post Hoc Tests					
Error: Between MS = .11234, df = 20.000					
Potency	{1}	{2}	{3}	{4}	{5}
	1.2857	1.9143	1.1077	1.3200	1.4500
4CH		0.076512	1.000000	1.000000	1.000000
9CH	0.076512		0.011094	0.109721	0.405271
15CH	1.000000	0.011094		1.000000	1.000000
30CH	1.000000	0.109721	1.000000		1.000000
200CH	1.000000	0.405271	1.000000	1.000000	

Table 4.13

The Bonferroni test for Treatment 6 (Molybdenum + Allopurinol) shows only a significant difference ($p < 0.05$) between the 9CH and the 15CH potencies.

4.9 DATA TREATMENT 7: ABA + Molybdenum + Allopurinol.

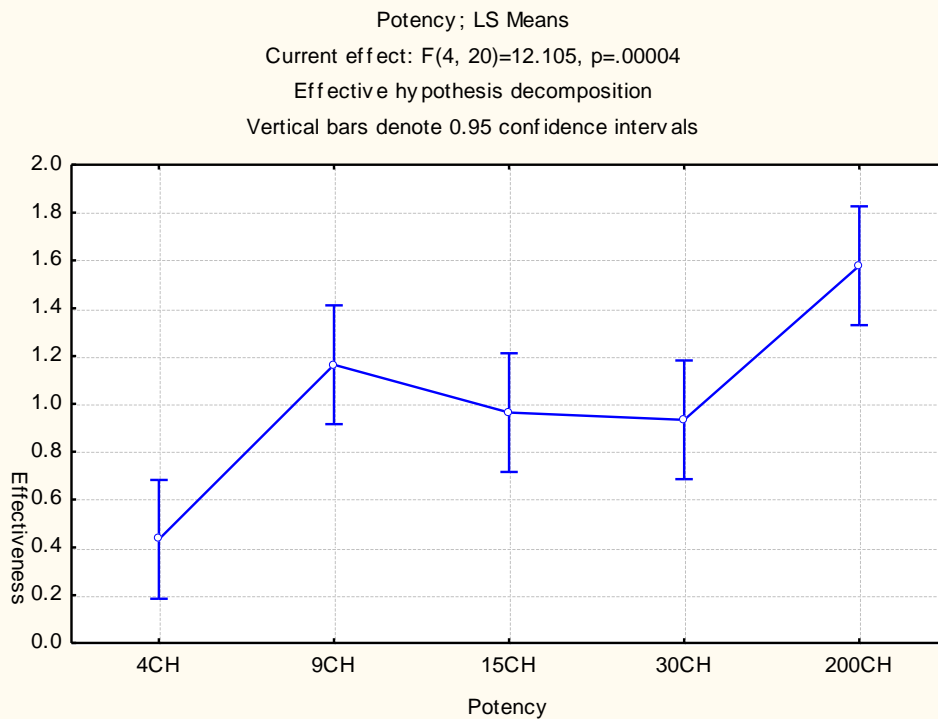
ANOVA Results 2:

Univariate Tests of Significance for Effectiveness (DATA)

Effect	Univariate Tests of Significance for Effectiveness (DATA) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. Of Freedom	MS	F	p
Intercept	25.72232	1	25.72232	362.8753	0.000000
Potency	3.43227	4	0.85807	12.1051	0.000037
Error	1.41769	20	0.07088		

Table 4.14

Potency; LS Means Treatment 7: ABA + Molybdenum + Allopurinol.



Graph 4.11

Graph 4.11 shows that for Treatment 7 (ABA + Molybdenum + Allopurinol) the 200CH potency was the most effective potency.

The second most effective was the 9CH potency.

The 15CH and 30CH performed at roughly the same level of effectiveness.

The least effective was the 4CH potency.

