A comparison between the efficacy of radionically prepared gibberellic acid and Homoeopathically prepared gibberellic acid (GHP) on the germination rate and seedling development of barley seeds.

By

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Dissertation submitted in partial compliance with the requirements of the Master's Degree in Technology: Homoeopathy in the Faculty of Health Sciences at the Durban University of Technology

I Gerhard Kleingeld do declare that this dissertation is representative of my own work, both in conception and execution.

Signature of Student	**Date of signature**

APPROVED FOR FINAL SUBMISSION

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DEDICATION

I would like to dedicate my dissertation to my parents Estelle and Gerhard, my grandmother Dr Roux and my family for their incredible support throughout my studies.

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Abstract

Aim

The aim of this controlled, experimental study was to compare the biological activity of various homoeopathic potencies of gibberellic acid manufactured radionically (AMS transfer device) and conventionally (GHP) in terms of their respective influence on germination rate and seedling development of barley seeds; all the respective results being contrasted against those produced by the distilled water control.

Methodology

The research was completed by employing quantitative research techniques and followed true experimental design. Homoeopathically (Hahnemannian) prepared gibberellic acid followed the manufacturing guidelines of method 5a involving liquid preparations, as specified in the German Homoeopathic pharmacopoeia (GHP) (Benyunes 2005).

A second radionic 'equivalent' version of each of the Hahnemannian potencies was manufactured using the 'AMS wave transfer' device. Four sources of data were collected namely, germination count and rate, seedling development (root length), seedling dry mass, and number of seeds with measurable roots. All the data was collected and documented on a data collection sheet using Microsoft Excel. All the data was statistically analysed and subjected to analysis of variance (ANOVA) using GenStat Version 14 (VSN International, UK) at the 5% level of significance. The statistical data was used to produce a comparison between the different remedies and distilled water.

Results

All of the remedy treatment groups (Radionic 200c, Hahnemannian 200cH, Radionic 4c and Hahnemannian 4cH) displayed suppressive effects (to certain extents respectively) on seed growth and development in comparison to the control group (distilled water). The control group displayed greater seedling development in comparison to all remedy treatment groups which was most evident in the **average root lengths** and **high vigour seed lot root lengths** having longer roots than all remedy treatment groups. The control group also displayed a higher **number of seeds with measurable roots** compared to all the remedy treatment groups in both total number of seeds and in the seeds accounted for in the high vigour lots. This suggests that all Homoeopathic remedies irrespective of potency or manufacture method (Radionic or Hahnemmanian) had similar suppressive effects on root growth and seedling development and this suppressive effect was in turn not evident in the control group.

Conclusion

The experiment results suggest that radionically manufactured homoeopathic remedies (AMS wave transfer device) have similar biological effects (suppressive effects) to the equivalent Hahnemannian manufactured homoeopathic remedies, although further research in this field is necessary to confirm these findings the results from this study are supportive of the use of radionically prepared remedies in homoeopathic practice.

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Table of abbreviations

AMS	The Advanced Medical Systems
c	Centesimal scale
cH	Centesimal scale Hahnemannian
CH2	Methylene group
CH3	Methyl group
CV	Coefficients of variation
D or X	Decimal scale
GA3	Gibberellic acid
GHP	German Homoeopathic Pharmacopoeia
HGA3	Homoeopathically prepared Gibberellic acid
H2O	Water molecule
LSD	Least Significant Difference
ml	Millilitre
n	Number
NMR	Nuclear Magnetic Resonance
OH	Hydroxyl group
TZ	Tetrazolium Chloride

Definitions of terms

Arndt Schulz Law

This law states that small doses stimulate, moderate doses inhibit (interfere with biochemical pathways) and large doses destroy or kill. This law is used in connection to the mechanism of action of highly diluted Homoeopathic substances (Kayne 2006).

Avogadro's number

Avogadro's number is the concentration level of a dilution at which no molecules remain in solution the point is exceeded. Once a substance is diluted past the 12c or 24X level, no molecules of the original solute is considered to remain in solution (Kayne 2006).

Centesimal potency

This is a scale used (denoted 'c') which refers to the number of successive dilutions (1:100). The first potency contains one hundredth part of the base substance and is succussed a select number of times. Each of the succeeding potencies contains one hundredth part of the preceding potency and is succussed a select number of times (Gaier 1991).

Decimal potency

This is a scale used (denoted 'D' or 'X') which refers to the number of successive dilutions (1:10). The first potency contains one tenth part of the base substance and is succussed a select number of times. Each of the succeeding potencies contains one tenth part of the preceding potency and is succussed a select number of times (Gaier 1991).

Gibberellic acid

Gibberellic acid (GA3) is a chemical or plant hormone that stimulates the growth of seeds. Gibberellic acid (GA3) belongs to a larger gibberellin group which consists of plant hormones that have similar physiological action (Copeland and McDonald 2001).

Hormesis

Hormesis can be defined as a concept in which stimulatory effects can be seen in biological organisms after these organisms have been exposed to a low concentration (dose) of a known toxic substance (Oberbaum and Cambar 1994).

Law of similars

Homoeopathic remedies are prescribed according to the Law of Similars which was established by the founder of Homoeopathy, Samuel Hahnemann. This law allows Homoeopathic practitioners to connect symptoms of disease or ill health with substances that produce similar symptoms when tested on a healthy person (O'Reilly 1996).

Pharmacopoeia

A book published by a jurisdiction body which contains all details of medicinal manufacture including their formulas, dosages, manufacture requirements and standards of purity (Martin 2007).

Potency

Potency is the level of dilution of a substance which has been succussed at each de-concentration level during the preparation (potentization) process (Gaier 1991).

Potentisation

Potentisation or Dynamization is a process which includes dilution of the prepared substance and a selected amount of successions at each de-concentration level. The potentisation process increases the therapeutic strength of the remedy produced (Kayne 2006).

Radionic device

A radionic device is a device used to duplicate or re-create an existing homoeopathic remedy or treatment. In the case of the “AMS” device, a bipolar input and output cup is used for transmission and storage of electromagnetic signals from the homoeopathic remedy to blank vehicle (AMS 2004).

Remedy

A healing medicinal treatment that cures or will relieve a disease or disorder (Martin 2007).

Succussion

An action forming part of the potentisation process in which a substance in solution is vigorously shaken in a downwards motion ending in a sudden impact (Kayne 2006).

Vigour (of the seeds)

Seed vigour is a term used which includes various properties that determine the level of physiological potential of the seeds and the ability of the seeds to develop. The vigour of the seeds is usually an indication of the seeds ability to germinate and grow (Copeland and McDonald 2001).

Water Clusters

Water clusters are individual groups of specifically arranged structural bonds between water molecules which have inherent frequencies. The structural arrangements of the water molecules (Hydrogen bonds) are what enable it to store information (AMS 2004).

Chapter 1

Introduction

1.1 Introduction

Radionically prepared homeopathic remedies are widely used by practitioners and anecdotal evidence suggests that radionically prepared remedies are as effective as those prepared by conventional means; however no literature could be sourced which measures or confirms the biological activity of such remedies. There are various radionic devices on the market that claim to produce remedies with the same effects as their corresponding conventionally manufactured (Hahnemannian) homoeopathic remedies and the question has been raised whether the biological effects of these radionic remedies are similar to the 'corresponding' conventional remedies when applied in practice.

Allsopp (2010) stated that further studies into radionic potencies are needed and that biological activity of radionically produced remedies should be thoroughly investigated and compared with that of the equivalent Hahnemannian produced remedies.

Homoeopathic remedies are used successfully by many practitioners around the world but some may criticise remedy production methods and the efficacy of the remedies claiming that the improvement after administration of the remedies are due to the perception (placebo effect) of patients. Therefore it is ideal to eliminate human perception (possible placebo effect) in a study. This can be achieved by using plant models to demonstrate that homoeopathic preparations are biologically active and it would support the validity of homoeopathic remedy preparation methods (Hamman, Koning and Him Lok 2001).

Healthy plant models have shown to be a very useful approach to investigate basic research questions about the specificity of homeopathic preparations (Majewsky *et al*. 2009). This methodological model using seedlings is the most frequently used experimental plant model in homoeopathy. There are several advantages of using plant models for homoeopathic research such as eliminating the placebo effect and avoiding the ethical implications associated with human studies, in addition plant methodological models allow testing of hypotheses in limited time frames (Majewsky *et al*. 2009).

In this study double blind, randomised study methods have been implemented ensuring the most valid and un- biased results as the researcher cannot affect the random treatment groups with biased methods as he is unaware of the treatment used on treatment groups. A control group is also included in the study design as further comparative and re-enforcing results.

Radionically prepared homoeopathic remedies are manufactured by using a device which creates an “energy pattern” that mimics/duplicates the therapeutic action and properties of the original remedy substances. The creation of radionic remedy ‘equivalents’ that correspond to the remedies from the homoeopathic Materia Medica can be used therapeutically (Franks 2000).

Theories on how remedy information is maintained in radionic form are mostly based on the principle that water has memory.

Water consists of H2O molecules that continuously form bonds between each other and within these bonds a structural framework can be observed. In water there are electric dipoles and many individual groups of specifically arranged water molecules called clusters which have inherent frequencies. The structural arrangement of the water molecules is what enables it to store information. Although it was previously believed that the hydrogen bonds are too weak and unstable, it is now known that water has a crystalline liquid component with hydrogen bonds that are connected very firmly (AMS 2004).

When the device is activated it transmits the specific frequency of the original remedy to the water in the receiving output pole of the device. The water clusters will rearrange until they are exactly in resonance with the signal provided from the original remedy. However this new cluster pattern is considered weakly linked and more energy is required (in the form of succussions) to form strong hydrogen bonds in crystalline liquid form (AMS 2004).

Although literature comparing the remedy structure and chemical composition of radionically prepared homeopathic remedies with that of conventionally prepared equivalents exists, no literature around the biological activity of radionically prepared homoeopathic remedies could be sourced, similarly no literature comparing biological activity of radionically prepared remedies with that of conventionally prepared ones have been identified.

The biological activity of conventionally manufactured (Hahnemanian) gibberellic acid 4CH, 15CH, 30CH and 200CH was demonstrated by Hamman, Koning and Him Lok (2001) in terms of their effect on germination and development of barley seedlings. Similarly the biological activity of other plant hormones in various homeopathic dilutions has been demonstrated using similar seed models including wheat. Bruni (2001) conducted a study on the effectiveness of high dilutions of abscisic acid on barley endosperm half-seeds and stated that his study demonstrated the feasibility and value of the barley endosperm half-seed model as an attractive experimental model to establish the effects of ultra-high dilutions (Homoeopathic remedies) as the seeds have shown sensitivity to high dilutions of abscisic acid and gibberelic acid.

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It is expected that the research will be of interest to practitioners using radionic devices or who are planning to make use thereof. It may also be helpful to students, academics and researchers in the field of homoeopathy and radionic remedies.

1.2 Aim and objectives

The aim of this controlled, experimental study was to compare the biological activity of various potencies of gibberellic acid manufactured radionically (AMS transfer device) and conventionally (GHP) in terms of their respective influence on germination rate and seedling development of barley seeds in addition all the respective remedies were compared to a control (Distilled water) to determine the significance of the results.

The individual objectives were as follows

1) To determine the influence of various potencies of gibberellic acid manufactured conventionally (GHP) on germination rate and seedling development of barley seeds.

2) To determine the influence of various potencies of gibberellic acid manufactured radionically (AMS transfer device) on germination rate and seedling development of barley seeds.

3) To determine the influence of distilled water (negative control) on germination and seeding development of barley seeds.

4) To compare the respective influence of gibberellic acid (manufactured conventionally and radionically) and distilled water on germination rate and seedling development of barley seeds.

Although it is not a primary objective, assessing the difference in biological affect that potency (4cH and 200cH) had on the seed development was also relevant and important.

1.3 Hypothesis

1) It is hypothesised that there is a difference in biological effect between radionically prepared gibberellic acid and conventionally prepared homoeopathic gibberellic acid (GHP) on the germination rate and seedling development of barley seeds.

2) It is hypothesised that there will be a significant difference in biological effect between the conventional homoeopathic remedies and the control (distilled water).

The researcher assumed that biological activity would be evident in the Hahnemannian remedy as seen in previous studies but if biological activity would be seen from the radionic remedy groups was to be established.

Chapter 2

Literature Review

2.1 A brief history of homoeopathic potentisation

Potentisation, which may also be referred to as dynamisation forms part of the precise method of the Homoeopathic remedy production process and involves dilution and succession. The potentisation process increases the therapeutic strength of the remedy (Kayne 2006).

Samuel Hahnemann, the founder of homoeopathy formulated the laws and principles on which homoeopathy is based and developed all the guidelines for the manufacturing of homoeopathic remedies applying the method of potentisation. Hahnemann's potentisation process can be described as the active process of imparting the pharmacological message of the original substance into a suitable vehicle by exposing the original substance to serial dilutions and succussions (or trituration) in the selected vehicle at each level of de-concentration. This manufacturing process increases the physical solubility of the drug while also increasing the therapeutic activity in its use as a homoeopathic remedy (Gaier 1991).

Homoeopathic remedies are prescribed according to the Law of Similars which was established by the founder of Homoeopathy, Samuel Hahnemann. This law allows Homoeopathic practitioners to connect symptoms of disease or ill health with substances that produce similar symptoms when tested on a healthy person. These substances however need to have been prepared using the Homoeopathic potentisation process before it can be used effectively as a treatment (O'Reilly 1996).

For example: The remedy Apis mellifica is produced from a honey bee including its venom. This venom causes swelling, redness and pain of an affected area in a person when introduced in crude substance form (after being stung) but when it is prepared in homoeopathic form it has the opposite effect in that it prevents or decreases redness, swelling and pain in the affected part.

During his early experimentation Hahnemann discovered that when smaller doses are used the toxicological effect decreases while maintaining and enhancing the therapeutic effect. In these experiments Hahnemann ingested crude doses of Cinchona bark (containing quinine) which produced similar symptoms (including fever attacks) to the disease picture of malaria. The Law of Similars (like

cures like) is one of the fundamental principles in homoeopathy and through the application of the principle it became clear that when a substance (for instance quinine) is taken in large crude doses it produces the toxicological symptoms (fever) of the disease but if it is taken in small potentised doses, it could cure the symptoms it produced. Hahnemann's final potentisation methods and views on dose were arrived at gradually, after years of experimentation (Barthel 1991).

These small doses may fall below or above Avogadro's number depending on the potency. The limit of Avogadro's number is reached at the homeopathic centesimal potency scale of 12 cH. Homeopathic potencies below 12cH may still contain molecules of the original crude substance in solution, but in the higher potency ranges (greater than 12cH) no molecule of the original crude substance is expected to remain (Gaier 1991).

The science behind the process of potentization and production of effective remedies (even in the absence of any molecule of the original substance) is based on the fact that water molecules have a changeable structural network and inherent memory. The structure of water will be discussed further in 2.2 (Radionics in homoeopathy) as the theory behind homoeopathic remedy manufacture and radionic remedy manufacture share similarities.

In homoeopathic science it is claimed that during the potentisation process the original remedy information is integrated and dynamically maintained within the more dilute solution (Resch and Guttman 1987). During the potentisation process the crude substance is exposed to a process of serial dilutions using either water, alcohol or a combination of both in solution. The pharmacological properties of the original crude substance are transferred to all successive potencies and the succussion process activates and incorporates the latent curative powers. The vital curative properties of all of the crude substances used are only observed after potentisation (Gaier 1991).

Hahnemann developed a method of potentisation in which separate vials are used for each successive step in the manufacturing process. All of the vials are filled with ninety-nine drops of a desired percentage alcohol then one drop of the relevant substance is added to the first vial and the vial goes through the succession process. Hahnemann could not come to final decision on the number of succussions used in his manufacturing process but succession numbers are established by which pharmacopoeia is used and the number always remain consistent throughout the entire process. Whenever the Hahnemannian method of potentisation is used to produce remedies it is indicated by using the H symbol after the deconcentration level (ie 30 cH). Hahnemann attributes the powerful effect of the remedies which may be very dilute to the way in which the remedies are prepared as the remedies become more potent by using the potentisation method (Kayne 2006).

There are different potencies used in homoeopathy and manufacturing methods may depend on the individual potency needed. The manufacturing processes of the first two classes are similar and the centesimal class was used to prepare the treatments for this study.

These classes are:

Decimal

This is a scale used (denoted 'D' or 'X') which refers to the number of successive dilutions (1:10). The first potency contains one tenth part of the base substance and is succussed a select number of times. Each of the succeeding potencies contains one tenth part of the preceding potency and is succussed a select number of times (Gaier 1991).

Centesimal

This is a scale used (denoted 'c') which refers to the number of successive dilutions (1:100). The first potency contains one hundredth part of the base substance and is succussed a select number of times. Each of the succeeding potencies contains one hundredth part of the preceding potency and is succussed a select number of times (Gaier 1991).

All of the methods for manufacturing of different base substances into homoeopathic potencies can be found in the German homoeopathic pharmacopoeia (GHP) (Benyunes 2005).

The Arndt Schulz Law is an important guideline which assists in understanding the effect that dose has on living organisms. This law states that small doses stimulate, moderate doses inhibit (interfere with biochemical pathways) and large doses destroy or kill. The Law of Cure is another fundamental principle of Homoeopathy and is associated with the effects that different dose levels present. This law states: "*The quantity of action necessary to effect a change in nature is the least possible, and the decisive amount is always the minimum*" (Kayne 2006).

Both these Laws support the hypothesis that potentised Homoeopathic treatments could stimulate biological activity in living organisms (in this case plants) and when the current study is considered, the biological effect would be suppressive in nature according to the Law of Similars (gibberellic acid in its crude form stimulates germination of seeds).

