THE EFFECT OF LIGHT AND HEAT ON 4CH AND 200CH POTENCIES OF GIBBERELLIC ACID AS A PROMOTER OF GERMINATION IN BARLEY SEED (*HORDEUM VULGARE*), MEASURED BY A GERMINATION INDEX

BY

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I, Hayley Pieterse, do hereby declare that this minidissertation represents my own work both in concept and execution

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Dedicated to my parents, Dan and Gaye Pieterse

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ABSTRACT

The purpose of this investigation was to evaluate the effects of light and heat on homoeopathic dilutions of gibberellic acid ($cHGA_3$) as measured on *Hordeum vulgare* (barley) seed germination.

Gibberellin is found naturally in the embryo, and is known to break dormancy and promote germination. An exogenous source of gibberellic acid (GA_3) will promote germination further. This study used GA_3 in two homoeopathic dilutions, 4cH and 200cH, to assess the activity of GA_3 at this dilution level.

A previous study showed that application of $cHGA_3$ 4cH and 200cH to barley seeds had a biological effect. This study intended to extend these results by assessing the effect of various external influences on the homoeopathic dilution. There is uncertainty in homoeopathic fields regarding the effect of various external influences on homoeopathic dilutions. The exposed dilutions were tested on the seed model to ascertain whether they maintained their biological action on seed germination after exposure, thus demonstrating the effect of the external influences on the homoeopathic dilution.

Dilutions of $cHGA_3$ 4cH and 200cH were exposed to light from 60 Watt and 200 Watt incandescent light bulbs, respectively, for 24 hours in clear glass bottles. Similar dilutions were exposed to 20°C and 60°C in a water bath for 24 hours. Seeds were imbibed in 12,5ml of the relevant treatment solutions for 24hours. Treatments solutions consisted of a positive control (GA_3 $0.5gL^{-1}$), a negative control (distilled water), unexposed controls ($cHGA_3$ 4cH and 200cH) and exposed dilutions. There were three replicates of 100 seeds in each group. A germination index, root and shoot lengths and dry mass determined seed growth.

Results were inconsistent with previous research; therefore the experiment was repeated, using only the germination index as the measurement criterion.

Data were analysed statistically by Independent Analysis of Variance (SPSS-version 9.0). The results indicated that the only significant difference was between water and 4cH with regards to root length. No significant differences were detected among the other measurements. Since there were no differences between water and $cHGA_3$ 4cH or 200cH for other measurements, further comparisons between exposed and $cHGA_3$ could not be made. With regards to the effects of 4cH on root length, statistically significant differences were noted on seeds treated with $cHGA_3$ 4cH exposed to 60°C when compared to unexposed $cHGA_3$ 4cH. Possible reasons were put forward as to how 60°C may affect the activity of $cHGA_3$ with regards to root length. It was postulated that the heat may cause decoherence in oscillations of the water molecules, resulting in the loss of information of the original solute. The results obtained were not strong enough to make conclusive comments on the effect of light and heat on homoeopathic dilutions.

Errors were noted in the seed model, which did not lead to optimal conditions for seed growth, such as the method of storage of seeds and the volume of liquid used for imbibition. These conditions may have led to increased variability and therefore inconclusive data. It was concluded that the basic seed model used needs to be further analysed and changed accordingly before different influences could be tested more effectively.

Further research in the area of external influences on homoeopathic dilutions needs to be conducted in order to clarify these effects to the manufacturers and end users of homoeopathic medicines.

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GA_3: gibberellic acid

cH: centesimal dilution

$cHGA_3$: homoeopathically prepared gibberellic acid

$cHGA_3$ - 60W: homoeopathically prepared gibberellic acid exposed to 60 Watt light

$cHGA_3$ - 100W: homoeopathically prepared gibberellic acid exposed to 100 Watt light

$cHGA_3$ - 20°C: homoeopathically prepared gibberellic acid exposed to 20°C

$cHGA_3$ - 60°C: homoeopathically prepared gibberellic acid exposed to 60°C

DEFINITION OF TERMS

Antidote

Antidote (homoeopathic) refers to a substance, which, by virtue of its similarity in bioenergetic effects, neutralizes the field of influence of competing substances, 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).

Homoeopathy ("Similar suffering")

Homoeopathy utilizes the natural Law of Similars, the principle that whatever can harm can also heal. Minute, safe doses are prescribed when a patient

exhibits the same symptoms as a larger dose of the same substance would have produced (Hayfield 1993:9).

Mother tincture

The starting point for the manufacture of most homoeopathic medicines. Constituents are extracted from a suitable source material and combined with an alcoholic solution (Gaier 1991: 354-355).

Potency

Through the homoeopathic preparation process, information is transferred to the solvent. It is assumed that the information is greater and the substance more powerful the more often the process of agitation and dilution takes place (Andersch and Endler 1994). The potency is displayed as a 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 (Gaier 1991:432).

Potentisation

Process of stepwise dilution of a substance in water or a water-alcohol mixture, and input of energy by agitation (succussing) between the dilution steps. Non-water soluble substances are triturated with lactose. Dilutions diluted in steps of 1:10 are named "D", and dilutions in steps of 1:100 are named "C" (Andersch and Endler, 1994).

Seed

A structure that contains at least an embryo and usually a supply of stored nutrients. Seeds are typically dry, dormant and resistant to environmental stress (Mauseth, 1988: 477).

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CHAPTER ONE

INTRODUCTION

The principles and practice of homoeopathy are mysterious to many people, inside and outside of the field and are subject to a great deal of vagueness and conflicting opinions. There are many issues and ways of practicing on which there is incomplete agreement among homoeopaths. These difficulties include selection of medicine, administration of the medicine, and storage of the medicine. These uncertainties result in an apparent lack of structure of homoeopathy as a healing discipline, which does not always bode favourably in the scientific world.

Homoeopathic dilutions are subtle substances due to their method of manufacture and potentisation, which involves serial centesimal dilution and succussion. A dilution greater than the 12^{th} centesimal no longer contains any molecules of the original substance (Gaier, 1991:47-48).

One of the uncertainties relating to the manufacture of homoeopathic medicines is how to store the medicines, due to their subtle nature. It is thought that exposure to various influences will negate the therapeutic power of the medicine. Most commonly mentioned are the effects of light and heat (Dancu, 1996:172-173; Adams, 1996:31; Kayne, 1997:181). It is thought that light or heat may interfere with the homoeopathic dilution because of the nature of the substance. Concerns on the effect of light were expressed early by Hahnemann in Aphorism 267 of the Organon (6^{th} edition) (Boericke translation, 1996:285-286). Dancu, however, does not think one should be too rigid about possibilities of negating the therapeutic power of homoeopathic medicines (1996:173). Thus, patients and homoeopaths alike are left with different experts expressing different concerns on storage.

It becomes difficult to conduct patient -based trials to clarify these unknowns as many results have been attributed to "placebo effect" (Elmiger, 1998:171) or to

the power of suggestion (Aubin and Pichard, 1983:91). For this reason, research has increasingly been focused on the development of laboratory and plant-based models for determining the effects of homeopathic medicines. Plants are a reliable source for testing the efficacy of homoeopathic dilutions as there are no placebo effects (Pelikan and Unger, 1971), and no ethical implications (Hopkins, 1998:107). Several studies have been carried out in order to test the efficacy of homoeopathic medicine in the agricultural context. (*e.g.* Pelikan and Unger, 1971; Bornoroni, 1991; Pongratz and Endler, 1994; Hopkins, 1998; Steele, 1999 and Him Lok, 2001).

For general acceptance by conventional science, experimental results must be reproducible. Scofield (1984) stressed that if promising experimental work in the field of homoeopathy was repeated and further developed, it would add to its validity and would be accepted more easily by medical and scientific fields.

This study tested the effects of light and heat on homoeopathic dilutions, utilizing a plant model. Gibberellic acid, a plant growth hormone that stimulates germination, was used to elicit germination in barley seeds, which are highly sensitive to this hormone (Moore, 1995:423).

This research was based on a previous study by Him Lok (2001) in which a barley seed germination model was used. That study showed statistically significant biological effects of homoeopathically prepared gibberellic acid in various dilutions on the germination of barley seed.

The activity of the homoeopathically prepared dilutions exposed to light and heat was assessed. The results were used to help clarify the controversy around the effects of light and heat on homoeopathic medicines.

1.1 Aim of the study

The purpose of this controlled study was to evaluate the effect of external influences on dilutions of 4cH (10^{-8}) and 200cH (10^{-400}) gibberellic acid using a plant model, *i.e.* the germination performance of whole barley seeds, as measured by means of a germination index.

1.2 Statement of the objectives

1.2.1 The first objective

The first objective was to evaluate the efficacy of an unexposed Hahnemannian dilution of gibberellic acid 4cH on barley seed (*Hordeum vulgare*) as measured by germination.

1.2.2 The second objective

The second objective was to evaluate the efficacy of an unexposed Hahnemannian dilution of gibberellic acid 200cH on barley seed (*Hordeum vulgare*) as measured by germination.

1.2.3 The third objective

The third objective was to evaluate the effect of a 60 Watt incandescent lamp on dilutions of gibberellic acid 4cH as measured by germination of barley seed (*Hordeum vulgare*).

1.2.4 The fourth objective

The fourth objective was to evaluate the effect of a 60 Watt incandescent lamp on dilutions of gibberellic acid 200cH as measured by germination of barley seed (*Hordeum vulgare*).

1.2.5 The fifth objective

The fifth objective was to evaluate the effect of a 100 Watt incandescent lamp on dilutions of gibberellic acid 4cH as measured by germination of barley seed (*Hordeum vulgare*).

1.2.6 The sixth objective

The sixth objective was to evaluate the effect of a 100 Watt incandescent lamp on dilutions of gibberellic acid 200cH as measured by germination of barley seed (*Hordeum vulgare*).

1.2.7 The seventh objective

The seventh objective was to evaluate the effect of 20°C on dilutions of gibberellic acid 4cH as measured by germination of barley seed (*Hordeum vulgare*).

1.2.8 The eighth objective

The eighth objective was to evaluate the effect of 20°C on dilutions of gibberellic acid 200cH as measured by germination of barley seed (*Hordeum vulgare*).

1.2.9 The ninth objective

The ninth objective was to evaluate the effect of 60°C on dilutions of gibberellic acid 4cH as measured by germination of barley seed (*Hordeum vulgare*).

1.2.10 The tenth objective

The tenth objective was to evaluate the effect of 60°C on dilutions of gibberellic acid 200cH as measured by germination of barley seed (*Hordeum vulgare*).

1.3 The Hypotheses

1.3.1 The first hypothesis

It was hypothesized that homoeopathic serial dilution of unexposed gibberellic acid 4cH would have a biological effect on barley seed germination.

1.3.2 The second hypothesis

It was hypothesized that homoeopathic serial dilution of unexposed gibberellic acid 200cH would have a biological effect on barley seed germination.

1.3.3 The third hypothesis

It was hypothesised that a 60 Watt incandescent light would have a negating effect on gibberellic acid 4cH in the germination of barley seeds.

1.3.4 The fourth hypothesis

It was hypothesised that a 60 Watt incandescent light would have a negating effect on gibberellic acid 200H in the germination of barley seeds.

1.3.5 The fifth hypothesis

It was hypothesised that a 100 Watt incandescent light would have a negating effect on gibberellic acid 4cH in the germination of barley seeds.

1.3.6 The sixth hypothesis

It was hypothesised that a 100 Watt incandescent light would have a negating effect on gibberellic acid 200H in the germination of barley seeds.

1.3.7 The seventh hypothesis

It was hypothesised that 20°C would have a negating effect on gibberellic acid 4cH in the germination of barley seeds.

1.3.8 The eighth hypothesis

It was hypothesised that 20°C would have a negating effect on gibberellic acid 200cH in the germination of barley seeds.

1.3.9 The ninth hypothesis

It was hypothesised that 60°C would have a negating effect on gibberellic acid 4cH in the germination of barley seeds.

1.3.10 The tenth hypothesis

It was hypothesised that 60°C would have a negating effect on gibberellic acid 200cH in the germination of barley seeds.

1.4 The benefits of the study

This study aimed to benefit the users of homeopathic medicines, the homoeopathic practitioners and homoeopharmaceutical companies in clarifying the effect of light and heat on homoeopathic dilutions.

CHAPTER TWO

REVIEW OF RELATED LITERATURE

2.1 Homoeopathy and high dilutions

2.1.1 Introduction

Homoeopathic medicine is prepared by serial dilution and succussion (vigorous shaking) of the original substance. It was discovered that progressive dilutions were not as toxic as the crude substance (Kayne, 1997:26). As the substance gets diluted, so it becomes more powerful on a therapeutic level. An example is common salt. At a dilution level of one part in a million (3cH), it is effective in the treatment of many conditions, such as violent and prolonged sneezing (Kayne, 1997:27).