Bonferroni test; variable Effectiveness (DATA)

Treatment 7: ABA + Molybdenum + Allopurinol

Bonferroni test; variable Effectiveness (DATA)					
Probabilities for Post Hoc Tests					
Error: Between MS = 0.07088, df = 20.000					
Potency	{1}	{2}	{3}	{4}	{5}
	.43333	1.1636	.96364	.93333	1.5778
4CH		0.003199	0.050466	0.075804	0.000013
9CH	0.003199		1.000000	1.000000	0.231472
15CH	0.050466	1.000000		1.000000	0.016024
30CH	0.075804	1.000000	1.000000		0.010535
200CH	0.000013	0.231472	0.016024	0.010535	

Table 4.15

The Bonferroni test for Treatment 7 (ABA + Molybdenum + Allopurinol) shows a significant difference ($p < 0.05$) between the 200CH potency and all the other potencies, except the 9CH.

There is also a significant difference ($p < 0.05$) noted between the 4CH and the 9CH.

CHAPTER FIVE

DISCUSSION

Failure of a seed to germinate or inappropriate germination, such as in pre-harvest or precocious germination, is a worldwide concern. What triggers either event is a complex interaction of hormones, enzymes and metabolic reactions. Trying to understand the process which causes these events to occur is sometimes as difficult as trying to understand what causes a person to become ill.

With regards to plants and people, homoeopathy offers a potential solution to restoring the correct functioning to the organism.

At the outset of this research, it was hypothesised that there would be measurable effects on the germination of barley seeds as a result of treatment with homoeopathic dilutions of specific substances. All conditions were set as optimal for germination, the measurable criterion being radicle emergence.

Treatment 1 (ABA) at the 4CH dilution level (10^{-8}) showed a stimulatory effect on germination, which is the opposite to the action of ABA in its unpotentised state. This action however was altered at an even higher dilution, with the 15CH dilution being the most inhibitory to germination. However, a 4CH dilution still contains a considerable amount of the original ABA, which raises the question as to why it would have acted in a stimulatory way. The 15CH dilution (10^{-30}) which according to Avagadro's law contains nothing of the original ABA, acted most

similarly to ABA in its unpotentised state. This requires further investigation to see if a trend emerges.

In Treatment 2 (Molybdenum), the 9CH dilution showed a statistically significant stimulatory action on seed germination. As it is thought molybdenum through its action on ABA metabolism, inhibits seed germination, this is a significant finding. The law of similars and the isopathic principle supports what was found statistically, that a substance will have the opposite effect when used in homeopathic dilution. Interestingly the 200CH dilution (10^{-400}) caused the most inhibition of seed germination. The trend emerging is that the action of the potencies changes with dilution.

Treatment 3 (Allopurinol) showed the 4CH dilution to again be the most effective in stimulating germination, as in Treatment 1. The 15CH and 200CH were also the most inhibitory as was seen in the previous two treatments. The trend thus far seems to be the lower potencies stimulating and the higher potencies inhibiting germination. The interesting deviation from this is the 30CH dilution, which seems to stimulate germination the most, as was clearly seen in Graph 4.2.

Treatment 4 (ABA + molybdenum), the first combination treatment, showed slightly different results to the single treatments, with regards to the 4CH dilution. Instead of stimulating germination, it was the most inhibitory of all the potencies.

The 30CH, however performed consistently with the previous treatments, in that it stimulated seed germination significantly, in fact in this combination treatment it was the most stimulatory to germination of all the potencies. The 15CH and 200CH dilution also performed consistently with the previous treatments and caused decreased germination with respect to the control.

Treatment 5 (ABA + Allopurinol) showed the same results as the previous treatment. Only the 30CH was significantly effective in stimulating germination. All other potencies including the 4CH and 9CH dilutions, inhibited germination to some degree.

Treatment 6 (Allopurinol + molybdenum) reflected a change in the effectiveness of the 30CH dilution, with the 9CH showing the most stimulation of germination. This is still consistent with Treatments 1, 2 and 3 where the lower potencies were more stimulatory. Also consistent with all the other treatments, the 15CH dilution had the most inhibitory effect on germination.