Hormesis is another concept used to describe the stimulatory effects that low concentration (dose) substances may have on biological systems. Hormesis can be defined as a concept in which stimulatory effects can be seen in biological organisms after these organisms have been exposed to a low concentration (dose) of a known toxic substance. It should however be noted that this concept may not apply to some ultra-high diluted Homoeopathic treatments as hermetic effects act at much higher concentrations than can be seen from some Homoeopathic substances (Oberbaum and Cambar 1994).

This concern may have been addressed as many studies (including the study done by Hamman, Koning and Him Lok 2001) have demonstrated biological effects on living organisms.

2.2 Radionics in homeopathy

Radionically prepared homoeopathic remedies are manufactured by duplicating the dynamic medicinal properties of the original Hahnemannian produced remedy (at the input pole of the radionic device) into a suitable vehicle (at the output pole of the radionic device) and this newly formed substance (radionic remedy) should in theory mimic the therapeutic action of the original remedy from which it was produced. The practitioner is able to use instrumentation to produce such remedies using a conventional Hahnemannian remedy as a template (Franks 2000; AMS 2004).

Radionic remedies could prove to be beneficial in homoeopathic practice for various reasons and may be an attractive method of remedy manufacture if proven to be effective. Radionic remedies may be a cost effective and time saving alternative as practitioners will be able to manufacture required remedies immediately for patient use without having to stock or order more costly alternatives from manufacturers. This will be possible as only a single Hahnamannian remedy is required to produce many radionic equivalents. Radionic devices are relatively new to the homoeopathic industry and scepticism regarding the effectiveness of radionically prepared remedies exists among practitioners as not many studies have been conducted to prove treatment results and radionic remedy manufacture does not directly follow the strict guidelines given by Hahnemann.

One of the few studies on radionic remedies was that of Allsopp (2010); Hahnemannian and radionically prepared potencies of *Natrum muriaticum* were compared using Nuclear Magnetic Resonance (NMR) Spectroscopy. This study focused on measuring the physical aspects of the different remedies namely the chemical shift and relative integration values of H2O, OH, CH2 and CH3. Allsopp determined that there were differences in the physico-chemical properties of the respective correlating potencies of radionic and Hahnemannian produced remedies but suggested that differing physico-chemical properties did not necessarily indicate differing biological and therapeutic activity of the two groups of remedies.

In this regard Allsopp suggested that biological activity of radionically produced remedies should be thoroughly investigated and compared with that of equivalent Hahnemannian produced remedies.

The Advanced Medical Systems (AMS) Wave Transfer device was developed by Dr W.Ludwig of the Institute for Biophysics in Tauberbischofsheim (Germany). This radionic device is used to duplicate an existing homoeopathic remedy; transmitting it to a blank vehicle (the radionic version [copy] of the original remedy). The device uses a bipolar input and output cup for transmission and storage of electromagnetic signals from the homoeopathic remedy to the blank vehicle. The bipolar cup creates an almost interference free signal transmission process (AMS 2004).

Theories on how remedy information is maintained in radionic form are mostly based on the principle that water has memory, this same principal also forms part of some theories on the method of action of Hahnemannian homoeopathic remedies.

Water consists of H2O molecules that continuously form bonds between each other and within these bonds a structural framework can be observed. In water there are electric dipoles and many individual groups of specifically arranged water molecules called clusters which have inherent frequencies. The structural arrangement of the water molecules is what enables it to store information. Although it was previously believed that the hydrogen bonds are too weak and unstable, it is now known that water has a crystalline liquid component with hydrogen bonds that are connected very firmly (AMS 2004).

When the device is activated it transmits the specific frequency of the original remedy to the water in the receiving output pole of the device. The water clusters will rearrange until they are exactly in resonance with the signal provided from the original remedy. However this new cluster pattern is considered weakly linked and more energy is required (in the form of succussions) to form strong hydrogen bonds in crystalline liquid form (AMS 2004).

Elia (2007) stated that in the last decade investigations have been done to determine if the water molecules in homoeopathically prepared medicines are structurally different from a physicochemical point of view to the initial water used to produce the homoeopathic medicines. It is understood that these preparations don't contain different molecules but many experimental results indicate a difference in structural framework between the water molecules of initial water and the homoeopathic medicines created from it.

Elia (2007) also stated that the experimental methodologies used in their investigation were well established physicochemical methodologies and were chosen as they were the most effective methods among the viable options. The tests conducted were flux calorimetry, conductometry, pHmetry and galvanic cell electrode potential; the findings in the study done by Elia (2007) support the concepts behind both radionic remedy production and Hahnemannian homoeopathic remedy production.

Radionic devices are relatively new to the homoeopathic industry and different radionic device variations exist. It is important to note that these devices may have vastly different methods of remedy production and theories on the method of action which may not be as scientifically based as the (AMS) Wave Transfer device. The current research conducted using the (AMS) Wave Transfer device can therefore only be compared to radionic devices following the same method and principles in remedy production.

2.3 Homoeopathic research utilising plant testing models

Healthy plant models have shown to be a very useful approach to investigate basic research questions about the specificity of homeopathic preparations. This methodological model using seedlings is the most frequently used experimental plant model in homoeopathy. There are several advantages of using plant models for homoeopathic research including the vital aspect of eliminating the placebo effect and avoiding the ethical implications associated with human studies. In addition plant methodological models allow testing of hypotheses in limited time frames and can be conducted using limited financial resources. Plant models also present the added advantage of easy experimental repetition (Majewsky *et al*. 2009).

Gibberellic acid (GA3) is a chemical or plant hormone that stimulates the growth of seeds. Gibberellic acid (GA3) belongs to a larger gibberellin group which consists of plant hormones that have similar physiological action. In the normal seed growth process Gibberellin is released by the embryo and circulates towards the endosperm layer of the seed. Here the Gibberellin activates the enzyme amylase which causes the conversion of starch into sugar. Sugar can then be used as energy by the seed to stimulate protein synthesis which is required for the seed to sprout. Gibberellic acid (GA3) is a chemical commercially used to stimulate and increase plant growth. The increased growth effects of Gibberellic acid can be seen on root growth, stem growth, increased plant size (larger fresh and dry weight) and can be used to improve growth of nutrient deficient plants (Copeland and McDonald 2001).

Therefore gibberelic acid is a plant hormone that stimulates growth in the seeds and according to the Law of Similars, if gibberelic acid is prepared in homoeopathic potency it should have a suppressive effect on seed growth.

Seed vigour played an important role in this study and this concept should be understood. The vigour of the seeds is usually an indication of the seeds ability to germinate and grow. Seed vigour is a term used which includes various properties that determine the level of physiological potential of the seeds and the ability of the seeds to develop. When seeds have decreased ability to develop and perform the physiological functions required to grow they are considered physiologically aged and are of lower vigour (ie medium and low vigour seeds). High vigour seeds are most responsive and should show superior development to medium and low vigour seeds (Copeland and McDonald 2001). Medium and low vigour seeds used in this study have undergone a physiological aging process.

Hamman, Koning and Him Lok (2001) stated that even though homoeopathic remedies contain very few or no molecules of the original substance, gibberelic acid in homoeopathic potency stimulated biological activity in the seeds; homoeopathically prepared giberellic acid in various potencies (4cH, 15cH, 30cH and 200cH) were applied to barley seeds of low, medium and high vigour levels and a comparison was made (seedling mass, root length and shoot length) with that of distilled water (control). The high-vigour seeds imbibed in homoeopathically prepared gibberelic acid at 200cH level produced larger seeds by mass compared to the other input variables in the study and homoeopathically prepared gibberelic acid in all potencies used demonstrated significant measurable differences compared to that of the control. The Homoeopathically prepared gibberellic acid 4cH potency was found to inhibit the rate of germination of low-vigour seeds.

Hamman, Koning and Him Lok (2001) stated that since endogenous gibberelic acid is contained in whole barley seeds, it may have been possible that effects of the homoeopathically prepared gibberelic acid would be evident in germination studies. However the study done by Hamman, Koning and Him Lok (2001) indicated that biological activity is possible when using whole barley seeds where previous studies done mostly used de-embryonated half barley seeds. Bruni (2001) conducted a study on the effectiveness of high dilutions of abscisic acid on barley endosperm half-seeds. His study demonstrated the feasibility and value of the barley endosperm half-seed model as an attractive experimental model to establish the effects of ultra-high dilutions (Homoeopathic remedies) as the seeds showed sensitivity to high dilutions of abscisic acid and gibberelic acid. Steele (1999) demonstrated that dilutions of gibberellic acid at a potency of 4cH are biologically active as it stimulated the synthesis of alpha-amylase in de-embryonated half-barley seeds.

Evans (2008) conducted a study where the effectiveness of homoeopathically prepared abscisic acid was tested on the germination of barley seeds and in this study it was evident that the homoeopathic remedies produced distinct biological effects in the barley seeds.

Homoeopathic potencies have displayed biological effects in past studies utilising various plant models which include wheat and maize.

Homoeopathic potencies of *Arsenicum album* 45D have displayed statistically significant biological effects on wheat seed development and the study was conducted utilising two independent research groups. Even though both of the research groups found that biological effects were evident on seed development it was concluded that more studies using similar plant models should be conducted to identify factors which affect size and direction of the results as the specific results on various output variables were not identical between these independent research groups. It is therefore important to repeat Homoeopathic studies utilising plant models as reproducibility of results has been an issue (Lahnstein *et al*. 2009).

Homoeopathic potencies of the herbicidal substance known as 2,4-D displayed biological effects on maize seed development as significant differences were evident in root and shoot development. It is also important that further studies be conducted utilising plant models to determine the effect that different potencies have on seed development as more knowledge on the mechanism of action of potencies is needed (Dragicevic *et al.* 2013).

Majewsky et al. (2009) conducted a study in which the literature on basic homoeopathic research involving biological plant models was reviewed. Majewsky et al. (2009) concluded that plant models seem to be a useful method to investigate the effectiveness of homoeopathic remedies and recommended that more biological plant studies with varying potentisation techniques be conducted. Majewsky et al. (2009) stated that a range of potency levels should also be used in future study designs and that reproducibility be investigated. It was also recommended that systematic negative control experiments be used to maintain stability throughout the experiment.

The purpose of this study is to determine if there is a difference in biological effect between Hahnemannian produced remedies and Radionically produced remedies by using the seedling model. The use of the seedling model in this trial can be justified as an effective method to determine remedy efficacy as many previous studies have delivered significant biological responses.

Chapter 3

Materials and methods

3.1 Introduction

The research was completed by employing quantitative research techniques and followed true experimental design.

This study followed the guidelines of previous research done using homoeopathically (Hahnemannian) prepared gibberellic acid in various potencies and a barley seed germination model (Hamman, Koning and Him Lok 2001). However, in this study an additional independent variable (Radionically prepared Gibberellic acid) was included alongside homoeopathically (Hahnemannian) prepared gibberellic acid and distilled water was applied as the control.

3.2 Manufacture of test substances

All the test substances (radionic and Hahnemannian) were manufactured by the researcher in the Homoeopharmaceutics laboratory located at the Department of Homoeopathy at Durban University of Technology. All the test substances were also manufactured under supervision. All lab equipment was thoroughly cleaned and final dispensing (500ml) bottles were autoclaved to prevent contamination.

The starting substance gibberellic acid, 90% (GA3) was manufactured by Acros Organics, New Jersey, USA and sourced through Labchem (Pty) Ltd. Edenvale, Gauteng. The gibberellic acid used in this study comprised of 90% gibberellic acid (GA3) and 10% potassium hydroxide and was selected due to its successful use in previous homoeopathic studies including that of Hamman, Koning and Him Lok (2001).

Homoeopathically (Hahnemannian) prepared gibberellic acid followed the manufacturing guidelines of method 5a involving liquid preparations, as specified in the German Homoeopathic pharmacopoeia (GHP) (Benyunes 2005). A detailed diagram depicting the remedy manufacturing process is included as Appendix F. To manufacture the first centesimal potency (1cH), one part of the material used (gibberelic acid 90%) was dissolved in 99 parts of the liquid vehicle (distilled water) and succussed ten times. No alcohol was used in the manufacturing process as alcohol may have stimulatory or inhibitory effects on the germination performance of barley seeds (Hamman, Koning and Him Lok 2001).

According to the German Homoeopathic Pharmacopoeia the second centesimal potency is made with one part of the 1cH and 99 parts of the liquid vehicle (distilled water). All subsequent potencies were produced following the same formula and using ten succussions except for the final Hahnemannian potencies which required one hundred succussions (Hamman, Koning and Him Lok 2001).

Remedies were applied to the seed lots in 4c and 200c (dilution of 1:108 and 1:10400 respectively) potencies which included dilutions both above and below Avogadro's number. Each of the Hahnemannian remedies were produced in the Homoeopharmaceutics laboratory at DUT and a second radionic 'equivalent' version of each of the Hahnemannian potencies was manufactured using the 'AMS wave transfer' device. The two respective Hahnemannian potencies of GA3 were denoted HGA3 4cH and HGA3 200cH respectively.

A sample drawn from each of the conventionally manufactured Hahnemannian prepared remedies was placed in the AMS wave transfer device input pole to create an equivalent radionic remedy (using distilled water) in the output pole. Each radionically prepared remedy was then removed from the output pole and needed to be succussed twenty times according to the user manual (AMS 2004). The radionically prepared 'equivalent' remedies produced were in corresponding homoeopathic potency to that of the Hahnemannian 'equivalents' i.e. in 4cH and 200cH potency and denoted as RGA3 4c and RGA3 200c.

Each treatment (Hahnemannian 4cH and 200cH and Radionic 4c and 200c) was produced in final volumes of 400ml for administration to barley seeds and the distilled water control was sampled from the same batch of distilled water used for the manufacture of the Hahnemannian and radionically prepared remedies.

3.3 Pre-experimental tests and preparations

Samples from the seed batches were used to perform various tests (Including *Tetrazolium Chloride* (TZ) tests) to determine if the seeds in the batches were of adequate quality. These tests were done according to the guide lines in the International seed testing association (*International Rules for Seed Testing* 2012) and the seeds were found to be of good quality.

The vigour of the seeds is usually an indication of the seeds ability to germinate and grow. Seed vigour is a term used which includes various properties that determine the level of physiological potential of the seeds and the ability of the seeds to develop. When seeds have decreased ability to develop and perform the physiological functions required to grow they are considered physiologically aged and are of lower vigour (ie medium and low vigour seeds) (Copeland and McDonald 2001). Medium and low vigour seeds used in this study have undergone a physiological aging process.

All the seeds initially used were high vigour seeds and samples from the high vigour batch were used to create the lower vigour seeds.

Medium vigour seeds were created by putting randomly selected high vigour seeds through an 8 hour imbibition process which caused a certain level of physiological aging and as a result decreased the responsiveness of these seeds. Low vigour seeds were created by putting randomly selected high vigour seeds through a 24 hour imbibition process which caused an even greater level of physiological aging.

3.4 Experimental procedure

3.4.1 Experimental site

The research experiment was conducted in the Seed Physiology Laboratory School of Agricultural, Earth and Environmental Science at UKZN Pietermaritzburg. Barley seeds (*Hordeum vulgare L*.) were supplied by the School of Agricultural, Earth and Environmental Science - University of KwaZulu-Natal. All laboratory work including taking care of the seeds on a daily basis was conducted by the researcher under supervision from the School of Agricultural, Earth and Environmental Science staff.

3.4.2 Experimental grouping and stratification

Each individual remedy (4cH, 200cH, Radionic 4c and Radionic 200c) and distilled water (control) was manufactured in 400ml volumes and applied to their own individual groups of barley seed lots (*Hordeum vulgare L*.) comprising 90 seeds per group (30 seeds each of low, medium and high vigour respectively) with a total of 450 seeds. The 30 seeds of each individual treatment and vigour group were divided into three replications of ten seeds for both statistical analysis purposes and to provide space needed for the growth of the seeds.

	Treatment 1 (RGA3 200c) (n=90)	**Treatment 2 (HGA3 200cH) (n=90)**	**Treatment 3 (RGA3 4c) (n=90)**	**Treatment 4 (Distilled water control) (n=90)**	**Treatment 5 (HGA3 200cH) (n=90)**
High vigour	Rep 1 (n=10) Rep 2 (n=10) Rep3 (n=10)	Rep 1 (n=10) Rep 2 (n=10) Rep3 (n=10)	Rep 1 (n=10) Rep 2 (n=10) Rep3 (n=10)	Rep 1 (n=10) Rep 2 (n=10) Rep3 (n=10)	Rep 1 (n=10) Rep 2 (n=10) Rep3 (n=10)
Medium vigour	Rep 1 (n=10) Rep 2 (n=10) Rep3 (n=10)	Rep 1 (n=10) Rep 2 (n=10) Rep3 (n=10)	Rep 1 (n=10) Rep 2 (n=10) Rep3 (n=10)	Rep 1 (n=10) Rep 2 (n=10) Rep3 (n=10)	Rep 1 (n=10) Rep 2 (n=10) Rep3 (n=10)
Low vigour	Rep 1 (n=10) Rep 2 (n=10) Rep3 (n=10)	Rep 1 (n=10) Rep 2 (n=10) Rep3 (n=10)	Rep 1 (n=10) Rep 2 (n=10) Rep3 (n=10)	Rep 1 (n=10) Rep 2 (n=10) Rep3 (n=10)	Rep 1 (n=10) Rep 2 (n=10) Rep3 (n=10)

Table 3.1 A summary of experimental design

3.4.3 Experimental methodology

The barley seeds were placed in petri dishes (9cm), lined with no. 1 Watmann filter paper and imbibed with one of the five treatments mentioned in Table 3.1. The seeds were placed within a dark growth chamber at 20^{o}C and more treatment was applied after 24 hours. Each petri dish was inspected once per day (24 hour intervals) to ensure moisture content was adequate and more treatment was applied to the petri dishes every day to ensure continuous treatment stimulatory effects and to prevent them from drying out.