2.1.2 Ultra high dilutions

An ultra high dilution is defined as a homoeopathically prepared dilution containing infinitesimally small quantities of the original substance. The concentration ratio of solute to solvent is generally beyond Avogadro's number (6.022×10^{-23}), *i.e.* 12cH (Andersch and Endler, 1994). As a dilution passes Avogadro's number, it is thought that no single molecule of the original solute is left. This has been one of the major reasons why homoeopathy is not readily accepted in medical and scientific fields (Scofield, 1984; Linde *et al.*, 1997).

The effect of shaking is believed to activate the healing properties of the medicine. A substance that is diluted and succussed is known as a potency (Scofield, 1984). Scofield (1984) reviews and discusses studies that attempt to measure the physical difference between potentised dilutions and the vehicle. These included the use of u.v. spectra, conductivity measurements and i.r. analysis by Heintz (1941) and NMR spectroscopy by Smith and Boericke (1966, 1968). Much of this work shows evidence of activity of progressive dilutions. Scofield comments that the work is generally not presented with statistics.

2.1.3 Homoeopathic method of preparing dilutions

Initially, two methods of potentisation were devised: decimal and centesimal (Kayne, 1997:49). The decimal scale is made up by diluting one part of the original substance with 9 parts of the diluent (alcohol or water). The bottle is succussed and labeled 1X. One part of this dilution is then added to 9 parts of diluent to make up the 2X and so on. Similarly adding one part to 99 parts of the diluent makes up the centesimal dilution (cH). The potency is said to increase with increased dilution, therefore the more dilute a substance is, the higher the potency. Later, a third method of potentisation was developed. This was the quinquagenimillesimal or LM scale in which there is a 1:50 000 dilution at each level. Insoluble substances are triturated with lactose powder up to 4cH where they become soluble, and then made according to the above procedures. (Kayne, 1997:52.) Dilutions used commonly by homoeopaths range from 1:100 to $1:10^{20\ 000}$ (Kayne, 1997:26; Table 2.1).

Table 2.1: Homoeopathic centesimal potencies (Kayne, 1997:50).

Dilution	Concentration	Centesimal potency
1:100	10^{-2}	1cH
1:10 000	10^{-4}	2cH
1:1000 000	10^{-6}	3cH
$1:10^{12}$	10^{-12}	6cH
$1:10^{30}$	10^{-30}	15cH
$1:10^{60}$	10^{-60}	30cH
$1:10^{400}$	10^{-400}	200cH
$1:10^{2000}$	10^{-2000}	1000cH or M
$1:10^{20\,000}$	$10^{-20\,000}$	10 000cH or 10M

2.1.4 Scientific investigation and theories of ultra high dilutions, with reference to homoeopathic medicines

As the substances are so dilute, it becomes difficult to comprehend or explain how homoeopathic medicines work. Most medicines are prescribed in dilution levels past Avogadro's number. This means that the solution is not easily evaluated in terms of its chemical composition. Various theories have been proposed to explain the transfer and storage of the dilute solute in the solvent, and the mode of action of these medicines.

Kayne (1999:49-50) reviews some of these theories. Theories on ultra high dilutions attempt to explain the storage of the substance in high dilutions and are based on the formation of "sustained static and dynamic structures in water". There have been a range of attempts to establish some understanding of the processes of information transfer and storage. It has been suggested by Kayne (1997:49) that the structure of the solvent molecules may change during succussion. The structure is electrochemically changed so as to receive an imprint of the structure of the substance.

Theories regarding the microstructure of water and changes in the molecular properties of water by substances dissolved therein is proposed by Antonchenko and Ilyin (1992). The relationship between processes occurring in dissipative water structures and homoeopathic preparations is shown. Each substance creates a different hydration shell structure. These clusters consist of water molecules organized as one-dimensional chains. The proton transfer along hydrogen-bonded chains in these structures has a stabilizing effect. The conditions for the stability of dissipative structures are connected with charge transfer processes. During the first succussion, there is a flux of charged particles and the structures acquire some stability. The water acquires new characteristics conditioned by the specific dissolved substance. This results in homoeopathic medicines displaying a characteristic frequency spectrum of radiation.

A further theory has been put forward by Anagnostatos (1994), which focuses on local order formation in liquids using the clathrate model. The structure is developed by an externally-induced fluctuation (shaking). The structure, in the form of a cluster, is thought to lead to the formation of clathrates (expanded clusters enclosing smaller cluster clathrates inside). The clathrates provide the mould for the reproduction of the original cluster structure, hence for the replication of a certain order. The local order parameters, or potential carriers of distinct information, are the characteristic clathrate bond angles and bond lengths.

Berezin (1994) proposes an ordered framework, which is made up of the interaction of atomically identical, isotopically different atoms. The sequence of isotopic diversity becomes the core order parameter associated with information storage. Berezin's theory does not indicate how order is created.

The molecular concept is extended by Resch and Gutmann (1991) by introducing the concept of a "supermolecular system organization" of liquid water. This concept describes the continuous relationships and changes in the quality of

water molecules during the potentisation process. Molecules at and near the interface provide first line communication with the environment and exert the greatest influence on the liquid phase. The interface consists of liquid water and its contacting gaseous phase in which continuous interactions occur. Dissolved gas molecules in liquid water have an influence on the oscillating pattern of the whole liquid. The oscillations of the gas molecules must come in harmony with those of the inner surfaces of the liquid, which provide static boundary conditions for the oscillations of both the gas molecules and the water molecules. Interactions of the oscillating gas and water molecules influence each other. The dissolved gas molecules act as synchronization nodes and have the ability to take over structural information from the solution and to maintain it dynamically within their oscillating behaviour. Addition of solutes to liquid alters the whole solution structure, and the oscillation pattern is modified and dynamically maintained by all the molecules. The original "mother substance" information is integrated into and dynamically maintained within the more dilute solution. Succussion leads to the transfer of structural information from the solute molecules to the gas molecules and integration thereof into their dynamics. Hence potentisation leads to improvement in the system organization and therefore the precision of the remedy information.

Kayne (1997:174) concludes in his review that there is a possible connection between the structure of water and the characteristics of homoeopathic preparations. Most are theories or hypotheses, and little is known about the behaviour of water.

2.1.5 The effects of external influences on the stability of homoeopathic dilutions

There is great controversy regarding the effects of external influences on the stability of homoeopathic medicines. Concern exists regarding possible negation of the potency of stored homoeopathic medicines. Homoeopaths have different views on these effects, depending on their experience. Much of this information

seems to be anecdotal and there is little objective evidence in this field (Quinn, 2000).

The most common external influences that allegedly negate homoeopathic medicines are electromagnetic radiation, for example, light and heat (Dancu, 1996:172-173; Adams, 1996:31; Kayne, 1997:181). Storage conditions recommended for homoeopathic medicine are temperatures below 25°C, and protected from light (Natura, n.d; Heel, n.d and Pharma Natura, n.d). Some manufacturers also store products away from strong odours (Leivers, 2000).

There are many questions regarding how homoeopathic medicines should be stored, and the influences that may affect them. Some literature suggests it is essential to take these possibilities of negation into consideration (Adams, 1996:30). Hahnemann writes in Aphorism 267 of the Organon (6th edition), "store away from sunlight" (Boericke translation, 1996:285-286). He goes on to say that the substances will lose their medicinal powers if not stored away from sunlight. This comment from Hahnemann reveals the main reason behind the concern about storage - that the therapeutic powers of homoeopathic medicine can possibly be negated. Other sources suggest that the medicines are sensitive to external sources, but that there is no need to be rigid, only to be aware of the possibilities (Dancu, 1996:173). If homoeopathic medicines are stored "correctly" it has been suggested that they have the potential to last indefinitely (Adams, 1996:30; Kayne, 1997:75).

Perhaps the reason that homoeopaths think that extreme influences can interfere with homoeopathic medicine is that the medicine itself is an extremely subtle substance. Most theories regarding the mechanism of action of ultra high dilutions are based on biophysical rather than biochemical explanations. These theories assert that such dilutions are active because of the resulting energetic properties rather than their physical composition (Towsey and Hasan, 1995; Resch and Gutman, 1991:191-213; Antochenko and Ilyin 1992).

In an attempt to explain the nature of a homoeopathic medicine, Vithoulkas (2000:89-94) relates the medicine to the patient that it cures. Field-measuring devices and Kirlian photography have demonstrated active electromagnetic fields associated with living organisms. Electromagnetic fields are characterized by vibration as electrons oscillate at a specific frequency. These fields are dynamic and change with the onset of illness. As all substances possess characteristic electromagnetic fields, a homoeopathic dilution having a "vibration rate" which most closely matches that of the patient during illness, will facilitate healing. When the vibration rates of the patient and remedy are matched, resonance occurs, just as one tuning fork can stimulate vibration of another of identical frequency. This increase of the electromagnetic field of the patient is at precisely the frequency needed to bring about cure.

This hypothesis raises the question of whether another source of energy (for example, light or heat) can interfere with or negate the energetic (therapeutic) pattern of homoeopathic medicine.

Most concern appears to be around light and heat (Adams, 1996:30; Dancu, 1996:172; Hahnemann, 1996:285-286; Kayne, 1997:181; Natura, n.d and Pharma Natura, n.d). For this reason, light and heat were examined in this study.

2.2.1 Electromagnetic Radiation

All life is affected by electromagnetic radiation (EMR). On a fundamental level, energy from the sun is absorbed as EMR by plants, which convert it into the chemical energy used by animals, including humans. Modern communications and medical services also depend on EMR. Other examples are fuels, microwaves to warm food, television, radios, cameras and nuclear physics. (Beiser, 1987:27.)

Radiant energy is transmitted by electromagnetic waves. The forms and effects may be different, but all forms of electromagnetic radiation share two aspects: a particle aspect and a wave aspect. Electromagnetic waves are regarded as waves because under suitable circumstances they exhibit properties of waves *i.e.*, diffraction, interference, and polarization. Under other circumstances, electromagnetic waves behave as though they consist of streams of particles. (Beiser, 1987:45.)

The properties of EMR are described in terms of a number of parameters. The *wavelength* is the distance between consecutive identical points on a wave and the *frequency* is the number of crests of the wave that pass by a point every second. The frequency is measured in hertz (Hz): 1 hertz = 1 wave crest/second. The relationship between *speed of light* (c), *wavelength* (λ) and *frequency* (f) is: $f = c/\lambda$, or $c = f\lambda$. Therefore, frequency is inversely proportional to the wavelength. Light with a smaller wavelength has a higher frequency, and a longer wavelength has a lower frequency. (Strobel, 2001.)

Table 2.2 shows various groups of electromagnetic radiation, arranged according to their frequencies. The visible spectrum is a narrow band, and contains the range of frequencies to which the human eye responds (Schneider and Schneider, 1988:239).

Table 2.2 The electromagnetic spectrum (Hecht, 1987:69)

BAND NAME	ENERGY LEVEL	FREQUENCY (hertz)	WAVELENGTH (meters)
GAMMA RAYS	4000eV	3×10^{17}	10^{-9}
X RAYS	400eV	3×10^{17}	10^{-9}
ULTRAVIOLET	40eV	3×10^{16}	10^{-8}
VISIBLE LIGHT	4eV	3×10^{15}	10^{-7}
INFRARED	0.4eV	$3 \times 10^{14} - 10^{12}$	$10^{-6} - 10^{-4}$
MICROWAVES	4×10^{-3} eV	$3 \times 10^{11} - 10^{9}$	$10^{-4} - 0.1$
RADIO FREQUENCY	4×10^{-5} eV	$3 \times 10^{8} - 10^{3}$	$1.0 - 10^{5}$

2.2.1.1 Light

The spectrum of electromagnetic radiation covers an extremely broad range of frequency and wavelength. Visible light consists of electromagnetic waves with wavelengths ranging from 400 to 700nm, and frequencies of 7.5 to 4.3 X 10^{14} Hz (Sears *et al,* 1987:813).

A classical understanding of light requires an understanding of electric and magnetic fields. Electrical charges and magnets alter the region of space around them, called a force field. An electric charge or magnet responds to the field produced by a distant object. Electrical and magnetic fields are intimately related to each other. A changing electric field creates a magnetic field; similarly, a changing magnetic field creates electrical field. Thus changing electric and magnetic fields trigger each other and these changing fields move at a speed equal to the speed of light. Electric and magnetic fields oscillate together, perpendicular to each other, and the electromagnetic wave moves in a direction perpendicular to both the fields. Light, electricity and magnetism are manifestations of electromagnetic radiation. (Strobel, 2001.)

Light, as a form of electromagnetic radiation, has a dual nature. It propagates through space in a wavelike fashion and yet can display particle-like behaviour during its interaction with matter (Hecht, 1987:33). According to the wave theory, light waves spread out from a source in the way ripples spread out on the surface of a lake when a stone falls into it. The energy carried by the light is spread continuously throughout the wave pattern.

