

THE EFFECT OF CAMPHOR, NAPHTHALENE (MOTH BALLS),  
AND ELECTROMAGNETIC RADIATION ON 4CH AND 200CH  
HOMOEOPATHIC POTENCIES OF GIBBERELIC ACID AND  
THEIR SUBSEQUENT ABILITY TO PROMOTE GERMINATION  
OF BARLEY SEED (*HORDEUM VULGARE*), AS MEASURED BY  
A GERMINATION INDEX

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I, Claire Stubbs, do hereby declare that this dissertation represents my own  
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Dedicated to my family for their love and support over the years and to  
Casper for all his help and patience in instructing me in the finer workings of  
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## ABSTRACT

This study investigated the effect of camphor, naphthalene (mothballs), cellular phones and televisions on 4cH and 200cH homoeopathic dilutions of gibberellic acid ( $GA_3$ ). The ability of treated dilutions to promote germination of barley seed, (*Hordeum vulgare* cv. Stirling, ex Sensako, Western Cape, South Africa, 2000 harvest) was measured by a germination index.

The addition of  $GA_3$  to the barley seed stimulates the aleurone layer to start producing  $\alpha$ -amylase, an enzyme responsible for the breakdown of starch. The starch then supplies the energy required for seedling growth. By the addition of homoeopathically prepared dilutions of  $GA_3$  (HGA<sub>3</sub>), Him Lock (2001) showed that there was an increase in germination and growth of the seedling, proving that ultra-high dilutions do have a biological effect. This project extended that of Him Lock by using her model as a means of testing possible negation of homoeopathic dilutions.

A series of experiments was conducted using possible negating substances to homoeopathic dilutions. The experiment consisted of 7 treatment groups (including a positive control and a negative control group). Group 1 was exposed to camphor, Group 2 was exposed to naphthalene (mothballs), Group 3 was exposed to a cellular phone, and Group 4 was exposed to a television set. Group 5 was unexposed HGA<sub>3</sub> only (positive control) and Group 6 was  $GA_3$  ( $0.5\text{g/L}^{-1}$ ) only (positive control). Group 7 was distilled water only (negative control). Groups 1 –5 were divided into two subgroups to



represent the 2 levels of dilution selected, 4cH and 200cH. Group 6 and 7 were the controls. Each subgroup consisted of three repetitions. This amounted to 36 repetitions in total. Each repetition consisted of 5 petri dishes with 20 seeds. This amounted to 3600 seeds in total ( $2 \times 12 \times 3 \times 100$ ).

Each group of 100 seeds was placed in a numbered petri dish lined with two filter papers, moistened with one of the various treatment solutions and incubated for 24 hours in a dark growth chamber set at a constant temperature. After 24 hours the imbibed seeds were removed from the petri dishes and placed on moistened filter paper, in groups of 20, in 9cm petri dishes. The petri dishes were then replaced in the growth chamber for 7 days. They were checked daily, the number of germinated seeds (radicle  $>1\text{mm}$ ) was recorded, and dead or deformed seeds were removed but also recorded. On day 7 all the germinated seedlings were measured for root and shoot length as well as seedling dry mass.

Data was analyzed statistically by means of the One-way ANOVA. The results showed no real difference between the homoeopathic unexposed dilutions and the other controls. This meant that the growth of the seedlings imbibed with the homoeopathic dilutions could not be attributed to the biological activity of the homoeopathic dilutions. An interesting trend, however, was noticed in each parameter with the seedlings that were imbibed in the dilutions that were exposed to the camphor and mothballs. This was inhibition of growth of the seedlings, indicating that these substances had somehow influenced the dilutions in a way that caused inhibition.

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TABLE OF ABBREVIATIONS

GA<sub>3</sub> – Gibberellic acid

cH – centesimal Hahnemann

EMR – Electromagnetic Radiation

RF – Radio frequency

$\alpha$ -amylase – Alpha amylase

HGA<sub>3</sub> – Homoeopathically prepared gibberellic acid

## THE DEFINITION OF TERMS

### **Avogadro's number**

This a constant representing the number of molecules in a mole of any substance and is equivalent to  $6.02254 \times 10^{23} \text{ mol}^{-1}$  (Majerus, 1991).

### **Germination**

The process whereby the embryo resumes the growth activities that were suspended during quiescence or dormancy, and during which new genetic programs are initiated (Jann and Amen 1977:8). The beginning or resumption of growth by a spore, seed, bud, or other structure (Raven *et al* 1992:746).

### **Plant hormone**

Plant hormones are organic compounds made in one part of a plant and transported to another, where they elicit a response (Moore *et al* 1995:411).

A chemical substance produced in minute amounts in one part of an organism, from which it is transported to another part of that organism on which it has a specific effect (Raven *et al* 1992:747).

### **Homoeopathy**

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

## **The law of similars**

The law of similars "is the similarity between the toxicological action of a substance and therapeutic action.... The same things which cause the disease cure it" (Jouanny 1993:12)

## **Dilution**

The volume of solvent in which a given amount of solution is dissolved (Anon 1990:97).

## **Antidotes**

Antidote (homoeopathic) refers to a substance, which by virtue of its similarity in bioenergetic effects, neutralizes the field of influence of the competing substance, concealing its effects. The antidoting effect is exerted directly upon the organism, although it takes place indirectly between drugs by neutralization (Gaier 1991:39-40).

## **Electromagnetic Radiation**

The successive series of oscillations in the strengths of electrical and magnetic fields associated with light, microwaves, gamma rays, ultraviolet rays, infrared rays, and the like (Brady and Holum 1993:G-6).

## **Hormesis**

Biphasic medicinal dose/response action; describes the reversed biological effects in various ranges of concentration of the same medicinal agent (Gaier, 1991:275).

## **Potency**

1. The especially produced capability in a medicine to effect a dynamis in the appropriate patient (Gaier 1991:432).
2. The number appearing after the name of the homoeopathic substance, indicating the number of times the substance has been diluted and succussed or triturated, e.g. gibberellic acid 200cH.

## **Trituration**

A process peculiar to Homoeopathy, which reduces, according to scale, the inert substance in a crude form to a state of physical solubility, physiological assimilability and therapeutic activity (Hopkins, 1999).

## **Dynamization**

Sequence of dilution and shaking or a process in which we increase the relative quantity of solvent in contact with active substance is increased (Zacharias, 1997).

## **Potentisation**

Preparation of Homoeopathic dilutions using dynamisation, trituration and succussion (Gaier, 1991:441).

## **Succussion**

The vigorous action of shaking a liquid homoeopathic solution in its bottle where each stroke ends in a jolt, usually against the palm of the opposite hand (Gaier, 1991:532).

**Superscripted numbers = Internet reference**



# CHAPTER 1

## INTRODUCTION

### 1.1 Introduction

Hahnemann developed homoeopathy to improve the life of his patients.

Life is dependent on food, and food chains. At the beginning and end of every food chain are plants. Plants give life to the planet, and this life begins with germination. Without plants there would be no life.

This study investigated one aspect of seed germination – the germination of barley in the presence of the plant hormone gibberellic acid ( $GA_3$ ). The aim was to investigate the effect of certain potentially neutralizing influences on the capacity of homoeopathic dilutions of  $GA_3$  (4cH and 200cH) to promote the germination of barley seeds, as measured by a germination index.

$GA_3$  plays a crucial role in promoting germination in barley seeds.  $GA_3$  is released by the embryo and acts on cells of the endosperm aleurone layer, causing *de novo* synthesis of  $\alpha$ -amylase. This enzyme catalyses the hydrolysis of starch in the endosperm to produce sugar, which is used as an energy source for the growing seedling. (Moore, 1995: 424-423.)

Him Lock (2001) investigated the effect of ultra high dilutions of  $GA_3$  on the germination of barley, as measured by a germination index, and found the dilutions to be biologically active. This study extends that of Him Lock (2001), by testing the reproducibility of the reported effects and by investigating additional factors determining the efficacy of the homoeopathic dilutions. A

parallel study by Pieterse (in progress) also evaluated additional influences on the homoeopathic dilutions, using the same model.

Several studies have been carried out in order to test the efficacy of homoeopathic medicine in the agricultural context.

Jones and Jenkins (1983), showed that *Pulsatilla* in varying dilutions had an effect on sunflower seeds, by increasing growth. Later Steffan (in Shulte 1999) repeated this work, using yeast, and confirmed the results of Jones and Jenkins.

A randomized laboratory trial was conducted by Betti *et al.* (1994), where homoeopathic dilutions of Arsenicum album (decimal potencies 23-45) were tested for their effect on seed germination. The experimental results showed that differences among the treatment groups could not be explained as a mere effect of intrinsic seed variability.

Investigations into the biological effects of homoeopathic treatments (Sulphur, Nitric acid and camphor in 3cH, 9cH, 15cH and 30cH potencies) on lettuce seed germination, as measured by a germination index, were conducted by Hopkins (1998). He concluded that biological effects (promotion or suppression of germination, depending on the treatment) were evident, represented by the significant difference of effect between treatments.

Experiments using oat coleoptiles pretreated with highly diluted solutions of  $\text{CaCO}_3$  (the homoeopathic medicine Calcium carbonate), were conducted by Bornoroni (1991). The coleoptiles were cultured in the presence of indoleacetic acid (IAA), a member of the auxin class of plant hormones. He noticed a statistically significant increase in growth in the coleoptiles that were pretreated with Calcium carbonate 5cH, as compared to those that were treated with IAA only. Homoeopathically prepared IAA increased the growth in the coleoptiles.

The present study along with that of Pieterse (2001, in progress) expanded on that by Him Lock (2001). It also set out to test objectively common assumptions regarding neutralization of homoeopathic dilutions, using a plant model.

## **1.2 Aim of the study**

The aim of this study was to investigate the effects of camphor, naphthalene (mothballs), cellular phones and television exposure on 4cH and 200cH homoeopathic dilutions of gibberellic acid, in terms of the subsequent ability of the dilutions to promote germination of barley seeds (*Hordeum vulgare*), as measured by a germination index.

## **1.3 Statement of the objectives**

### **1.3.1 The first objective**

The first objective was to evaluate the effect of exposure to camphor on 4cH and 200cH homoeopathic dilutions of gibberellic acid, in terms of the

subsequent ability of the dilutions to promote germination of barley seeds (*Hordeum vulgare*), as measured by a germination index.

### **1.3.2 The second objective**

The second objective was to evaluate the effect of exposure to naphthalene (mothballs) on 4cH and 200cH homoeopathic dilutions of gibberellic acid, in terms of the subsequent ability of the dilutions to promote germination of barley seeds (*Hordeum vulgare*), as measured by a germination index.

### **1.3.2 The third objective**

The third objective was to evaluate the effect of exposure to cellular phones on 4cH and 200cH homoeopathic dilutions of gibberellic acid, in terms of the subsequent ability of the dilutions to promote germination of barley seeds (*Hordeum vulgare*), as measured by a germination index.

### **1.3.3 The fourth objective**

The fourth objective was to evaluate the effect of exposure to television on 4cH and 200cH homoeopathic dilutions of gibberellic acid, in terms of the subsequent ability of the dilutions to promote germination of barley seeds (*Hordeum vulgare*), as measured by a germination index.

## **1.4 Statement of the hypotheses**

All hypotheses are stated in the null form

### **1.4.1 The first hypothesis**

It is hypothesized that camphor exposure will have a neutralizing effect on 4cH homoeopathic dilution of gibberellic acid in terms of the subsequent ability of this dilution to promote germination of barley seeds, as measured by a germination index.

### **1.4.2 The second hypothesis**

It is hypothesized that camphor exposure will have a neutralizing effect on 200cH homoeopathic dilution of gibberellic acid in terms of the subsequent ability of this dilution to promote germination of barley seeds, as measured by a germination index.

### **1.4.3 The third hypothesis**

It is hypothesized that naphthalene (mothballs) exposure will have a neutralizing effect on 4cH homoeopathic dilution of gibberellic acid in terms of the subsequent ability of this dilution to promote germination of barley seeds, as measured by a germination index.

### **1.4.4 The fourth hypothesis**

It is hypothesized that naphthalene (mothballs) exposure will have a neutralizing effect on 200cH homoeopathic dilution of gibberellic acid in terms

of the subsequent ability of this dilution to promote germination of barley seeds, as measured by a germination index.

#### **1.4.5 The fifth hypothesis**

It is hypothesized that electromagnetic radiation originating from cellular phone exposure will have a neutralizing effect on 4cH homoeopathic dilution of gibberellic acid in terms of the subsequent ability of this dilution to promote germination of barley seeds, as measured by a germination index.

#### **1.4.6 The sixth hypothesis**

It is hypothesized that electromagnetic radiation originating from cellular phone exposure will have a neutralizing effect on 200cH and homoeopathic dilution of gibberellic acid in terms of its effect on the germination of barley seeds, as measured by a germination index after exposure to this substance.

#### **1.4.7 The seventh hypothesis**

It is hypothesized that exposure to electromagnetic radiation originating from a television set will have a neutralizing effect on 4cH homoeopathic dilution of gibberellic acid in terms of its effect on the germination of barley seeds, as measured by a germination index after exposure to this substance.

#### **1.4.8 The eighth hypothesis**

It is hypothesized that exposure to electromagnetic radiation originating from a television set will have a neutralizing effect on 200cH homoeopathic dilution of

gibberellic acid in terms of its effect on the germination of barley seeds, as measured by a germination index after exposure to this substance.

### **1.5 The implications of the study**

The implications of this study are practical and theoretical. It has long been the concern of both manufacturer and practitioner, that stronger external influences are capable of negating the subtle homeopathic dilutions. Of particular concern are strong odours and more recently electromagnetic radiation. It has been demonstrated that substances with strong smelling odours, namely camphor and mothballs, are capable in some way of affecting the homeopathic dilutions. From a theoretical point of view, these results will have obvious implications for the storage of homeopathic medication. Exposure of dilutions to electromagnetic radiation showed very little effect, and most interestingly television 200cH seemed to have a stimulatory effect on the dilutions. Biological activity of homeopathic preparations could not be confirmed conclusively due to confounding factors.

### **1.6 The benefits of the study**

The main benefit of this study is the demonstration that homeopathic dilutions can be influenced by external influences. These findings will then benefit practitioner, patient, and manufacturer alike, in the storage of homeopathic dilutions, extending their shelf life. Another benefit, is additional evidence that homeopathic dilutions have an effect on plants, extending research into homeopathic agriculture.

## CHAPTER 2

### REVIEW OF RELATED LITERATURE

#### 2.1 Seed germination

Germination is the initiation of the process of growth from a seed into a viable plant, and begins with imbibition. Imbibition is the absorption of water by the seed, as a result of its low water potential. The absorbed water triggers metabolic changes in the seed, which cause growth. The thin outer layer of the endosperm - the aleurone layer - produces the enzyme  $\alpha$ -amylase, as well as other enzymes, which catalyze the hydrolysis of starch stored in the endosperm. If the embryo is dissected out before water is absorbed, no  $\alpha$ -amylase is produced. This indicates that the embryo is responsible for sending a chemical signal to the aleurone cells. Such chemical signals or messengers are the plant hormones. Germination is normally defined by seed physiologists as the emergence of a radical through the seed coat. (Campbell, 1993:742-743.)