3.4.4 Data sources and collection

Four sources of data was collected namely, germination count and rate, seedling development (root length), seedling dry mass, and number of seeds with measurable roots.

3.4.4.1 Seed development

Seed development was determined by measuring the root lengths of the seeds in each group and by accounting for the number of seeds with measurable roots for each group. Seeds were measured by straightening the root next to a measuring ruler and all the seeds that presented with root lengths of 1mm or longer were included in the data collection. Seed development was determined 7 days after the start of imbibition.

3.4.4.2 Seedling mass

Seedling dry mass was determined using the dry oven method exposing the seeds to 60^{o}C for 30 hours as previously done by Hamman, Koning and Him Lok (2001). After germination rate and seed development was established, each seed replicate was inserted into a labelled envelope which was then sealed and all seed envelopes were inserted into the 60 degree dry oven for 30 hours. After 30 hours had passed, seed dry weight was established by using electronic scales.

3.4.4.3 Germination rate

Germination rate was taken by counting the number of seeds in each group and replicate that showed radical protrusion and development of a root. Germination count was taken at 4, 24 and 48 Hour intervals. Final germination count was taken 7 days after the start of imbibition and the germination rate determined.

3.4.5 Data analysis

All the data was collected and documented on a data collection sheet using Microsoft Excel. All the data was statistically analysed and a comparison between the different remedies and distilled water was made.

Data was analysed in a similar fashion to that done by Hamman, Koning and Him Lok (2001), by analysing it as a 5 (incubation medium: control vs HGA3 at 4cH vs HGA3 at 200cH vs RGA3 at 4c vs RGA3 at 200c) X 3 (seed quality: High vs medium vs lower) factorial treatment classification. Data collected was subjected to analysis of variance (ANOVA) using GenStat Version 14 (VSN International, UK) at the 5% level of significance.

Chapter 4

Results

A variety of dependant “output” variables have been investigated in this study which include root length, amount of measurable seeds, germination rate and seedling weight. Most of these variables showed significant statistical differences between treatment groups. Shoot length was also originally included as a variable to be measured but very few seeds produced shoots due to complications during the experimental process. All of the remaining variables produced viable data and details of the complications which only affected minor aspects of the experimental procedure will be discussed in Chapter 5

4.1.1 Number of seeds with measurable roots

All the seeds that presented with root lengths of 1mm or longer were measured and included in the data collection. The collected data was statistically analysed and used to form a comparison between the treatment groups. Seeds that failed to germinate or that had roots shorter than 1mm did not form part of the root length comparison (4.2) but was accounted for and proved very relevant in the number of seeds with measurable roots comparison.

The number of seeds with measurable roots was not originally included as one of the main variables to consider but during the experimental procedure it became evident that it would be one of the most important variables to include in the data analysis as significant differences in barley seed development between treatment groups were evident. Table 4.1. presents all the measurable seed data.

The total number of seeds for each treatment group that had a root of measurable length (1mm and longer) was documented and all vigour levels were included.

Treatment	High	Medium	Low	Total
Radionic 200c	7	12	0	19
Hahnemannian 200cH	8	7	6	21
Radionic 4c	9	9	6	24
Control (Distilled water)	18	11	3	32
Hahnemannian 4cH	8	12	6	26

Table 4.1. A comparison between the Number of seeds with measurable roots for each treatment group and across all vigour levels.

The control (Distilled water) group displayed a significantly higher number of measurable seeds in total compared to the other remedy treatment groups (18.75% higher number of seeds than the closest treatment group). This suggests that all Homoeopathic remedies irrespective of potency or manufacture method had similar suppressive effects on root growth and seedling development and this suppressive effect was in turn not evident in the control group.

Data captured for the total number of seeds with measurable roots displayed similar results between remedy treatments of the same potency level. The Radionic 4c and Hahnemannian 4cH remedy treatments were relatively similar in number (24 and 26 respectively at 6.25% difference to each other) indicating similar suppressive effects of the potency on seed development regardless of the manufacturing method. The Radionic 200c and Hahnemannian 200cH remedy treatments were relatively similar in number as well (19 and 21 respectively at 6.25% difference) again indicating similar suppressive effects of the potency regardless of manufacturing method. These results indicated that the 200c treatments were more suppressive on the amount of seeds that developed in comparison to 4c treatments.

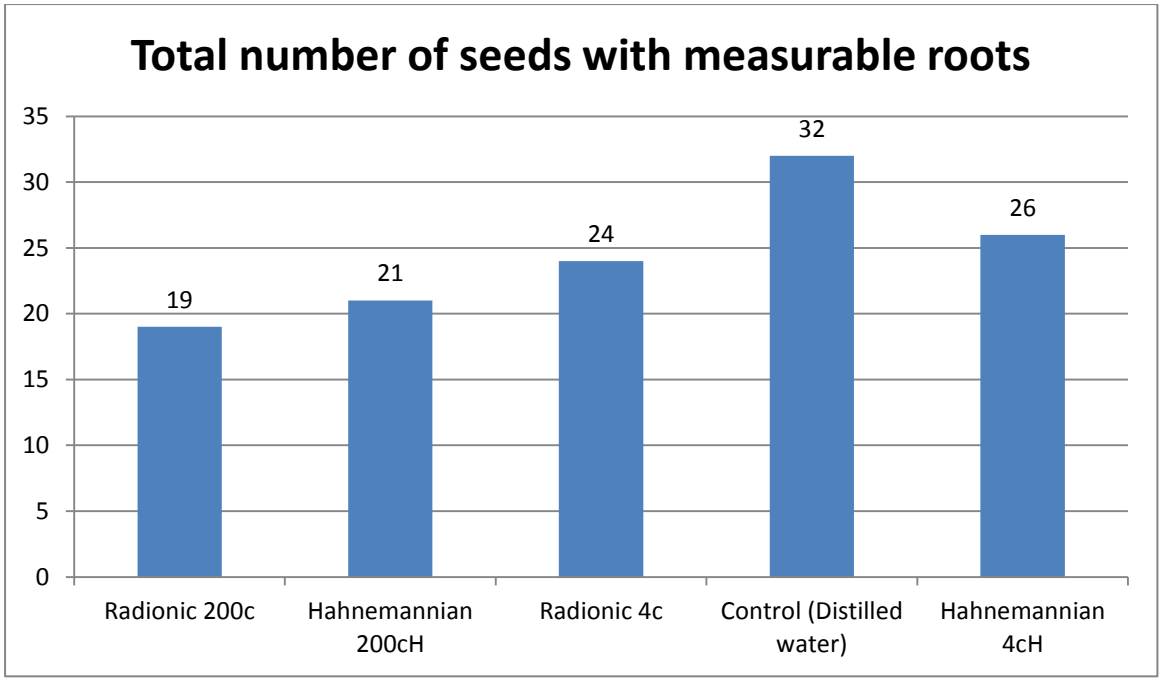

Fig 4.1.1 A comparison between the number of seeds with measurable roots for each treatment group including all three vigour groups. The specific amount of seeds per treatment group is indicated.

A comparison between the average number of seeds of each treatment group is displayed in Fig 4.1.2. and the results closely resemble that of the total number of seeds with measurable roots (Fig 4.1.1)

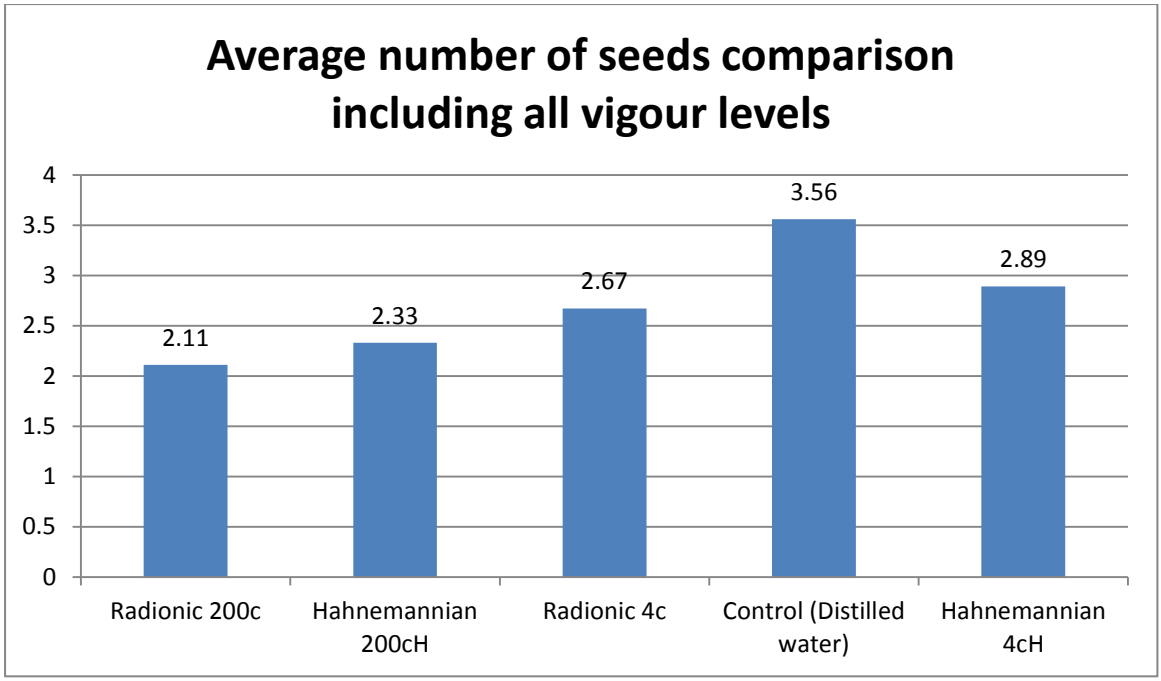

Fig 4.1.2. A comparison between the average root lengths of each treatment group including all vigour levels.

A statistical comparison of the number of seeds with measurable roots between all treatment and vigour groups is displayed in Fig 4.1.3. A statistical method (Duncan's multiple range test) was used to analyse the data and determined whether there was a significant difference between treatment results (at 5% significance). The analysis indicated that the high vigour control group presented with the highest number of seeds with measurable roots and was significantly different compared to the other treatment groups (33.3% higher average than the closest treatment group).

The medium vigour seed lot displayed a higher average (3.4) compared to the high vigour lots (3.33) which suggests that suppressive effects of all the treatments were more evident in the more conventional high vigour lots as high vigour seeds are expected to produce stronger seed growth than medium vigour seeds (High vigour seed growth is discussed in 4.1.2). This suppressive effect is also confirmed again as the control group in the high vigour lots displayed a higher number of measurable seeds than the control group in the medium vigour lot, as would normally be expected when comparing vigour lots free of treatment effects.

The low vigour seed lot displayed the lowest average number of seeds (1.4) which was expected due to lower seed vitality. Statistical variances between all treatments are indicated in Fig 4.1.3.

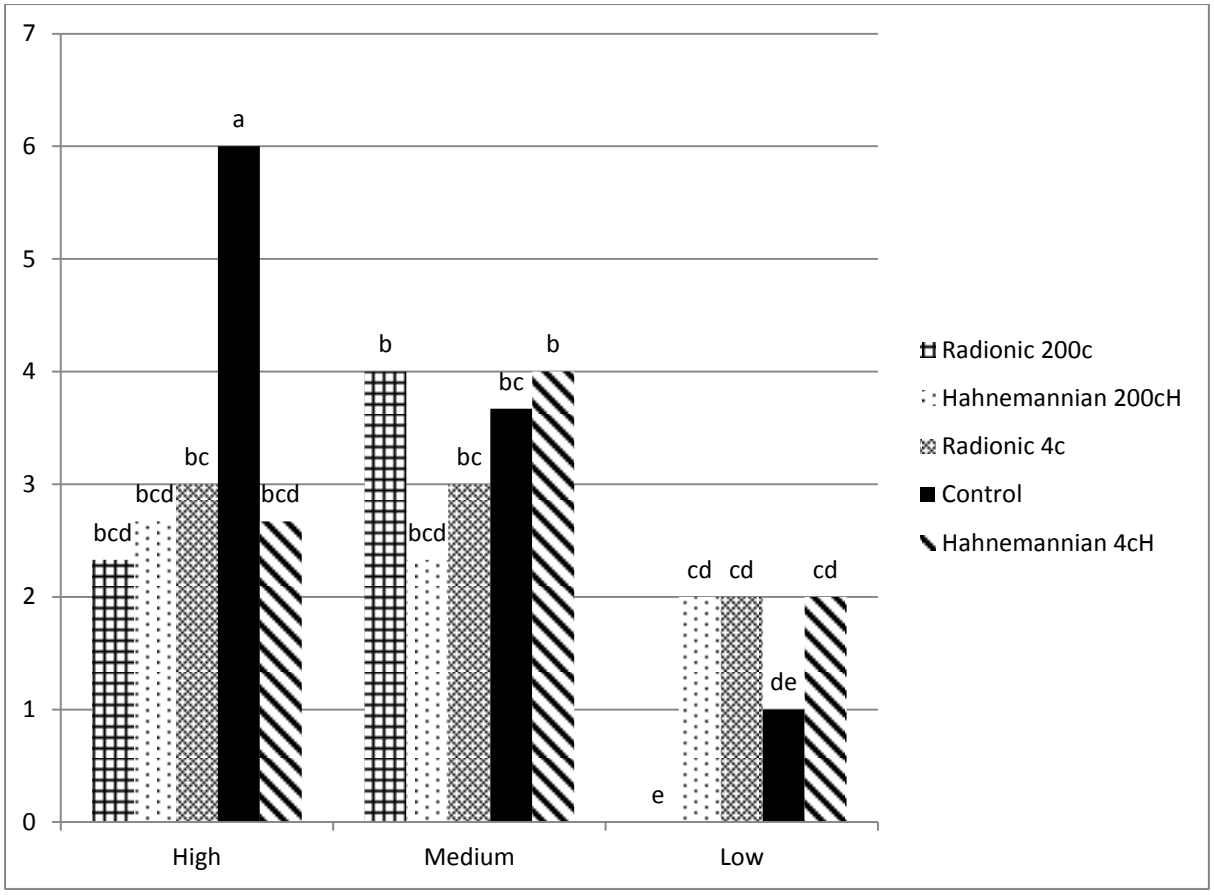

Fig 4.1.3. A statistical comparison of the number of seeds with measurable roots between all treatment and vigour groups. All means columns with the same letters above are not significantly different from each other. For example, all columns with the letters bcd are within the same level of significance to each other and all columns with b are significantly different to these bcd columns but within the same level of significance to all columns with b.

4.1.2 Number of seeds with measurable roots (high vigour)

High vigour seeds have not been through an alteration or preparation process to affect their responsiveness and were used in the most natural form possible. Therefore the high vigour seeds could be expected to produce the most relevant results of all the seed groups in the experiment.

There were three replicates of 10 (30 total) high vigour seeds per treatment group. There was a significantly higher amount of high vigour measurable seeds found in the control group (Distilled water) compared to all other remedy treatment groups (50% higher number of seeds than the closest treatment group). This significant difference is also indicated in the statistical comparison in Fig 4.1.3. and Fig 4.1.4 which demonstrates the significant suppressive effect that all of the remedy treatment groups had on seed development.

The remedy treatment groups (Radionc 200c, Hahnemannian 200cH, Radionic 4c and Hahnemannian 4cH) displayed relatively small differences to each other (although the higher amount of seeds displayed by Radionic 4c is considered significant) with regards to the number of high vigour measurable seeds produced after treatment and all of them showed a distinct significant difference compared to the control group seeds.

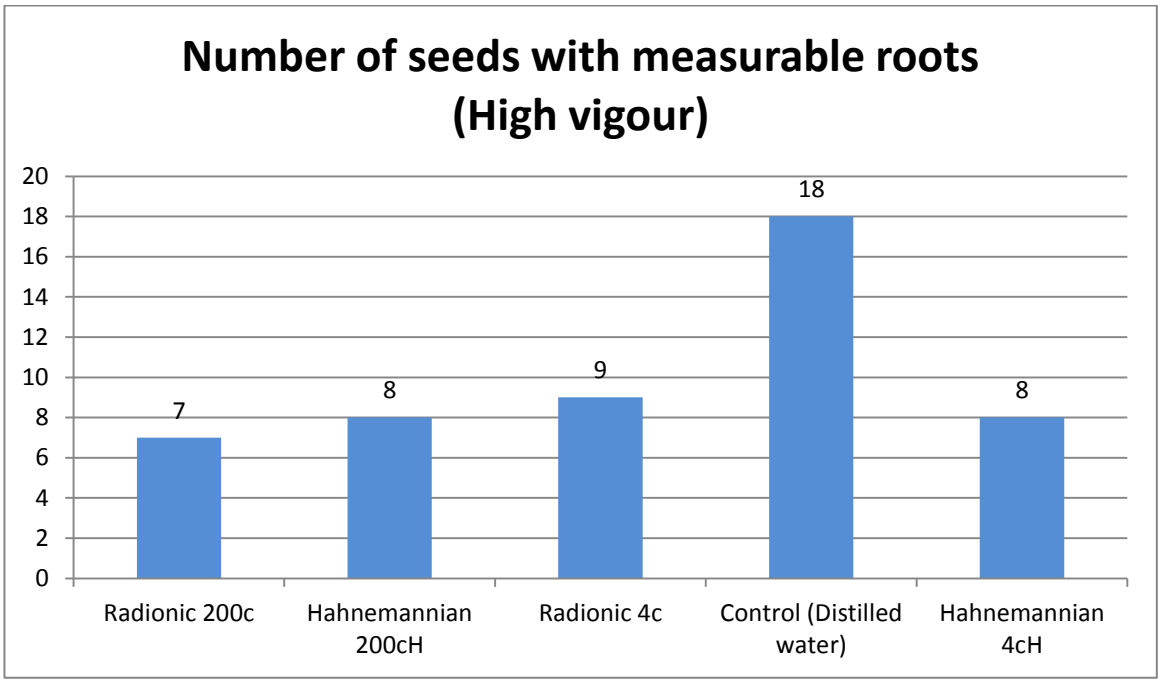

Fig 4.1.4 A comparison of the number of measurable seeds between all treatment groups but including high vigour seeds only.