According to the quantum theory, light consists of "particles" of energy (photons) and leaves a source as a series of localized bursts of energy. Photons have several properties that distinguish them from other subatomic particles. There are no restrictions on the number of photons that can exist in a region. The photon has a zero rest mass, and therefore large numbers of low-energy photons can be present in a beam of light. The energy transported by a large number of

photons is equivalent to the energy transferred by a classical electromagnetic wave. (Hecht, 1987:34.) Each photon has an energy proportional to its frequency *f*, according to the relationship, $E = hf$, where *h* is known as Plank's constant. In other words, quantum energy is proportional to frequency (Hecht, 1987:9).

Thus light has a dual character in which the wave theory of light and the quantum theory of light complement each other. Neither theory is complete and each can only provide the explanation for certain effects (Beiser, 1987:59-60).

In an incandescent light bulb, the filament is a coil of fine tungsten wire. When current passes through the filament, its temperature increases as a result of the electrical resistance of the wire. This heat energy is radiated as EMR. Once the temperature rises to about 3000°C, the radiation contains enough visible wavelengths (between 400nm and 700nm) so that the filament appears "white-hot" (Sears *et al*, 1987:381,838). Electrons are randomly accelerated and undergo frequent collisions, resulting in a broad emission spectrum called thermal radiation (Hecht, 1987:72). The intensity of a 100W bulb is $8W/m^2$ and that of a 60W bulb is $4,77W/m^2$ (Govender, 2001). These were the intensities examined in this study.

2.2.1.2 Heat

Heat is a form of energy, also called thermal energy, which can be obtained from electrical, light, chemical and nuclear energy, as well as mechanical work. Heat is defined as the thermal energy transferred from a region of high temperature to a region of low temperature via atomic collisions. When heat has been transferred to a body, it is called internal energy. The internal energy is related to the kinetic and potential energy of molecules inside the body. (*Physics manual 1* of Technikon Natal, n.d.)

Temperature is a quantitative measure in which numerical values are assigned to various degrees of hotness or coldness. Different devices act as thermometers (Sears *et al*, 1987: 341). When two bodies that are not in thermal equilibrium are placed in contact, their temperatures change by means of heat transfer. Heat flow is energy transfer from one body to another at a different temperature. The temperature of a body can be changed by adding or taking heat away. Every body at a temperature above 0°C emits electromagnetic radiation or radiant energy. At a temperature of 20°C, all energy is carried by infrared waves with wavelengths longer than those of visible light. As the temperature rises, the wavelengths become shorter. In the case of a radiant light source (e.g. a light bulb), the energy, which is radiated as heat and light, is supplied electrically (Sears *et al*, 1987: 381-382,363).

2.2.2 Possible effects of light and heat on homoeopathic dilutions

As discussed above (2.1.4), water consists of a lattice structure in which there is storage of information. If energy in the form of light or heat is supplied, it could be absorbed by the lattice structure, which becomes unstable and breaks up, therefore losing stored information. Once a core structure is destroyed, it cannot reform. The water molecule, which stores the information of the homoeopathic medicine, is rendered useless for storage of information. (Govender, 2001.)

2.3 Seeds as model systems for investigation of homoeopathic dilutions

Plant models have been used to investigate the efficacy of homoeopathic dilutions. Some examples include studies on the germinability of wheat (*Triticum durum*) by Betti *et al.* (1994), of lettuce seeds (*Lactuca sativa* L.) by Hopkins (1998), and on barley (*Hordeum vulgare*) germination by Steele (1999) and Him Lok (2001). This study used a biological model involving the germination of barley seeds.

2.3.1 Seed germination

Germination refers to the beginning of growth of seeds. Germination of most seeds occurs if water and oxygen are available at a suitable range of temperatures. Germination begins with a massive entry of water into the seed via imbibition. This imbibition is essential in reawakening the metabolic activity of the resting seed (Strasburger, 1965:220). The water causes the seed to swell which puts strain on the seed coat. The seed coat eventually becomes progressively softened by absorbing water resulting in rupture (Duddington, 1970:25). During the early stages of germination, the embryo produces gibberellins, which diffuse to the aleurone layer of the endosperm. In response to gibberellins, the aleurone cells produce enzymes that catalyze the conversion of starch to sugar. This in turn is used as an energy source for the growing seedling. (Curtis, 1983:637.)

2.3.2 Plant hormones

Almost all communication in a plant occurs via hormones. Hormones are chemical messengers produced in very low concentrations and active in another part of the organism. Several hormones may be involved in generating a particular response (Mader, 1996:572). There are five major classes of plant hormones. Growth promoters include auxin, gibberellins and cytokinins. Growth inhibitors include abscisic acid and ethylene. The primary example of gibberellins is gibberellic acid (GA_3), which promotes stem elongation and releases buds and seeds from dormancy. (Mader, 1996:583; Duddington, 1970:111.) Gibberellic acid was used as the test substance in the present study.

2.3.2.1 Gibberellins and gibberellic acid

Gibberellins were isolated and identified by Japanese botanists investigating a disease of rice plants caused by the fungus *Gibberella fujikuroi.* Their identity was established by 1939. (Moore *et al*, 1995:422.) Gibberellins have at least two

separate promotive actions on seed germination: (1) on reserve food mobilization, and (2) on embryo growth (Karssen *et al,* 1989:356).

Gibberellic acid is synthesized via the mevalonic acid pathway. Out of the more than eighty gibberellins isolated from plants and fungi, all have an interlocking ring structure and one or more carboxyl groups that impart acidic properties to the molecule. Gibberellins (GA) are assigned subscripts to distinguish them from each other. The most intensively studied gibberellin is GA_3, which is isolated from *Gibberella fujikuroi.* (Moore *et al*, 1995:422.)

Plant growth regulators are used in the commercial sector to increase crop yields (Moore, 1995:423). Gibberellins are used to stimulate seed germination and seedling growth of some grains, beans and fruits (Mader, 1996:576).

2.3.3 Barley seed

Barley is a monocotyledon of the *Gramineae* family (grasses and cereals). Barley seed is sensitive to GA_3, and there is a well-established role for gibberellins in the mobilization of seed nutrient reserves in the family Gramineae. (Lea and Leegood, 1993:264.) Barley seeds were utilized for this study. A previous study on the role of homoeopathically-prepared GA_3 in germination also used barley seeds as the experimental system (Him Lok, 2001).

2.3.4 The role of gibberellic acid in barley seed germination

GA_3 acts as a chemical messenger in the breaking of seed dormancy (Mader, 1996:574) and in promotion of germination (Lea and Leegood, 1993:264). Although the relationship between these phenomena and the endogenous hormonal metabolism of the embryo remains unknown, gibberellin is found in the embryo and plays an essential role in barley seed germination (Lea and Leegood, 1993:264). It is possible to duplicate the effect of the embryo on the endosperm by treatment of embryo-free endosperm with GA_3 (Karssen *et al,*

1989; Baskin and Baskin, 1998:29). Simple experiments provide evidence for the role of GA_3 in the germination of barley seeds. If the embryo is removed (the source of GA), the endosperm is not broken down. Similarly, the addition of GA_3 to embryoless seeds results in the breakdown of the endosperm starch to sugar. (Moore, 1995:423.) Barley seeds with intact embryos were used for this study.

2.4 Homoeopathic research using agricultural models

There are many experiments showing the effect of homoeopathic dilutions of plant growth factors on seeds and plants. Agriculturally-based research is important for homoeopathy as it is objective and placebo effects are avoided (Hopkins, 1998).

Areas of agriculturally-based homeopathic research have been reviewed by Scofield (1984). He stresses the need for positive results to be repeated and extended. This adds to the validity of the research and makes it more readily accepted by medical and scientific fields.

Pelikan and Unger (1971) showed the effects of decimal dilutions of silver nitrate (8X-19X) on the growth of wheat seedlings. The length of the seedlings was measured, and the average length determined. This research provided evidence that potentised substances have a statistically significant effect on plant growth. The larger differences were shown to be significant relative to one another and to the water control.

In a further study, Betti *et al.* (1994) conducted a randomized laboratory trial to study the effect of homoeopathic decimal potencies of Arsenicum album (23X-45X) on the germination of wheat. The percentage of germinated seeds was calculated as a function of time and compared with a distilled water control group. The homoeopathically prepared dilution had a statistically significant effect on the germination of wheat, which could not be explained as an effect of intrinsic seed variability. Three treatment groups showed a significance level of

less than 1% and another was below 5% significance. Significant differences between treatments, analysed by means of a one-factor analysis of variance, were not found when assessing average germination time.

Betti et al (1997) clarified and extended the results from their 1994 study. The 1997 study was conducted under similar conditions, with the aim of ascertaining whether homoeopathic dilutions had an effect on seed germination and growth. Seeds were subjected to a material (poisonous) dose of Arsenicum album, and then treated with 45X (10^{-45}) dilution of the same substance. It was found that the homoeopathic dilution had a positive effect on wheat seedling growth.

Pongtatz and Endler (1994) studied the effects of three different dilutions of silver nitrate ($1:10^{24}$, $1:10^{25}$ and $1:10^{26}$) on the germination of wheat seedlings. Significant enhancement of growth was found in two ($1:10^{24}$ and $1:10^{25}$) of the dilutions used when measured in terms of germination rates (compared using the chi-square test) as well as of the mean stalk length (compared using one way variance analysis).

Homoeopathic dilutions of $CaCO_3$ were tested for synergistic effect on the indoleacetic acid (IAA) induced growth of segments of oat coleoptiles (Bornoroni, 1991). During the rapid growth phase, the coleoptiles were cultured in the presence of IAA, a member of the auxin class of plant hormones. Groups pretreated with 5cH of $CaCO_3$ prior to the addition of the growth hormone IAA showed a statistically significant increase in growth when compared to groups treated with IAA alone. The methodology used to prepare the homoeopathic solutions and the statistical methods used were not described.

Hopkins (1998) studied the efficacy of homoeopathic medicines on the percentage germination of lettuce seeds (*Lactuca sativa* L.) by applying homoeopathically prepared treatments (Sulphur, Nitric Acid and Camphor) of potencies 3cH, 4cH, 15cH and 30cH to whole seeds. The homoeopathically prepared dilutions produced statistically significant biological effects.

Hopkins (1998) states that his " investigation served to support the employment of germinability trials as a means of assessing effect of homoeopathic medicines". Zacharias and Zacharias (1997) maintain that biological experiments should be the main source of information about homoeopathic phenomena, due to simplicity, compared to clinical trials, and their reproducibility. Betti *et al.* (1994), stress the difficulty of testing homoeopathic dilutions on biological systems due to their complexity and other variables related to the environment. Betti *et al.* (1994) proposes that seed germination models should be used as they are simple and these experiments show the most direct effects.

Steele (1999) investigated the effect of ultra high dilutions of gibberellic acid on the synthesis of α-amylase in de-embryonated halves of barley seed. Phadebas Amylase Test tablets (Pharmacia Diagnostics AB, Sweden) were used to assess α-amylase activity. The GA_3 was prepared in homoeopathic dilutions 9cH,15cH, 30cH and 200cH in the traditional Hahnemannian method with succussion and serial dilution without succussion. Steele's results indicated a significant difference between both treatment groups and the controls, and no significant difference between the treatment groups in terms of their ability to produce α-amylase. He concluded that ultra high dilutions of gibberellic acid are biologically active, and that succussion as per the homoeopathic method is not a significant factor underlying this capability. However, Balding (2001), who had based her methodology on Steele's findings, discovered a technical error with regards to the proportion of incubation buffer used in the control groups compared to the treatment groups as used by Steele. This rendered any comparison with the control groups unreliable. Steele's research was valuable as it laid the groundwork for other homeopathic research using the model of barley seeds and gibberellic acid (Him Lok, 2001; Balding, 2001 and Stubbs, 2001).

The present study is based on an investigation reported by Him Lok (2001), which showed that homoeopathically prepared dilutions of GA_3 ($cHGA_3$) resulted in an increased germination rate of barley seeds when compared to untreated controls. She tested $cHGA_3$ at a range of potencies: *viz.* 4cH, 15cH, 30cH, and 200cH. High, medium, and low vigour seeds were tested. The potential of $cHGA_3$ as an alternative to GA_3 for enhancing germination performance of barley seeds was also assessed.

There were two parts to the experiment. In the first phase, the effect of $cHGA_3$ on the germination rate of barley seeds was investigated using time to 50% germination (T_{50}) in hours as a measure of germination. Barley seeds were allowed to imbibe in 12.5ml of each treatment solution or either 12.5ml of water or GA_3 at 0.5g L^{-1}, the latter two treatments acting as negative and positive controls respectively. The cumulative number of germinated seeds was assessed every 4 hours. Seeds imbibed in $cHGA_3$ growth media germinated faster than the negative control group. Germination was fastest for seeds imbibed in GA_3 ($0.5gL^{-1}$).