#### 2.2 Plant hormones

Plant hormones are organic substances that are produced in one tissue, in minute amounts, and transported to another tissue to have an effect (Raven *et al.* 1992: 545). Five classes of plant hormones have been discovered, viz. auxin, cytokinins, gibberellins, abscisic acid, and ethylene. In general, hormones control growth and development by affecting the division, elongation, and differentiation of cells. Each hormone has its own effect depending on where it is produced, its concentration, the site of action, and the developmental stage of the plant. Hormones act by altering gene



expression, activating enzymes, or changing the properties of the membranes of the cells. The plant growth inhibitory hormones are abscisic acids and auxins, which control root formation and growth. Gibberellins and cytokinins are plant growth promoters, and regulate protein synthesis, stem elongation and organ differentiation. (Campbell, 1993:759.)

### 2.3 Gibberellins and gibberellic acid

Gibberellic acid (see Figure 2.1) was first discovered in Asia during investigations into a disease of rice plants, which caused plants to grow long and spindly and then topple before they could mature. In 1926 E. Kurosawa, a Japanese scientist, discovered that a fungus of the genus *Gibberella* caused the disease. By 1930 it was discovered that the fungus caused hyperelongation of the stems by secreting a chemical, which was named gibberellin. In the last thirty years more than 70 gibberellins have been discovered, all slight variations of a common molecular theme. (Campbell, 1993:762.)

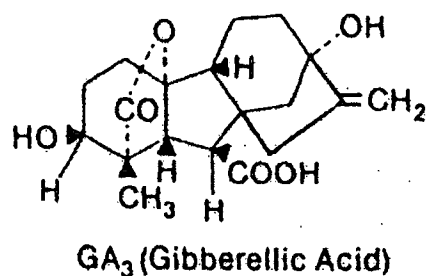


Figure 2.1 Gibberellins (Campbell, 1993:759)

Gibberellins are produced in roots and young leaves. They are responsible for growth in both leaves and stems, but have little effect on the growth of the

roots. In the stem, they promote elongation. Fruit growth is another area of development where gibberellins are involved, along with auxins. Lastly, gibberellins are also involved in germination, the developmental area on which this study is based. The gibberellins are the probable link between environmental cues and initiation of growth of the embryo. (Campbell, 1993:763.) The most widely used gibberellin, both experimentally and commercially, is gibberellic acid (GA<sub>3</sub>). Commercially, gibberellins are used to elongate stems of sugarcane, and to increase the rate of fermentation during the brewing process of beer (Lewis and Young, 1995:82).

#### **2.4 Barley**

Barley seeds were used in this study because they are highly sensitive to GA<sub>3</sub> (Foskett, 1994:447, Karssen, 1995:354). Many studies on the role of GA<sub>3</sub> in  $\alpha$ -amylase synthesis and the germination of seeds have utilized barley seeds (e.g.: Payley, 1960; Chrispeels and Varner, 1967; Takashi *et al.* 1988; Steele, 1999; Him Lock, 1999; reviewed by Ziegler, 1995). The variety of barley seed utilized in this study was *Hordeum vulgare* Stirling (ex-Sensako, Western Cape, 2000 harvest). Barley is a monocotyledon of the *Graminae* family (grasses). Barley is used extensively in the brewing of beer and whiskey, in breakfast cereals, and in animal feed. Barley also has medicinal properties, especially as a nutritive drink and supplement for those that do not follow a regular balanced diet. It is also used medicinally in febrile conditions and catarrhal conditions of the respiratory and urinary systems. (Greive, 1931:84.)

## 2.5 The role of GA<sub>3</sub> and α-amylase in barley seed germination

The aleurone layer of barley seeds is rich in proteins. At germination, the embryo releases gibberellins which diffuse into the aleurone cells and stimulate them to produce hydrolytic enzymes. One of these enzymes is α-amylase which catalyses the hydrolysis of stored starch into sugars. These are then absorbed by the scutellum and transported to growing regions of the embryo. (Raven *et al.* 1992:557.) GA<sub>3</sub> causes the cells of the aleurone layers to produce proteins that stimulate the release of enzymes which break down the cellulose walls of the aleurone cells, whereupon α-amylase is released (see Figure 2.2) (Moore, 1995:424-423). GA<sub>3</sub> is the only known agent that is capable of activating the α and β-enzymes (Payley, 1960). Salts such as calcium, magnesium, and potassium (Eastwell *et al.*, 1982 ) regulate the GA<sub>3</sub> - enzyme system. During germination, the embryo and the scutellum are the most likely source of GA<sub>3</sub>. GA<sub>3</sub> has two separate promotive actions in seed germination - to mobilize food reserves and to promote embryo growth (Karssen *et al.*, 1995:354)

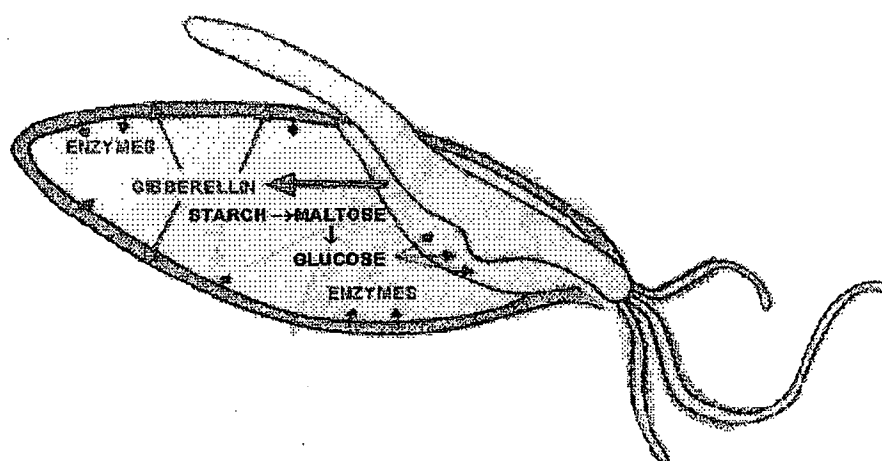


Figure 2.2 The mobilization of food reserves in barley (Barnes and Poor, 1994:8)

## 2.6 Seed quality

In farming and malting, the quality of seeds is very important. Good quality seeds yield stronger, better plants after germination. The quality of seeds is measured in terms of vigour. High vigour seeds are characterized by more vigorous uniform germination and seed development than are low vigour seeds. Seed vigour is therefore the ability of the seed to produce a healthy, viable plant. (Seed vigour report, 2001.)<sup>1</sup> High vigour implies that there will be uniform germination despite the changing environmental conditions. There are many variables that can influence seed vigour. These include genetics; environment and nutrition; stage of maturity at harvesting; seed size; weight; specific gravity; mechanical integrity; deterioration and aging; seedborne pathogens; dormancy; treatments and processing and crop protection products. (Seed vigour report, 2001.)<sup>1</sup>

The rate of germination is related to seed vigour. Seeds of high vigour germinate rapidly whereas those of low vigour germinate slowly. For this study, a seed germination test was performed to determine the percent germinability of the seeds accurately (See Appendix 1). The reason for this was to ensure that the seeds used were of medium to high vigour, and thereby to ensure optimum seed germination under experimental conditions. Results reported by Him Lock (2001) showed the greatest biological activity in medium to high vigour seeds. Him Lock (2001) however does not state how the vigour was tested.

## **2.7 Germination Index**

Many parameters are used when measuring the germination index of seeds. The parameters used by Him Lock (2001) were also used in this study. Root and shoot length, percentage germination (the percentage of seeds that actually germinated in the course of the seven days) was determined, and seedling dry mass was measured. The Walker Simmons equation (1988:769-775) was then used to calculate the final germination index (see 3.6.6). The same equation was used by Hopkins (1999) in a similar study.

## **2.8 Homoeopathic agricultural research**

Scientific demonstration of the efficacy of homoeopathic dilutions has long been the goal of many homoeopathic researchers. Many of these demonstrations have been obtained through clinical trials on patients, but such trials raise issues of ethics and the placebo effect (Linde, 1997). To avoid these two issues, homoeopathic researchers have used agricultural plants as model systems. Plants are thought to lack the same level of consciousness that humans have, therefore they should not be influenced by placebo effects, and research on plants avoids ethical problems. (Kayne, 1991; Hopkins, 1999; Him Lock, 2001.)

Schofield (1984) reviews the use of homoeopathy in agriculture. Two of these studies are described here. Kolisko and Kolisko (1978) conducted intensive research into the effect of homoeopathic dilutions on germination of different plants, predominately wheat. This research revealed that lower dilutions promoted growth, higher dilutions inhibited growth, and still higher dilutions

stimulated growth. Boyd (1941, 1942) conducted experiments using micro-doses of mercuric chloride that showed statistically significant effects on diastase activity. However, Schofield concluded that not many of the studies done on plants subjected data to statistical analysis and many gave no indication of why the research was being conducted. The failure to identify the rationale of the experiments seriously detracts from the value of these studies.

Jones and Jenkins (1983) showed that *Pulsatilla* in varying dilutions up to 13cH had an effect on yeast and wheat seedlings, by increasing growth. This work was later repeated using yeast (Steffan, in Schofield, 1984).

Betti *et al.* (1994) conducted a randomized laboratory trial where homoeopathic dilutions of Arsenicum album (decimal potencies 23 to 45) were tested for their effect on seed germination. The percentage germination was compared with germination in a distilled water group. Base solution of Arsenicum album completely inhibited growth. A comparison between potentised distilled water and Arsenicum album  $10^{-30}$  mol/L, showed that the distilled water had a greater stimulatory effect than did the dilution of Arsenicum album. The experimental results showed that differences between treatment groups could not be explained as an effect of intrinsic seed variability. Comparison of average germination times did not yield significant results.

Bornoroni (1999) pretreated oat coleoptiles with highly diluted solutions of  $\text{CaCO}_3$  (the homoeopathic medicine Calcium carbonate). The coleoptiles

were cultured in the presence of indoleacetic acid (IAA), a member of the auxin class of plant hormones. A statistically significant increase in growth was observed in the coleoptiles that had been pretreated with Calcium carbonate 5cH, as compared to those had been treated with IAA only. Homoeopathically prepared IAA increased the growth in coleoptiles. However, Bornoroni did not specify the methodology used in the preparation of homoeopathic solutions, or the statistical method used.

Hopkins (1998) investigated the biological effects of homoeopathic treatments (Sulphur, Nitric acid and Camphor in 3cH, 9cH, 15cH and 30cH potencies) on lettuce seed germination. A germination index was calculated using the Walker Simmons equation, then analyzed using the nested design model. Significant biological effects (promotion or suppression of germination, depending on the treatment) were recorded. He also found camphor to be stimulatory in potency. Hopkins recommended that the number of potencies levels used be increased in future studies, and that the effects of plant growth substances or plant growth regulators prepared according to homoeopathic principles be further investigated. The present study aimed to investigate the effect of camphor in crude form, as was recommended by Hopkins (1998). In doing so, the present study follows the principles of the study done by Hopkins (1998).

Steele (1999) investigated the effect of ultra-high dilutions of gibberellic acid on the synthesis of  $\alpha$ -amylase in de-embryonated halves of barley seed.

The  $\alpha$ -amylase activity was assessed using Phadebas amylase tablets (Pharmacia Diagnostics AB, Sweden). He used the 4<sup>th</sup>, 9<sup>th</sup>, 15<sup>th</sup>, 30<sup>th</sup> and 200<sup>th</sup> centesimal dilutions prepared in two ways – traditional Hahnemannian with succussion, and straight serial dilution without succussion. His results indicated a significant difference between both treatment groups and controls, and no significant difference between the treatment groups in terms of their ability to produce  $\alpha$ -amylase. He concluded that ultra-high dilutions of gibberellic acid are biological active, and that succussion as per the homoeopathic method is not a significant factor underlying this capability. However, a technical error in the proportion of incubation buffer used in the control groups compared to the treatment groups casts doubt on the results reported and renders comparison with the control groups unreliable. His research was valuable because it laid the groundwork for other homoeopathic research using the model of barley seeds and gibberellic acid. The principles are being retested in current investigations (Him Lock, 2001; Bruni, 2000; Couchman, 2001; Balding, 2001; Pieterse, 2001; Stubbs, 2001).

Him Lock (2001) conducted experiments on barley seeds, using potentised gibberellic acid (4cH, 15cH, 30cH, and 200cH). She investigated the effects of these potency levels on the germination performance of low, medium and high vigour seeds. Measures of germination included germination percentage, root and shoot lengths, and seedling dry mass. In the tests run on the high vigour seeds the control group (water) showed a higher germination than the normal GA<sub>3</sub> control group. The 15cH potency level showed the highest germination among the homoeopathic dilutions, followed closely by 4cH, 30cH, and 200cH



all having significantly similar means. In the test run on the medium vigour seeds, it was found that the potencies of 4cH, 30cH and 200cH had greater effects than the control (water). These findings indicated the biological activity of ultra-high homoeopathic dilutions. Her results showed that there was an increased growth in all the parameters among the medium and high vigour seeds but only in root length among the low vigour seeds. This research provides evidence that ultra-high homoeopathic dilutions do have an effect on plants, and forms the basis of the present study.

## **2.9 Preparations of homoeopathic dilutions**

Homoeopathic dilutions are considered to be specific stimulants. Their use is dominated by the notion of the infinitesimal dose (Jouanny, 199:81).

Hahnemann developed the process of potentisation, diluting the dilution beyond its toxicological point, thus giving the patient very small doses, so that he could treat his patients in a gentler manner (Boericke, 1997).

Homoeopathic dilutions are prepared from three main sources: vegetable, mineral, and animal extracts. Special care is taken in the preparation of these substances. (Jouanny; 1993:81) If the original substance is soluble, then one part is added to either ninety-nine or nine parts of distilled water or alcohol.

Each dilution is then vigorously shaken by firmly beating it against the palm of a hand or a hard surface (see Figure 2.3). This results in a 1C ( $1:10^{-100}$ ) or 1D ( $1:10^{-10}$ ) dilution respectively. A dilution of 1/100 is designated by a Roman numeral of C (centesimal) and a 1/10 is designated by a Roman numeral of D or X (decimal). If the centesimal dilution is prepared in strict accordance to

rules laid down by Hahnemann, then the designation is "cH", indicating a Hahnemannian centesimal dilution. (see Figure 2.9). Insoluble base substances are firstly triturated in lactose, until they reach solubility. They are then diluted in the same way as soluble substances. (Jouanny, 1983:82-83) Liquid preparations used in this study were prepared in accordance to Method 5a as set out by the German Homoeopharmacopoeia (British Homoeopathic Association, 1985:22).

Dilutions commonly used by homoeopaths range from 4C and 4D ( $1:10^{-8}$  and  $1:10^{-4}$ ) to as high as 1000C ( $1:10^{-2000}$ ) and beyond. At 12C it is assumed that there are no longer any of the original particles left in the dilution, as this is now beyond Avogadro's number (Gaier, 1991:47-48). This preparation process is known as potentisation or dynamization.