4.1.3 Number of seeds with measurable roots (Medium and Low vigour)

Similar numbers of measurable seeds were found in the medium vigour groups for most treatment groups (including the control) with the exception of Hahnemannian 200cH treatment which displayed a slightly lower number of 7 seeds compared to the other treatments which averaged 11 seeds.

The Low vigour seed group had the least amount of measurable seeds and was most severely affected by experimental complications. The low vigour seeds showed poor seed growth regardless of treatment applied. The results are displayed in table 4.1 and the results were deemed more relevant when included in the total of all the seeds rather than this specific isolated group. A Statistical comparison between each individual group is displayed in Fig 4.1.3.

4.2 Root length comparison

4.2.1 Root length of germinated seeds

All the seeds that presented with root lengths of 1mm or longer were measured and included in the data collection. The collected data was statistically analysed and used to form a comparison between the treatment groups. Seeds that failed to germinate or that had roots shorter than 1mm did not form part of the root length comparison but was accounted for and proved very relevant in the number of seeds with measurable roots comparison.

An average root length comparison between all treatment and vigour groups is displayed in Fig 4.2.1 and significant differences between individual treatment groups were noted in all vigour groups. It should however be noted that the statistical analysis of the root lengths (comparing all groups) presented with a high CV% value (coefficients of variation) of 126.8% which indicated that an inconsistency between some of the replicates can be found. The cause of the high CV% was a single well developed seed in the low vigour group (Hahnemannian 4cH replicates) which was not a true reflection of the rest of the low vigour seeds. There were a few uncharacteristic seeds found in the medium vigour (Radionic 200c replicates) group as well which also contributed to the high CV% figure.

Even though the high vigour seed lot's CV% was also considered high (75.4%), it presented with a much more stable seed lot and replicates than both medium and low vigour lots. The significance between individual high vigour treatments was established and a separate comparison on the high vigour lot is presented in 4.2.2.

Radionic 200c (low vigour seed lot) presented with the highest average root length 8.87mm due to an uncharacteristic anomalous seed in this specific group. The control group (Distilled water) in the high vigour lot presented with a significantly higher root length average than all other groups which can be seen in the statistical comparison in Fig 4.2.1(Excluding low vigour Radionic 200c). Most of the remaining treatment group root length averages were not considered to be significantly different from each other due to the CV% and the least significant difference (LSD) statistical figures (which affect statistical significance) being high for the overall root length comparison.

The high vigour seed lot had an average root length of 3.36 which was 3.3% higher than medium vigour (3.25) and 17.56% higher than low vigour lots (2.77).

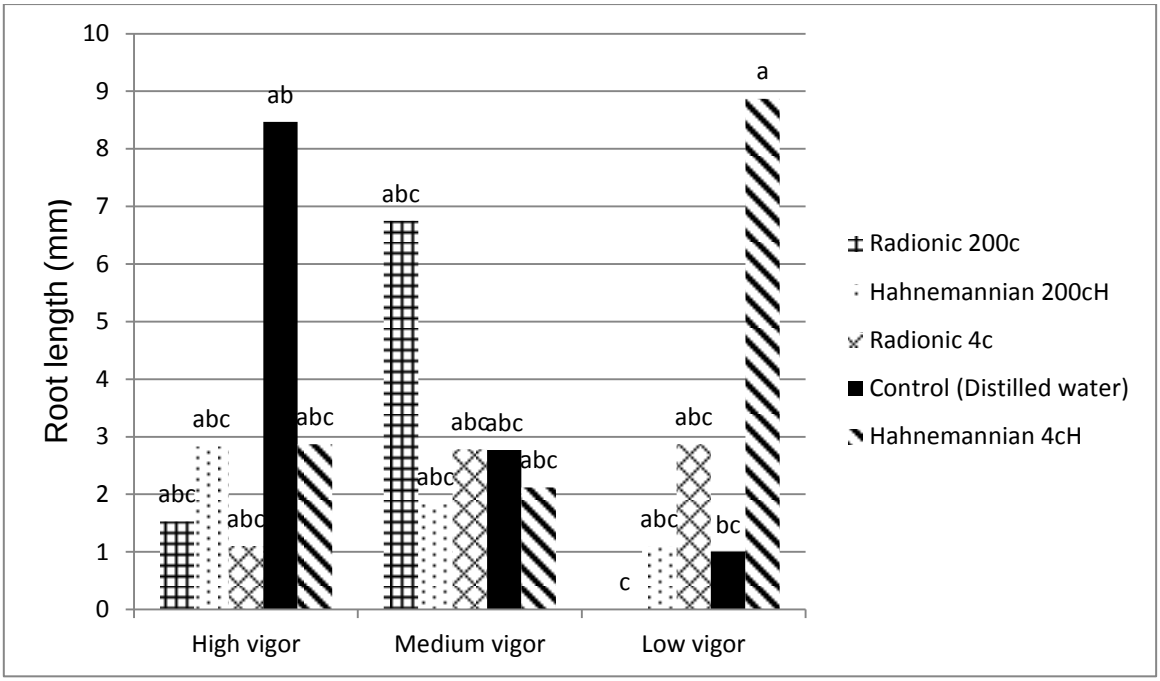

Fig 4.2.1. A statistical comparison of the root lengths (measured in mm) between all treatment and vigour groups. All means columns with the same letters above are not significantly different from each other. For example, all columns with the letters abc are within the same level of significance to each other and all columns with ab are significantly different to these abc columns but within the same level of significance to all columns with ab.

4.2.2 Root length comparison (High vigour)

High vigour seeds have not been through an alteration or preparation process to affect their responsiveness and were used in the most natural form possible. Therefore the high vigour seeds could be expected to produce the most relevant results of all the seed groups in the experiment.

The high vigour seed lot presented with more stable replicates than both medium and low vigour lots and a statistical root length comparison between treatment groups is displayed in Fig 4.2.2. The control group (distilled water) displayed a significantly higher root length average of 8.47mm which was a 66.12% higher root length average than the closest treatment group (Hahnemannian 4cH at 2.87mm)

Both Hahnemannian 4cH and Hahnemannian 200cH treatments presented with similar root length averages and were considered to not be significantly different (0.47% difference) from each other as indicated by Fig 4.2.2. Both Radionic 200c and Radionic 4c treatments were considered to not be significantly different from each other (at 5% difference) but were considered to be significantly different to both Hahnemannian treatment groups.

The high vigour root length comparison suggests that both Hahnemannian prepared treatments have similar suppressive effects on root development and both radionic treatments have similar suppressive effects on root development. These suppressive effects were not evident in the control group as the root development was clearly superior to the remedy treatment groups. The control group also produced the longest root under the High vigour seed lots and produced some of the few seeds that had measurable shoots

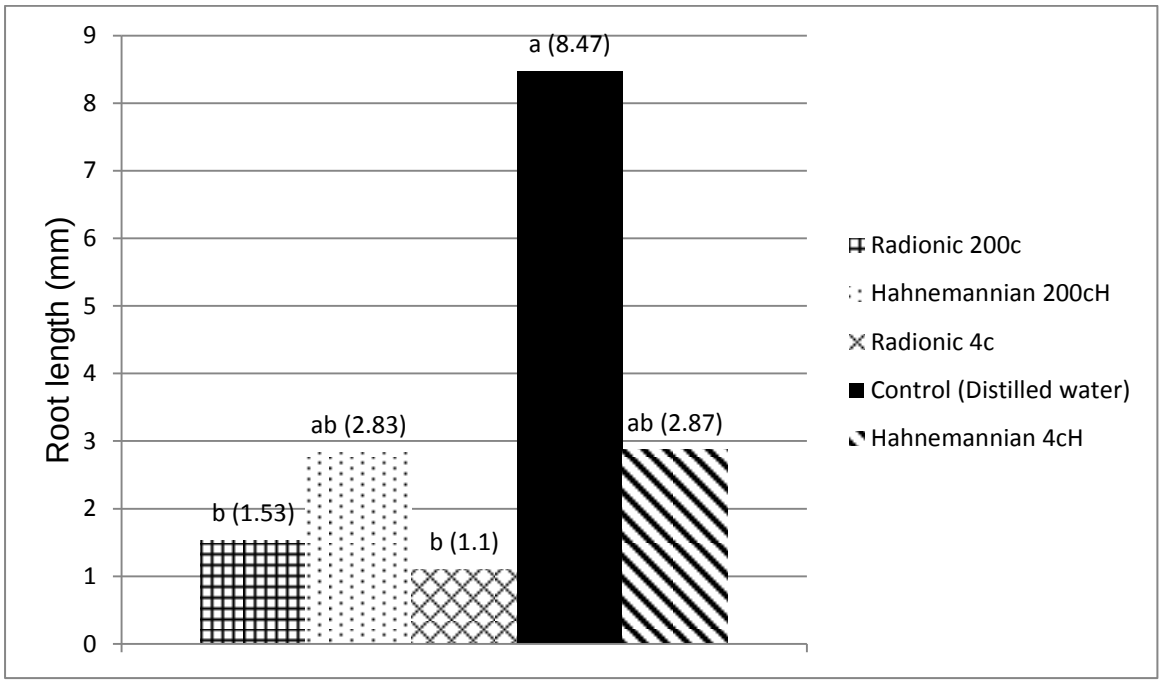

Fig 4.2.2 A statistical root length comparison (measured in mm) between treatment groups of the high vigour seed lot. All means columns with the same letters above are not significantly different from each other. For example, all columns with the letters ab are within the same level of significance to each other and all columns with b are significantly different to these ab columns but within the same level of significance to all columns with b.

4.3.1 Seed dry weight

Barley Seed dry weight was determined by placing them in a dry oven after full seed development had taken place and roots had been measured as explained in section 3.4.3.3 in the Methodology chapter.

Small differences in dry seed weight was evident between the treatment groups, however the vigour of the seeds played a much greater role in affecting the dry weight of the seeds. The average dry weight of all treatment groups are compared in Fig 4.3.1. The CV% (10.4%) and the LSD figures (0.047) were considered adequate and the replicates were stable.

The dry weight figures indicated that Hahnemannian 200cH had the lowest dry weight and Radionic 4c presented with the highest dry weight average. Some of the figures are considered significantly different to each other but the experimental complication may have played a larger role in affecting the dry weight of the seeds (compared to the effects that treatments had on the seeds) due to the difficulty in separating the fungal growth around seed capsules before weighing the seeds. This added weight from fungal growths may have caused inconsistent weight characteristics between seed batches weighed due to the difficulty in manually removing fungal growths equally and consistently from each seed.

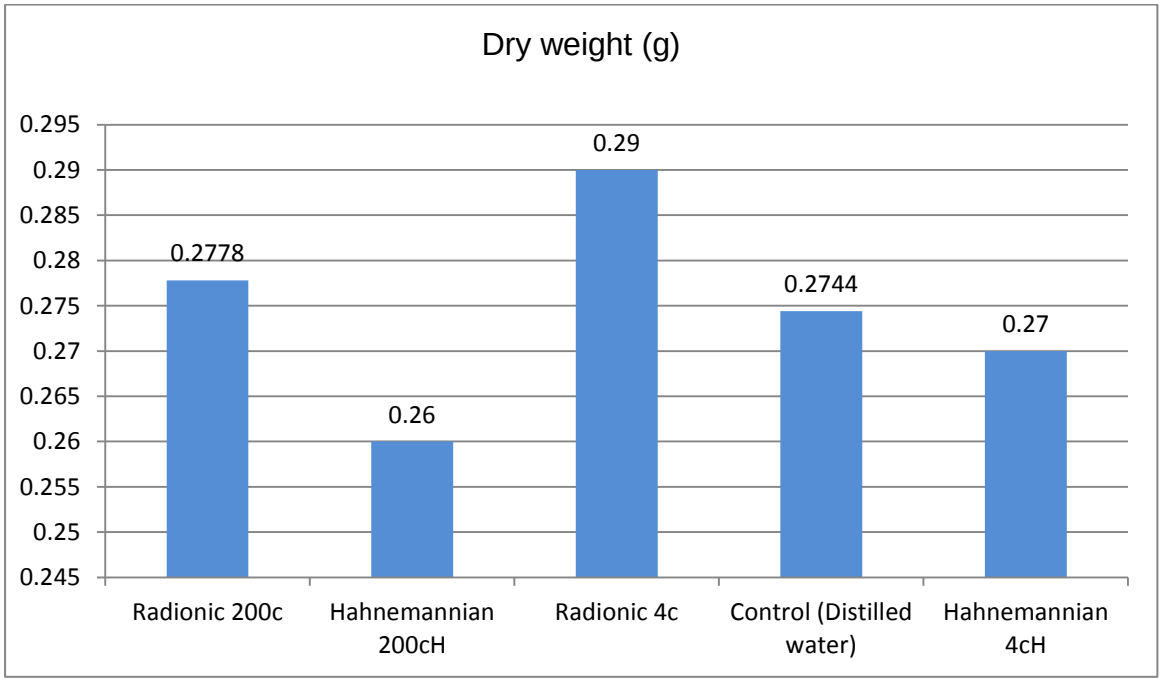

Fig 4.3.1 An average dry weight comparison between treatment groups, measured in grams.

4.3.2 Seed dry weight comparison between all treatment and vigour groups

The dry weight of all the seed groups throughout all vigour levels is statistically compared in Fig 4.3.2. The weight of the individual treatment groups displayed relatively similar dry weight compared to other treatment groups in the same vigour level which was supported by the fact that the vigour of the seeds played a strong role in their final weight. The high vigour seed lot produced the highest average seed lot weight (0.2953g) followed by medium vigour (0.2860g) and low vigour lots (0.2420g).

The treatment with the highest weight average in the high vigour seed lot indicated an 11.69% higher weight than the lowest weight in the high vigour lot with remaining groups all being close to the average for their vigour group. All statistical differences between individual groups are displayed in Fig 4.3.2.

The control group also did not display vastly different seed weights compared to other treatment groups in the same vigour which suggests that there were no evident suppressive treatment effects on seed weight.

Standard errors and coefficients of variation (CV%) for dry weight was 10.4% which is within acceptable levels indicating that the replications were consistent.

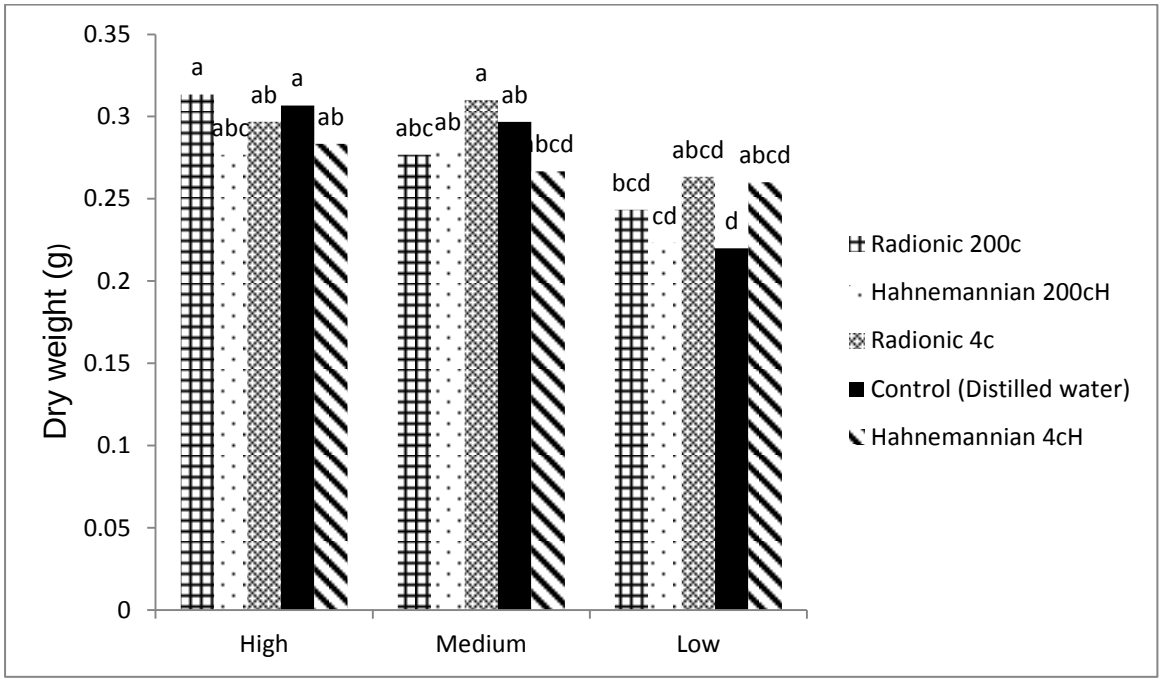

Fig 4.3.2. A seedling dry weight comparison (measured in grams) between all treatment and vigour groups. All means columns with the same letters above are not significantly different from each other. For example, all columns with the letters abc are within the same level of significance to each other and all columns with ab are significantly different to these abc columns but within the same level of significance to all columns with ab.

4.4 Germination rate

Germination rate was taken at various time intervals by counting the number of seeds per group that showed radical protrusion and development of a root. Seeds of all vigour levels were included in the data analysis.

After the seeds had been incubated for 4 hours the germination count showed similar development between the control and both the 200c remedies. After 4 hours both of the 4c remedies indicated slightly slower levels of germination compared to the other treatment groups which suggests that the 4c remedy potency had a suppressive effect on germination rate of the seeds. No significant differences were found between radionic and Hahnemannian remedy groups of the same potency at the 4 hour interval. All of the germination data captured can be found in table 4.4 and the 4hour interval comparison is displayed in Fig 4.4.1.