The second phase of experiments assessed the effect of $cHGA_3$ on seedling development, as measured by a germination index. The seeds were imbibed in treatment solutions and then allowed to germinate. Final germination counts were taken 7 days after start of imbibition, at which time the number of normal, abnormal and dead seeds were assessed. Shoot and root lengths were measured and seedling dry mass was determined. Root length of seeds imbibed in $cHGA_3$ 4cH, 30cH and 200cH solutions did not differ significantly from those of the water-treated control. The $cHGA_3$ dilution 15cH stimulated root development more successfully than either GA_3 ($0.5gL^{-1}$) or the control (water). The $cHGA_3$ dilution had no effect on shoot growth across all seed vigour levels and potency levels. Seedling mass was significantly higher for all $cHGA_3$ imbibed seeds compared to seedling mass of the water-treated control and of GA_3 ($0.5gL^{-1}$) imbibed seeds. The heaviest seedlings were developed from $cHGA_3$ 200cH imbibition.

Him Lok (2001) concluded that biological activity was evident in $cHGA_3$ 4cH, 15cH, 30cH, and 200cH potencies. All $cHGA_3$ solutions stimulated seedling growth when high vigour seeds were used. Unfortunately, specific statistical values were not stated.

2.5 Aims of this study

The present study used the seed germination model described by Him Lok (2001) for investigating the effects of external influences on homoeopathic dilutions. The aims of the study were:

(i) to evaluate the reproducibility of results reported by Him Lok (2001);

(ii) to assess the activity of homoeopathic dilutions following exposure to light and heat.

CHAPTER THREE

RESEARCH MATERIALS AND METHODS

3.1 Introduction

The study was carried out at the School of Life and Environmental Sciences, George Campbell Building, University of Natal, Durban, South Africa.

3.2 Summary of the experimental protocol

- Homoeopathic and non-homoeopathic dilutions were prepared.
- Homoeopathic dilutions were exposed to various external influences.
- Seeds were placed into petri dishes in groups of 100.
- Treatment solutions were added to petri dishes and these were then incubated in a dark growth chamber at 20° C for 24 hours.
- After 24 hours, seeds were removed from the treatment solutions.
- Seeds were placed into petri dishes in groups of 20. One filter paper was placed below and one above the seeds, and the petri dishes were placed in the growth chamber at 20°C for a further 7 days.
- Germinated (radicle >1mm), dead or deformed seeds were counted daily.
- On day 7, root and shoot lengths of all germinated seedlings were measured.
- After the final reading on day 7, seedlings were dried in an oven at 80°C for 4 hours, and the dry mass recorded.
- Data were analysed by ANOVA (SPSS, version 9,0) to test for significance of differences among experimental treatments.

3.3 Study population

Barley seeds (*Hordeum vulgare* Stirling) were obtained from Caledon Farmers Cooperative, Western Cape, South Africa. Seeds were obtained directly from Sensako, rather than a seed supplier, to ensure that the seed had not been treated with any exogenous substance. A total of 3600 seeds were utilised.

3.4 Design

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

Table 3.1 Design of experimental groups. (See 3.6 for experimental specifications.)

	TREATMENT SOLUTIONS	DILUTION LEVEL	CONCENTRATION
1.	Homoeopathic gibberellic acid exposed to light from a 60 Watt bulb ($cHGA_3$ - 60W)	4cH	2.6×10^{-10} mol/L
		200cH	2.6×10^{-402} mol/L
2.	Homoeopathic gibberellic acid exposed to light from a 100 Watt bulb ($cHGA_3$ - 100W)	4cH	2.6×10^{-10} mol/L
		200cH	2.6×10^{-402} mol/L
3.	Homoeopathic gibberellic acid exposed to heat of 20°C ($cHGA_3$ - 20°C)	4cH	2.6×10^{-10} mol/L
		200cH	2.6×10^{-402} mol/L
4.	Homoeopathic gibberellic acid exposed to heat of 60°C ($cHGA_3$ - 60°C)	4cH	2.6×10^{-10} mol/L
		200cH	2.6×10^{-402} mol/L
5.	Homoeopathic gibberellic acid unexposed ($cHGA_3$)	4cH	2.6×10^{-10} mol/L
		200cH	2.6×10^{-402} mol/L
6.	Gibberellic acid (positive control) 0.5g L^{-1}		1.3×10^{-3} mol/L
7.	Distilled water (negative control)		N/A

Groups 1-5 consisted of 2 subgroups representing the 2 levels of dilution selected for this study, *viz.* 4cH (10^{-8} dilution) and 200cH (10^{-400} dilution). These dilution levels are selected because they span Avogadro's dilution limit (12cH or 10^{-24}), and are the lowest and highest dilution levels used by Him Lok (2001). Groups 6 and 7 served as controls. This amounted to 10 subgroups and 2 groups, *i.e.* 12 groups in total.

Each group consisted of 3 replicates, each comprising 100 seeds. This amounts to 3600 seeds in total (12 x 3 x 100 seeds).

3.5 Preparation of the homoeopathic dilutions of gibberellic acid (cHGA$_3$)

Gibberellic acid (GA_3), isolated from *Gibberella fujikuroi* fungus (Moore, 1995:422), was obtained from Sigma-Aldrich (Pty) Ltd, South Africa. The potassium salt of GA_3 (KGA_3), empirical formula $C_{19}H_{21}O_6K$, molecular weight 384.5) was used, as it is readily soluble in water.

Homeopathic dilutions of gibberellic acid were prepared by the researcher, as per Steele (1999:38), under laminar flow conditions in the homoeopharmaceutical laboratory of the Department of Homoeopathy, Technikon Natal, Durban. All glassware was sterilised by rinsing with distilled water and then baking in an oven set at 160°C for 2 hours. The stock solution was made up by combining one part KGA_3 (0.25g) with 99 parts (24.75ml) of distilled water, forming a 1:100 (10^{-2}) solution of KGA_3. From this solution the centesimal dilutions were made up in accordance with 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 instead of alcohol to avoid the possible intrinsic effect alcohol may have on 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).

3.6 Exposure of the $cHGA_3$ dilutions to external influences

The following experimental specifications arose from discussions with Hopkins (2001) and Govender (2001) at Technikon Natal, Durban.

Group 1:

Group 1 was exposed to a 60 Watt incandescent lamp (Philips) for 24 hours. The dilution was made up to 100ml and exposed to the light source in a 100ml clear glass bottle. The bottle was placed at 90° to the light source at a distance of 50cm on a laminar flow bench with the airflow on to reduce heat accumulation from the light. The room in which the laminar flow bench was situated had all external light sources removed by blocking up light cracks with black tape. The temperature of the dilution was recorded with a digital thermometer at 24 hours. A separate bottle, containing distilled water and exposed to identical conditions was used to measure the temperature to prevent contamination of the homoeopathic dilution. The dilution was transferred to an amber bottle after exposure.

Group 2:

Group 2 was exposed to a 100 Watt incandescent lamp (Philips) for 24 hours. Conditions were otherwise identical to Group 1.

Group 3:

Group 3 was exposed to a temperature of 20°C for 24 hours. The dilution was made up to 100ml in an amber glass bottle and placed in a temperature-controlled water bath. The water surface of the water bath was at the same level as the top of the liquid inside the bottle. The water bath was covered and was situated in a darkened laminar flow room as above.

Group 4:

Group 4 was exposed to a temperature of 60°C for 24 hours. Conditions were otherwise identical to Group 3.

3.7 Series one: Imbibition and germination

One hundred seeds were placed in petri dishes (9cm) lined with two filter papers (Schleicher and Schuell, diameter 90 mm), and imbibed with 12.5ml of treatment solution (see Table 3.2) and placed in a growth chamber at 20°C for 24 hours in the dark. After 24 hours, seeds were removed from the petri dishes and transferred by hand into new petri dishes (20 seeds per petri dish) with one filter paper above and below the seeds. The filter paper was moistened with 5ml of deionised water. The seeds were incubated for a further 7 days in a dark growth chamber at 20°C. Germination counts were taken daily and the number of normal, abnormal and dead seedlings were recorded, according to ISTA (1999:155). Refer to Appendix A. A further 1ml of deionised water was added to each petri dish on day 3. Final germination counts and seedling root and shoot lengths were recorded 7 days after imbibition. Seedling dry mass was determined in groups of 20 seeds by drying the seeds in an oven at 80°C for 48 hours.

Germination was expressed as the number of seeds germinated in the form of a germination index as described by Walker-Simmons (1988). The index is explained in Appendix B.

3.8 Series two of the germination index

The same experiment was repeated, due to anomalous results, with measurements taken only for the germination index. Conditions were identical to those in series one.

3.9 Statistical analysis

One-way (independent) Analysis of Variance (ANOVA) was used for the data analysis with the assumption of homogenous variables. The data were compared in three groups. The control group consisted of GA_3 ($0.5gL^{-1}$), water, $cHGA_3$ - 4cH and $cHGA_3$ - 200cH. The two treatment groups consisted of firstly $cHGA_3$ - 4cH exposed to light and heat, secondly $cHGA_3$ - 200cH exposed to light and heat. Groups were compared to each other in turn (pairwise). The mean for each replicate of seeds was calculated. There were three replicates.

3.9.1 Intergroup comparisons

Intergroup comparisons were performed between the control group, between the control and all $cHGA_3$ - 4cH groups, between the control and all $cHGA_3$ - 200cH groups and between all $cHGA_3$ - 4cH and $cHGA_3$ - 200cH groups for root, shoot length, germination index and dry mass.

3.10 Statistical model

The statistical model used for the multifactorial ANOVA experiment was as follows:

$Y_{ijk} = \mu+A_i+B_j+A_iB_j+\varepsilon_{ijk}$

where

μ is the common effect

A_i is the effect of methods

B_j is the effect of dilution levels

A_iB_j is the interaction effect between methods and dilution levels

ε_{ijk} is the random error

$i = 1, ...,5$ = number of "treatments"

$j = 1,2$ = number of dilution levels

$k = 1, ...,3$ = number of "replications"

3.10.1 Procedure: The one-way ANOVA to test main effects for significance

The effects of methods and dilution levels were tested for significance. In each case, the null hypothesis stated that there were no differences between the categories of the main effects. The alternative hypothesis stated that there were differences between the treatment groups. The null hypothesis was rejected if the p-value was smaller than the level of significance, α, of the test. It was accepted if the p-value was equal to or greater than the level of significance, α, of the test. In this study, the level of significance, α, was fixed at the 0.05 level. This is the significance level typically accepted when comparing biological effects or parameters.

3.10.2 Statistical analysis comparing the two series of the germination index

A t- test was used to compare the two sets of data. The level of significance was fixed at $\alpha = 0.05$.

3.11 Statistical package

The statistical package SPSS for Windows (version 9.0) was used for data analysis.

CHAPTER FOUR

RESULTS

4.1 The criteria governing the admissibility of the data

The data utilised was root and shoot length, dry mass and germination index. Radical protrusion was the measure for germination. A weighted germination index was calculated using the observational data (see 3.7).

4.2 Statistical analysis of results

H_0: null hypothesis

H_1: alternative hypothesis

Decision rule: at the $\alpha = 0.05$ level of significance.

Reject H_0 if $p<\alpha$

Accept H_1 if $p \geq \alpha$

4.2.1 Comparison groups

a) Control group: Gibberellic acid (GA_3) ($0.5gL^{-1}$), water, $cHGA_3$ - 4cH and $cHGA_3$ – 200cH.

b) 4cH group: All 4cH treatment groups.

c) 200cH group: All 200cH treatment groups.

4.3 Results from the one-way analysis of variance: Measurement of root length

Table 4.1 One-way Analysis of Variance intergroup comparison between control group with regard to root length.

	Sum of Squares	Degrees of Freedom	Mean Square	F-ratio	Significance level
Between Groups	8.002	3	2.667	10.260	.004
Within Groups	2.080	8	.260		
Total	10.082	11			

Table 4.1 shows that p=0.004, <0.05 which means that the null hypothesis is rejected at the 5% level of significance.

Conclusion: There was a difference between the control groups with regard to root length at the 5% level of significance.

Table 4.2 One-way Analysis of Variance intergroup comparisons between control groups and all $cHGA_3$ - 4cH treatment groups with regard to root length.

	Sum of Squares	Degrees of Freedom	Mean Square	F-ratio	Significance level
Between Groups	16.967	7	2.424	5.835	.002
Within Groups	6.647	16	.415		
Total	23.613	23			

Table 4.2 shows that p=0.002, <0.05 which means that the null hypothesis is rejected at the 5% level of significance.

Conclusion: There was a difference between the control groups and $cHGA_3$ - 4cH with regard to root length at the 5% level of significance.

Table 4.3 One-way Analysis of Variance intergroup comparisons between control groups and all $cHGA_3$ – 200cH treatment groups with regard to root length.