According to Zacharias *et al.* (1997), dynamization postulates that medicinal properties can be transferred from an active medium to an inert medium, and that the transfer of these properties is stimulated by succussion. The efficiency and quality of transference depends on the degree of dynamization. Dynamization can be defined as a step-like sequence of dilution and shaking, and is a process in which the relative quantity of solvent in contact with the active substance is steadily increased. Dilution is essential because an inert solution is only capable of receiving medicinal properties until saturation and dilution avoids saturation. Thus, dynamization requires dilution to avoid saturation and succussion to stimulate the transfer of medicinal substances.

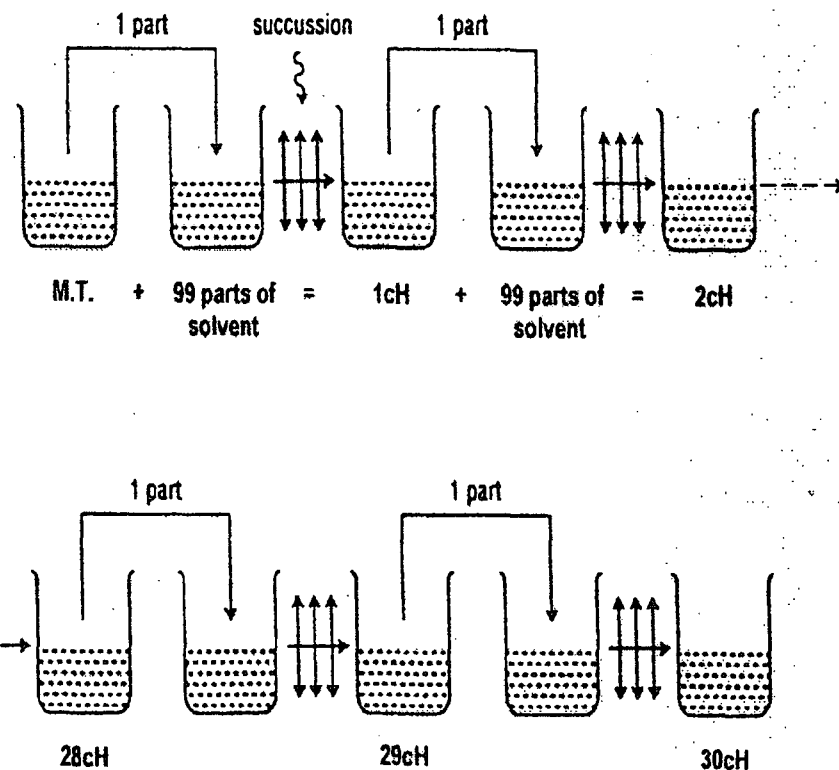


Figure 2.3 Centesimal Hahnemannian Potentisation Method (Gaier, 1991:456).

## 2.10 Theories of storage and transfer of information in homoeopathic dilutions

### 2.10.1 Endler

According to Endler (1989), the medicinal substance imprints on the water a water molecular polymer of various sizes and configurations during the succussion phase of dilution. These polymers are then capable of self-replication. The author links the ability of the medicinal substance to transfer information to the existence of clusters of the water molecules enclosing dissolved gas molecules. These gas molecules vibrate to maintain the structure of the fluid. With the introduction of another substance, the gas molecules take on the new vibrational pattern, by swinging or rotation, and

hence they maintain the new structure of the dilution. These gas molecules vibrate in a frequency range similar to electromagnetic fields.

#### 2.10.2 Anagnostatos

Anagnostatos (1999) proposes a three step model to explain how aqueous solutions are capable of transferring and maintaining information. He postulates that in the first step small clusters of the pharmaceutical substance are formed, surrounded by shells of hydrogen bonded molecules of the solvent (clathrates). In the second step an externally induced fluctuation (succussion) causes the small clusters to move out of their clathrates, and a new clathrate is formed around the relocated cluster (mantle-clathrate) and the initial clathrate (core clathrate), that has shrunk due to the loss of the inner cluster. The third step proposes that at this stage none of the original substance is left and the role of the small cluster for the dilutions and succussions to follow is taken on by the compact core clathrate. The structure of the clathrate is considered the fingerprint of the drug. The local parameters or potential carriers of distinct information are then the characteristic clathrate bond lengths and angles. (Schulte, 1999.)

#### 2.10.3 Del Giudice

Del Giudice (in Shulte, 1994) showed that, in condensed matter, electromagnetic fields could become "trapped" to form coherent regions. Matter with coherent regions is far more stable than matter that does not contain coherent regions. Del Giudice showed that an externally applied electromagnetic field could create 'metastable' extended polarization fields in

a coherently moving system of water. He proposed that it is possible to create low frequency long-lived polarization fields in water, without the use of an original substance (base substance) or to alter an already existing coherent fields by applying an external field. It is suggested that the mere existence of these coherence phases within domains serve as the message carrier.

#### 2.10.4 Xu and Bishop

Xu and Bishop (in Shulte, 1999) proposed that information could be extracted from a dynamic system by a series of phase space data, then reconstructed and stabilized by feedback mechanisms but of smaller amplitudes. This model has yet to be verified experimentally.

#### 2.10.5 Schulte and Endler

Schulte and Endlers' dynamic model consists of coherent stable excitations of groups of atoms, which form "stable aggregations of rigid or loosely bound molecules". These excitations are treated as 'quasi-particles' and the 'quasi-particle' formation can be predicted and measured as 'fingerprints' in their respective energy spectra. (Shulte,1999:157.)

#### 2.10.6 Resch and Gutmann

Resch and Gutmann (1991:191-213) proposed the Supermolecular System of Organization of Liquid Water, in which the liquid is broken down into four hierarchal levels. The first level comprises the molecules at the surface of the liquid, which communicate with the environment and exert the greatest influence on the total liquid. The second level is the 'inner surface' which is the

water molecules that surround the hydrophobic solutes or gas molecules dissolved in the liquid, and have a decisive influence on the oscillating pattern of the liquid. The oscillations of the gas molecules are in harmony with the 'inner surface', and are thus modified by the oscillations of the 'inner surface'. The gas molecules are then capable of taking on the structural information from the solution and preserving it. Thus, the gas molecules have been named 'synchronization nodes'. The third level is the water molecules that surround the hydrophilic solutes in the water, called 'solvation spheres'. By addition of hydrophilic solutes, the bond lengths and angles of the 'solvation spheres' are altered, and hence the oscillating pattern is also altered. The fourth level is the normal water molecules. The 'inner surface' and the 'solvation spheres' are called the dynamic components as they interact and change according to the solution, whereas the other two levels are static. Resch and Gutmann go on to explain how potentiation changes the system so that the solution is capable of maintaining the medicinal information. When the original substance is added to the pure solvent, the structure of the solvent is changed. This is because the original substance is the more static component and the pure solvent is the more dynamic, such that the static aspects of the original substance alter the structure of the solvent, causing the system to oscillate differently. The new oscillations are taken up by the dynamic aspects of the solvent. Thus the information from the original substance is transmitted to the solvent. As more solvent is added and the original substance diluted, the medicinal information is not lost because the information is retained by the dynamic aspects of the solvent. Agitation or succussion allows for a bigger interface between the original substance and

solvent, and thus allows for greater transfer of information from one to the other.

### **2.11 Neutralization**

Homoeopathic dilutions are subtle substances due to their method of manufacture and potentisation, which involves serial centesimal dilution and succussion. It is maintained that dilutions greater than the 12th centesimal no longer contain any molecules of the original substance (Gaier, 1991:47-48). Most theories regarding the mechanisms of ultrahigh dilutions are based on the biophysical rather than the biochemical properties of the dilution, meaning that their ability to act is because of energetic properties and not chemical properties (Towsey and Hasan, 1995; Jones and Jenkins, 1981; Resch and Gutman, 1991:191-213; Bellavite and Signorini, 1995:243-3001; Antochenko and Ilyin, 1992). This then raises the question of whether another source of energy could interfere with or negate the energetic pattern of homoeopathic dilutions. Strong smelling substances (such as camphor and mothballs), as well as sunlight, heat and electromagnetic radiation are the most common external influences suspected of neutralising homoeopathic dilutions (Dancu, 1996:172-173; Adams, 1996:56-57; Kayne, 1997:181; Vithoulkas, 1980:264; Helios, 2001). In this investigation, the ability of camphor, naphthalene (mothballs) and electromagnetic radiation to neutralise homoeopathic dilutions of GA<sub>3</sub> was evaluated.

### 2.11.1 Camphor

Essential oils are strong smelling compounds usually extracted from plants, where "essential" means they give off a distinct "essence" or smell. Camphor is an essential oil belonging to the group terpenoids. Terpenoids are oxygen containing analogs of terpenes. Camphor has a fragrant, penetrating odour and tastes cool. (Jenkins and Hartung, 1941.) In homoeopathy it is known as the "universal" antidote (Kayne, 1997:121).

### 2.11.2. Naphthalene

Naphthalene is a benzene hydride. It is composed of two benzene type rings, and is the traditional component of mothballs. It has the formula  $C_{10}H_8$  (Brady and Holum, 1993:1051). Traditionally it is placed in cupboards to repel moths and prevent clothes from being eaten by the moth larvae (Brady and Holum, 1993:364). Naphthalene is also an essential oil, therefore it is substance with a strong odour. Many homoeopathic texts warn against storing dilutions near mothballs because of their strong odour (Helios, 2001).

### 2.11.3 Electromagnetic radiation (EMR)

All matter is made up of atoms. In 1911 Rutherford proposed a model showing that at the center of every atom is a nucleus that has weight and density.

Circulating the nucleus are small particles called electrons. The negatively charged electrons are held in place around the nucleus by electrostatic forces caused by its attraction to the positive protons found in the nucleus. Whenever an electron changes its velocity EMR is produced. EMR has both wave and particle properties. Electrons moving from a higher to a lower energy level in



an atom will radiate an energy wave of a particular frequency and wavelengths (Pain, 1998:201-202). By going from a higher to a lower energy state a quantum of electromagnetic energy is emitted. The magnitude of the quantum is directly proportional to the difference between the higher and lower energy states (Britannica.com, 2001)<sup>3</sup>.

EMR comprises electric and magnetic fields propagating at the speed of light  $c$  through empty space. The electric and magnetic fields change magnitude and direction each second. This rate of change is called the frequency  $\nu$ , measured in cycles per second. The frequency of a wave is related to its speed  $c$  and wavelength  $\lambda$ . Wavelengths are defined as the distance between successive crests or troughs (Strobel, 2000)<sup>7</sup>. The speed of electromagnetic radiation of all kinds is the same universal constant, that is exactly  $c = 299,792,458$  meters per second. The formula used to describe EMR is  $c = \lambda \nu$ . The great variation in magnitude of the various parts of the spectrum means that the sources, interactions with matter and detectors also vary correspondingly. (Young, 1992: 936.) The electric and magnetic fields are always perpendicular to each other and at right angles to the direction of propagation. (Strobel, 2000)<sup>7</sup>.

EMR varies from very small frequencies to very high frequencies, this range being known as the electromagnetic radiation spectrum. This extremely large range of frequencies is shown in Figure 2.4, together with the common names used for its various parts, or regions. General properties shared by all forms of EMR are that they can all travel through empty space at the speed of light

which is constant in space.

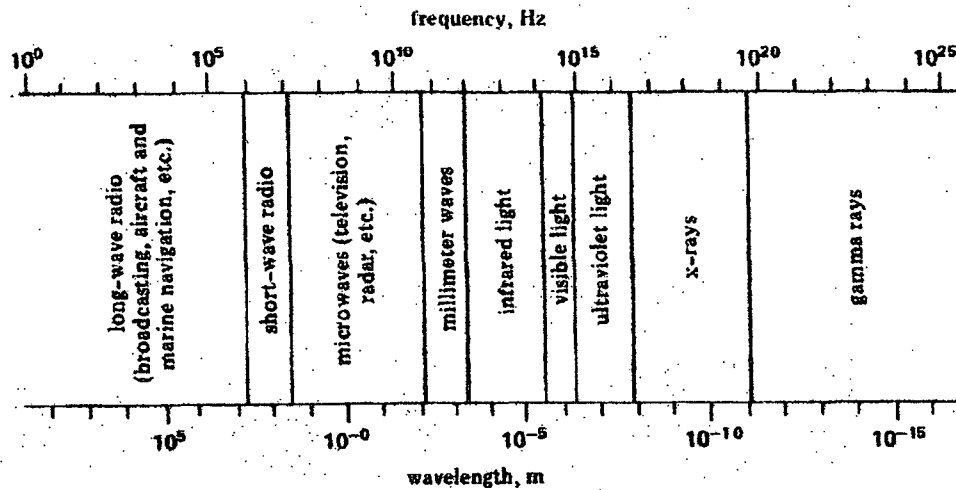


Figure 2.4. The Electromagnetic spectrum (Pain, 1998:202)

### 2.11.3.1 Forms of EMR

There are many forms of EMR, depending on the frequency. The frequencies range from low levels to high levels of frequency as seen in the spectrum. The two frequencies used in this present study were sourced from radio waves and microwaves.

Radio waves are used to transmit sound messages or communication information. The information is imposed on the electromagnetic carrier as an amplitude modulation (AM) or frequency modulation (FM) or in a digital form (Pulse modulation). Therefore transmission is not a single-frequency electromagnetic wave but rather a frequency band whose width is proportional

to the information density. The width is about 5MHz for a high definition television. (Britannica.com, 2001.)<sup>3</sup>

A television tube is a cathode ray tube. The inside of the tube is coated with a phosphor, which glows when struck by electrons. At the rear of the tube is an electron gun that shoots a beam of electrons onto the phosphor coating. The beam of electrons is then directed around the tube using electromagnetic coils or electrically charged metal plates. This then forms a "glow-picture" of the band frequency being received by the cathode ray tube. (Gibbs, 1990: 412.)

When these electrons are accelerated to strike the phosphor coating there is a potential for the development of X-rays. These X-rays are capable of escaping the receiver cabinet or picture tube, making TV sets a potential hazard (Center for Devices and Radiological Health, 1999)<sup>5</sup>. This radiation can go through most building materials, such that the TV set is surrounded by a field of electromagnetic radiation regardless of its position in a room (Biomagnetics, 2001).<sup>2</sup>

The microwave region extends from 1,000 to 300,000MHz. They are the principle carriers of telegraphic data transmissions and the beams can be directed like searchlights between earth-based stations and satellites. (Gibbs, 1996: 221; Britannica.com, 2001)<sup>3</sup> Cellular phones emit radiation in the microwave region when in use and in the radio wave region when on stand-by mode (Center for Devices and Radiological Health, 1999)<sup>5</sup>. Most cellular phones in South Africa function at 800Mhz – 1000Mhz. The fact that they operate at such high levels of radiation has become a concern to public

health. The electromagnetic waves generated from cellular phones penetrate directly into the body. Some of the influences of electromagnetic radiation, especially from cellular phones are thought to include headaches, dim vision, brain tumors, decreased reproductive ability, fetal deformities and, miscarriages in the case of pregnant women. (Zeropa, 2001.)

Other forms of EMR include infrared radiation, in the region of  $10^{12}$  and  $5 \times 10^{14}$  Hz), visible radiation (the most common source of electromagnetic radiation), ultraviolet radiation, X rays, and gamma rays.