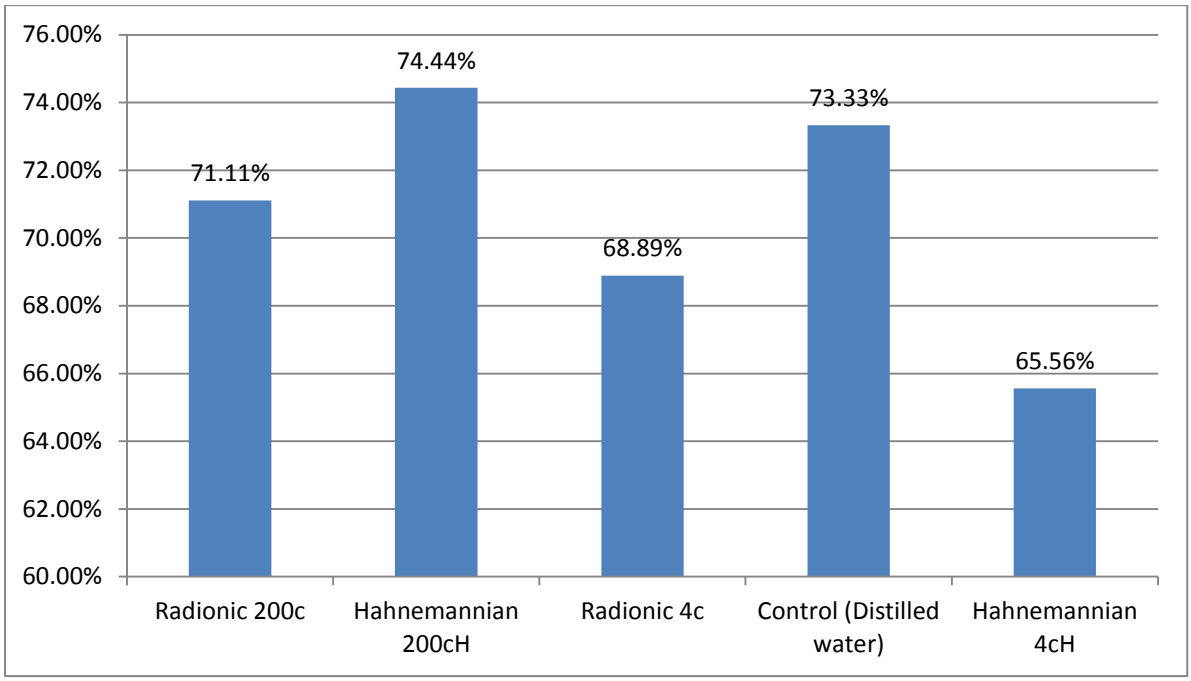

Fig 4.4.1 A Germination count comparison between treatment groups at 4 hours.

All of the treatment groups indicated similar germination rates to each other after 24 hours and were not significantly different to each other. This suggests that even though slight differences could be seen at the initial stages of germination, the remedy treatment groups had no significant effect on germination rate at the 24 hour mark.

There was no significant difference in germination rate found between treatment groups at the 48 hour interval but both 200c potencies displayed the highest germination count indicating similar effect of potency level regardless of manufacturing method. All numbers are indicated in table 4.4.

The 48 hour interval can also be regarded as the closest data to final germination as complications during the experimental procedure prevented a reliable final germination count (after 7 days) from being possible.

Treatment	**4 hours**	**24 hours**	**48 hours**
Radionic 200c	71.11 %	86.67 %	90.00 %
Hahnemannian 200cH	74.44 %	88.89 %	90.00 %
Radionic 4c	68.89 %	84.44 %	87.78 %
Control (Distilled water)	73.33 %	84.44 %	86.67 %
Hahnemannian 4cH	65.56 %	85.56 %	85.56 %

Table 4.4 The Germination count over various time intervals.

All of the statistical data is included and can be found in the Appendix section (Appendix A-E)

Chapter 5

Discussion

5.1 Introduction

Homoeopathic remedies are prescribed according to the Law of Similars which was established by the founder of Homoeopathy, Samuel Hahnemann. This law allows Homoeopathic practitioners to connect symptoms of disease or ill health with substances that produce similar symptoms when tested on a healthy person. These substances however need to have been prepared using the Homoeopathic potentization process before it can be used effectively as a treatment (O'Reilly 1996).

For example: The remedy *Apis mellifica* is produced from a honey bee including its venom. This venom causes swelling, redness and pain of an affected area in a person when introduced in crude substance form (after being stung) but when it is prepared in homoeopathic form it has the opposite effect in that it prevents or decreases redness, swelling and pain in the affected part.

In Homoeopathic science it is claimed that during the Homoeopathic potentization process the original remedy information is integrated and dynamically maintained within the more dilute solution (Resch and Gutmann 1987). The scientific theories on how this manufacturing process integrates the curative properties are discussed in 2.1 and 2.2.

Previous research has demonstrated the biological action of homoeopathic treatments on plants and Hamman, Koning and Him Lok (2001) stated that even though homoeopathically prepared treatments are highly diluted, they still produce biological activity i.e. they demonstrated various effects producing measurable data throughout the respective barley seed germination study. Biological activity was evident in Him Lok's study and such activity was largely supported by the current study as biological activity was evident in most of the data sections collected and most significant in terms of number of seeds with measurable roots and root length data sections. The study done by Hamman, Koning and Him Lok (2001) however; presented with greater dry weight of most seed groups treated with homoeopathic treatments compared to the control (distilled water) and this specific biological activity was not evident in the current study.

A study done by Steele (1999) also demonstrated that gibberelic acid prepared in homoeopathic potencies had a biological effect on barley seeds as these potencies stimulated alpha-amylase synthesis in de-embryonated barley seeds.

The biological activity of conventionally manufactured (Hahnemannian) Gibberellic acid 4cH, 15cH, 30cH and 200cH was demonstrated by Hamman, Koning and Him Lok (2001) in terms of their effect on germination and development of barley seeds. Healthy plant models have shown to be a very useful approach to investigate basic research questions about the specificity of homeopathic preparations (Majewsky *et al*. 2009). As gibberellic acid is the substance used in these experimental models, it is important to understand the physical properties of gibberellic acid and the method in which it is used in Homoeopathic research.

Gibberellic acid (GA3) is a chemical or plant hormone that stimulates the growth of seeds. Gibberellic acid (GA3) belongs to a larger gibberellin group which consists of plant hormones that have similar physiological action. In the normal seed growth process gibberellin is released by the embryo and circulates towards the endosperm layer of the seed. Here the gibberellin activates the enzyme amylase which causes the conversion of starch into sugar. Sugar can then be used as energy by the seed to stimulate protein synthesis which is required for the seed to sprout. Gibberellic acid (GA3) is a chemical commercially used to stimulate and increase plant growth. The increased growth effects of gibberellic acid can be seen on root growth, stem growth, increased plant size (larger fresh and dry weight) and can be used to improve growth of nutrient deficient plants (Copeland and McDonald 2001).

Therefore gibberelic acid is a plant hormone that stimulates growth in the seeds and according to the Law of Similars, if gibberelic acid is prepared in homoeopathic potency it should have a suppressive effect on seed growth. The suppressive effects on seed development were clear in the results and were especially evident in the number of seeds with measurable roots and root length development which supports the Law of Similars.

Seed vigour played an important role in this study and this concept should be understood. The vigour of the seeds is usually an indication of the seeds ability to germinate and grow. Seed vigour is a term used which includes various properties that determine the level of physiological potential of the seeds to develop. When seeds have decreased ability to develop and perform the physiological functions required to grow they are considered physiologically aged and are of lower vigour (ie medium and low vigour seeds). High vigour seeds are most responsive and should show superior development to medium and low vigour seeds. Medium and low vigour seeds used in this study have undergone a physiological aging process which was discussed in chapter 3 (Copeland and McDonald 2001).

A variety of dependant “output” variables have been investigated in this study which include root length, number of seeds with measurable roots, germination rate and seedling weight. Most of these variables showed significant statistical differences between respective treatment groups.

Radionic devices are relatively new to the homoeopathic industry and different radionic device variations exist. It is important to note that such variations in devices result in vastly different methods of remedy production; in this regard and theories behind how these remedies conduct their biological stimulation may differ to that of the (AMS) Wave Transfer device used in this study. The research conducted using the (AMS) Wave Transfer device can therefore only be compared to radionic devices following the same methodologies and principles in remedy production.

Scepticism regarding the effectiveness of radionically prepared remedies exists among practitioners as not many studies have been conducted to prove treatment results and radionic remedy manufacture does not directly follow the strict guidelines given by Hahnemann. However; radionic remedies could prove to be beneficial in Homoeopathic practice for various reasons and may be an attractive method of remedy manufacture if proven to be effective. Radionic remedies may be a cost effective and time saving alternative as practitioners will be able to manufacture required remedies immediately for patient use without having to stock or order more costly alternatives from manufacturers. This will be possible as only a single Hahnamannian remedy is required to produce many radionic equivalents.

5.2 Complications

The germination process of the seeds followed the guidelines from the methodology implemented in the study done by Hamman, Koning and Him Lok (2001) which stated that it was preferred to avoid using alcohol in the remedy manufacture process as it may have a stimulatory or inhibitory effect on the seeds and may affect the overall germination process.

During the initial germination count taken in the petri dishes, all of the seed batches displayed healthy conditions and root development. However after the seeds had been placed in the germination towels (after 24 hours) the seeds gradually became affected by fungal growth. All of the treatment groups were equally affected but the vigour of the seeds determined the severity and extent of fungal growth. Low vigour was most severely affected due to the increased initial moisture content caused by the manufacturing process and high vigour seeds were least affected.

Limited data from the initial trial was collected but a second trial was conducted with minor changes in the germination model to limit the effect that fungal spores would have on the trial. The second trial was conducted in petri dishes only as the germination towels were suspected of promoting fungal growth. These changes were necessary to limit fungal complications as alcohol could not be used as a preventative measure.

The second trial also displayed fungal growth but to a far lesser extent than the first trial and most of the output variables data could be collected. The data used in this dissertation is exclusively from the second trial and the first trial was only used for comparison purposes.

Even though the original data set had experienced high levels of contamination by fungal growth, similarities were found in the control group producing longer roots and more seeds with measurable roots compared to all the remedy treatment groups. The data from the first trial is included in Appendix G.

Shoot length was also originally included as one of the variables that would be measured but very few seeds produced shoots which made a valid comparison between the shoot lengths of all the treatment groups impossible. The causative factor behind the poor response of shoot growth was most likely fungal contamination; however the effect of other unknown variables cannot be excluded.

All of the data collected can be regarded as valid as the fungal contamination was distributed evenly across all seed batches and the fungal spores showed equal effects on seed batches of the same vigour regardless of treatment applied.

5.3.1 Number of seeds with measurable roots

All the seeds that presented with root lengths of 1mm or longer were measured and included in the data collection. The collected data was statistically analysed and used to form a comparison between the treatment groups. Seeds that failed to germinate or that had roots shorter than 1mm did not form part of the root length comparison (4.2) but was accounted for and proved very relevant in the number of seeds with measurable roots comparison.

The number of seeds with measurable roots was a comparison not originally included as one of the main variables to consider but during the experimental procedure it became evident that it would be one of the most important variables to include in the data analysis as the control group (Distilled water) presented with a higher number of seeds with measurable roots than all of the remedy treatment groups in the high vigour lot and in total.

The control (Distilled water) group displayed a significantly higher number of seeds with measurable roots in total compared to the other remedy treatment groups (18.75% higher number of seeds than

the closest treatment group). The high vigour seed lot also displayed a significant difference between the number of seeds in the control group (50% higher number of seeds than the closest treatment group) compared to all the remedy treatment groups. This suggests that all Homoeopathic remedies irrespective of potency or manufacture method had similar suppressive effects on root growth and seedling development and this suppressive effect was in turn not evident in the control group.

These findings are also supported when seed vigour is taken into consideration. Normally it is expected that high vigour seed lots will have superior seed germination compared to medium vigour lots and low vigour lots and are thus expected to display the lowest germination results. The results indicated that the low vigour lot produced the lowest average (1.4) as expected but the medium vigour seed lot displayed a higher average (3.4) compared to the high vigour lots (3.33).

These results indicate that the remedy treatment groups had significant suppressive effects on the high vigour seeds that were not evident in the high vigour control group (which presented with an expected average of 6) and that suppressive effects of all the remedy treatments were more evident in the more conventional high vigour lots as medium and low vigour control group results did not display the same level of difference to the remedy treatment groups in their respective vigour lots. It is therefore evident that the high vigour seed lot had a lower average compared to the medium vigour lot due to suppressive effects caused by the treatments (A statistical comparison of the number of seeds with measurable roots between all treatment and vigour groups is displayed in Fig 4.1.3)

The results from the number of seeds with measurable roots comparison (including all vigour levels) indicated similarities in effect between remedy treatments of the same potency level. The Radionic 4c and Hahnemannian 4cH remedy treatments were relatively similar in number (24 and 26 respectively at 6.25% difference to each other) suggesting similar suppressive effects of the potency on seed development regardless of the manufacturing method. The Radionic 200c and Hahnemannian 200cH remedy treatments were relatively similar in number as well (19 and 21 respectively at 6.25% difference) again suggesting similar suppressive effects of the potency regardless of manufacturing method. These results suggest that the 200c treatments were more suppressive on the amount of seeds that developed in comparison to 4c treatments and this suppressive effect was in turn not evident in the control group.

5.3.2 Number of seeds with measurable roots (High vigour comparison)

High vigour seeds have not been through an alteration or preparation process to affect their responsiveness and were used in the most natural form possible. Therefore the high vigour seeds could be expected to produce the most relevant results of all the seed groups in the experiment. It is also important to note that the high vigour seed lots were less affected by fungal complications compared to the other vigour lots. In the high vigour seed lot the control group (Distilled water) displayed a significant difference (as indicated in Fig 4.1.3 and 4.1.4) in the number of seeds compared to all the remedy treatment groups (50% higher number of seeds than the closest treatment group).

These results indicate a significant difference in effect between the control group and remedy treatment groups. This difference suggests that all Homoeopathic remedies (regardless of potency or manufacture method) had similar suppressive effects to one another on root growth and seedling development and this suppressive effect was in turn not evident in the control group.

It was expected to see much greater seedling development than was displayed in the High vigour seed lot (3.33 average) and this poor development can be attributed to the suppressive effects seen on seed development from remedy treatment groups. The control group (Distilled water) did not have any treatment effects and therefore was the only group in the high vigour lot that presented with what can be considered relatively normal seed development under the conditions.

The remedy treatment groups (Radionc 200c, Hahnemannian 200cH, Radionic 4c and Hahnemannian 4cH) displayed relatively small differences to each other (although the higher amount of seeds displayed by Radionic 4c is considered significant) with regards to the number of developed high vigour seeds after treatment and all of them displayed a distinct difference compared to the control group seeds. This difference again reinforces the suggestion that all Homoeopathic remedies (regardless of potency or manufacture method) had similar suppressive effects to one another on seed growth.

5.4.1 Root length of germinated seeds

All the seeds that presented with root lengths of 1mm or longer were measured and included in the data collection. The collected data was statistically analysed and used to form a comparison between the treatment groups. Seeds that failed to germinate or that had roots shorter than 1mm did not form part of the root length comparison but was accounted for and proved very relevant in the number of seeds with measurable roots comparison.

An average root length comparison between all treatment and vigour groups is displayed in Fig 4.2.1 and significant differences between individual treatment groups were noted in all vigour groups. It should however be noted that the statistical analysis of the root lengths (comparing all groups) presented with a high CV% value (coefficients of variation) of 126.8% which indicated that an inconsistency between some of the replicates can be found. The main cause of the high CV% was a single well developed seed (outlier) in the low vigour group (Hahnemannian 4cH replicates) which was not a true reflection of the rest of the low vigour seeds. There were a few uncharacteristic seeds (outliers) found in the medium vigour (Radionic 200c replicates) group as well which also contributed to the high CV% figure.

Radionic 200c (low vigour seed lot) presented with the highest average root length 8.87mm due to an uncharacteristic anomalous seed in this specific group. The control group (Distilled water) in the high vigour lot presented with a significantly higher root length average than all other groups which can be seen in the statistical comparison in Fig 4.2.1 (Excluding low vigour Radionic 200c). Most of the remaining treatment group root length averages were not considered to be significantly different from each other due to the CV% and the least significant difference (LSD) statistical figures (which affect statistical significance) being high for the overall root length comparison.

Him Lok (2001) stated that biological effects of homoeopathic treatments were evident on root length development. Him Lok (2001) found that Homoeopathically prepared gibberelic acid in 15cH potency affected root development in medium vigour seed lots and 4cH, 15cH, 30cH and 200cH displayed effects on high vigour seed mass. These previous results are comparable in the sense that significant differences were also displayed in this current study and are especially evident in the high vigour lot.

5.4.2 Root length comparison (High vigour)

High vigour seeds have not been through an alteration or preparation process to affect their responsiveness and were used in the most natural form possible. Therefore the high vigour seeds could be expected to produce the most relevant results of all the seed groups in the experiment.

High vigour seeds also proved to be a responsive lot in a previous study (Hamman, Koning and Him Lok 2001) in which it was found that all of the remedies used (4cH, 15cH, 30cH and 200cH) displayed effects on high vigour seed weight. These previous results are comparable in the sense that significant differences were also displayed in this current study and are especially evident in the high vigour lot.