	Sum of Squares	Degrees of Freedom	Mean Square	F-ratio	Significance level
Between Groups	13.586	7	1.941	3.796	.013
Within Groups	8.180	16	.511		
Total	21.766	23			

Table 4.3 shows that p=0.013, <0.05 which means that the null hypothesis is rejected at the 5% level of significance.

Conclusion: There was a difference between the control groups and $cHGA_3$ - 200cH with regard to root length at the 5% level of significance.

Table 4.4 One-way Analysis of Variance intergroup comparisons between and all $cHGA_3$ - 4cH and $cHGA_3$ - 200cH treatment groups with regard to root length.

	Sum of Squares	Degrees of Freedom	Mean Square	F-ratio	Significance level
Between Groups	8.423	7	1.203	1.805	.155
Within Groups	10.667	16	.667		
Total	19.090	23			

Table 4.4 shows that p=0.155, >0.05 which means that the null hypothesis is accepted at the 5% level of significance.

Conclusion: There was no difference between $cHGA_3$ - 4cH and $cHGA_3$ - 200cH with regard to root length at the 5% level of significance.

4.3.1 Tests of between-subjects effects (Analysis of Variance table)

The analysis of variance procedure showed that there were significant differences between groups. A Post Hoc test was carried out to identify which groups were significantly different.

Table 4.5 Post Hoc test between subjects (root length)

Tukey HSD		**Significance level**
GA_3 (control)	WATER	.01
	$CHGA_3$ - 200cH	.026
	$CHGA_3$ - 4cH - 20°C	.038
	$CHGA_3$ - 4cH - 60°C	.006
	$CHGA_3$ - 200cH - 20°C	.039
WATER	$CHGA_3$ - 4cH	.015
$cHGA_3$ - 4cH	$CHGA_3$ - 200cH	.04
	$CHGA_3$ - 4cH - 60°C	.01
$cHGA_3$ - 4cH - 60°C	$CHGA_3$ - 4cH - 100W	.026

Table 4.5 shows that comparisons of the following seed treatment showed significant differences in the mean root length of seedlings:

a) Between Gibberellic acid ($0.5g/L^{-1}$) and water; at the 5% level of significance.

b) Between Gibberellic acid ($0.5g/L^{-1}$) and $cHGA_3$ - 200cH; at the 5% level of significance.

c) Between Gibberellic acid ($0.5g/L^{-1}$) and $cHGA_3$ - 4cH - 20°C; at the 5% level of significance.

d) Between Gibberellic acid ($0.5g/L^{-1}$) and $cHGA_3$ - 4cH - 60°C; at the 5% level of significance.

e) Between Gibberellic acid ($0.5g/L^{-1}$) and $cHGA_3$ - 200cH - 20°C; at the 5% level of significance.

f) Between water and $cHGA_3$ - 4cH; at the 5% level of significance.

g) Between $cHGA_3$ - 4cH and $cHGA_3$ - 200cH; at the 5% level of significance.

h) Between $cHGA_3$ - 4cH and $cHGA_3$ - 4cH - 60°C; at the 5% level of significance.

i) Between $cHGA_3$ - 4cH - 60°C and $cHGA_3$ - 4cH - 100W; at the 5% level of significance.

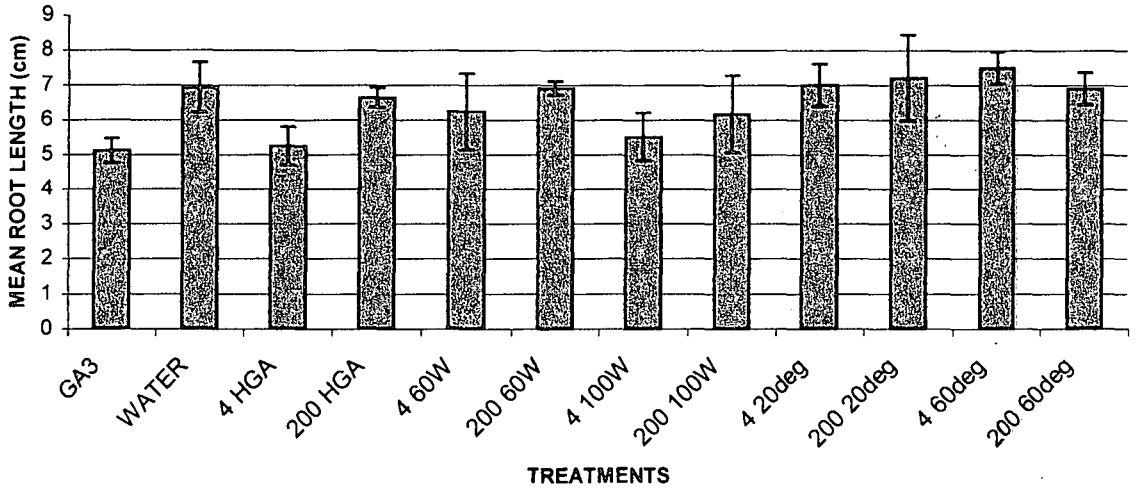

Figure 4.1 Bar chart representing the mean root length of all control and treatment groups. Error bars represent one standard deviation around the mean. 'GA3' refers to GA_3 ($0.5gL^{-1}$), '4 HGA' and '200 HGA' to $cHGA_3$ - 4cH and $cHGA_3$ – 200cH respectively. '60 W' and '100 W' refers to those dilutions exposed to 60 Watt and 100 Watt light, similarly '20 deg' and '60 deg' to those dilutions exposed to 20°C and 60°C.

4.4 Results from the one-way analysis of variance: Measurement of shoot length

Table 4.6 One-way Analysis of Variance intergroup comparison between control group with regard to shoot length.

	Sum of Squares	Degrees of Freedom	Mean Square	F-ratio	Significance level
Between Groups	5.743	3	1.914	3.324	.077
Within Groups	4.607	8	.576		
Total	10.3492	11			

Table 4.6 shows that p=0.077, >0.05 which means that the null hypothesis is accepted at the 5% level of significance.

Conclusion: There was no difference between the control groups with regard to shoot length at the 5% level of significance.

Table 4.7 One-way Analysis of Variance intergroup comparisons between control groups and all $cHGA_3$ - 4cH treatment groups with regard to shoot length.

	Sum of Squares	Degrees of Freedom	Mean Square	F-ratio	Significance level
Between Groups	9.500	7	1.357	2.803	.042
Within Groups	7.747	16	.484		
Total	17.246	23			

Table 4.7 shows that p=0.042, <0.05 which means that the null hypothesis is rejected at the 5% level of significance.

Conclusion: There was a difference between the control groups and $cHGA_3$ - 4cH with regard to shoot length at the 5% level of significance.

Table 4.8 One-way Analysis of Variance intergroup comparisons between control groups and all $cHGA_3$ – 200cH treatment groups with regard to shoot length.

	Sum of Squares	Degrees of Freedom	Mean Square	F-ratio	Significance level
Between Groups	13.143	7	1.878	3.625	.016
Within Groups	8.287	16	.518		
Total	21.430	23			

Table 4.8 shows that p=0.016, <0.05 which means that the null hypothesis is rejected at the 5% level of significance.

Conclusion: There was a difference between the control groups and $cHGA_3$ - 200cH with regard to shoot length at the 5% level of significance.

Table 4.9 One-way Analysis of Variance intergroup comparisons between and all $cHGA_3$ - 4cH and $cHGA_3$ - 200cH treatment groups with regard to shoot length.

	Sum of Squares	Degrees of Freedom	Mean Square	F-ratio	Significance level
Between Groups	8.925	7	1.275	2.991	.033
Within Groups	6.820	16	.426		
Total	15.745	23			

Table 4.9 shows that p=0.033, <0.05 which means that the null hypothesis is rejected at the 5% level of significance.

Conclusion: There was a difference between $cHGA_3$ - 4cH and $cHGA_3$ - 200cH with regard to shoot length at the 5% level of significance.

4.4.1 Tests of between-subjects effects (Analysis of Variance table)

The analysis of variance procedure showed that there were significant differences between groups. A Post Hoc test was carried out to identify which groups were significantly different.

Table 4.10 Post Hoc test between subjects (shoot length)

Tukey HSD		**Significance level**
GA_3 (control)	$CHGA_3$ – 200cH - 100W	.024
	$CHGA_3$ - 4cH	.006
	$CHGA_3$ - 200cH	.04
	$CHGA_3$ - 4cH - 60 W	.005
	$CHGA_3$ - 4cH - 60°C	.009
$cHGA_3$ - 200cH - 60W	$CHGA_3$ - 200cH - 100W	.046

Table 4.10 shows that comparisons of the following seed treatment showed significant differences in the mean shoot length of seedlings:

a) Between Gibberellic acid ($0.5g/L^{-1}$) and $cHGA_3$ - 200cH - 100W; at the 5% level of significance.
b) Between Gibberellic acid ($0.5g/L^{-1}$) and $cHGA_3$ - 4cH; at the 5% level of significance.
c) Between Gibberellic acid ($0.5g/L^{-1}$) and $cHGA_3$ - 200cH; at the 5% level of significance.
d) Between Gibberellic acid ($0.5g/L^{-1}$) and $cHGA_3$ - 4cH - 60W; at the 5% level of significance.
e) Between Gibberellic acid ($0.5g/L^{-1}$) and $cHGA_3$ - 4cH - 60°C; at the 5% level of significance.
f) Between $cHGA_3$ - 200cH - 60W and $cHGA_3$ - 200cH - 100W; at the 5% level of significance.

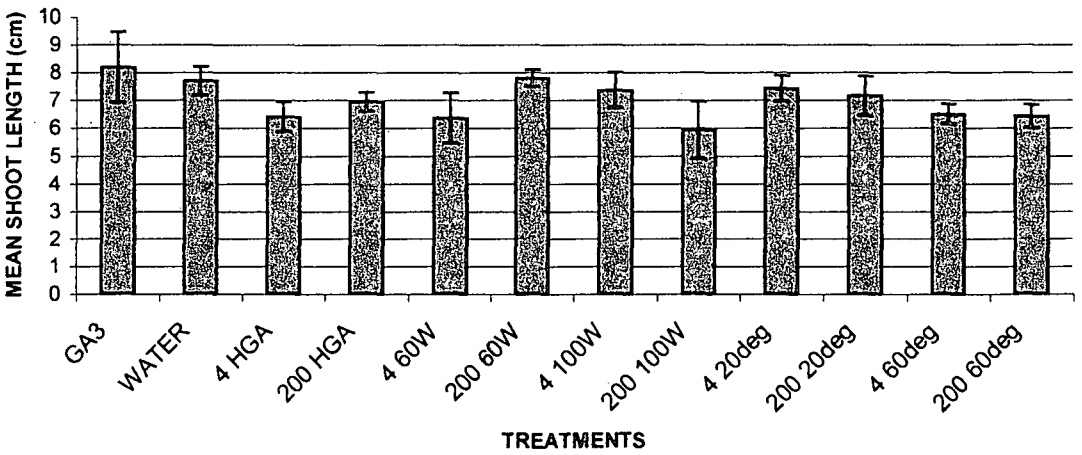

Figure 4.2 Bar chart representing the mean shoot length of all control and treatment groups. Error bars represent one standard deviation around the mean. Treatment designations as for Figure 4.1.

4.5 Results from the one-way analysis of variance: Germination index- SERIES ONE.

Table 4.11 One-way Analysis of Variance intergroup comparison between control group with regard to the germination index.

	Sum of Squares	Degrees of Freedom	Mean Square	F-ratio	Significance level
Between Groups	7.142	3	2..381	4.282	.064
Within Groups	4.447	8	5.559		
Total	.116	11			

Table 4.11 shows that p=0.064, >0.05 which means that the null hypothesis is accepted at the 5% level of significance.

Conclusion: There was no difference between the control groups with regard to the germination index at the 5% level of significance.

Table 4.12 One-way Analysis of Variance intergroup comparisons between control groups and all $cHGA_3$ - 4cH treatment groups with regard the germination index.

	Sum of Squares	Degrees of Freedom	Mean Square	F-ratio	Significance level
Between Groups	7.914	7	1.131	2.868	.038
Within Groups	6.306	16	3.941		
Total	.142	23			

Table 4.12 shows that p=0.038, <0.05 which means that the null hypothesis is rejected at the 5% level of significance.

Conclusion: There was a difference between the control groups and $cHGA_3$ – 4cH with regard to the germination index at the 5% level of significance.

Table 4.13 One-way Analysis of Variance intergroup comparisons between control groups and all $cHGA_3$ – 200cH treatment groups with regard to the germination index.

	Sum of Squares	Degrees of Freedom	Mean Square	F-ratio	Significance level
Between Groups	8.876	7	1.268	2.965	.034
Within Groups	6.842	16	4.276		
Total	.157	23			

Table 4.13 shows that p=0.034, <0.05 which means that the null hypothesis is rejected at the 5% level of significance.

Conclusion: There was a difference between the control groups and $cHGA_3$ - 200cH with regard to the germination index at the 5% level of significance.