## **2.12 Summary**

Homoeopathic dilutions are very subtle substances due to the procedure of manufacture. It is proposed that their healing properties can be attributed to the energy retained by the water and alcohol used to produce them. Because of this, it is thought that they can be neutralized by any stronger entity. Initially concern centered on entities such as strong odors. However, very little research has been carried out to test this theory. As homoeopathy moved into the 21<sup>st</sup> century, new potentially neutralizing entities appeared, such as electromagnetic radiation. It has been suggested that the frequencies of radiation are capable of disturbing the energy of the dilutions and therefore rendering them useless. Again, this was never tested experimentally. This study was undertaken to test these theories empirically, using a plant model.

## **CHAPTER 3**

### **RESEARCH MATERIALS AND METHODS**

#### **3.1 Research location**

This study was carried out at the School of Life and Environmental sciences, George Campbell building, University of Natal, Durban.

#### **3.2 Experimental protocol**

3.2.1 Preparation of homoeopathic and non – homoeopathic dilutions (see 3.7.1)

3.2.2. Homoeopathic dilutions were exposed to various external influences.

3.2.3 Germinability trials were run on two different seed lots, to test for viability. The seed lot of higher vigour was identified for use in the experiment.

3.2.4 Prepared the petri dishes with the different treatment solutions.

Incubated petri dishes in dark growth chamber that was set at 20°C for 24 hours (see 3.7.4-3.7.5).

3.2.5 After 24 hours the seeds were removed from the treatment solutions and placed onto moistened (with deionised water) filter paper in a clean petri dish that was then replaced in growth chamber for 7 days.

3.2.6 Every day the germinated seeds were counted (radicle>1mm) and the dead or deformed seeds were removed. All these figures were recorded.

On day seven the germinated seedlings were measured for root and shoot lengths.

3.2.7 The seedlings were placed on a tray and put into an oven that was set at 80°C for 48 hours. After removal from the oven the seedlings were weighed and this data was recorded.

3.2.8 Data was analyzed using the One way ANOVA.

### 3.3 Study population

A total of 3600 whole barley seeds (*Hordeum vulgare*) were used.

### 3.4 Study design

The study consisted of 7 treatment groups (including a positive control group and a negative control group), as follows:

Group 1 was exposed to camphor in an enclosed chest for the period of 72 hours (Steele 2001);

Group 2 was exposed to mothballs (naphthalene) in an enclosed chest for a period of 72 hours (Steele, 2001);

Group 3 was exposed to a cellphone (Nokia 5110) receiving a call for a period of 2 hours (Hargreaves, 1999)<sup>6</sup>;

Group 4 was exposed to a Sony 61cm Trinitron colour TV for a period of 72 hours (Ross, 2000);

Group 5 was unexposed homoeopathic dilutions of gibberellic acid (HGA<sub>3</sub>);

Group 6 was gibberellic acid of a concentration of 0.5g/L<sup>-1</sup> (positive control).

The same concentration was used by Him Lock (2001);

Group 7 was distilled water only (negative control).

See 3.7.1 for experimental specifications.

Groups 1 - 5 consisted of 2 subgroups representing the 2 levels of dilution that were selected for this study, 4cH ( $1:10^{-8}$ ) and 200cH ( $1:10^{-400}$ ). These dilution levels were selected because they span Avagadro's dilution limit (12cH), and

are the highest and lowest levels used by Him Lock (2001). This amounted to 12 subgroups in total.

Each subgroup consisted of 3 repetitions. This amounted to 36 repetitions in total.

Each repetition comprised 5 petri dish with 20 seeds. This amounted to 3600 seeds in total ( $2 \times 12 \times 3 \times 100$ ).

### **3.5 Photographic record**

Photographs were taken of each treatment group on days two and seven, to record the differences in growth of the seedlings.

### **3.6 Materials**

#### **3.6.1 Gibberellic acid**

Gibberellic acid, isolated from *Gibberella fujikuroi* fungus, (Moore 1995:422) was obtained from Sigma-Aldrich (Pty) Ltd., South Africa). This form is the potassium salt of GA<sub>3</sub> (C<sub>19</sub>H<sub>21</sub>O<sub>6</sub>K, molecular weight 384.5g.mol<sup>-1</sup>) which is readily soluble in water. Potassium hydroxide (used to produce the potassium salt) does not reduce the effect of GA<sub>3</sub> and has no effect on the synthesis of  $\alpha$ -amylase (Cairns, 1998; as cited by Steele 1999).

#### **3.6.2 The seed**

Seeds were obtained from two different sources, viz Sensako (Western Cape, South Africa) and SP Brown and company (Kwa-Zulu Natal, South Africa).

Seed-lots of 100 seeds were chosen from each group and germinated to test which had the greatest percentage germination (See Appendix 1). Both groups showed very high percentage germination. The Sensako batch was selected because Him Lock (2001) used seeds from the same source. The species used was *Hordeum vulgare* Stirling, 2000 harvest. Three kilograms of seeds were obtained and from this 3600 whole seeds were selected.

### **3.6.3 Laboratory chemicals**

- 3.6.3.1 Deionised water (Water Still Model W14S, J.Biddy Science Products Limited).
- 3.6.3.2 Camphor 200g. (Deer Brand refined camphor tablets. Made in China. Obtained from Pick n Pay, Durban, South Africa).
- 3.6.3.3 Naphthalene 200g. Brand: Hoption & Son (118 Umbilo Road, Durban, South Africa).

### **3.6.4 Laboratory equipment**

- 3.6.4.1 Petri dishes (9cm) × 180.
- 3.6.4.2 Schleicher & Schuell filter papers (9cm) × 720.
- 3.6.4.3 Latex gloves.
- 3.6.4.4 Automatic pipettes with disposable tips (Socorex, Swiss): 1 × 0.5ml; 1 × 5ml.
- 3.6.4.5 Electronic balance (Mettler) × 1.
- 3.6.4.6 Growth chamber (Gallenkamp) × 1.
- 3.6.4.7 Freezer set at -20°C (Ocean) × 1.
- 3.6.4.8 50ml amber glass round screw top bottles × 195.



- 3.6.4.9 100ml amber glass square screw top bottles × 10.
- 3.6.4.10 Sony 60cm Trinitron® colour TV (Model KV-G25SA, Frequency: 100Hz-8KHz).
- 3.6.4.11 Nokia 5110 cell phone (800-1000MHz).
- 3.6.4.12 Medicine chest. Wooden 44×35×10cm with mirrored front.

### **3.7 Methodology**

Methods used were per Him Lock (2001), Association of Official Seed Analysts (1995), International Seed Testing Association (2000) and Steele (1999:38-42).

#### **3.7.1 Preparation of homoeopathic dilutions of GA<sub>3</sub>**

Homoeopathic dilutions of GA<sub>3</sub> were prepared as per Steele (1999:38) under laminar flow conditions in the homoeopharmaceutical laboratory of the Department of Homoeopathy, Technikon Natal, Durban. All glassware was sterilized by rinsing with distilled water and then baked in an oven at 160°C for 2 hours. The stock solution was made up by combining one part KGA<sub>3</sub> (0.25g) with 99 parts (24.75ml) distilled water, forming a 10<sup>-1</sup> solution of KGA<sub>3</sub>. From this solution the centesimal dilutions were made up according to the Method 5a ("solutions") of the German Homoeopathic Pharmacopoeia (British Homoeopathic Association 1985:20-21). This is the Pharmacopoeia most commonly used by homoeopathic pharmaceutical manufacturers in South Africa. Dilutions were made up in distilled water, rather than alcohol to avoid any intrinsic effect which alcohol may have had on the plant hormone activity (Cairns 1998 as cited by Steele 1999:39). Dilutions required for experimental

purposes (4cH and 200cH) were made up in 100ml volumes (1ml of the preceding dilution mixed with 99ml of distilled water).

### **3.7.2 Exposure of the GA<sub>3</sub> dilutions to external influences**

Group 1: 100ml of GA<sub>3</sub> in an open amber glass screw top bottle was placed directly alongside 200g of crude camphor for a period of 72 hours, in a sealed medicine chest (see 3.6.4.12 for size specifications) (Steele, 2001).

Group 2: 100ml of GA<sub>3</sub> in an open amber glass screw top bottle was placed directly alongside an open packet (200g) of mothballs for a period of 72 hours, in a sealed medicine chest (see 3.6.4.12 for size specifications) (Steele, 2001).

Group 3: 100ml of GA<sub>3</sub> in a sealed clear glass screw top bottle was placed alongside the antenna of a Nokia 5110 cell phone (which was receiving a call) for a period of 2 hours (Hargreaves 1999).

Group 4: 100ml of GA<sub>3</sub> in a sealed clear glass screw top bottle was placed on top of a Sony 60cm colour TV for a period of 72 hours (Ross, 2000).

### **3.7.3 Preparation of the seed**

3600 seeds were selected from the Sensako batch of seeds. Damaged and deformed seeds were excluded.

#### **3.7.4 Preparation of the petri dishes**

Each petri dish was lined with two 9cm filter papers and marked with the group, subgroup and repetition number. The seeds were divided into groups of 100, and placed in the petri dishes. The filter papers in each petri dish were moistened with 12.5ml of the relevant treatment solution. Each subgroup was placed into a sealed box.

#### **3.7.5 Incubation of the seeds**

The petri dishes were placed into a growth chamber set at 20°C and incubated in the dark for 24 hours. After 24 hours the seeds were removed from the petri dishes using forceps. Seeds were exposed to treatment solutions during imbibition because the focus of this project was on the processes within the seeds once the solutions had been imbibed. Each group of 100 seeds was divided into groups of 20 and placed onto clean filter paper, in clean petri dishes. Another filter paper was placed over the seeds, which was then moistened with 5 ml deionised water. The petri dishes were placed back into the growth chamber for a further seven days. On day three and five a further 3ml of water was added to prevent desiccation of the seeds.

The seeds were counted daily, and those that had germinated (radicle >1mm) or were dead and deformed were counted and recorded. Dead and deformed seeds were removed to ensure that they did not contaminate those that were still germinating and growing. Germinated seeds that had become contaminated (infected with fungus) were recorded in the index as

germinated, but were removed and were therefore not included in the final dry count and seedling dry mass.

### 3.7.6 The germination index

On day 7, all seeds were removed from the incubation chambers and counted. The following information was recorded as per Him Lock (2001)

- ◆ Standard germination: The number of normal seedlings within a seed-lot was recorded.

- ◆ Germination percentage: the accumulated number of seeds with radicle greater than or equal to 1mm were measured.

- ◆ Seedling development: length of roots and shoots at final count (day 7) were measured as an indication of rate of seedling development.

- ◆ Seedling dry mass. After the final count all seedlings were placed onto trays to bake in an oven at 80°C for a period of 48 hours, after which they were weighed.

- ◆ A germination index (GI) was calculated, using the Walker Simmons equation (1988: 769-775), as shown below.

$$GI = \frac{(7 \times n_1 + 6 \times n_2 + \dots 1 \times n_7)}{\text{Total days} \times \text{Total Seeds}}$$

where  $n_1, n_2 \dots n_7$  are the numbers of seeds that germinated on the first, second and subsequent days until the seventh day, respectively; 7, 6 ... 1 are weights given to the number of germinated on the first, second and subsequent days respectively. The maximum GI is 1.

### **3.8 Data analysis**

#### **3.8.1 Statistical methods**

The One-way ANOVA method (between independent groups) was used for data analysis in this study.

##### **3.8.1.1 Procedure 1: Inter group comparison between all control groups with regard germination percentage**

$H_0$ : there is no difference between the controls.

$H_1$ : there is a difference between the controls.

The decision rule:

Reject if the P-value  $< \alpha$

Accept if the P-value  $\geq \alpha$ .

In this study the level of significance,  $\alpha$ , was fixed at the 0.05 level.

##### **3.8.1.2 Procedure 2: Inter group comparison between all control groups with regards to germination index**

$H_0$ : there is no difference between the controls.

$H_1$ : there is a difference between the controls.

The decision rule:

Reject if the P-value  $< \alpha$

Accept if the P-value  $\geq \alpha$ .

In this study the level of significance,  $\alpha$ , was fixed at the 0.05 level.

### **3.8.1.3 Procedure 3: Inter group comparison between all controls with regard to root length**

$H_0$ : there is no difference between the controls.

$H_1$ : there is a difference between the controls.

The decision rule:

Reject if the P-value  $< \alpha$

Accept if the P-value  $\geq \alpha$ .

In this study the level of significance,  $\alpha$ , was fixed at the 0.05 level.

### **3.8.1.4 Procedure 4: Inter group comparison between controls and 4cH treatment groups with regards to root length**

$H_0$ : there is no difference between the controls and the 4cH treatment groups.

$H_1$ : there is a difference between the controls and the 4cH treatment groups.

The decision rule:

Reject if the P-value  $< \alpha$

Accept if the P-value  $\geq \alpha$ .

In this study the level of significance,  $\alpha$ , was fixed at the 0.05 level.

### **3.8.1.5 Procedure 5: Inter group comparison between controls and 200cH treatment groups with regards to root length**

$H_0$ : there is no difference between the controls and the 200cH treatment groups.

$H_1$ : there is a difference between the controls and the 200cH treatment groups.

The decision rule:

Reject if the P-value  $< \alpha$

Accept if the P-value  $\geq \alpha$ .

In this study the level of significance,  $\alpha$ , was fixed at the 0.05 level.

#### **3.8.1.6 Procedure 6: Inter group comparison between 4cH and 200cH treatment groups with regards to root length**

$H_0$ : there is no difference between the 4cH and 200cH treatment groups.

$H_1$ : there is a difference between the 4cH and 200cH treatment groups.

The decision rule:

Reject if the P-value  $< \alpha$

Accept if the P-value  $\geq \alpha$ .

In this study the level of significance,  $\alpha$ , was fixed at the 0.05 level.

#### **3.8.1.7 Procedure 7: Inter group comparison between all controls with regards to shoot length**

$H_0$ : there is no difference between the controls.

$H_1$ : there is a difference between the controls.

The decision rule:

Reject if the P-value  $< \alpha$

Accept if the P-value  $\geq \alpha$ .

In this study the level of significance,  $\alpha$ , was fixed at the 0.05 level.

**3.8.1.8 Procedure 8: Inter group comparison between controls and 4cH treatment groups with regards to shoot length**

$H_0$ : there is no difference between the controls.

$H_1$ : there is a difference between the controls.

The decision rule:

Reject if the P-value  $< \alpha$

Accept if the P-value  $\geq \alpha$ .

In this study the level of significance,  $\alpha$ , was fixed at the 0.05 level

**3.8.1.9 Procedure 9: Inter group comparison between controls and 200cH treatment groups with regards to shoot length**

$H_0$ : there is no difference between the controls.

$H_1$ : there is a difference between the controls.

The decision rule:

Reject if the P-value  $< \alpha$

Accept if the P-value  $\geq \alpha$ .

In this study the level of significance,  $\alpha$ , was fixed at the 0.05 level

**3.8.1.10 Procedure 10: Inter group comparison between all controls with regards to seedling dry mass**

$H_0$ : there is no difference between the controls.