The statistical analysis of the root lengths (comparing all groups) presented with a high CV% value (coefficients of variation) of 126.8% which indicated that an inconsistency between some of the replicates can be found. The low and medium vigour lots were the cause of the high CV% and the high vigour lot (75.4%) was therefore analysed separately to form a more relevant comparison. Even though the high vigour seed lot's CV% was also considered high, it presented with a much more stable seed lot and replicates than both medium and low vigour lots. A statistical root length comparison between treatment groups is displayed in Fig 4.2.2.

The control group (distilled water) displayed a significantly higher root length average of 8.47mm which was a 66.12% higher root length average than the closest treatment group (Hahnemannian 4cH at 2.87mm). These results strongly suggest that the remedy treatment groups all had suppressive effects as the extent of the difference in root development between the control group and remedy treatment groups were large and most of the remedy treatment groups displayed results that were relatively similar to each other.

Both Hahnemannian 4cH and Hahnemannian 200cH treatments presented with similar root length averages and were considered to not be significantly different (0.47% difference) from each other as indicated by Fig 4.2.2. Both Radionic 200c and Radionic 4c treatments were considered to not be significantly different from each other (at 5% difference) but were considered to be more suppressive on root length and significantly different to both Hahnemannian treatment groups.

The high vigour root length comparison suggests that both Hahnemannian prepared treatments have similar suppressive effects on root length development and both radionic treatments have similar suppressive effects on root length development. These results also suggest that manufacturing method played a role in determining the level of suppression on root development even though it is considered a small (while remaining significant) difference when the control group results are considered. These suppressive effects were not evident in the control group as the root development was considerably greater compared to the remedy treatment groups

The control group also produced the longest root under the High vigour seed lots and produced more seeds that had measurable shoots which further support the lack of suppressive effects displayed by the control group compared to remedy treatment groups.

The high vigour seed lot had an average root length of 3.36 which was 3.3% higher than medium vigour (3.25) and 17.56% higher than low vigour lots (2.77). High vigour lots are expected to produce better root development than medium and low vigour lots and the fact that the high vigour lot displayed an average close to the medium vigour lot indicates that suppressive effects are more evident in the high vigour lot compared to the other lots.

5.5 Seed dry weight

Barley Seed dry weight was determined by placing them in a dry oven after full seed development had taken place and roots had been measured.

Small differences in dry seed weight was evident between the treatment groups, however the vigour of the seeds played a much greater role in affecting the dry weight of the seeds. The CV% (10.4%) and the LSD figures (0.047) were considered adequate and the replicates were stable.

High vigour seeds also proved to be a responsive lot in a previous study done by (Hamman, Koning and Him Lok 2001) in which it was found that all of the remedies used (4cH, 15cH, 30cH and 200cH) displayed effects on high vigour seed weight. All of the remedy treatment groups in Him Lok's study produced seeds with higher seed weight than the control group (With the exception of 30cH in the medium vigour group).

The dry weight figures indicated that Hahnemannian 200cH had the lowest dry weight and Radionic 4c presented with the highest dry weight average. Some of the figures are considered significantly different to each other but the fungal complication may have played a larger role in affecting the dry weight of the seeds compared to the treatments due to the difficulty in separating the fungal growth around seed capsules before weighing the seeds. This added weight from fungal growths may have caused inconsistent weight characteristics between seed batches weighed due to the difficulty in manually removing fungal growths equally and consistently from each seed.

The dry weight of all the seed groups throughout all vigour levels is statistically compared in Fig 4.3.2. The weight of the individual treatment groups displayed relatively similar dry weight compared to other treatment groups in the same vigour level which was supported by the fact that the vigour of the seeds played a strong role in their final weight. The high vigour seed lot produced the highest average seed lot weight (0.2953g) followed by medium vigour (0.2860g) and low vigour lots (0.2420g).

The treatment with the highest weight average in the high vigour seed lot indicated an 11.69% higher weight than the lowest weight in the high vigour lot with remaining groups all being close to the average for their vigour group.

The control group also did not display vastly different seed weights (even though individual treatments were considered statistically different) compared to other treatment groups in the same vigour which suggests that there were no evident suppressive treatment effects on seed weight.

5.6 Germination rate

Germination rate was taken at various time intervals by counting the number of seeds per group that showed radical protrusion and development of a root. Seeds of all vigour levels were included in the data analysis.

After the seeds had been incubated for 4 hours the germination count showed similar development between the control and both the 200c remedies. After 4 hours both of the 4c remedies indicated slightly slower levels of germination compared to the other treatment groups which suggests that the 4c remedy potency had a suppressive effect on germination rate of the seeds initially. No significant differences were found between radionic and Hahnemannian remedy groups of the same potency at the 4 hour interval.

All of the treatment groups indicated similar germination rates to each other after 24 hours and were not significantly different to each other. This suggests that even though slight differences could be seen at the initial stages of germination, the remedy treatment groups had no significant effect on germination rate at the 24 hour mark.

There was no significant difference in germination rate found between treatment groups at the 48 hour interval but both 200c potencies displayed the highest germination count indicating similar effect of potency level regardless of manufacturing method.

The 48 hour interval can also be regarded as the closest data to final germination as complications during the experimental procedure prevented a reliable final germination count (after 7 days) from being possible. There is no significant difference indicated between treatment groups at the final germination count which suggests that there is no difference in effect between any of the treatment groups including the control group.

5.7 Comparative summary

Allsopp (2010) conducted a study which compared various potencies of Hahnemannian produced remedies and their corresponding radionically produced remedies in terms of their physico-chemical properties. The study concluded that Hahnemannian and radionic remedies were significantly different to each other and corresponding potencies displayed NMR spectra results with different physico-chemical structure. Allsopp (2010) also stated that the manufacture method played a role in the structural formation of the remedies and that different production methods should be researched to develop manufacturing methods that ensure standardisation of remedies.

Although all remedy treatment groups indicated biological (suppressive) activity on root development in the current study, significant differences in suppressive effect were noted between Hahnemannian and radionic treatments in the root length comparison (high vigour). These differences in biological effect can be linked to correlating structural differences (physico-chemical differences) found between Hahnemannian and radionic treatments in Allsopp's study. It should however be noted that these differences were not consistent throughout all the variables in the current study.

Hamman, Koning and Him Lok (2001) conducted a study which demonstrated that Homoeopathically produced gibberellic acid of various potencies are capable of stimulating biological activity in barley seeds. It was found that the Homoeopathically prepared gibberellic acid produced larger organisms (measured by dry weight) throughout all vigour levels compared to the control group (distilled water) seeds. The 200 cH Homoeopathic treatment displayed the strongest stimulatory effects as it produced significantly larger organisms (measured by dry weight) than the control group throughout all vigour levels.

The current study displayed similarities to the study done by Hamman, Koning and Him Lok (2001) and most previous studies using the barley seed model in that clear biological effects of remedy treatments were evident. The output variables that displayed the most significant biological effects in the current study were the root length and the number of seeds with measurable roots (the number of seeds with measurable roots is an additional variable to the current study). Hamman, Koning and Him Lok's (2001) study presented with a root length comparison which did not indicate statistical differences to control results except for the 15 cH remedy treatment which stimulated root development in the medium vigour seed group resulting in longer roots than the control. Contrastingly however, in the current study all remedy treatment groups displayed suppressive effects on root development compared to the control as would be expected according to the Law of Similars.

All of the remedy treatment groups in Hamman, Koning and Him Lok's (2001) study produced seeds with higher seed weight than the control group (With the exception of 30cH in the medium vigour group). Such findings were however not demonstrated in the current study; but it should be noted that fungal complications could have affected the dry weight of seed batches. Small differences in dry seed weight was evident between the treatment groups in the current study, however the vigour of the seeds played a much greater role in affecting the dry weight of the seeds. The weight of the individual treatment groups displayed relatively similar dry weight compared to other treatment groups in the same vigour level which was supported by the fact that the vigour of the seeds played a strong role in their final weight.

The Law of Similars supports the suppressive effects on seed development produced by Homoeopathic treatment groups in the current study. These effects are in certain aspects contrasted by Hamman, Koning and Him Lok's (2001) study as stimulatory effects were seen on root development (15cH group only) and higher seed dry weights were evident in most remedy treatment groups.

These stimulatory effects are however not supported by the Law of Similars and could have been caused by other factors. A previous plant study conducted by Dragicevic *et al.* (2013) concluded that it is also important that further studies be conducted utilising plant models to determine the effect that different potencies have on seed development as more knowledge on the mechanism of action of potencies is needed. This statement may be relevant as different potencies may have different effects and the potency that had stimulatory effect on root development in Hamman, Koning and Him Lok's (2001) study was 15cH which was not one of the potencies used in the current study as a wider potency scale (potencies that were above and below Avogadro's number) needed to be included.

The stimulatory effects on seed dry weight seen in Hamman, Koning and Him Lok's (2001) study were also not evident in the current study; however fungal complications may have affected the dry weight of the seeds in the current study and according to the Law of Similars one would expect lower seed weight.

Even though biological effects were evident in both Hamman, Koning and Him Lok's (2001) study and the current study, the size and direction of results differ to an extent. Lahnstein *et al.* (2009) stated that Homoeopathic studies need to be repeated to identify factors which affect size and direction of the results as the specific results on various output variables in their study was not identical between the independent research groups. It is therefore important that the current study be repeated to further support the findings.

As previously stated, radionic devices are relatively new to the homoeopathic industry and scepticism regarding the effectiveness of radionically prepared remedies exists among practitioners as not many studies have been conducted to prove treatment results and radionic remedy manufacture does not directly follow the strict guidelines given by Hahnemann. The results from the current study suggest that radionically prepared remedies have similar biological effects compared to the corresponding Hahnemannian remedies which addresses the concerns for the effective use of these remedies in Homoeopathic practice. These findings (along with future confirmative studies) could have implications in the radionic remedy manufacture industry and Homoeopathic practice as more practitioners may be interested in the benefits that this method of remedy production can bring to their practice.

Suppressive effects on seed development was evident from radionic and Hahnemannian gibberellic acid treatment groups in the current study and plant models have proven to be responsive test models (using Homoeopathic remedies) as biological activity was evident in the current study and past studies. These findings could have implications in the field of agriculture and agronomy as various 'Homoeopathic' dilutions of plant hormones such as gibberellic acid appear to have significant effects on plant development (Steele 1999; Bruni 2001; Hamman, Koning and Him Lok 2001; Evans 2008; Majewsky *et al*. 2009) while proving to be a cost effective option when radionic manufacturing methods are utilised for larger scale use in agriculture.

Gibberellic acid was used in the current study and had suppressive effects on seed development and these effects were supported by the Law of Similars. It stands to reason that other Homoeopathic substances could have significant stimulatory effect on plant growth although further research is needed in radionic fields and Homoeopathic treatment effects on plants.

5.8 Hypothesis discussion

1) It was hypothesised that there would be a difference in biological effect between radionically prepared gibberellic acid and conventionally prepared gibberellic acid (GHP) on the germination rate and seedling development of barley seeds. Although radionic 200c and radionic 4c treatments were considered to be more suppressive on root length development and significantly different to both Hahnemannian treatment groups, these differences were small when the control group results are considered and radionic treatments did not consistently display these differences in relation to Hahnemannian treatments throughout all variables. The potency rather than manufacturing method (radionic or Hahnemannian) determined the level of suppression in the number of seeds with measurable roots comparison. Therefore the hypothesis was rejected due to the similarity of biological effects between radionic and Hahnemannian treatments displayed in other variables (majority).

2) It was hypothesised that there would be a significant difference in biological effect between the conventional homoeopathic remedies and the control (distilled water). This Hypothesis was accepted as significant differences in biological effect between all homoeopathic remedies and the control were evident in the root length comparison and the number of seeds with measurable roots comparison. The suppressive effects of the all the remedy treatment groups were especially evident in the high vigour seed lots where the control group displayed a large difference in seed development compared to the remedy treatment groups (conventional Hahnemannian remedies and radionic remedies) supporting the hypothesis.

Chapter 6

Conclusion

There are various radionic devices on the market that claim to produce remedies with the same effects as their corresponding conventional (Hahnemannian) homoeopathic remedies and the question has been raised weather the biological effects of these radionic remedies are in fact similar to their 'equivalent' conventionally manufactured remedies when applied in Homoeopathic practice.

Therefore the main objective in this study was to determine if there was a difference in biological activity between remedies produced radionically and those produced using the conventional Hahnemannian method; this objective was operationalized using an established seedling model in which the effect of Homoeopathically (Hahnemannian) prepared gibberellic acid (GHP) of varying potency and the effect of radionic 'equivalent' remedies were compared in terms of the germination rate and seedling development of barley seeds.

The seedling model was ideal as there are several advantages of using plant models for homoeopathic research including the vital aspect of eliminating the placebo effect while assessing the performance of substances by quantitative methods. Seedling models also provide advantages due to easy experimental repetition and avoiding the ethical implications associated with human studies (Majewsky *et al.* 2009).

The individual objectives of the study are stated below and a discussion on the results and answers to the objectives follows.

1) To determine the influence of various potencies of gibberellic acid manufactured conventionally (GHP) on germination rate and seedling development of barley seeds.

2) To determine the influence of various potencies of gibberellic acid manufactured radionically (AMS transfer device) on germination rate and seedling development of barley seeds.

3) To determine the influence of distilled water (negative control) on germination and seeding development of barley seeds.

4) To compare the respective influence of gibberellic acid (manufactured conventionally and radionically) and distilled water on germination rate and seedling development of barley seeds.

All of the remedy treatment groups (Radionc 200c, Hahnemannian 200cH, Radionic 4c and Hahnemannian 4cH) displayed suppressive effects of varying degree on seed growth and development, contrastingly the control group (distilled water) displayed greater seedling development in comparison to all remedy treatment groups which was most evident in **the high vigour seed lot root lengths** which displayed a longer root average than all remedy treatment groups. The control group also displayed a higher **number of seeds with measurable roots** compared to all the remedy treatment groups in both total number of seeds and in the seeds accounted for in the high vigour lots. This suggests that all Homoeopathic remedies irrespective of potency or manufacture method (radionic or Hahnemmanian) had similar suppressive effects on root growth and seedling development and this suppressive effect was in turn not evident in the control group.

When considering the number of seeds with measurable roots it is evident that the total seeds of both Hahnemannian 4ch and Radionic 4c groups delivered very similar results to each other and the same was noted for Hahnemannian 200cH and Radionc 200c treatments. The 200c potencies displayed greater suppressive effects compared to the 4c treatments. These findings suggest that all Homoeopathic remedies regardless of potency have suppressive effects on seed development but the level of potentization does have an effect on the extent of suppression seen on seed development. It should be noted however that all the results of the remedy treatment groups were relatively similar to each other when compared to the distinct difference displayed by the control group results.

In the high vigour seed lot it was evident that the control group (distilled water) presented with a significantly higher root length average of 8.47mm which was a 66.12% higher root length average than the closest treatment group (Hahnemannian 4cH at 2.87mm). These results strongly suggest that the remedy treatment groups all had suppressive effects as the extent of the difference in root development between the control group and remedy treatment groups were large and most of the remedy treatment groups displayed results that were relatively similar to each other. These suppressive effects on root length by the treatment groups were similar to and supported by the findings in the Number of seeds with measurable roots comparison.

Hahnemannian 4cH and Hahnemannian 200cH treatments presented with similar root length averages (high vigour lot) and were considered to not be significantly different (0.47% difference) from each other as indicated by Fig 4.2.2. Both radionic 200c and radionic 4c treatments were considered to not be significantly different from each other (at 5% difference) but were considered to be more suppressive and significantly different to Hahnemannian treatment groups.

The high vigour root length comparison suggests that both Hahnemannian prepared treatments have similar suppressive effects on root length development and both radionic treatments have similar suppressive effects on root length development. These results also suggest that manufacturing method played a role in determining the level of suppression on root development even though it is considered a small (while remaining significant) difference when the control group results are considered. These suppressive effects were not evident in the control group as the root development was substantially greater compared to the remedy treatment groups.

The control group did not display vastly different seed lot weights compared to other treatment groups of the same vigour which suggests that there were no evident treatment effects on seed weight. The dry weight average of the High vigour group was the highest and the low vigour group was the lowest indicating that vigour had a much greater effect on seed mass than the respective treatments treatment.

All of the treatment groups indicated similar germination rates to each other after 24 hours and were not significantly different to each other. This suggests that even though slight differences could be seen at the initial stages of germination, the remedy treatment groups had no significant effect on germination rate at the 24 hour mark.

There were no significant differences in germination rate found between treatment groups at the 48 hour interval but both 200cH potencies displayed the highest germination count indicating similar effect of potency level regardless of manufacturing method. This effect that the remedy potency had on germination rate was also evident in the number of seeds with measurable roots comparison.

Final concluding remarks and applications of the findings

There are similarities in the results from the Number of seeds with measurable roots and the average root lengths of the high vigour treatments which reinforces the findings that

1. The control group (Distilled water) did not display the same suppressive effects on seed development as were evident from the various remedy treatment groups.

2. Both radionically prepared remedies and Hahnemannian (GHP) prepared remedies displayed similar biological (suppressive) effects on seed development.

3. Potency and manufacturing method affect seed development to a certain extent as statistical differences were displayed by both factors, however these differences were relatively small compared to the control (distilled water) group results and both potency and manufacturing method displayed greater suppressive effect compared to one another depending on the output variable tested (root length or number of seeds with measurable roots)

The experiment results suggest that radionically manufactured remedies (using the AMS Wave Transfer Device) have similar biological effects to the 'equivalent' Hahnemannian manufactured homoeopathic remedies of the same potency. Although further research is needed these findings are encouraging and may support the use of this device as a method of remedy manufacture in clinical practice.

Recommendations

1. In scientific research it is considered good practice to repeat existing studies to strengthen the results and findings. It is therefore recommended to conduct future research using similar methodology.