Table 4.1 4 One-way Analysis of Variance intergroup comparisons between and all $cHGA_3$ - 4cH and $cHGA_3$ - 200cH treatment groups with regard to the germination index.

	Sum of Squares	Degrees of Freedom	Mean Square	F-ratio	Significance level
Between Groups	1.931	7	2.759	1.038	.444
Within Groups	4.253	16	2.658		
Total	6.184	23			

Table 4.14 shows that p=0.444, >0.05 which means that the null hypothesis is accepted at the 5% level of significance.

Conclusion: There was no difference between $cHGA_3$ - 4cH and $cHGA_3$ - 200cH with regard to the germination index at the 5% level of significance.

4.5.1 Tests of between-subjects effects (Analysis of Variance table)

The analysis of variance procedure showed that there were significant differences between groups. A Post Hoc test was carried out to identify which groups were significantly different.

Table 4.15 Post Hoc test between subjects (germination index 1)

Tukey HSD		**Significance level**
GA_3 (control)	WATER	.048
	$CHGA_3$ - 200cH	.038
	$CHGA_3$ - 4cH - 60 W	.038
	$CHGA_3$ - 200cH - 60°C	.018

Table 4.15 shows that comparisons of the following seed treatment showed significant differences in the germination index (series one):

a) Between Gibberellic acid ($0.5g/L^{-1}$) and water; at the 5% level of significance.
b) Between Gibberellic acid ($0.5g/L^{-1}$) and $cHGA_3$ - 200cH; at the 5% level of significance.
c) Between Gibberellic acid ($0.5g/L^{-1}$) and $cHGA_3$ - 4cH - 60W; at the 5% level of significance.
d) Between Gibberellic acid ($0.5g/L^{-1}$) and $cHGA_3$ - 200cH - 60°C; at the 5% level of significance.

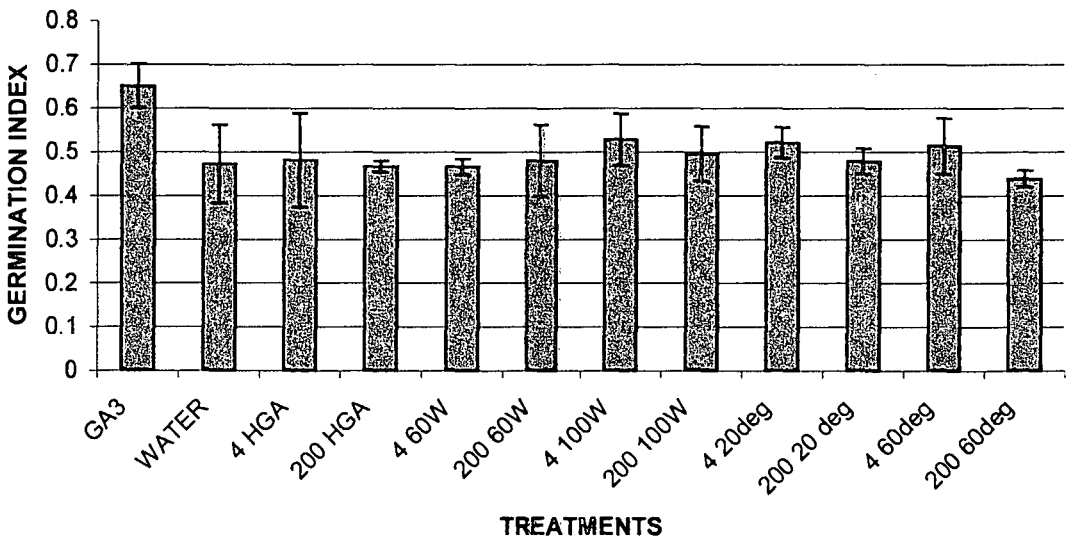

Figure 4.3 Bar chart representing the mean germination (series one) expressed by a germination index of all control and treatment groups. Error bars represent one standard deviation around the mean. Treatment designations as for Figure 4.1.

4.6 Results from the one-way analysis of variance: Dry mass

Table 4.16 One-way Analysis of Variance intergroup comparison between control group with regard to dry mass.

	Sum of Squares	Degrees of Freedom	Mean Square	F-ratio	Significance level
Between Groups	40.188	3	13.396	20.700	.000
Within Groups	5.177	8	.647		
Total	45.365	11			

Table 4.16 shows that p=0.000, <0.05 which means that the null hypothesis is rejected at the 5% level of significance.

Conclusion: There was a difference between the control groups with regard to dry mass at the 5% level of significance.

Table 4.17 One-way Analysis of Variance intergroup comparisons between control groups and all $cHGA_3$ - 4cH treatment groups with regard to dry mass.

	Sum of Squares	Degrees of Freedom	Mean Square	F-ratio	Significance level
Between Groups	82.822	7	11.832	4.870	.004
Within Groups	38.873	16	2.430		
Total	121.695	23			

Table 4.17 shows that p=0.004, <0.05 which means that the null hypothesis is rejected at the 5% level of significance.

Conclusion: There was a difference between the control groups and $cHGA_3$ - 4cH with regard to dry mass at the 5% level of significance.

Table 4.18 One-way Analysis of Variance intergroup comparisons between control groups and all $cHGA_3$ – 200cH treatment groups with regard to dry mass.

	Sum of Squares	Degrees of Freedom	Mean Square	F-ratio	Significance level
Between Groups	58.485	7	8.355	12.842	.000
Within Groups	10.409	16	.651		
Total	68.895	23			

Table 4.18 shows that p=0.000, <0.05 which means that the null hypothesis is rejected at the 5% level of significance.

Conclusion: There was a difference between the control groups and $cHGA_3$ - 200cH with regard to dry mass at the 5% level of significance.

Table 4.19 One-way Analysis of Variance intergroup comparisons between and all $cHGA_3$ - 4cH and $cHGA_3$ - 200cH treatment groups with regard to dry mass.

	Sum of Squares	Degrees of Freedom	Mean Square	F-ratio	Significance level
Between Groups	32.001	7	4.572	1.879	.140
Within Groups	38.928	16	2.433		
Total	70.929	23			

Table 4.19 shows that p=0.140, >0.05 which means that the null hypothesis is accepted at the 5% level of significance.

Conclusion: There was no difference between $cHGA_3$ - 4cH and $cHGA_3$ - 200cH with regard to dry mass at the 5% level of significance.

4.6.1 Tests of between-subjects effects (Analysis of Variance table)

The analysis of variance procedure showed that there were significant differences between groups. A Post Hoc test was carried out to identify which groups were significantly different.

Table 4.20 Post Hoc test between subjects (dry mass)

Tukey HSD		**Significance level**
GA_3 (control)	WATER	.001
	$cHGA_3$ - 4cH	.001
	$cHGA_3$ - 200cH	.001
	$cHGA_3$ - 4cH - 20°C	.026
	$cHGA_3$ - 4cH - 60°C	.034
	$cHGA_3$ - 4cH - 60W	.001
	$cHGA_3$ - 4cH - 100W	.007
	$cHGA_3$ - 200cH - 20°C	.004
	$cHGA_3$ - 200cH - 60°C	.001
	$cHGA_3$ - 200cH - 60W	.000
	$cHGA_3$ - 200cH - 100W	.000

Table 4.20 shows that comparisons of the following seed treatment showed significant differences in the dry mass of seedlings:

a) Between Gibberellic acid ($0.5g/L^{-1}$) and water; at the 5% level of significance.
b) Between Gibberellic acid ($0.5g/L^{-1}$) and $cHGA_3$ - 4cH; at the 5% level of significance.
c) Between Gibberellic acid ($0.5g/L^{-1}$) and $cHGA_3$ - 200cH; at the 5% level of significance.
d) Between Gibberellic acid ($0.5g/L^{-1}$) and $cHGA_3$ - 4cH - 20°C; at the 5% level of significance.
e) Between Gibberellic acid ($0.5g/L^{-1}$) and $cHGA_3$ - 4cH - 60°C; at the 5% level of significance.
f) Between Gibberellic acid ($0.5g/L^{-1}$) and $cHGA_3$ - 4cH - 60W; at the 5% level of significance.
g) Between Gibberellic acid ($0.5g/L^{-1}$) and $cHGA_3$ - 4cH - 100W; at the 5% level of significance.
h) Between Gibberellic acid ($0.5g/L^{-1}$) and $cHGA_3$ - 200cH - 20°C; at the 5% level of significance.
i) Between Gibberellic acid ($0.5g/L^{-1}$) and $cHGA_3$ - 200cH - 60°C; at the 5% level of significance.

j) Between Gibberellic acid ($0.5g/L^{-1}$) and $cHGA_3$ - 200cH - 60W; at the 5%
level of significance.

k) Between Gibberellic acid ($0.5g/L^{-1}$) and $cHGA_3$ - 200cH - 100W; at the 5% level of significance.

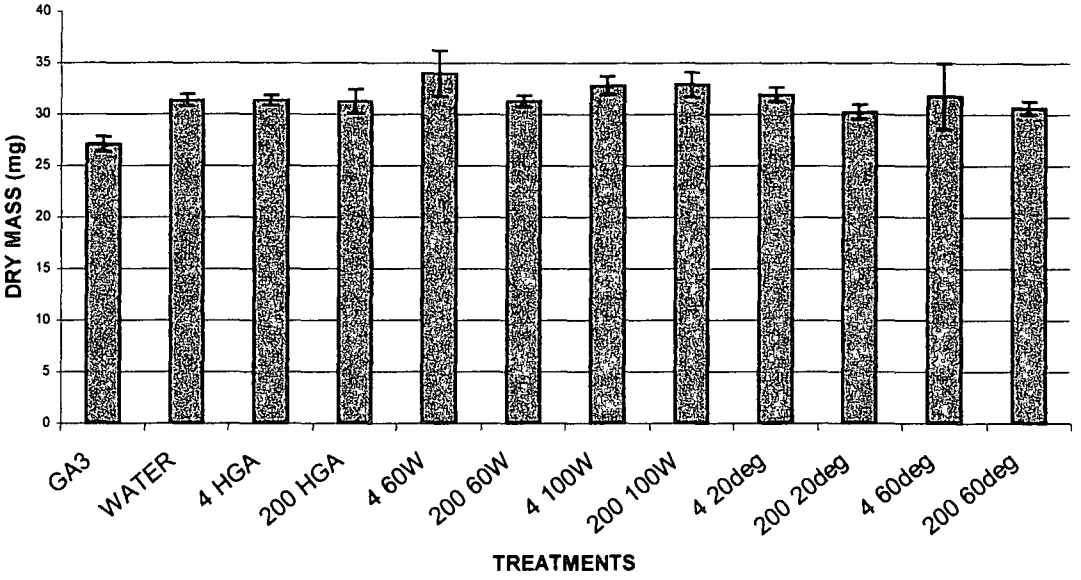

Figure 4.4 Bar chart representing the mean dry mass of all control and treatment groups. Error bars represent one standard deviation around the mean. Treatment designations as for Figure 4.1.

4.7 Results from the one-way analysis of variance: Germination index- SERIES TWO.

Table 4.21 One-way Analysis of Variance intergroup comparison between control groups with regard to the germination index – SERIES TWO

	Sum of Squares	Degrees of Freedom	Mean Square	F-ratio	Significance level
Between Groups	7.065	3	2.667	15.007	.001
Within Groups	1.255	8	.260		
Total		11			

Table 4.21 shows that p=0.001, <0.05 which means that the null hypothesis is rejected at the 5% level of significance.

Conclusion: There was a difference between the control groups with regard to the germination index at the 5% level of significance.

Table 4.22 One-way Analysis of Variance intergroup comparisons between control groups and all $cHGA_3$ - 4cH treatment groups with regard to the germination index – SERIES TWO.

	Sum of Squares	Degrees of Freedom	Mean Square	F-ratio	Significance level
Between Groups	57.47	7	2.424	12.81	.000
Within Groups	10.12	16	.415		
Total	67.95	23			

Table 4.22 shows that p=0.000, <0.05 which means that the null hypothesis is rejected at the 5% level of significance.

Conclusion: There was a difference between the control groups and $cHGA_3$ - 4cH with regard to the germination index at the 5% level of significance.

Table 4.23 One-way Analysis of Variance intergroup comparisons between control groups and all $cHGA_3$ – 200cH treatment groups with regard to the germination index – SERIES TWO.

	Sum of Squares	Degrees of Freedom	Mean Square	F-ratio	Significance level
Between Groups	13.586	7	1.941	3.796	.013
Within Groups	8.180	16	.511		
Total	21.766	23			

Table 4.23 shows that p=0.013, <0.05 which means that the null hypothesis is rejected at the 5% level of significance.

Conclusion: There was a difference between the control groups and $cHGA_3$ - 200cH with regard to the germination index at the 5% level of significance.