$H_1$ : there is a difference between the controls.

The decision rule:

Reject if the P-value  $< \alpha$

Accept if the P-value  $\geq \alpha$ .



In this study the level of significance,  $\alpha$ , was fixed at the 0.05 level.

### **3.8.2 Statistical package**

The statistical package SPSS for Windows (version 9) was used for data entry and analysis.

## CHAPTER FOUR

### THE RESULTS

#### 4.1 Introduction

The One way ANOVA was used in this study, along with the Tukey HSD tests for multiple comparisons.

The parameters measured in this study were the same as those used by Him Lock (2001). These were germination percentage, root lengths, shoot lengths, seedling dry mass, and germination index. For each parameter, an ANOVA was performed between the controls. If there was a difference between the controls, showing biological activity of the homoeopathic dilutions, then further one way ANOVA tests were performed between the controls (water and GA<sub>3</sub>) and treatments. Multiple comparison Tukey HSD test were performed to ascertain where the differences lay.

The level of significance for each hypothesis was fixed at  $\alpha = 0.05$ . The null hypothesis was accepted if the P value was greater than or equal to  $\alpha = 0.05$  and rejected if the P value was less than  $\alpha = 0.05$ . A rejection indicted a difference between groups whereas an acceptance indicated that there was no difference.

Photographs were also taken to record the differences in appearance of the seedlings between the controls and the treatments, and between the individual treatments, at each stage of the process. (See Plates 4.1 – 4.7)

**4.2 Criteria for the admissibility of the data**

Only the data collected from this experiment were accepted for use in the results chapter. The data used were collected in the manner that was described in Chapter Three.

**4.3 Germination percentage**

**4.3.1 Inter group comparison between all controls**

Table 4.1 One way ANOVA comparison between all controls with regard to germination percentage

	Sum of squares	df	Mean Square	F	P
Between Groups	527.583	3	175.861	3.308	.078
Within Groups	425.333	8	53.167		
Total	952.917	11			

Table 4.1 shows that within these groups there was no statistical difference found, as P is greater than 0.05. The lack of difference between the controls means that the controls could not be compared to the treatments as the unexposed homoeopathic dilutions did not differ in effect to that of water alone.

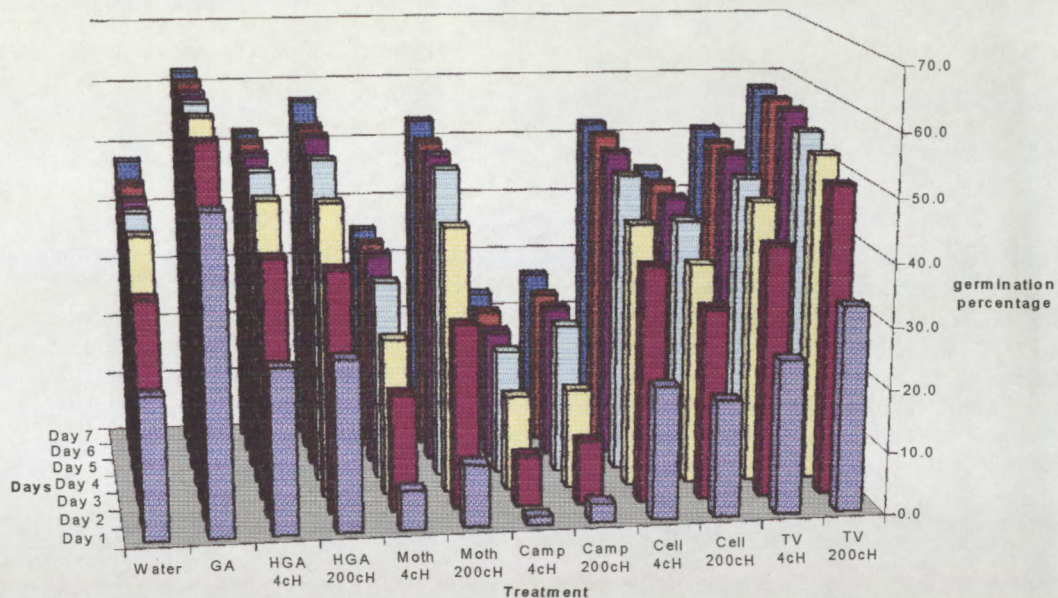


Figure 4.1 Bar chart showing germination percentage over the seven days of germination. GA = GA<sub>3</sub> (0.5g/L-1) control; HGA = HGA<sub>3</sub> control the homoeopathically prepared dilutions; Moth = the homoeopathic dilutions exposed to moth balls; Camp = the homoeopathic dilutions exposed to camphor; cell = the homoeopathic dilutions exposed to the cellular phone and TV = the homoeopathic dilutions exposed to the television set.

Figure 4.1 shows percent germination over the seven days of the trial. This is merely a seedling count and does not convey the rate of germination. It shows interesting trends over the seven days, such as the drop in germinability of the seeds from 99% in the germinability test to 48% (see the bars for seeds treated with water alone) as well as the inhibition of germination by the dilutions exposed to the moth balls and camphor.



## 4.4 Germination Index

### 4.4.1 Inter group comparison between all controls

Table 4.2 One way ANOVA between all controls with regard to germination index

	Sum of squares	df	Mean Square	F	P
Between Groups	5.891E-02	3	1.964E-02	6.076	<b>.019</b>
Within Groups	2.585E-02	8	3.232E-03		
Total	8.476E-02	11			

Table 4.2 shows that the P value was less than the level of significance, indicating that there was a difference between the controls.

Table 4.3 Tukey's multiple comparison between controls with regard to germination index

		P	H <sub>0</sub>
Water	GA (0.5g/L)	<b>.014</b>	<b>Reject</b>
	HGA 4cH	.665	Accept
	HGA 200cH	.494	Accept
GA (0.5g/L)	HGA 4cH	.070	Accept
	HGA 200cH	.109	Accept
HGA 4cH	HGA 200cH	.989	Accept

As is shown in Table 4.3 the difference was between the water and GA<sub>3</sub> (0.5g/L<sup>-1</sup>) controls groups. With no significant difference between the homoeopathic HGA<sub>3</sub> controls and the other two controls, the results of this test indicate that the homoeopathic dilutions were not active and for this reason no comparisons could be made with the treatment groups.



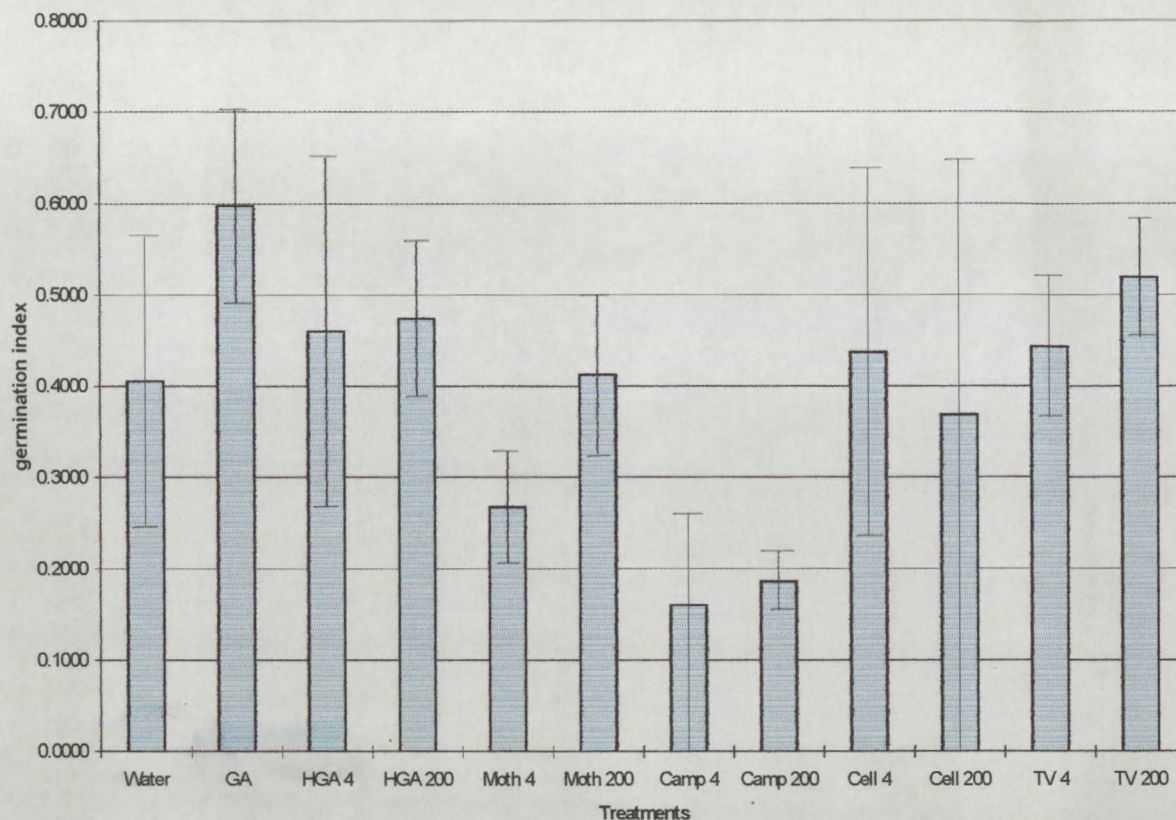


Figure 4.2 Bar chart showing germination index where GA = GA<sub>3</sub> (0.5g/L-1) control; HGA = HGA<sub>3</sub> control the homoeopathically prepared dilutions; Moth = the homoeopathic dilutions exposed to moth balls; Camp = the homoeopathic dilutions exposed to camphor; cell = the homoeopathic dilutions exposed to the cellular phone and TV = the homoeopathic dilutions exposed to the television set. Potencies are indicated as follows: 4 = 4cH; 200 = 200cH. Error bars show 95% confidence intervals.

Figure 4.2 once again reveals the trend of inhibited growth of seeds imbibed in the dilutions exposed to mothballs and camphor. Also noticeable is the low index for seeds imbibed in water and a relatively high index for the seeds imbibed in the 200cH dilution that was exposed to the television set.



## 4.5 Root Lengths

### 4.5.1 Inter group comparisons between all controls

Table 4.4 One way ANOVA between all controls with regard to root lengths

	Sum of squares	df	Mean Square	F	P
Between Groups	10.689	3	3.563	11.311	.003
Within Groups	2.520	8	.315		
Total	13.209	11			

In Table 4.4 the P value is less than the level of significance, indicating a difference between the control groups.

Table 4.5 Tukey's multiple comparisons between control with regard to root lengths

		P	H <sub>0</sub>
Water	GA (0.5g/L)	.093	Accept
	HGA 4cH	1.000	Accept
	HGA 200cH	.062	Accept
GA (0.5g/L)	HGA 4cH	.103	Accept
	HGA 200cH	.002	Reject
HGA 4cH	HGA 200cH	.056	Accept

Table 4.5 shows that the only difference was between GA<sub>3</sub> (0.5g/L<sup>-1</sup>) and HGA<sub>3</sub> 200cH. The HGA<sub>3</sub> 200cH, however, did not show a difference relative to water, indicating that the homoeopathic dilutions were not biologically active. Therefore no further comparisons with the treatment groups could be made. However the P value between water and HGA<sub>3</sub> 200cH is not much higher than 0.05, therefore it was suggested by the statistician (Thomas, 2001) that a more sensitive LSD multiple comparison be done.



Table 4.6 LSD multiple comparison between all controls with regard to root length

		P	H <sub>0</sub>
Water	GA (0.5g/L)	.025	Reject
	HGA 4cH	.944	Accept
	HGA 200cH	.016	Reject
GA (0.5g/L)	HGA 4cH	.027	Reject
	HGA 200cH	.000	Reject
HGA 4cH	HGA 200cH	.014	Reject

Table 4.6 shows that this test revealed a difference between water and the GA<sub>3</sub> (0.5g/L<sup>-1</sup>) control and the HGA<sub>3</sub> 200cH control, indicating that the HGA<sub>3</sub> 200cH dilution was biologically active as it was having an effect on the seeds that was statistically different from that of water.

#### 4.5.2 Inter group comparisons between controls and 4cH treatment groups

Table 4.7 One way ANOVA between controls and 4cH treatment groups with regard to root length

	Sum of squares	df	Mean Square	F	P
Between Groups	38.380	7	5.483	21.859	.000
Within Groups	4.013	16	.251		
Total	42.393	23			

Table 4.7 shows that between these groups there is a difference as the P value is less than the fixed level of significance.



Table 4.8 LSD multiple comparison between controls and 4cH treatment groups with regard to root length

		P	H <sub>0</sub>
Water	GA (0.5g/L)	.007	Reject
	HGA 4cH	.936	Accept
	HGA 200cH	.003	Reject
	Moth 4cH	.022	Reject
	Camphor 4cH	.000	Reject
	Cell 4cH	.631	Accept
	TV 4cH	.749	Accept
GA (0.5g/L)	HGA 4cH	.008	Reject
	HGA 200cH	.000	Reject
	Moth 4cH	.576	Accept
	Camphor 4cH	.000	Reject
	Cell 4cH	.019	Reject
	TV 4cH	.014	Reject
HGA 4cH	HGA 200cH	.003	Reject
	Moth 4cH	.026	Reject
	Camphor 4cH	.000	Reject
	Cell 4cH	.689	Accept
	TV 4cH	.810	Accept
HGA 200cH	Moth 4cH	.000	Reject
	Camphor 4cH	.000	Reject
	Cell 4cH	.001	Reject
	TV 4cH	.002	Reject
Moth 4cH	Camphor 4cH	.000	Reject
	Cell 4cH	.058	Accept
	TV 4cH	.043	Reject
Camphor 4cH	Cell 4cH	.000	Reject
	TV 4cH	.000	Reject
Cell 4cH	TV 4cH	.873	Accept

In Table 4.8 the camphor 4cH treatment group and the HGA<sub>3</sub> 200cH group show a statistical difference when compared with all the other groups. The mothball 4cH treatment group shows differences with all the groups except GA<sub>3</sub> (0.5g/L<sup>-1</sup>) and cell 4cH. The GA<sub>3</sub> (0.5g/L<sup>-1</sup>) group shows a difference with HGA<sub>3</sub> 4cH, HGA<sub>3</sub> 200cH, Cell 4cH, and TV 4cH groups.



#### 4.5.3 Inter group comparison between controls and 200cH treatment groups

Table 4.9 One way ANOVA between controls and 200cH treatment groups with regard to root length

	Sum of squares	df	Mean Square	F	P
Between Groups	30.490	7	4.356	13.541	.000
Within Groups	5.147	16	.322		
Total	35.636	23			

Table 4.9 shows that there was a statistical difference between these groups as the P value is less than the level of significance.