2. The seedling model applied in this study as it did in that of Hamman, Koning and Him Lok (2001) proved to be a useful method of measuring biological activity of homoeopathic remedies and it is recommended that future studies attempting to quantify such effects where applicable consider this method accordingly.

3. During the process of conducting any research unforeseen complications may arise and during this particular study fungal contamination of seeds had to be controlled by making small adjustments to the methodology. It is therefore recommended that future studies using similar methodology should find methods to limit and prevent fungal growth on seeds. These methods should however not have any effects on seed development or the study outcome.

4. Radionic devices are relatively new to the homoeopathic industry and very little research has been performed testing these devices. Different radionic device variations exist and it is important to note that these devices may have vastly different methods of remedy production and theories behind how these remedies produce their biological stimulatory effect (which may not be as scientifically based as the (AMS) Wave Transfer device). It is therefore important to compare the effects of different radionic device type's in future scientific studies and not to generalise the findings of this study to all radionics devices.

5. It may prove to be beneficial to conduct comparative Homoeopathic studies (utilising Hahnemannian and radionc remedies) using other plant based biological test beds (i.e. wheat) to determine if similar results are produced.

6. This study included potencies both above and below Avogadro's number. It could prove useful to compare the effects of selected potencies in a wider range of concentration, possibly up to LM potencies.

7. It is recommended that other suitable living organisms from the animal kingdom (i.e. tadpoles) be used to compare the effect of radionic and Hahnemannian remedies on their development.

8. It may prove beneficial to conduct further agricultural studies using homoeopathic remedies and to demonstrate the use of homeopathic remedies on crops as the current findings (and those of some previous studies) suggest possible agricultural application of homoeopathic potencies of plant hormones such as gibberellic acid.

References

Allsopp, C. 2010. A comparative study of Hahnemannian and radionically prepared potencies of Natrum muriaticum using nuclear magnetic resonance spectroscopy. Dissertation/Thesis. Available at: http://dut.summon.serialssolutions.com/2.0.0/link/0/ (Accessed 05 August 2014).

AMS. 2004. Wave transfer operation instructions. AMS GMBH. Tauberbischofsheim.

Benyunes, S. 2005. German Homoeopathic Pharmacopoeia (GHP). Stuttgart: Medpharm Scientific Publishers. Deutscher Apothekar Verlag.

Barthel, P. 1991. Hahnemann's legacy- the Q (LM) potencies, British Homoeopathic Journal 80: 112-121.

Bruni, R. 2001. A comparison of the relative effectiveness of high and ultra high dilutions of abscisic acid prepared by serial dilution and succussion as opposed to dilutions prepared by serial dilution alone, on the synthesis of alpha-amylase in barley endosperm half-seeds. Dissertation/Thesis. Available at: http://dut.summon.serialssolutions.com/2.0.0/link/0/ (Accessed 05 August 2014).

Copeland LO, McDonald MB. 2001. Principles of Seed Science and Technology, 4th ed. Massachusetts: Kluwer Academic Publishers.

Dragicevic, V. Spasic, M. Simic, M. Dumanovic, Z. and Nikolic, B. 2013. Stimulative influence of germination and growth of maize seedlings originating from aged seeds by 2,4-D potencies. Homeopathy (2013) 102, 179-186. Available at: http://www.sciencedirect.com (Accessed 05 August 2014).

Elia, V. Napoli, E. and Germano, R. 2007. The 'Memory of Water': an almost deciphered enigma. Dissipative structures in extremely dilute aqueous solutions. Homeopathy (2007) 96, 163–169. Available at: http://www.sciencedirect.com (Accessed 06 August 2014).

Evans, N. P. 2008. A study of the effectiveness of homoeopathically prepared dilutions of abscisic acid, molybdenum and allopurinol in inhibiting or promoting the germination of barley seeds (Hordeum vulgare). Dissertation/Thesis. Available at: http://dut.summon.serialssolutions.com/2.0.0/link/0/ (Accessed 06 August 2014).

Franks, N. 2000. Reflections on the Ether and some Notes on the Convergence between Homoeopathy and Radionics. Radionic Journal, 46(2): 4-21.

Gaier, H. C. 1991. Thorsons encyclopaedic dictionary of homoeopathy: the definitive reference to all aspects of homoeopathy. London: Thorsons.

Hamman, B. Koning, G. and Him Lok, K. 2001. The effect of Homoeopathically- Prepared Dilutions of Gibberelic acid (GA3) on the germination rate of barley seed. Master's degree in Technology: Hom, Durban institute of technology.

International Rules for Seed Testing 2012. 6 ed. The International Seed Testing Association (ITSA) Zurichstr. 50, CH-8303 Bassersdorf, Switzerland.

Kayne, S.B. 2006. Homeopathic Pharmacy, Theory and Practice. London: Elsevier Churchill Livingstone.

Lahnstein, L. Binder, M. Thurneysen, A. Frei-Erb, M. Betti, L. Peruzzi, M. Heusser, P. and Baumgartner, S. 2009. Isopathic treatment effects of Arsenicum album 45x on wheat seedling growth – further reproduction trials. Homeopathy (2009) 98, 198–207. Available at: http://www.sciencedirect.com (Accessed 05 August 2014).

Majewsky, V. Arlt, S. Shah, D. Scherr, C. Jager, T. Betti, L. Trebbi, G. Bonamin, L. Klocke,P. Baumgartner, S. 2009. Use of homeopathic preparations in experimental studies with healthy plants. Homeopathy (2009) 98, 228–243. Available at: http://www.sciencedirect.com (Accessed 30 September 2013).

Martin, E. A. 2007. Concise Medical Dictionary, 7th Edition. New York: Oxford University press Inc.

Oberbaum, M. Cambar, J. Hormesis: Dose-dependent reverse effects of low and very low doses. In: Endler PC, Schulte J (eds).1994. Ultra-high Dilution, Physiology and Physics. Dordrecht: Kluwer Academic Publishers.

O'Reilly, W.B. 1996. Organon of the Medical Art, 6th Edition. United States of America, Calif. Birdcage Books.

Resch, G. and Gutmann, V. 1987. Scientific foundations of homoeopathy. Berg am Starnberger See: Barthel & Barthel.

Steele, R. 1999. The effect of ultra high dilutions of gibberellic acid on the synthesis of a-amylase in de-embryonated halves of barley seed (Hordeum vulgare stirling). Dissertation/Thesis. Available at: http://dut.summon.serialssolutions.com/2.0.0/link/0/ (Accessed 06 August 2014).

Appendices

Appendix A: Number of seeds with measurable roots statistical analysis

Analysis of variance

Variate: Number_of_seeds

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	2.9778	1.4889	1.86	
Rep.*Units* stratum					
Treatment	4	11.2444	2.8111	3.52	0.019
Vigour	2	38.7111	19.3556	24.24	<.001
Treatment.Vigour	8	31.9556	3.9944	5.00	<.001
Residual	28	22.3556	0.7984		
Total	44	107.2444			

Message: the following units have large residuals.

Rep 3 *units* 10 1.64 s.e. 0.70

Tables of means

Variate: Number_of_seeds

Grand mean 2.71

Treatment	1	2	3	4	5
	2.11	2.33	2.67	3.56	2.89

Vigour	High vigor	Low vigor	Medium vigor

3.33	1.40	3.40

Treatment	Vigour	High vigor	Low vigor	Medium vigor
1		2.33	0.00	4.00
2		2.67	2.00	2.33
3		3.00	2.00	3.00
4		6.00	1.00	3.67
5		2.67	2.00	4.00

Standard errors of means

Table	Treatment	Vigour	Treatment Vigour
rep.	9	15	3
d.f.	28	28	28
e.s.e.	0.298	0.231	0.516

Standard errors of differences of means

Table	Treatment	Vigour	Treatment Vigour
rep.	9	15	3
d.f.	28	28	28
s.e.d.	0.421	0.326	0.730

Least significant differences of means (5% level)

Table	Treatment	Vigour	Treatment Vigour
rep.	9	15	3
d.f.	28	28	28
l.s.d.	0.863	0.668	1.494

Stratum standard errors and coefficients of variation

Variate: Number_of_seeds

Stratum	d.f.	s.e.	cv%
Rep	2	0.315	11.6
Rep.*Units*	28	0.894	33.0

```
  38  DELETE [REDEFINE=yes] _mean, _rep, _var, _resid, _rdf, _scode
  39  AKEEP [FACTORIAL=9] Treatment.Vigour; MEAN=_mean; REP=_rep;
VARIANCE=_var; RTERM=_resid;\
  40   STATUS=_scode
  41  IF _scode .in. !(1,2)
  42    AKEEP [FACTORIAL=9] #_resid; DF=_rdf
  43    AMCOMPARISON [PRINT=letter; METHOD=duncan; DIRECTION=descending;
PROB=0.05] Treatment.Vigour
```

Duncan's multiple range test

Treatment.Vigour

	Mean
4 High vigor	6.000 a
1 Medium vigor	4.000 b
5 Medium vigor	4.000 b
4 Medium vigor	3.667 bc
3 High vigor	3.000 bc
3 Medium vigor	3.000 bc
2 High vigor	2.667 bcd
5 High vigor	2.667 bcd
1 High vigor	2.333 bcd
2 Medium vigor	2.333 bcd
3 Low vigor	2.000 cd
5 Low vigor	2.000 cd
2 Low vigor	2.000 cd
4 Low vigor	1.000 de

1 Low vigor 0.000 e

```
  44  ELSE
  45    CAPTION !t('Multiple comparisons are available for tests other
than',\
  46    'Fisher''s LSD tests, only if all components of the term are
estimated',\
  47    'with equal efficiency and in the same stratum.')
  48  ENDIF
```

Appendix B: High vigour root length statistical analysis

GenStat Release 16.2 (PC/Windows 7) 30 July 2015 19:36:50

Registered to: University of KwaZulu-Natal

GenStat Sixteenth Edition

GenStat Procedure Library Release PL24.2

```
  1  SET [WORKINGDIRECTORY='C:/Users/212512302/Documents']
  2  "Data taken from file: '\
 -3  C:/Users/212512302/Documents/Root length of measurable seeds (High
vigor).xlsx\
 -4  '"
  5  DELETE [REDEFINE=yes] _stitle_: TEXT _stitle_
  6  READ [PRINT=*; SETNVALUES=yes] _stitle_
 10  PRINT [IPRINT=*] _stitle_; JUST=left
```

Data imported from Excel file: C:\Users\212512302\Documents\Root length of measurable seeds (High vigor).xlsx
on: 30-Jul-2015 19:39:27
taken from sheet "Sheet1", cells A2:D16

```
 11  DELETE [REDEFINE=yes] Treatment,Vigour_Level,Rep,\
 12  Root_length_mm_of_measurable_seeds
 13  UNITS [NVALUES=*]
 14  FACTOR [MODIFY=no; NVALUES=15; LEVELS=5; LABELS=*; REFERENCE=1]
Treatment
 15  READ Treatment; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
Treatment	15	0	5

```
  17  FACTOR [MODIFY=no; NVALUES=15; LEVELS=1; LABELS=!t('High')\
  18  ; REFERENCE=1] Vigour_Level
  19  READ Vigour_Level; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
Vigour_Level	15	0	1

```
  21  FACTOR [MODIFY=no; NVALUES=15; LEVELS=3; LABELS=*; REFERENCE=1] Rep
  22  READ Rep; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
Rep	15	0	3

```
  24  VARIATE [NVALUES=15] Root_length_mm_of_measurable_seeds
  25  READ Root_length_mm_of_measurable_seeds
```

Identifier	Minimum	Mean	Maximum	Values	Missing	
Root_length_mm_of_measurable_see	1.000	3.360	14.50	15	0	Skew

```
  27
  28  %PostMessage 1129; 0; 100001 "Sheet Update Completed"
  29  "General Analysis of Variance"
  30  BLOCK Rep
  31  TREATMENTS Treatment
  32  COVARIATE "No Covariate"
  33  ANOVA [PRINT=aovtable,information,means,%cv; FACT=32; CONTRASTS=7;
PCONTRASTS=7; FPROB=yes;\
  34   PSE=diff,lsd,means; LSDLEVEL=5] Root_length_mm_of_measurable_see
```

Appendix C: Root length statistical analysis

Analysis of variance

Variate: Root_length_mm_of_measurable_see

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	3.71	1.85	0.12	
Rep.*Units* stratum					
Treatment	4	49.33	12.33	0.78	0.545
Vigour_Level	2	2.99	1.50	0.10	0.910
Treatment.Vigour_Level	8	256.08	32.01	2.04	0.079
Residual	28	440.18	15.72		
Total	44	752.28			

Message: the following units have large residuals.

Rep 2 *units* 15	-7.81	s.e.	3.13
Rep 3 *units* 15	14.36	s.e.	3.13

Tables of means

Variate: Root_length_mm_of_measurable_see

Grand mean 3.13

Treatment	1	2	3	4	5
	2.76	1.92	2.25	4.08	4.62

Vigour_Level	High	Low	Medium
	3.36	2.77	3.25

Treatment	Vigour_Level	High	Low	Medium
1		1.53	0.00	6.75
2		2.83	1.10	1.83
3		1.10	2.87	2.78
4		8.47	1.00	2.77
5		2.87	8.87	2.12

Standard errors of means

Table	Treatment	Vigour_Level	Treatment Vigour_Level
rep.	9	15	3
d.f.	28	28	28
e.s.e.	1.322	1.024	2.289

Standard errors of differences of means

Table	Treatment	Vigour_Level	Treatment Vigour_Level
rep.	9	15	3
d.f.	28	28	28
s.e.d.	1.869	1.448	3.237

Least significant differences of means (5% level)

Table	Treatment	Vigour_Level	Treatment Vigour_Level
rep.	9	15	3
d.f.	28	28	28
l.s.d.	3.829	2.966	6.631

Stratum standard errors and coefficients of variation

Variate: Root_length_mm_of_measurable_see

Stratum	d.f.	s.e.	cv%
Rep	2	0.352	11.2
Rep.*Units*	28	3.965	126.8

```
  55  DELETE [REDEFINE=yes] _mean, _rep, _var, _resid, _rdf, _scode
  56  AKEEP [FACTORIAL=9] Treatment.Vigour_Level; MEAN=_mean; REP=_rep;
VARIANCE=_var; RTERM=_resid;\
  57   STATUS=_scode
  58  IF _scode.IN.!(1,2)
  59    AKEEP [FACTORIAL=9] #_resid; DF=_rdf
  60    AMCOMPARISON [METHOD=duncan; DIRECTION=descending; PROB=0.05]
Treatment.Vigour_Level
```

Duncan's multiple range test

Treatment.Vigour_Level

	Mean	
5 Low	8.867	a
4 High	8.467	ab
1 Medium	6.750	abc
3 Low	2.867	abc
5 High	2.867	abc
2 High	2.833	abc
3 Medium	2.783	abc
4 Medium	2.767	abc
5 Medium	2.120	abc
2 Medium	1.833	abc
1 High	1.533	abc
2 Low	1.100	abc
3 High	1.100	abc
4 Low	1.000	bc
1 Low	0.000	c

```
  61  ELSE
  62    PRINT !t('Multiple comparisons available only if all components of
the term',\
  63  'are estimated with equal efficiency and in the same stratum.');\
  64  JUST=left
  65  ENDIF
```

Analysis of variance

Variate: Root_length_mm_of_measurable_see

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	15.964	7.982	1.24	
Rep.*Units* stratum					
Treatment	4	105.129	26.282	4.10	0.043
Residual	8	51.343	6.418		
Total	14	172.436			

Message: the following units have large residuals.

Rep 2 *units* 4	4.61	s.e.	1.85

Tables of means

Variate: Root_length_mm_of_measurable_see

Grand mean 3.36

Treatment	1	2	3	4	5
	1.53	2.83	1.10	8.47	2.87

Standard errors of means

Table	Treatment
rep.	3
d.f.	8
e.s.e.	1.463

Standard errors of differences of means

Table	Treatment
rep.	3
d.f.	8
s.e.d.	2.068

Least significant differences of means (5% level)

Table	Treatment
rep.	3
d.f.	8
l.s.d.	4.770

Stratum standard errors and coefficients of variation

Variate: Root_length_mm_of_measurable_see

Stratum	d.f.	s.e.	cv%
Rep	2	1.263	37.6
Rep.*Units*	8	2.533	75.4

```
  35  DELETE [REDEFINE=yes] _mean, _rep, _var, _resid, _rdf, _scode
  36  AKEEP [FACTORIAL=9] Treatment; MEAN=_mean; REP=_rep; VARIANCE=_var;
RTERM=_resid;\
  37   STATUS=_scode
  38  IF _scode .in. !(1,2)
  39    AKEEP [FACTORIAL=9] #_resid; DF=_rdf
  40    AMCOMPARISON [PRINT=letter; METHOD=tukey; DIRECTION=descending;
PROB=0.05] Treatment
```

Tukey's 95% confidence intervals

Treatment

	Mean
4	8.467 a
5	2.867 ab
2	2.833 ab
1	1.533 b
3	1.100 b