Table 4.24 One-way Analysis of Variance intergroup comparisons between and all $cHGA_3$ - 4cH and $cHGA_3$ - 200cH treatment groups with regard to the germination index – SERIES TWO.

	Sum of Squares	Degrees of Freedom	Mean Square	F-ratio	Significance level
Between Groups	8.423	7	1.203	1.005	.259
Within Groups	10.667	16	.667		
Total	19.090	23			

Table 4.24 shows that p=0.259, >0.05 which means that the null hypothesis is accepted at the 5% level of significance.
Conclusion: There was no difference between $cHGA_3$ - 4cH and $cHGA_3$ - 200cH with regard to the germination index at the 5% level of significance.

4.7.1 Tests of between-subjects effects (Analysis of Variance table)

The analysis of variance procedure showed that there were significant differences between groups. A Post Hoc test was carried out to identify which groups were significantly different.

Table 4.25 Post Hoc test between subjects (germination index 2)

Tukey HSD		**Significance level**
GA_3 (control)	WATER	.005
	$CHGA_3$ - 200cH	.001
	$CHGA_3$ – cH - 60W	.011
	$CHGA_3$ - 4cH - 20°C	.002
	$CHGA_3$ - 4cH - 60°C	.018
	$CHGA_3$ - 200cH - 60°C	.015
$cHGA_3$ - 4cH	$CHGA_3$ - 4cH - 60W	.026
$cHGA_3$ - 200cH	$CHGA_3$ - 4cH - 100W	.017

Table 4.25 shows that comparisons of the following seed treatment showed significant differences in the germination index:

a) Between Gibberellic acid ($0.5g/L^{-1}$) and water; at the 5% level of significance.
b) Between Gibberellic acid ($0.5g/L^{-1}$) and $cHGA_3$ - 200cH; at the 5% level of significance.
c) Between Gibberellic acid ($0.5g/L^{-1}$) and $cHGA_3$ - 4cH - 60W; at the 5% level of significance.
d) Between Gibberellic acid ($0.5g/L^{-1}$) and $cHGA_3$ - 4cH - 20°C; at the 5% level of significance.

e) Between Gibberellic acid ($0.5g/L^{-1}$) and $cHGA_3$ - 4cH - 60°C; at the 5% level of significance.

f) Between Gibberellic acid ($0.5g/L^{-1}$) and $cHGA_3$ - 200cH - 60°C; at the 5% level of significance.

g) Between $cHGA_3$ - 4cH and $cHGA_3$ - 4cH - 60W; at the 5% level of significance.

h) Between $cHGA_3$ - 4cH - 100W and $cHGA_3$ - 200cH; at the 5% level of significance.

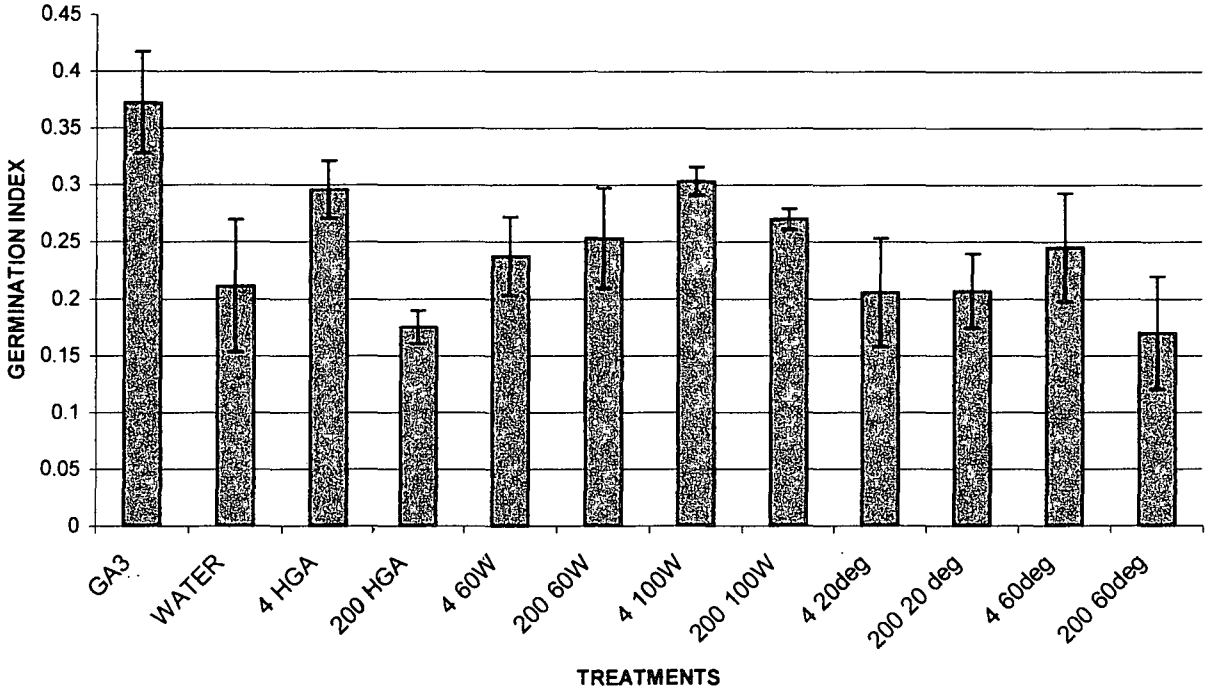

Figure 4.5 Bar chart representing the mean germination for series two expressed by a germination index of all control and treatment groups. Error bars represent one standard deviation around the mean. Treatment designations as for Figure 4.1.

4.8 T-Test comparing the germination indexes of two series

Table 4.26 T-test between two series of the germination index

		MEAN	STANDARD DEVIATION	SIGNIFICANCE LEVEL
GA_3 ($0.5gL^{-1}$)	GROUP 1	0.650667	0.050402	
	GROUP 2	0.372381	0.044775	p= 0.002
WATER	GROUP 1	0.472333	0.089584	
	GROUP 2	0.210952	0.058315	p= 0.013
$cHGA_3$ – 4cH	GROUP 1	0.480333	0.107221	
	GROUP 2	0.295714	0.025715	p= 0.044
$cHGA_3$ – 200cH	GROUP 1	0.466	0.013229	
	GROUP 2	0.174762	0.014662	p= 0.000
$cHGA_3$ – 4cH - 60W	GROUP 1	0.465667	0.018448	
	GROUP 2	0.237143	0.03467	p= 0.001
$cHGA_3$ – 200cH - 60W	GROUP 1	0.479333	0.082513	
	GROUP 2	0.253333	0.044294	p= 0.014
$cHGA_3$ – 4cH - 100W	GROUP 1	0.527333	0.059786	
	GROUP 2	0.302857	0.012372	p= 0.003
$cHGA_3$ – 200cH - 100W	GROUP 1	0.494667	0.062883	
	GROUP 2	0.27	0.009368	p=0.004
$cHGA_3$ - 4cH - 20°C	GROUP 1	0.520667	0.035529	
	GROUP 2	0.205238	0.047944	p= 0.001
$cHGA_3$ – 200cH - 20°C	GROUP 1	0.478	0.029103	
	GROUP 2	0.206667	0.033023	p= 0.000
$cHGA_3$ - 4cH - 60°C	GROUP 1	0.513	0.064156	
	GROUP 2	0.245238	0.047559	p=0.004
$cHGA_3$ – 200cH - 60°C	GROUP 1	0.438	0.019	
	GROUP 2	0.169571	0.142285	p=0.032

The overall T-TEST table (Table 4.26) shows the following:

a) In comparing both groups of gibberellic acid ($0.5gL^{-1}$): p=0.02, <0.05, which means that the null hypothesis is rejected.
Conclusion: the two groups are different from each other at the 5% level.

b) In comparing both groups of water: p=0.013, <0.05, which means that the null hypothesis is rejected.
Conclusion: the two groups are different from each other at the 5% level.

c) In comparing both groups of $cHGA_3$ - 4cH: p=0.044, <0.05, which means that the null hypothesis is rejected.
Conclusion: the two groups are different from each other at the 5% level.

d) In comparing both groups of $cHGA_3$ - 200cH: p=0.000, <0.05, which means that the null hypothesis is rejected.
Conclusion: the two groups are different from each other at the 5% level.

e) In comparing both groups of $cHGA_3$ - 4cH - 60W: p=0.001, <0.05, which means that the null hypothesis is rejected.
Conclusion: the two groups are different from each other at the 5% level.

f) In comparing both groups of $cHGA_3$ - 200cH - 60W: p=0.014, <0.05, which means that the null hypothesis is rejected.
Conclusion: the two groups are different from each other at the 5% level.

g) In comparing both groups of $cHGA_3$ - 4cH - 100W: p=0.003, <0.05, which means that the null hypothesis is rejected.
Conclusion: the two groups are different from each other at the 5% level.

h) In comparing both groups of $cHGA_3$ - 200cH - 100W: p=0.004, <0.05, which means that the null hypothesis is rejected.
Conclusion: the two groups are different from each other at the 5% level.

i) In comparing both groups of $cHGA_3$ - 4cH - 20°C: p=0.001, <0.05, which means that the null hypothesis is rejected.
Conclusion: the two groups are different from each other at the 5% level.

j) In comparing both groups of $cHGA_3$ - 200cH - 20°C: p=0.000, <0.05, which means that the null hypothesis is rejected.

Conclusion: the two groups are different from each other at the 5% level.

k) In comparing both groups of $cHGA_3$ - 4cH - 60°C: p=0.004, <0.05, which means that the null hypothesis is rejected.
Conclusion: the two groups are different from each other at the 5% level.

l) In comparing both groups of $cHGA_3$ - 200cH - 60°C: p=0.032, <0.05, which means that the null hypothesis is rejected.
Conclusion: the two groups are different from each other at the 5% level.

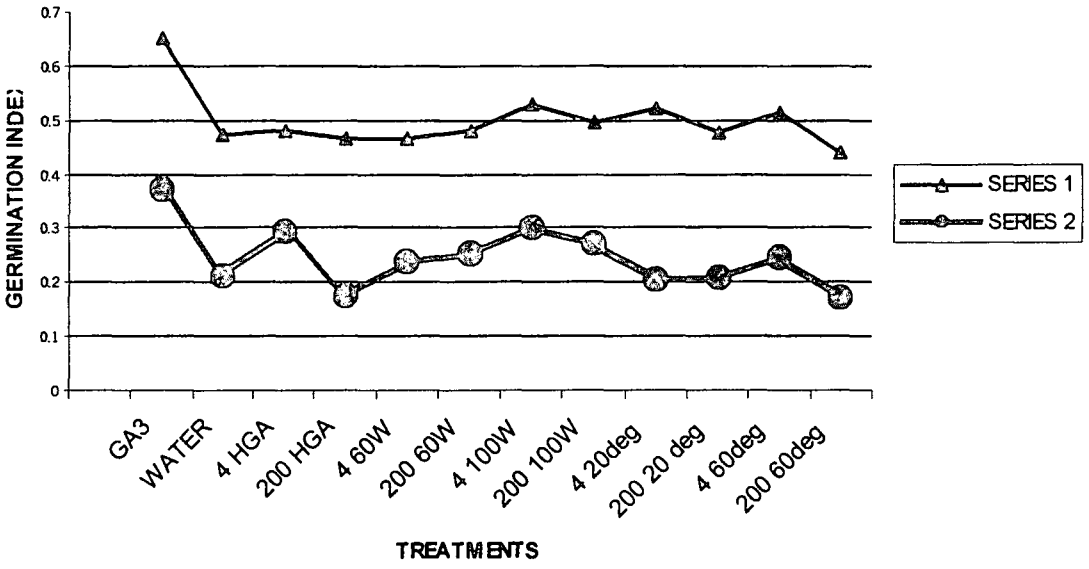

Figure 4.6 Comparison of germination index between the two series. Treatment designations as for Figure 4.1.

4.9 Germinability of seedlings

Table 4.27 Changes in % germinability over time. One freeze-thaw cycle between each stage.

1.	Germinabily test: Initial percentage before commencing with experiments	98%
2.	Experiment: Series one	53%
3.	Germinability test: 100 seeds imbibed in 12,5ml of water	27%
4.	Germinabilty test: 50 seeds imbibed in 12,5ml of water	38%
5.	Experiment: Series two	25%

CHAPTER FIVE

DISCUSSION OF THE RESULTS

The methodology used for this study was based on that of Him Lok (2001). Experimental conditions replicated those of Him Lok as closely as possible. The study was conducted concurrently to Stubbs (2001), which used the same experimental conditions. For this reason, similar results were expected for control groups, those treated with gibberellic acid (GA_3) ($0.5gL^{-1}$), water, homoeopathically prepared gibberellic acid ($cHGA_3$) 4cH and 200cH. The results of these studies varied, resulting in uncertainty regarding the reliability of this methodology and difficulty in interpreting the results.