Table 4.10 LSD Multiple comparison between controls and 200cH treatment groups with regard to root length

		P	H <sub>0</sub>
Water	GA (0.5g/L)	.015	Reject
	HGA 4cH	.944	Accept
	HGA 200cH	.008	Reject
	Moth 200cH	.000	Reject
	Camphor 200cH	.000	Reject
	Cell 200cH	.724	Accept
	TV 200cH	.621	Accept
GA (0.5g/L)	HGA 4cH	.017	Reject
	HGA 200cH	.000	Reject
	Moth 200cH	.117	Accept
	Camphor 200cH	.053	Accept
	Cell 200cH	.030	Reject
	TV 200cH	.040	Reject
HGA 4cH	HGA 200cH	.007	Reject
	Moth 200cH	.001	Reject
	Camphor 200cH	.000	Reject
	Cell 200cH	.777	Accept
	TV 200cH	.672	Accept
HGA 200cH	Moth 200cH	.000	Reject
	Camphor 200cH	.000	Reject
	Cell 200cH	.004	Reject
	TV 200cH	.003	Reject
Moth 200cH	Camphor 200cH	.672	Accept
	Cell 200cH	.001	Reject
	TV 200cH	.001	Reject
Camphor 200cH	Cell 200cH	.000	Reject
	TV 200cH	.001	Reject
Cell 200cH	TV 200cH	.887	Accept

Table 4.10 shows that both the mothball 200cH and camphor 200cH groups differ from all the other treatment groups except GA<sub>3</sub> (0.5g/L<sup>-1</sup>). The GA<sub>3</sub> (0.5g/L<sup>-1</sup>) group also differs from the cell and TV 200cH groups. The HGA<sub>3</sub> 200cH group shows a difference when compared with all the other groups.



#### 4.5.4 Inter group comparison between 4cH and 200cH treatment groups

Table 4.11 One way ANOVA between 4cH and 200cH treatment groups with regard to root length

	Sum of squares	df	Mean Square	F	P
Between Groups	30.570	7	4.367	16.960	.000
Within Groups	4.120	16	.258		
Total	34.690	23			

Table 4.11 shows that there was a difference between these groups as the P value is less than the level of significance.



4.12 Tukey's multiple comparison between 4cH and 200cH treatment groups with regard to root length

		P	H <sub>0</sub>
Moth 4cH	Camphor 4cH	.001	Reject
	Cell 4cH	.504	Accept
	TV 4cH	.415	Accept
	Moth 200cH	.298	Accept
	Camphor 200cH	.138	Accept
	Cell 200cH	.459	Accept
	TV 200cH	.551	Accept
Camphor 4cH	Cell 4cH	.000	Reject
	TV 4cH	.000	Reject
	Moth 200cH	.121	Accept
	Camphor 200cH	.256	Accept
	Cell 200cH	.000	Reject
	TV 200cH	.000	Reject
Cell 4cH	TV 4cH	1.000	Accept
	Moth 200cH	.008	Reject
	Camphor 200cH	.003	Reject
	Cell 200cH	1.000	Accept
	TV 200cH	1.000	Accept
TV 4cH	Moth 200cH	.006	Reject
	Camphor 200cH	.002	Reject
	Cell 200cH	1.000	Accept
	TV 200cH	1.000	Accept
Moth 200cH	Camphor 200cH	1.000	Accept
	Cell 200cH	.007	Reject
	TV 200cH	.009	Reject
Camphor 200cH	Cell 200cH	.003	Reject
	TV 200cH	.004	Reject
Cell 200cH	TV 200cH	1.000	Accept

Table 4.12 shows there was a difference between the camphor 4cH group and all the other treatment groups except the mothball and camphor 200cH groups. There was a difference between the mothball 4cH and cell 4cH groups. The camphor and mothball 200cH group also showed differences with the cell and TV 4cH and 200cH groups.



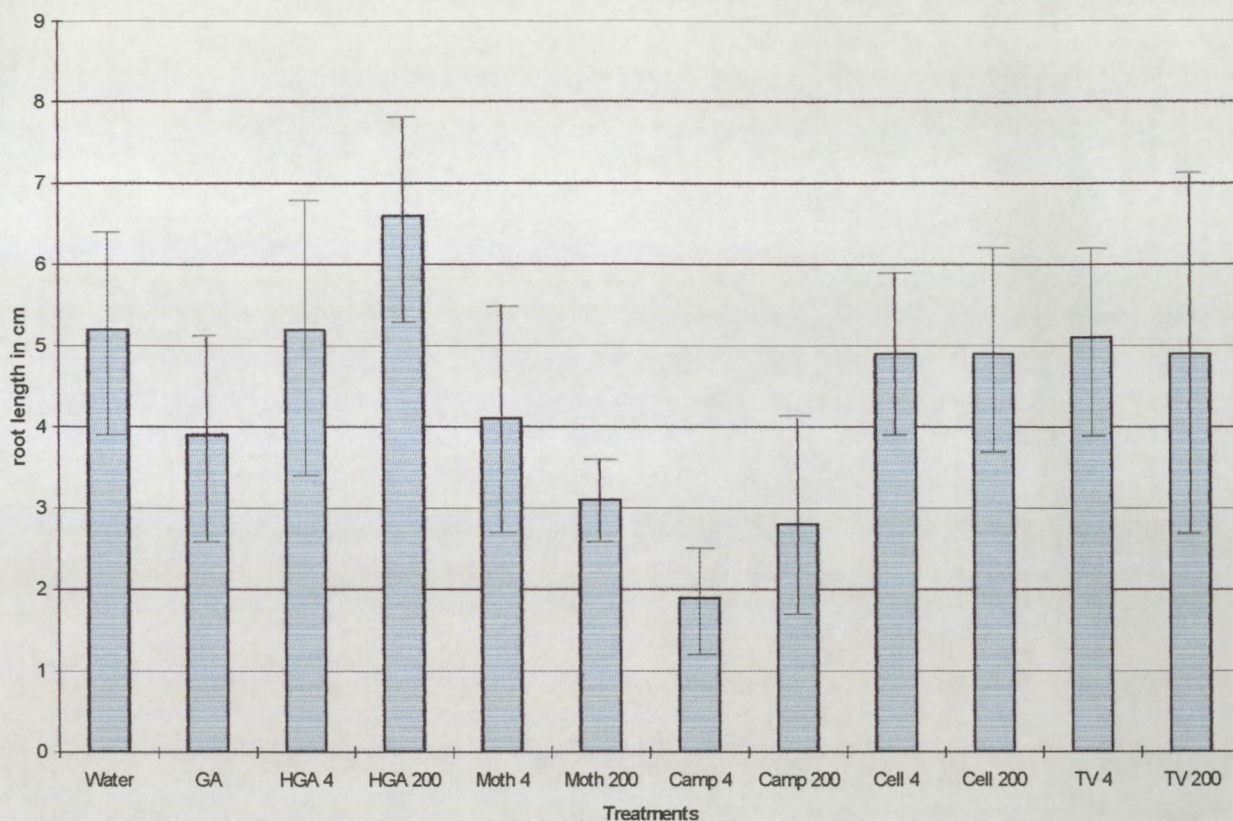


Figure 4.3 Bar chart of root length averages. Abbreviations as for figure 4.2. Error bars show 95% confidence intervals.

Trends to note in Figure 4.3 are the low root growth shown by the seeds imbibed in the  $GA_3$  ( $0.5g/L^{-1}$ ) solution, the high root growth shown by the seeds imbibed in the HGA<sub>3</sub> 200cH dilution, and the low root growth of the seeds imbibed in the homoeopathically prepared dilutions that were exposed individually to camphor and to mothballs respectively.



## 4.6 Shoot lengths

### 4.6.1 Inter group comparisons among all controls

Table 4.13 One way ANOVA between all controls with regard to shoot lengths

	Sum of squares	df	Mean Square	F	P
Between Groups	10.342	3	3.447	2.043	.186
Within Groups	13.500	8	1.687		
Total	23.842	11			

Table 4.13 shows the P value is greater than 0.05, therefore there were no differences between these groups. The lack of difference between the controls means that the controls could not be compared to the treatment groups. However the statistics have been included as the differences between the seeds imbibed in the dilutions exposed to mothballs and camphor, were very interesting.

### 4.6.2 Inter group comparison between controls and 4cH treatment groups

Table 4.14 One way ANOVA between controls and 4cH treatment groups with regard to shoot length

	Sum of squares	df	Mean Square	F	P
Between Groups	76.393	7	10.913	11.458	.000
Within Groups	15.240	16	.952		
Total	91.633	23			

Table 4.14 indicates that there was a difference between these groups.



Table 4.15 Tukey's multiple comparison between controls and 4cH treatment groups with regard to shoot length

		P	H <sub>0</sub>
Water	GA (0.5g/L)	.084	Accept
	HGA 4cH	.979	Accept
	HGA 200cH	.959	Accept
	Moth 4cH	.871	Accept
	Camphor 4cH	.002	Reject
	Cell 4cH	1.000	Accept
	TV 4cH	1.000	Accept
GA (0.5g/L)	HGA 4cH	.370	Accept
	HGA 200cH	.436	Accept
	Moth 4cH	.007	Reject
	Camphor 4cH	.000	Reject
	Cell 4cH	.078	Accept
	TV 4cH	.036	Reject
HGA 4cH	HGA 200cH	1.000	Accept
	Moth 4cH	.370	Accept
	Camphor 4cH	.000	Reject
	Cell 4cH	.974	Accept
	TV 4cH	.853	Accept
HGA 200cH	Moth 4cH	.311	Accept
	Camphor 4cH	.000	Reject
	Cell 4cH	.951	Accept
	TV 4cH	.794	Accept
Moth 4cH	Camphor 4cH	.026	Reject
	Cell 4cH	.887	Accept
	TV 4cH	.984	Accept
Camphor 4cH	Cell 4cH	.002	Reject
	TV 4cH	.005	Reject
Cell 4cH	TV 4cH	1.000	Accept

Table 4.15 shows that there was a difference between the seeds that were imbibed in the dilution exposed to camphor and all the other treatment groups. There was also a difference between the seeds imbibed in the 4cH dilution exposed to mothballs and the seeds that were imbibed in the GA<sub>3</sub> (0.5g/L<sup>-1</sup>) solution.



#### 4.6.3 Inter group comparison between controls and 200cH treatment groups

Table 4.16 One way ANOVA between controls and 200cH treatment groups with regard to shoot length

	Sum of squares	df	Mean Square	F	Sig.
Between Groups	69.978	7	9.997	7.668	.000
Within Groups	20.860	16	1.304		
Total	90.838	23			

Table 4.16 shows that there was a difference between these groups as the P value is less than the level of significance.



Table 4.17 Tukey's multiple comparison between controls and 200cH treatment groups with regard to shoot length

		Sig.	H <sub>0</sub>
Water	GA (0.5g/L)	.187	Accept
	HGA 4cH	.992	Accept
	HGA 200cH	.982	Accept
	Moth 200cH	.115	Accept
	Camphor 200cH	.101	Accept
	Cell 200cH	1.000	Accept
	TV 200cH	1.000	Accept
GA (0.5g/L)	HGA 4cH	.551	Accept
	HGA 200cH	.615	Accept
	Moth 200cH	.001	Reject
	Camphor 200cH	.001	Reject
	Cell 200cH	.147	Accept
	TV 200cH	.095	Accept
HGA 4cH	HGA 200cH	1.000	Accept
	Moth 200cH	.027	Reject
	Camphor 200cH	.023	Reject
	Cell 200cH	.978	Accept
	TV 200cH	.927	Accept
HGA 200cH	Moth 200cH	.022	Reject
	Camphor 200cH	.019	Reject
	Cell 200cH	.961	Accept
	TV 200cH	.891	Accept
Moth 200cH	Camphor 200cH	1.000	Accept
	Cell 200cH	.147	Accept
	TV 200cH	.221	Accept
Camphor 200cH	Cell 200cH	.130	Accept
	TV 200cH	.198	Accept
Cell 200cH	TV 200cH	1.000	Accept

Table 4.17 shows that the differences were between the seeds imbibed in the 200cH dilutions exposed to camphor and mothballs and the seeds imbibed in the 4cH and 200cH unexposed dilutions and the GA<sub>3</sub> (0.5g/L<sup>-1</sup>) solution.



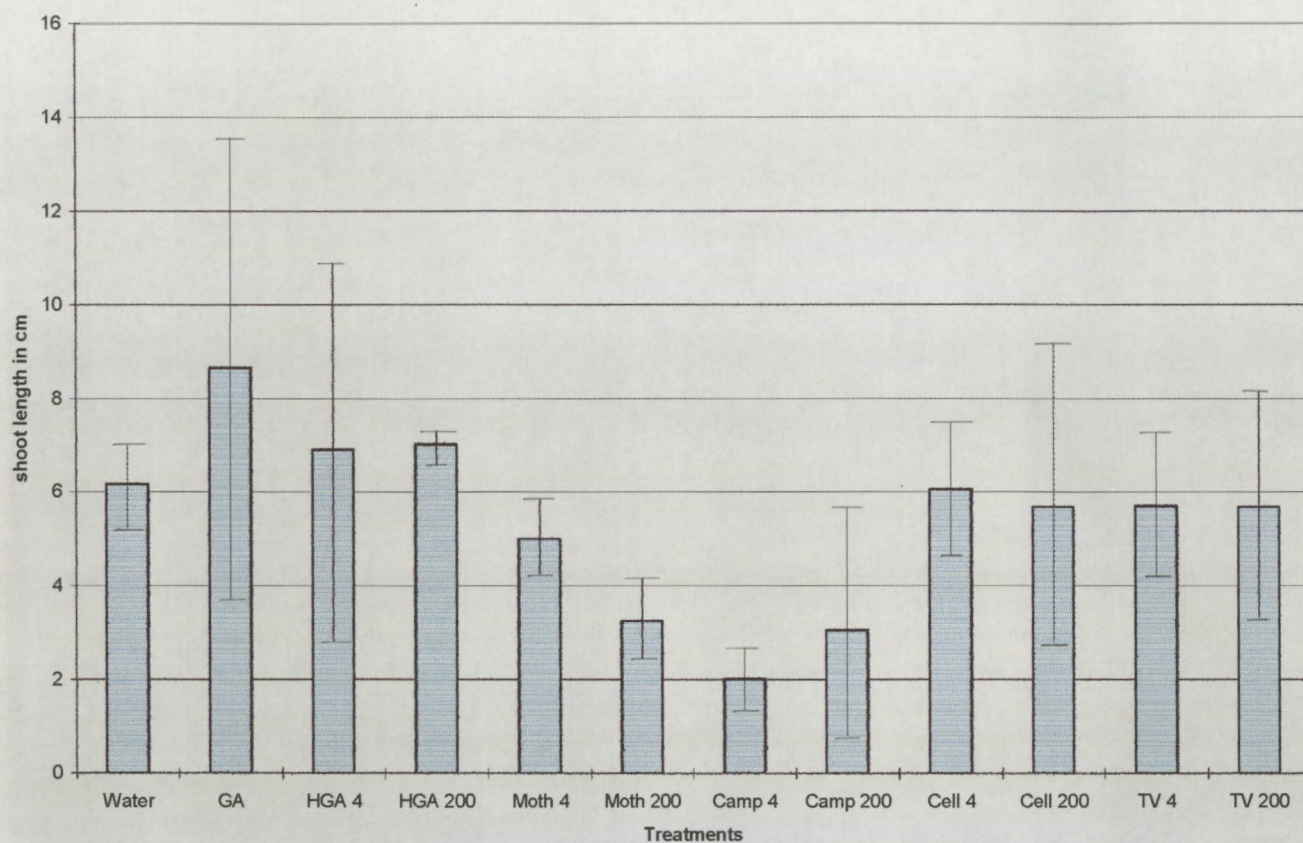


Figure 4.4 Bar chart of shoot length averages. Abbreviations as for Figure 4.2. Error bars show 95% confidence intervals.