Treatment 7 (ABA + allopurinol + molybdenum) was the only treatment combining three substances. The results from this treatment were slightly different to all the others. In this treatment, the 4CH dilution was the most inhibitory to germination, like in Treatment 4. Interestingly the 200CH dilution, which had seemed to be the second most inhibitory to germination of all the potencies, was the most stimulatory in this treatment. There was a statistically significant difference

between the effectiveness of the 4CH dilution and the 200CH dilution. This treatment particularly showed the efficacy of ultra high dilutions in being able to have biological effects, which directly challenges Avagadro's Law.

This treatment also proved the isopathic principle to be true, in that all three substances working together are known to inhibit germination and yet in homoeopathic dose they did the opposite.

Summary

- a) There are clear measurable and statistically significant effects for all treatments on barley seed germination.
- b) There are statistically significant differences between potencies and their effect on barley seed germination.
- c) As regards the treatment effect, the treatments containing allopurinol seemed to be the most effective in stimulating germination and, the treatments containing ABA seemed to be the most effective in inhibiting germination.
- d) The potency effect was clearly seen with the 15CH overall being the most inhibitory to germination and the 30CH the most stimulatory.

More repetition of plant studies are required to truly understand and evaluate the changing effect of homoeopathic treatments in different dilutions on the effect of plant growth. This broader base of research will ensure that homoeopathy is

regarded with increasing interest from the scientific world and that more work is done to either prove or disprove the findings.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

It is evident from the results of this study that all the treatments produced distinct biological effects in homoeopathic dilution. This means that all the hypotheses made at the outset of this research were supported by the results. Statistical differences were noted in all the treatments in certain dilutions and there were statistical differences between potencies within each treatment.

The results showed that the treatments with ABA either alone or in combination inhibited germination more than the other treatments and the treatments with allopurinol, alone or in combination (except treatment 7), showed a stimulatory response.

The results also provide evidence of the 30CH potency as having the most effect on promotion of germination of all the potencies, and the 15CH potency as having the most effect on inhibiting germination.

6.2 Recommendations

As plant studies done recently utilizing homoeopathic dilutions have shown there to be biological activity, (Hopkins, 1998, Him Lok, 2001, Bruni, 2001) more research is needed on germination of barley seeds in a less controlled environment than the petri dish, to see if it is possible to help farmers in the field to minimize pre-harvest sprouting and seed dormancy.

6.2.1 Recommendations for further research

- 1 The use of other plant types. Other plant types that show a sensitivity to ABA should be utilized to ensure the reliability of the results. This would also allow the use of a wider range of homoeopathic treatments in agriculture and possibly allow for them to be extrapolated into the commercial sector.
- 2 The establishment of the correct potency levels. There needs to be more definitive research on what effect certain potencies have on plant growth and seed germination. More studies will reveal which potencies stimulate or inhibit germination over a wider spectrum of treatments.

- 3 The effect of other plant hormones or recognised plant growth regulators in homoeopathic dilution. Other plant hormones need to be tested in homoeopathic dilutions to get a broader idea of the potential use of homoeopathy in improving the general functioning of the plants natural processes e.g. cytokinins, auxins.

- 4 The employment of other mineral substances or trace elements. It was mentioned in a study by Cairns *et al.* that tungsten was used with molybdenum to influence ABA metabolism. Another example is fluridone, which lowers ABA levels (Kermode, 1992:281). These could be tested in homoeopathic dilutions along with other possible minerals/elements found in the homoeopathic materia medica, which either stimulate or inhibit growth.

- 5 The employment of ABA in homoeopathic dilutions in plant processes other than germination. ABA is also involved in regulating the water balance of plants under water stress. ABA in homoeopathic dilution could be tested to see if it has the ability to influence this process.

- 6 The employment of field trials utilizing findings found in the laboratory. It is important to test the findings from laboratory work on a larger scale, such as in the field or tunnels, for there to be economic benefits to the agricultural industry.

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