5.1 Comparison between control groups

Seedling development of seeds imbibed in GA_3 ($0.5\ gL^{-1}$) *versus* water showed varying results. There was a significant increase between GA_3 ($0.5\ gL^{-1}$) and water control treatments only in the germination index. The mean root length and dry mass were significantly lower in seeds imbibed with GA_3 ($0.5\ gL^{-1}$) than those of seeds imbibed in water. Exogenous application of gibberellic acid appeared to increase the rate of germination, but not the size of the seedling.

A marked drop in germination was found in series one of the germination index when compared to initial germinability tests. Further germinability tests were conducted, including a repetition with an increase in the volume of water per seedling during imbibition. It was found that the germinability dropped further in seedlings imbibed in the initial quantity of water. Seeds imbibed with double the amount of water used in series one showed an increase in germination. This implies that an optimum quantity of water was not available to the seedlings in the experiments. The experiment was repeated with germination index as the measurement criteria, and there was a further drop in germination in series two of the germination index when compared to series one. This drop in germination is presumed to be due to the storage of the seeds. The seeds were stored in a freezer to break dormancy. Barley seeds

are orthodox seeds and therefore hold very little to no free water. They should not be susceptible to freezing damage. In this case, the barley seeds may have absorbed or imbibed some water during thawing and refreezing, leading to development of ice crystals within the seed. Crystals rupture the inner membranes, which results in leakage of constituents and reduced viability. (Erdey, 2001.)

There was a significantly increased rate of germination, as demonstrated by an increased germination index, in seeds imbibed in GA_3 (0.5 gL^{-1}) when compared to water in both experiments. This is in accordance with the known action of GA_3. There was no significant difference between $cHGA_3$ - 4cH and 200cH when compared to water in either experiment. This indicates that $cHGA_3$ did not significantly affect germination rate in this study. Despite the lack of significant differences among seed treatments, comparison of the results of the germination index in the two experiments, suggests a similar trend in the results of the two experiments (see Figure 4.6). Certain trends can be noted:

- An increased germination from seeds imbibed in $cHGA_3$ - 4cH compared to water is evident on the graph in series two, yet there is no significant difference to water. This difference was larger than in series one, possibly because the internal mechanism leading to germination had become less effective (presumably due to freezing damage), therefore it can be expected that addition of an external factor stimulating germination would show a more marked effect.
- Higher dilutions ($cHGA_3$ - 200cH) were less effective in promoting germination than lower dilutions ($cHGA_3$ - 4cH). This also applied to dilutions previously exposed to light or heat, with the exception to light at 60 Watt. Here the higher dilution ($cHGA_3$ - 200cH) showed a similar or slightly greater effect on the germination than $cHGA_3$ - 4cH.
- All the effects of the homoeopathic dilutions were slight and did not differ significantly from the negative control (water).

The optimum biologically active concentration of GA_3 (positive control) is 10^{-4}-10^{-5} mol/L in the presence of approximately 20mmol/L Ca^{2+} (Wilkins, 1984:42). The $cHGA_3$ - 4cH has a concentration of 2.6×10^{-10} mol/L, while the positive control has a concentration of approximately 1.3×10^{-3} mol/L. Thus the $cHGA_3$ - 4cH still contains molecules of the original substance and may have been exerting an effect through the biological properties of GA_3 present in solution, rather than through information stored in the solvent. Therefore this dilution does not provide conclusive evidence of the biological activity of homoeopathic dilutions.

With regards to root length, GA_3 (0.5 gL^{-1}) showed a significant inhibitory effect on seedling root development when compared to water. There was a significant increase in root length with seeds treated with water. $cHGA_3$ - 4cH (control group) showed a significantly reduced mean root growth when compared to water, whereas $cHGA_3$ - 4cH was not significantly different from GA_3 (0.5 gL^{-1}). This demonstrated that $cHGA_3$ - 4cH has an inhibitory effect on the development of root length to the same extent as GA_3 (0.5 gL^{-1}) when compared to water. $cHGA_3$ - 200cH showed no significant reduction of root length, demonstrating that $cHGA_3$ - 200cH has no effect when compared to water on root length. In a previous study by Him Lok it was also noted that GA_3 (0.5 gL^{-1}) had a significant inhibitory effect on root length. The concentration of GA_3, at 0.05gL^{-1} (~ 1.3×10^{-3}mol/L), is 10-100 times higher than the optimum level of GA_3. This excess can inhibit root growth, as seen by Him Lok and in the present study. Although 4cH is at a dilution level below Avogadros limit, with molecules of the original substance still remaining, the concentration is considerably lower ($5x\ 10^{-9}$g/L) than the GA_3 control ($5x\ 10^{-1}$g/L). This demonstrates the biological action at this dilution level.

With regards to shoot length, there was no significant difference between control groups. Imbibing seeds in $cHGA_3$ - 4cH and 200cH had no effect on shoot growth.

Similar results were found in the mean dry mass. GA_3 (0.5 gL^{-1}) had a significantly reduced dry mass relative to all other controls and treatment groups. Reduced seedling mass indicates utilization of seed reserves, hence

GA_3 promoted increased germination. The $cHGA_3$ dilution had no effect on the dry mass when compared to negative control (water), thus homoeopathic dilutions showed no biological effect on this parameter in this study.

5.2 Effects of light and heat

Results of this study showed that only $cHGA_3$ - 4cH had statistically significant results on root length when compared to water, therefore this was the only form of measurement that could be compared to the experimental groups that had been exposed to light and heat.

Treatment groups of $cHGA_3$ - 4cH - 60°C showed a significant increase in root length when compared to the $cHGA_3$ – 4cH (control). This suggests that temperature at 60°C has a negating effect on $cHGA_3$ - 4cH. Possible reasons why a temperature of 60°C may negate the effect of a homoeopathic dilution may be due to increasing kinetic energy of the system and hence increasing the movement of water molecules. This theory can be explained using the model formulated by Resch and Gutmann (1991). In this system, the water molecules maintain the structure of a homeopathic dilution. Any change in the structural information (by adding a solute) will be transferred throughout the system and integrated into the whole, interconnected, system (as discussed in 2.1.4). The molecules are never isolated but united within the continuous pattern. An addition of heat to this delicate structure may break the structure of the system by increasing the movement of the solute molecules, increasing the distances between them and altering their position relative to each other.

According to Resch and Gutmann (1991), shaking (succussion) facilitates the process of information storage when making up a homoeopathic dilution. This is a mechanical form of energy, which is perhaps why it does not have a detrimental effect of the structure of the water. Temperature affects the internal degree of freedom of the molecules as well as the surface tension, viscosity and physical properties of the water molecules. Heat breaks hydrogen bonds while shaking (succussion) is unlikely to do so.

The molecular interpretation of temperature can be explained according to a basic principle of classical physics called the theorem of equipartiton of energy (Serway 1998:454). This theory proposes that the energy of a system in thermal equilibrium is equally divided among all degrees of freedom. The 'degrees of freedom' refers to the way in which a molecule behaves (*i.e.* movement along the x,y,z axes). In this theory, temperature is proportional to the average kinetic energy of the molecules. As heat is introduced into the system, the change in temperature is gradual. A temperature gradient is set up within the liquid. This requires a finite time for equilibrium to be reached. An increased temperature results in increased kinetic energy of molecules within the liquid. As all molecules are interconnected, so all are affected. Since a temperature gradient is set up within the liquid, the kinetic energy of the molecules is not simultaneously increased throughout the liquid. Hence a decoherence in the oscillations of the molecules occurs, and hence information is lost. Equilibrium is eventually reached within the liquid but at a higher temperature, and the coherent structure initially present in the liquid is lost. (Govender, 2001.)

White light with a power rating of 60 Watt and 100 Watt respectively had no effect on $cHGA_3$ - 4cH.

5.3 Results from other studies

Results reported by Him Lok (2001) indicated that $cHGA_3$ - 4cH and 200cH had a statistically significant effect on root and shoot length when compared to water (see 2.4). A study by Stubbs (2001) showed no significant difference between groups treated with water and $cHGA_3$, when using root and shoot length, dry mass and germination index as measurement criteria. To clarify the lack of consistency, the experiment was repeated using the germination index aspect as the measurement criteria. This latter experiment showed no significant difference between $cHGA_3$ and water.

As there were varying results in the three independent studies, there is some doubt as to the validity and reliability of the methodology. All conditions for this experiment were similar to those of Stubbs (2001) and were conducted at

the same time, yet different results were obtained in control groups. This phenomenon is not readily explained.

In a study by Hopkins (1998), using a germinability trial similar to the one used in this study, on *Lactuca sativa* (lettuce seeds), homoeopathically prepared dilutions did show biological effects. This is contrary to what was found in this study with regards to the germination index used.

Future research needs to concentrate on the basic seed model used (see 6.2). It has been noted by Perry (1981:4) that the speed of germination and seedling growth frequently differs among seed lots. There are many unseen variables that may affect experiments. Only repeated repetitions by different researchers in other laboratories can fully clarify the effect of homoeopathic dilutions as measured by barley seed germination.

5.4 Summary

a) Results indicate that $cHGA_3$ - 4cH had a statistically significant effect on the root length of barley seedlings, supporting hypothesis 1.
b) There were statistically significant effects of 60°C on $cHGA_3$ - 4cH with regards to root length, thus supporting hypothesis 9.
c) Important observations were made regarding the use of a barley seed germination model in investigating the efficacy of homoeopathic dilutions.

6.2 Recommendations

1. The effects of an increased quantity of water and treatment solutions applied to the seeds should be compared.

2. The barley seeds should be stored in a cold room (4°C) rather than the freezer to prevent freezing-damage.

3. A lower dilution of gibberellic acid should be used as the control group.

4. The experiment should be repeated in other laboratories.

5. The study should be repeated after a few months to test the stability of the homoeopathic dilutions over a longer period of time.

6. Other possible forms of environmental influences, such as magnets, electricity, x-ray, sunlight and computers could be tested using the same model.

7. The effects of different types of dilutions *e.g.* decimal scale (1:10), and quinquagenimillesimal scale (1:50 000) could be tested.

8. Other dilution levels, below and above 200cH could be tested.

9. The dilutions could be exposed to different ranges and types of light and heat e.g. fluorescent light, oven heat.

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LIST OF APPENDIXES

APPENDIX A

(International Rules for Seed Testing. 1999. Seed Science and Technology. **27**: 155-162.)

1) Normal seedling growth was assessed in terms of the following:

a) **root system:** at least two seminal roots intact or with only slight defects:
e.g. - discoloured or necrotic spots.

b) **shoot system:**
the mesocotyl, intact or with only slight defects
e.g. - discoloured or necrotic spots
the coleoptile, intact or with only slight defects
e.g. - discoloured or necrotic spots
- loose twists
- split from one third or less from the tip the leaf intact, emerging through the coleoptile near the tip or with only slight defects

e.g. - discoloured or necrotic spots
- slightly damaged

c) **seedling:** all the essential structures normal as detailed above

2) Abnormal seedling growth was assessed in terms of the following:

a) **root system:** the seminal roots defective or insufficient
e.g. - stunted or stubby
- retarded
- only one or completely missing
- broken
- constricted
- spindly
- glassy
- decayed as a result of primary infection
- with negative geotropism

b) **shoot system:**
the mesocotyl, (where developed) defective
e.g. - broken
- decayed as a result of primary infection

the coleoptile, defective
e.g. - deformed (*e.g.* short and thick due to phyto-toxic effect)
- broken
- missing
- with the tip damaged or missing
- forming a loop or spiral
- tightly twisted
- strongly bent over
- split for more than one third of length from the tip
- split at the base
- spindly
- decayed as a result of primary infection

the leaf, defective:
e.g. - extending less than half-way up the coleoptile
- missing
- shredded or otherwise deformed

c) **seedling:** one or more of the essential structures abnormal as detailed above, or normal development prevented, because the seedling as a whole is defective

e.g. - deformed
- two fused together
- yellow or white
- spindly
- glassy
- decayed as a result of primary infection

Additional definitions:

Coleoptile:	The sheath enclosing and protecting the apex of the axis of the embryo and young seedling
Cotyledon:	The first leaf of a seedling
Embryo:	Rudimentary plant contained in a seed
Endosperm:	Nutritive tissue originating from fertilization and maintained as a storage tissue for food reserves
Mesocotyl:	The part of the seedling axis between the point of attachment of the scutellum and the coleoptile
Seminal roots:	The primary and secondary roots arising from the embryo axis
Scutellum:	a shield-shaped structure that is part of the cotyledon. Here nutrients are absorbed from the endosperm into the embryo

APPENDIX B

The Germination index of Walker-Simmons (1988) was used.

$$GI = \frac{(7 \times n1 + 6 \times n2 \ldots + 1 \times n7)}{\text{Total days x total seeds}},$$

where n1, n2 ...n7 are the number of seeds that germinate on the first, second and subsequent days until the seventh day, respectively. 7,6...1 are weights given to the number germinated on the first, second and subsequent days respectively. The maximum GI is 1.