Interesting trends evident from Figure 4.4 are the low shoot length averages of the seeds imbibed in dilutions exposed to mothballs and camphor respectively. Also noted again was the higher shoot length average of the seeds imbibed in  $GA_3 (0.5g/L^{-1})$  due to the promotion of growth.



## 4.7 Seedling dry mass

### 4.7.1 Inter group comparison between all controls

Table 4.18 One way ANOVA between all controls with regard to seedling dry mass

	Sum of squares	df	Mean Square	F	P
Between Groups	2.092E-05	3	6.972E-06	6.972	.013
Within Groups	8.000E-06	8	1.000E-06		
Total	2.892E-05	11			

In table 4.18 The P value = 0.13, indicating that there was a difference between these groups.

Table 4.19 Tukey's multiple comparison between controls with regard to seedling dry mass

		P	H <sub>0</sub>
Water	GA (0.5g/L)	.081	Accept
	HGA 4cH	1.000	Accept
	HGA 200cH	.414	Accept
GA (0.5g/L)	HGA 4cH	.081	Accept
	HGA 200cH	.009	Reject
HGA 4cH	HGA 200cH	.414	Accept

Table 4.19 shows that the GA<sub>3</sub> (0.5g/L<sup>-1</sup>) group differed from the HGA<sub>3</sub> 200cH group. There was no difference between the water and the homoeopathic dilutions to support a biological activity of the homoeopathic dilutions. Therefore no further comparisons could be performed between the controls and the treatment groups.



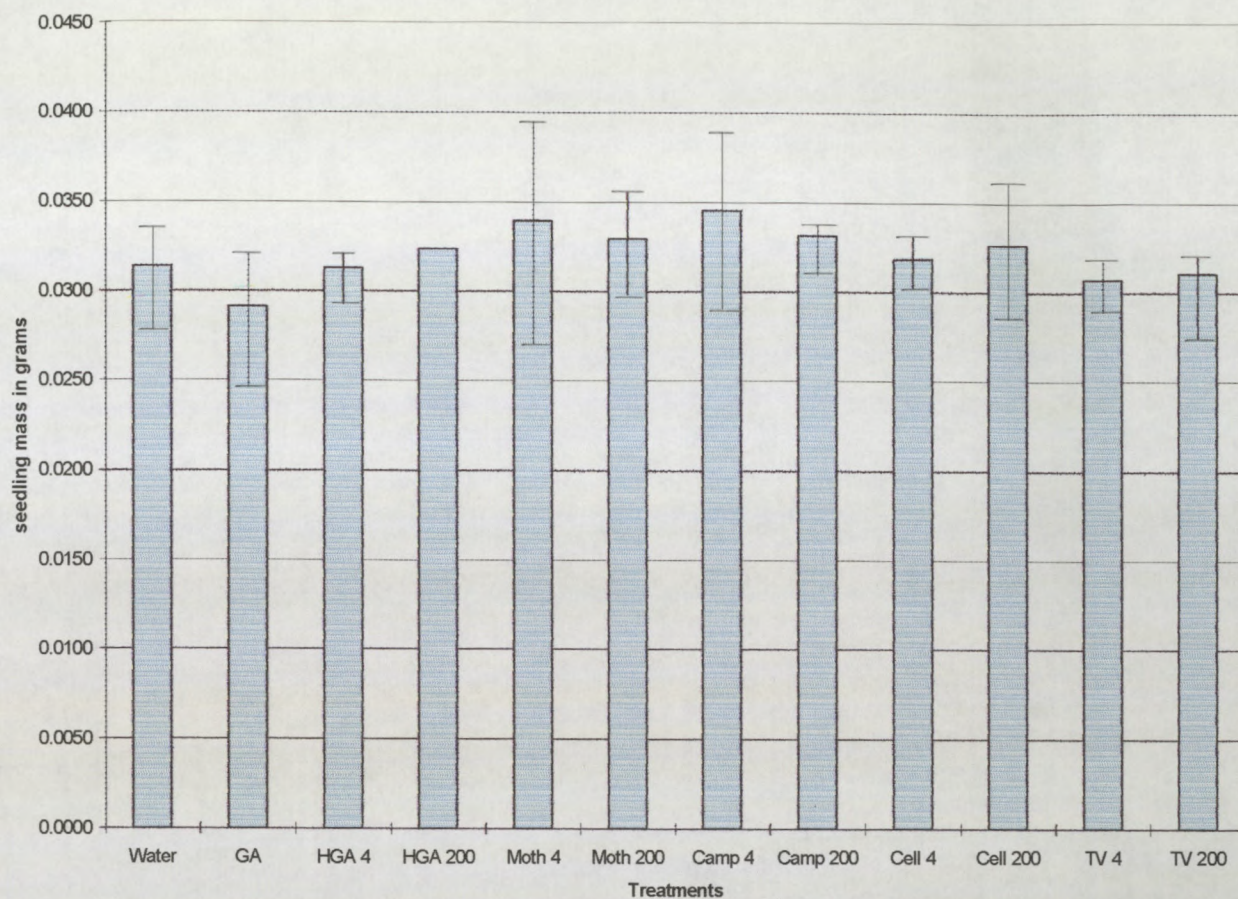


Figure 4.5 Bar chart of seedling dry mass averages. Abbreviations as for Figure 4.2. Error bars show 95% confidence intervals.



#### 4.8 Plates

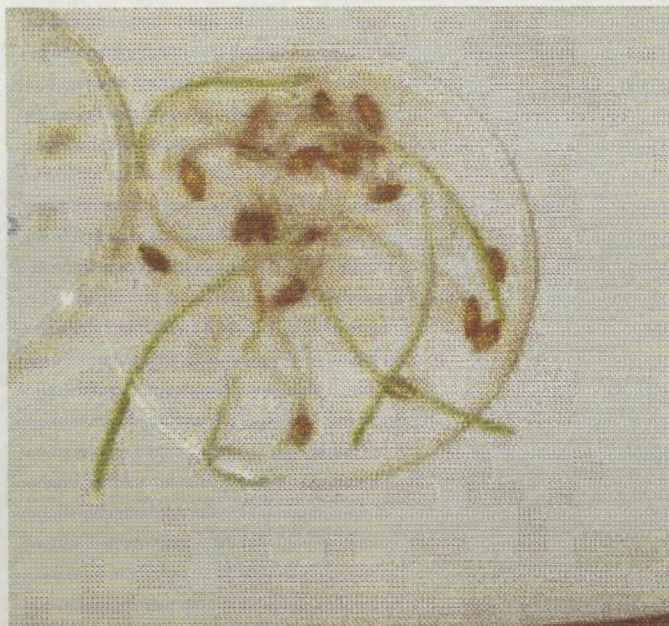


Plate 4.1 Seedlings from seeds imbibed in water alone. Day seven of the germination trial.



Plate 4.2 Seedlings from seeds imbibed in  $\text{GA}_3$  ( $0.5\text{g/L}^{-1}$ ) solution. Day seven of the germination trial





Plate 4.3 Seedlings from seeds imbibed in HGA<sub>3</sub> 200cH dilution. Day seven of the germination trial.

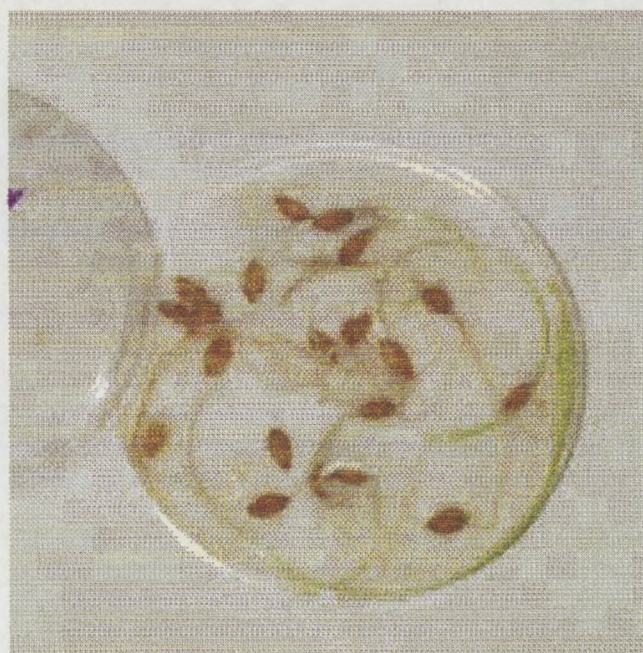


Plate 4.4 Seedlings from seeds imbibed in camphor 4cH dilution. Day seven of the germination trial.



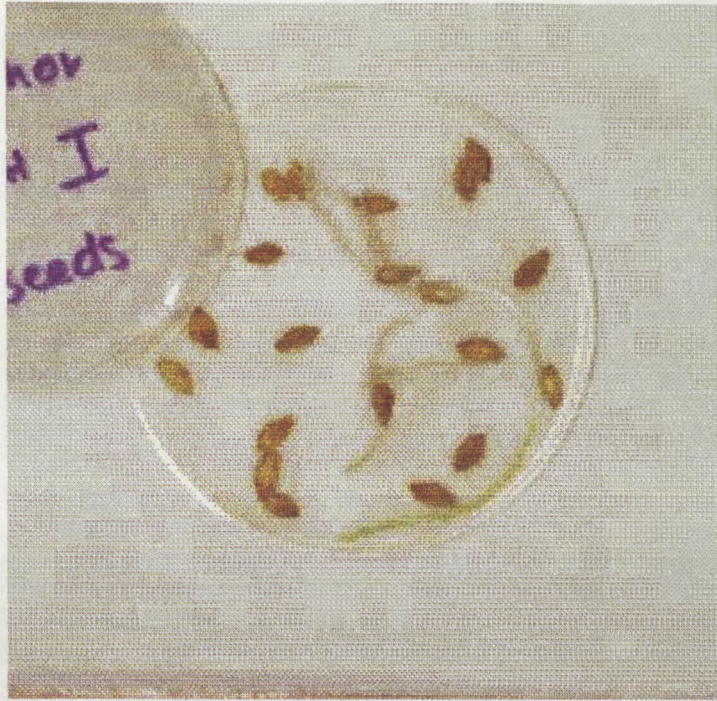


Plate 4.5 Seedlings from seeds imbibed in camphor 200cH dilution. Day seven of the germination trial.

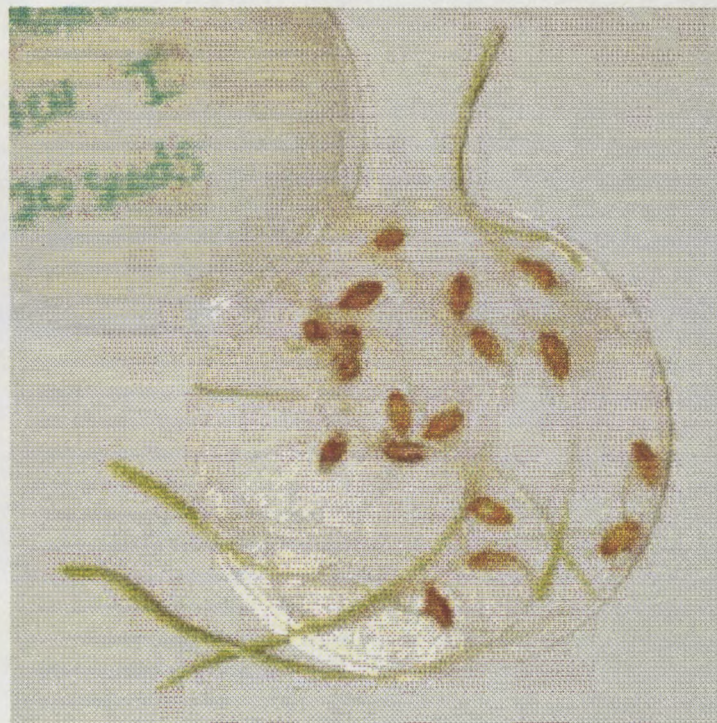


Plate 4.6 Seedlings from seeds imbibed in Mothball 4cH dilution. Day seven of the germination trial.





Plate 4.7 Seedlings from seeds imbibed in television 200cH dilution. Day seven of the germination trial.

## CHAPTER FIVE

### DISCUSSION OF THE RESULTS

#### 5.1 Germination percentage and germination index

As can be seen in Figure 4.1 the average germination percentage for water was only 48%, which is a marked drop from the initial 99% that was measured before the experiment was initiated. There are many factors that could have influenced the germinability of the seeds but the most likely would be the inappropriate storage of the seeds. The seeds were stored in a freezer, as this is an acceptable means of storage and sometimes used to break dormancy in orthodox seeds. It was also the method used by Steele (1999). However the seeds may have absorbed water during transport from the Western Cape to Durban or during thawing, so that on freezing, ice crystals formed in the seeds. These would have ruptured intracellular membranes, causing leakage of the intracellular contents. Such damage would lead to reduced vigour and increased fungal contamination, as observed.

As expected, seeds imbibed in  $GA_3$  ( $0.5g/L^{-1}$ ) solution showed the highest germination, as gibberellic acid promotes this process. This marked increase in growth can be seen in Figures 4.1 and 4.2. The increased size of the seedlings as a result of the gibberellic acid influence can be seen in Plate 4.2.

Statistically, there was no difference between the  $HGA_3$  (4cH and 200cH) dilutions and water. Despite the lack of statistical support for differences between controls and treatments, some interesting trends were noticed.

Seeds imbibed in the HGA<sub>3</sub> 4cH and 200cH dilutions showed greater mean germination percentages than did the seeds imbibed in water (see Figure 4.1 and 4.2). Seeds germinated in the 4cH dilutions that were exposed to camphor and mothballs showed markedly lower mean germination percentages than those of the other treatment groups. The seeds imbibed in the 200cH dilution that had been exposed to camphor showed a germination percentage similar to the seeds imbibed in the 4cH dilution, indicating that the camphor itself had a direct negative effect on germination of the seeds. Plates 4.4 and 4.5 show the smaller size of seedlings as a result of this inhibition.

Seeds imbibed in the 200cH dilution exposed to the television set had a germination percentage very similar to seeds germinated in the HGA<sub>3</sub> 200cH control dilution, a trend which can be seen in Figures 4.1 and 4.2. This finding indicates that the television exposure may have had a tendency to stimulate the growth of the seeds.

## **5.2 Root length**

The One way ANOVA test results showed no difference between the water and the HGA<sub>3</sub> control groups, indicating that the HGA<sub>3</sub> controls were not having an effect and therefore the biological activity of the homoeopathic treatment dilutions was questionable. On closer examination, however, it was noted that the P value differences between the water and the HGA<sub>3</sub> 200cH control groups were very close to the level of significance. It was therefore recommended that a more sensitive multiple comparison test be performed (Thomas, 2001). A LSD test showed that there was a significant difference

between the water and HGA<sub>3</sub> 200cH control groups. This difference can be seen in Figure 4.3. This meant that the HGA<sub>3</sub> 200cH dilution had a significant biological effect on root growth.

The seedlings that were exposed to the GA<sub>3</sub> (0.5g/L<sup>-1</sup>) solution showed a very poor root growth. This finding is similar to that of Him Lock (2001) and Pieterse (2001). The reason for the inhibited root growth is likely to be due to the concentration of the GA<sub>3</sub> solution, which was 10 to 100 times higher than the optimum that is needed ( $10^{-4}$  to  $10^{-5}$  mol/L in the presence of 20 mmol/L Ca<sup>2+</sup>) and excess GA<sub>3</sub> can cause inhibition of the roots (Wilkins, 1984:42). The concentration used in this experiment was the same used by Him Lock (2001) to ensure that the results between the studies were comparable. Clearly, the concentration of the GA<sub>3</sub> solution would have to be reconsidered in future applications of this experimental model.

In this study the HGA<sub>3</sub> 200cH dilution seemed to stimulate the growth of the roots. This finding is very interesting to homeopathy as it is known that when a substance is diluted beyond its toxicological point it has an opposite effect to what it would have in crude form, a principle known as hormesis (Gaier, 1991:275).

The EMR exposed dilutions all had increased root growth of the seedlings imbibed in these dilutions, although final root lengths were not significantly different from the HGA<sub>3</sub> and the water controls.

Seeds imbibed in the HGA<sub>3</sub> 4cH dilution had similar root lengths to those of the seeds imbibed in water only. Hence the HGA<sub>3</sub> 4cH dilution did not have an effect on seedling root growth.

Seeds imbibed in the 4cH dilution that had been exposed to camphor showed a difference when compared with all the other groups. From Figure 4.3 it can be seen that the roots of the seedlings grown in this dilution were much shorter than all the other groups including the GA<sub>3</sub> (0.5g/L<sup>-1</sup>) group (see Plate 4.3). Both the seed groups imbibed in the 200cH dilutions that had been exposed to camphor and mothballs showed differences relative to all the other treatment groups except the GA<sub>3</sub> (0.5g/L<sup>-1</sup>) group. These two dilutions inhibited root growth of the seedlings.

These results are very interesting. The HGA<sub>3</sub> 200cH dilution group exhibited a stimulatory biological effect. The camphor and mothball dilutions (4cH and 20cH) also exhibited biological effects, but in the opposite direction, *i.e.* inhibitory. This inhibitory effect is consistent with trends observed in all the other parameters.

The hypothesis being investigated in this study is that external influences can neutralize or negate homoeopathic dilutions (Dancu, 1996:172-173; Adams, 1996:56-57; Kayne, 1997:181; Vithoulkas, 1980:264; Helios, 2001).

In other words, it is suggested that an external influence can stop a homoeopathic dilution from working.

Results from the root length parameter in this study support this hypothesis, but go one step further. The action of the HGA<sub>3</sub> 200cH dilution is negated by exposure to camphor and mothballs in that the dilution no longer has a stimulatory effect. But it does not stop there - the external influences on the dilutions have a negative effect in themselves. Thus, the homoeopathic dilution is negated by virtue of this action of the external influence. The only way to explain this is to say that somehow the external influence has diffused the dilution and changed it, thus negating it.

This then raises the question of how the influence occurred. In this case, there was no direct contact between the camphor and mothballs and the homoeopathic dilution, and volatile oils are only sparingly soluble in water.

This suggests that only very low concentrations of the oils should have diffused into the dilutions. However, the characteristic odours of these volatile oils were noticed in the exposed dilutions when the stock bottles were opened to extract the aliquots required for experimental purposes. This would imply that molecules of these substances had markedly diffused into the HGA<sub>3</sub> dilutions during the period of exposure. This type of interaction warrants further investigation in a separate study.

### **5.3 Shoot length**

The seeds germinated in the control solutions showed similar shoot lengths to each other, with no statistical difference. This meant that the hypothesis of biological activity of the HGA<sub>3</sub> treatment dilutions could not be supported, and



no conclusions could be drawn regarding the influence of external influences on such activity.

When comparing average seedling shoot length, seeds imbibed in the 4cH dilution that had been exposed to camphor had the lowest average, suggesting that camphor itself had a possible inhibitory effect on the seeds (Figure 4.5 and Plate 4.4). This reflects results for germination percentage and germination index and root length. The seeds germinated in the 200cH dilution that was exposed to mothballs and camphor had very similar average shoot lengths. From these results it could be suggested that both camphor and mothballs had negatively affected the growth of the seedlings.

The seeds germinated in the EMR-exposed dilutions had average shoot lengths that were similar to each other and to water. There appears to be no inhibitory effect on the seeds by EMR.

#### **5.4 Seedling dry mass**

Visually there was a difference in seedling size between all the dilution groups (See Plates 4.1 – 4.7). However, statistically there was no difference between the weight of the seedlings in the control groups, meaning that the HGA<sub>3</sub> dilutions had the same effect as water. This indicated that it was not the GA<sub>3</sub> in the homoeopathic dilutions that was responsible for the growth of the seeds but rather the water content. Confirmation of the trend that camphor and mothballs inhibited growth can be seen in the higher average seedling masses for these groups. The higher average seedling masses was due to

the unused seed reserves in the seed, that then made these seedlings heavier than the fully grown seedlings (see Plates 4.4 - 4.7).

### **5.5 The experimental model**

The experimental model of Him Lock (2001) was used without modification to allow for comparisons of results between that study, the present one and the concurrent study of Pieterse (2001). It is clear that the experimental model, as used, suffered from a number of shortcomings. These include:

- (1) Incorrect storage of the seeds. This probably caused the vigour of the seeds to fall and facilitated fungal contamination.
- (2) Insufficient imbibing solution which would have caused a further decline in germination. The amount of imbibing solution used by Him Lock (2001) was 12.5ml per 100 seeds. Pieterse (2001) followed the same model, but ran a second germinability test using 12.5ml per 50 seeds and 12.5ml per 100 seeds. The germination rate for 12.5ml per 50 seeds was higher.
- (3) The concentration of the GA<sub>3</sub> solution was in excess of the optimum for seed germination and growth (Wilkins, 1984:42). This caused an inhibitory growth of the seeds especially the roots.
- (4) Incorrect handling of fungus-infected seeds. In the Him Lock model it was recommended that the fungally infected seeds be removed to prevent further contamination. This did not prevent further



contamination. Further research after completion of the experimental stage revealed that a more appropriate method would have been to remove the good seeds onto clean new filter paper at each count (Erdey, 2001). The reason behind this is that the fungus sends out rhizomes into the filter paper which remain after removal of infected seeds. Thus, the fungus continues to spread even after the apparent source of contamination has been removed

(5) Possible pre-existing fungal spores. In normal seed experiments, seeds are first washed in a solution of Jik<sup>®</sup> bleach to combat any prior fungal contamination. However this step was excluded in this study, because Jik<sup>®</sup> residue itself could have had a negating influence on the subtle homoeopathic dilutions. This could be investigated and possibly be a control for future experiments.

Due to these shortcomings, comparisons between Him Lock's study (2001) and this one are not very reliable. This model should be modified in light of the results of this study and that of Pieterse (2001) before being used in the future for the study of the biological effects of homoeopathic dilutions and the influences of other external influences on them.

## CHAPTER SIX

### CONCLUSIONS AND RECOMMENDATIONS

#### 6.1 Conclusions

As compared to water, the only unexposed homoeopathic dilution to demonstrate a significant biological effect was that of HGA<sub>3</sub> 200cH, on root length. The results presented here therefore provide limited support for the biological effect of homoeopathic dilutions.

The only treatment groups to demonstrate significant effects were the HGA<sub>3</sub> 200cH dilutions exposed to camphor and mothballs in all the parameters. They demonstrated a significant inhibitory effect as compared to the controls. One can conclude, therefore, that an external influence can have an effect on a homoeopathic dilution. However, from this study, it is not possible to conclude that the camphor and mothballs neutralized or negated the homoeopathic dilutions as such. It is possible that camphor or naphthalene diffused into the exposed homoeopathic dilutions and exerted their inhibitory effects directly.

Because of the confounding factors discussed in 5.5, these conclusions cannot be stated unequivocally.

## **6.2 Recommendations**

- 1) The model used for this study requires modification before being used in further studies. Any further research using the seed germination model should take into account comments contained in section 5.5.
- 2) The same external influences examined in this study should be examined by means of the Phadebas-amylase barley seed model as per Barnes and Blakeney (1974); Caines and de Villiers (1986); Bruni (2000); Couchman (2001); and Balding (2001).
- 3) The modified seed germination model, and/or the Phadebas model should be used to examine the effects of camphor and mothballs in themselves, ranging from crude form through different dilution potencies. This would build on findings from the present study and that of Hopkins (1998).

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## Home Germination Test (Nuss, 1997)<sup>7</sup>

### Equipment:

- 1) Germination Towel (Paper towel).
- 2) Zip-lock plastic bag.
- 3) Pencil.
- 4) Cool dark place to germinate seeds.

### Methodology:

- 1) The name of seed, date and follow up dates were written on the corner of the towel in pencil.
- 2) The towels were moistened, but not too much as this may have led to rotting of the seeds.
- 3) The number of seeds required were placed onto paper towels. (In this study 100 seeds were used, meaning that germination of 1 seed represented 1% germination). The seeds were placed 2 – 5 cm apart.
- 4) Another moistened towel was placed over the top and the towels were rolled up.
- 5) The rolled towels were then placed into plastic bags, to prevent desiccation.
- 6) The plastic bags were placed into an area that is dark and cool. (In this study a germination room, at 20°C was used).
- 7) Each day the moisture of the towels was monitored.

8) After 4 and 7 days the number of healthy sprouts with strong roots and shoots were counted. The germination percentage was then calculated.

Results:

Group 1: Seeds taken from the Sensako seed lot showed 99% germination of which 6% had fungal growth and 1% was dead.

Group 2: Seeds taken from SR Brown and Company seed lot, showed 100% germination and no fungal growth.



## Appendix 2

### Raw data

Root Length	Rep 1	Rep 2	Rep 3	Total seeds	Average
Water	174.6	278.5	279.2	140	5.2cm
GA <sub>3</sub> (0.5g/L <sup>-1</sup> )	208.9	236.4	240.4	176	3.9cm
HGA <sub>3</sub> 4cH	230.4	207.3	334.7	149	5.2cm
HGA <sub>3</sub> 200cH	421.2	328.1	325.4	163	6.6cm
Moth 4cH	152.5	129	142.4	103	4.1cm
Camp 4cH	38.3	56.2	49.4	75	1.9cm
Cell 4cH	276.8	228.8	237.2	151	4.9cm
TV 4cH	312.5	229.7	218.3	150	5.1cm
Moth 200cH	192.2	126.3	166.3	154	3.1cm
Camp 200cH	57.4	61.8	69.4	67	2.8cm
Cell 200cH	148	237.3	255.8	131	4.9cm
TV 200cH	329	248	525.1	168	4.9cm

Shoot length	Rep 1	Rep 2	Rep 3	Total seeds	Average
Water	217.2	314.9	331.1	140	6.2cm
GA <sub>3</sub> (0.5g/L <sup>-1</sup> )	478.7	440.8	603.6	176	8.7cm
HGA <sub>3</sub> 4cH	274.5	267.2	497.8	149	7.0cm
HGA <sub>3</sub> 200cH	400.7	359.7	370.5	163	6.9cm
Moth 4cH	172.1	177.5	166	103	5.0cm
Camp 4cH	40.9	57.2	52.5	75	2.0cm
Cell 4cH	344.4	288.7	281.1	151	6.1cm
TV 4cH	314.3	257.9	283.3	150	5.7cm
Moth 200cH	167.7	149.1	183.5	154	3.2cm
Camp 200cH	64.2	79.5	60.7	67	3.1cm
Cell 200cH	201.2	284.3	259.1	131	5.7cm
TV 200cH	367.3	302.7	283.5	168	5.7cm

Mass	Rep 1	Rep 2	Rep 3	Total seeds	Average
Water	1.1720	1.5086	1.7100	140	0.0314g
GA <sub>3</sub> (0.5g/L <sup>-1</sup> )	1.8303	1.7655	1.5280	176	0.0291g
HGA <sub>3</sub> 4cH	1.4264	1.4527	1.7751	149	0.0312g
HGA <sub>3</sub> 200cH	1.9269	1.6381	1.7102	163	0.0324g
Moth 4cH	1.1672	1.2535	1.0724	103	0.0339g
Camp 4cH	0.7349	1.0132	0.8397	75	0.0345g
Cell 4cH	1.8890	1.4045	1.5131	151	0.0318g
TV 4cH	1.7563	1.5222	1.3319	150	0.0307g
Moth 200cH	1.9739	1.3875	1.7108	154	0.0329g
Camp 200cH	0.5570	0.7126	0.9502	67	0.0331g
Cell 200cH	0.9202	1.4945	1.8643	131	0.0327g
TV 200cH	1.7435	1.6405	1.8331	168	0.0311g

Percentage	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Water	22.7	35.3	43.0	44.7	45.0	45.7	47.7
GA <sub>3</sub> (0.5g/L <sup>-1</sup> )	50.3	59.0	61.0	61.7	61.7	62.0	62.3
HGA <sub>3</sub> 4cH	26.3	41.0	48.0	50.7	51.7	52.0	52.0
HGA <sub>3</sub> 200cH	27.3	39.0	47.3	52.3	54.3	54.3	57.0
Moth 4cH	6.3	19.0	25.3	32.3	34.7	34.7	35.0
Camp 4cH	1.3	8.3	15.3	20.3	21.3	22.3	23.3
Cell 4cH	21.3	38.0	42.7	48.7	50.7	52.0	52.3
TV 4cH	24.7	40.7	45.7	47.7	50.0	50.3	51.0
Moth 200cH	10.0	29.7	43.0	50.3	51.0	51.3	53.3
Camp 200cH	3.0	10.3	16.0	24.3	25.0	25.0	26.3
Cell 200cH	18.7	31.0	36.0	41.0	43.0	43.7	44.3
TV 200cH	33.0	50.0	53.0	55.0	57.0	57.3	57.7

Germ Index	Rep 1	Rep 2	Rep 3	Average
Water	0.3500	0.3914	0.4757	.04057
GA <sub>3</sub> (0.5g/L <sup>-1</sup> )	0.6200	0.6229	0.5486	0.5971
HGA <sub>3</sub> 4cH	0.3986	0.4343	0.5457	0.4595
HGA <sub>3</sub> 200cH	0.5129	0.4486	0.4600	0.4738
Moth 4cH	0.2657	0.2929	0.2443	0.2676
Camp 4cH	0.1286	0.1457	0.2057	0.1600
Cell 4cH	0.5243	0.3643	0.4214	0.4367
TV 4cH	0.4700	0.4500	0.4086	0.4429
Moth 200cH	0.4100	0.3786	0.4486	0.4124
Camp 200cH	0.1843	0.2000	0.1743	0.1862
Cell 200cH	0.2514	0.3757	0.4771	0.3681
TV 200cH	0.4957	0.5129	0.5471	0.5186