```
  41  ELSE
  42    CAPTION !t('Multiple comparisons are available for tests other
than',\
  43    'Fisher''s LSD tests, only if all components of the term are
estimated',\
  44    'with equal efficiency and in the same stratum.')
  45  ENDIF
```

Appendix D: Dry weight statistical analysis

Analysis of variance

Variate: Dry_weight_g

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.0066178	0.0033089	4.04	
Rep.*Units* stratum					
Treatment	4	0.0043333	0.0010833	1.32	0.286
Vigour_Level	2	0.0243378	0.0121689	14.87	<.001
Treatment.Vigour_Level	8	0.0069067	0.0008633	1.05	0.421
Residual	28	0.0229156	0.0008184		
Total	44	0.0651111			

Tables of means

Variate: Dry_weight_g

Grand mean 0.2744

Treatment	1	2	3	4	5
	0.2778	0.2600	0.2900	0.2744	0.2700

Vigour_Level	High	Low	Medium
	0.2953	0.2420	0.2860

Treatment	Vigour_Level	High	Low	Medium
1		0.3133	0.2433	0.2767
2		0.2767	0.2233	0.2800
3		0.2967	0.2633	0.3100
4		0.3067	0.2200	0.2967
5		0.2833	0.2600	0.2667

Standard errors of means

Table	Treatment	Vigour_Level	Treatment Vigour_Level
rep.	9	15	3
d.f.	28	28	28
e.s.e.	0.00954	0.00739	0.01652

Standard errors of differences of means

Table	Treatment	Vigour_Level	Treatment Vigour_Level
rep.	9	15	3
d.f.	28	28	28
s.e.d.	0.01349	0.01045	0.02336

Least significant differences of means (5% level)

Table	Treatment	Vigour_Level	Treatment Vigour_Level
rep.	9	15	3
d.f.	28	28	28
l.s.d.	0.02762	0.02140	0.04785

Stratum standard errors and coefficients of variation

Variate: Dry_weight_g

Stratum	d.f.	s.e.	cv%
Rep	2	0.01485	5.4
Rep.*Units*	28	0.02861	10.4

```
 126  DELETE [REDEFINE=yes] _mean, _rep, _var, _resid, _rdf, _scode
 127  AKEEP [FACTORIAL=9] Treatment.Vigour_Level; MEAN=_mean; REP=_rep;
VARIANCE=_var; RTERM=_resid;\
 128   STATUS=_scode
 129  IF _scode .in. !(1,2)
 130    AKEEP [FACTORIAL=9] #_resid; DF=_rdf
 131    AMCOMPARISON [PRINT=letter; METHOD=duncan; DIRECTION=descending;
PROB=0.05] Treatment.Vigour_Level
```

Duncan's multiple range test

Treatment.Vigour_Level

	Mean
1 High	0.3133 a
3 Medium	0.3100 a
4 High	0.3067 a
3 High	0.2967 ab
4 Medium	0.2967 ab
5 High	0.2833 ab
2 Medium	0.2800 ab
1 Medium	0.2767 abc
2 High	0.2767 abc
5 Medium	0.2667 abcd
3 Low	0.2633 abcd
5 Low	0.2600 abcd
1 Low	0.2433 bcd
2 Low	0.2233 cd
4 Low	0.2200 d

```
 132  ELSE
 133    CAPTION !t('Multiple comparisons are available for tests other
than',\
 134    'Fisher''s LSD tests, only if all components of the term are
estimated',\
 135    'with equal efficiency and in the same stratum.')
 136  ENDIF
```

```
 137  "General Analysis of Variance"
 138  BLOCK Rep
 139  TREATMENTS Treatment*Vigour_Level
 140  COVARIATE "No Covariate"
 141  ANOVA [PRINT=aovtable,information,means,%cv; FACT=32; CONTRASTS=7;
PCONTRASTS=7; FPROB=yes;\
 142   PSE=diff,lsd,means; LSDLEVEL=5] Fresh_weight_g
```

Appendix E: Germination rate statistical analysis

GenStat Release 16.2 (PC/Windows 7) 07 September 2014 12:29:55

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GenStat Sixteenth Edition

GenStat Procedure Library Release PL24.2

```
   1  SET [WORKINGDIRECTORY='C:/Users/212512302/Documents']
   2  "Data taken from file: '\
  -3  C:/Users/212512302/Documents/Gerhard Batch 2 seed data for
analysis.xlsx'"
   4  DELETE [REDEFINE=yes] _stitle_: TEXT _stitle_
   5  READ [PRINT=*; SETNVALUES=yes] _stitle_
   9  PRINT [IPRINT=*] _stitle_; JUST=left
```

Data imported from Excel file: C:\Users\212512302\Documents\Gerhard Batch 2 seed data for analysis.xlsx
on: 7-Sep-2014 12:30:11
taken from sheet "germination over time", cells A2:F136

```
  10  DELETE [REDEFINE=yes]
Treatment,Vigour_Level,Rep,Time_hrs,Germination_count,\
  11  Germination_%
  12  UNITS [NVALUES=*]
  13  FACTOR [MODIFY=no; NVALUES=135; LEVELS=5; LABELS=*; REFERENCE=1]
Treatment
  14  READ Treatment; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
Treatment	135	0	5

```
  19  FACTOR [MODIFY=no; NVALUES=135; LEVELS=3;
LABELS=!t('High','Low','Medium')\
  20  ; REFERENCE=1] Vigour_Level
  21  READ Vigour_Level; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
Vigour_Level	135	0	3

```
  26  FACTOR [MODIFY=no; NVALUES=135; LEVELS=3; LABELS=*; REFERENCE=1] Rep
  27  READ Rep; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
Rep	135	0	3

```
  32  FACTOR [MODIFY=no; NVALUES=135; LEVELS=!(4,24,48); LABELS=*\
  33  ; REFERENCE=1] Time_hrs
  34  READ Time_hrs; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
Time_hrs	135	0	3

```
  39  VARIATE [NVALUES=135] Germination_count
  40  READ Germination_count
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Germination_count	5.000	8.156	10.00	135	0

```
  45  VARIATE [NVALUES=135] Germination_%
  46  READ Germination_%
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Germination_%	50.00	81.56	100.0	135	0

```
  53
  54  %PostMessage 1129; 0; 100001 "Sheet Update Completed"
  55  "General Analysis of Variance"
  56  BLOCK Rep
  57  TREATMENTS Vigour_Level*Treatment*Time_hrs
  58  COVARIATE "No Covariate"
  59  ANOVA [PRINT=aovtable,information,means,%cv; FACT=32; CONTRASTS=7;
PCONTRASTS=7; FPROB=yes;\
  60   PSE=diff,lsd,means; LSDLEVEL=5] Germination_%
```

Analysis of variance

Variate: Germination_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	57.78	28.89	0.88	
Rep.*Units* stratum					
Vigour_Level	2	6137.78	3068.89	93.92	<.001
Treatment	4	484.44	121.11	3.71	0.008
Time_hrs	2	8093.33	4046.67	123.84	<.001
Vigour_Level.Treatment	8	2862.22	357.78	10.95	<.001
Vigour_Level.Time_hrs	4	22.22	5.56	0.17	0.953
Treatment.Time_hrs	8	240.00	30.00	0.92	0.506
Vigour_Level.Treatment.Time_hrs	16	200.00	12.50	0.38	0.983
Residual	88	2875.56	32.68		
Total	134	20973.33			

Message: the following units have large residuals.

Rep 1 *units* 30	12.67	s.e.	4.62
Rep 1 *units* 45	12.67	s.e.	4.62

Tables of means

Variate: Germination_%

Grand mean 81.56

Vigour_Level	High	Low	Medium
	89.33	72.89	82.44

Treatment	1	2	3	4	5
	82.59	84.44	80.37	81.48	78.89

Time_hrs	4	24	48
	70.67	86.00	88.00

Vigour_Level	Treatment	1	2	3	4	5
High		88.89	92.22	91.11	83.33	91.11
Low		72.22	71.11	67.78	84.44	68.89
Medium		86.67	90.00	82.22	76.67	76.67

Vigour_Level	Time_hrs	4	24	48
High		78.67	93.33	96.00
Low		62.00	78.00	78.67
Medium		71.33	86.67	89.33

Treatment	Time_hrs	4	24	48
1		71.11	86.67	90.00
2		74.44	88.89	90.00
3		68.89	84.44	87.78
4		73.33	84.44	86.67
5		65.56	85.56	85.56

Vigour_Level	Treatment	Time_hrs	4	24	48
High	1		76.67	93.33	96.67
	2		83.33	96.67	96.67
	3		80.00	93.33	100.00
	4		73.33	86.67	90.00
	5		80.00	96.67	96.67
Low	1		60.00	76.67	80.00
	2		60.00	76.67	76.67

	3	56.67	73.33	73.33
	4	80.00	86.67	86.67
	5	53.33	76.67	76.67
Medium	1	76.67	90.00	93.33
	2	80.00	93.33	96.67
	3	70.00	86.67	90.00
	4	66.67	80.00	83.33
	5	63.33	83.33	83.33

Standard errors of means

Table	Vigour_Level	Treatment	Time_hrs	Vigour_Level Treatment
rep.	45	27	45	9
d.f.	88	88	88	88
e.s.e.	0.852	1.100	0.852	1.905

Table	Vigour_Level Time_hrs	Treatment Time_hrs	Vigour_Level Treatment Time_hrs
rep.	15	9	3
d.f.	88	88	88
e.s.e.	1.476	1.905	3.300

Standard errors of differences of means

Table	Vigour_Level	Treatment	Time_hrs	Vigour_Level Treatment
rep.	45	27	45	9
d.f.	88	88	88	88
s.e.d.	1.205	1.556	1.205	2.695

Table	Vigour_Level Time_hrs	Treatment Time_hrs	Vigour_Level Treatment Time_hrs
rep.	15	9	3

d.f.	88	88	88
s.e.d.	2.087	2.695	4.667

Least significant differences of means (5% level)

Table	Vigour_Level	Treatment	Time_hrs	Vigour_Level Treatment
rep.	45	27	45	9
d.f.	88	88	88	88
l.s.d.	2.395	3.092	2.395	5.355

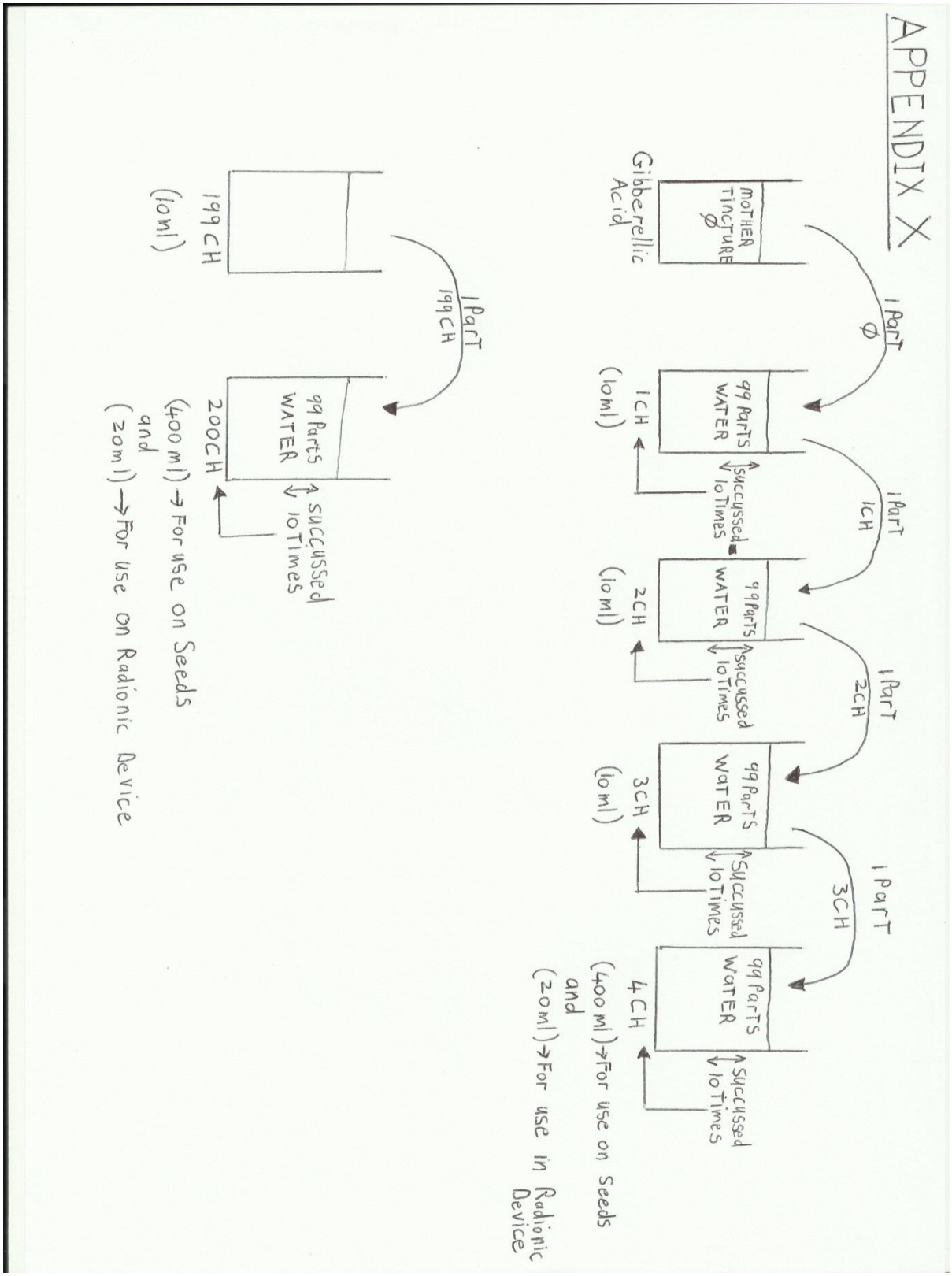
Table	Vigour_Level Time_hrs	Treatment Time_hrs	Vigour_Level Treatment Time_hrs
rep.	15	9	3
d.f.	88	88	88
l.s.d.	4.148	5.355	9.275

Stratum standard errors and coefficients of variation

Variate: Germination_%

Stratum	d.f.	s.e.	cv%
Rep	2	0.801	1.0
Rep.*Units*	88	5.716	7.0

Appendix F: Remedy manufacture process



Appendix G: First trial data

Treatment	Vigour Level	Rep	Number of seeds with measurable roots	Root length (mm) Average of measurable seeds	Root length (mm) Average of all 50 seeds
1	High	1	5	12.2	1.22
1	High	2	4	1.25	0.1
1	High	3	2	1.5	0.06
1	Medium	1	0	0	0
1	Medium	2	0	0	0
1	Medium	3	0	0	0
1	Low	1	0	0	0
1	Low	2	0	0	0
1	Low	3	0	0	0
2	High	1	0	0	0
2	High	2	0	0	0
2	High	3	2	5	0.2
2	Medium	1	2	6.5	0.26
2	Medium	2	1	2	0.04
2	Medium	3	1	1	0.02
2	Low	1	1	1	0.02
2	Low	2	2	7.5	0.3
2	Low	3	2	6.5	0.26
3	High	1	3	2	0.12
3	High	2	4	4	0.32
3	High	3	4	10	0.8
3	Medium	1	0	0	0
3	Medium	2	0	0	0
3	Medium	3	0	0	0
3	Low	1	0	0	0
3	Low	2	0	0	0
3	Low	3	0	0	0
4	High	1	5	4.8	0.48
4	High	2	4	3.5	0.28
4	High	3	4	3.25	0.26
4	Medium	1	5	8	0.8
4	Medium	2	3	7.6	0.46
4	Medium	3	3	8.3	0.5
4	Low	1	6	11.5	1.38
4	Low	2	3	3	0.18
4	Low	3	3	5.3	0.32
5	High	1	3	6.3	0.38
5	High	2	4	2.25	0.18
5	High	3	5	4.6	0.46

5	Medium	1	4	10.5	0.84
5	Medium	2	1	1	0.02
5	Medium	3	2	1	0.04
5	Low	1	0	0	0
5	Low	2	0	0	0
5	Low	3	0	0	0

Germination over time (data from first trial continued)

Treatment	Vigour Level	Rep	Time (hours)	Germination count	Germination %
1	High	1	4	45	90
1	High	2	4	45	90
1	High	3	4	47	94
1	Medium	1	4	44	88
1	Medium	2	4	41	82
1	Medium	3	4	42	84
1	Low	1	4	42	84
1	Low	2	4	41	82
1	Low	3	4	40	80
2	High	1	4	49	98
2	High	2	4	46	92
2	High	3	4	45	90
2	Medium	1	4	41	82
2	Medium	2	4	43	86
2	Medium	3	4	42	84
2	Low	1	4	35	70
2	Low	2	4	38	76
2	Low	3	4	42	84
3	High	1	4	48	96
3	High	2	4	45	90
3	High	3	4	46	92
3	Medium	1	4	42	84
3	Medium	2	4	43	86
3	Medium	3	4	43	86
3	Low	1	4	41	82
3	Low	2	4	45	90
3	Low	3	4	41	82
4	High	1	4	50	100
4	High	2	4	47	94
4	High	3	4	46	92
4	Medium	1	4	43	86
4	Medium	2	4	46	92
4	Medium	3	4	45	90
4	Low	1	4	43	86
4	Low	2	4	41	82
4	Low	3	4	40	80
5	High	1	4	45	90
5	High	2	4	47	94
5	High	3	4	46	92
5	Medium	1	4	45	90

5	Medium	2	4	43	86
5	Medium	3	4	45	90
5	Low	1	4	43	86
5	Low	2	4	43	86
5	Low	3	4	42	84
1	High	1	24	47	94
1	High	2	24	48	96
1	High	3	24	47	94
1	Medium	1	24	44	88
1	Medium	2	24	41	82
1	Medium	3	24	44	88
1	Low	1	24	44	88
1	Low	2	24	44	88
1	Low	3	24	44	88
2	High	1	24	49	98
2	High	2	24	47	94
2	High	3	24	48	96
2	Medium	1	24	42	84
2	Medium	2	24	44	88
2	Medium	3	24	45	90
2	Low	1	24	41	82
2	Low	2	24	40	80
2	Low	3	24	45	90
3	High	1	24	49	98
3	High	2	24	45	90
3	High	3	24	48	96
3	Medium	1	24	42	84
3	Medium	2	24	44	88
3	Medium	3	24	43	86
3	Low	1	24	43	86
3	Low	2	24	45	90
3	Low	3	24	42	84
4	High	1	24	50	100
4	High	2	24	47	94
4	High	3	24	47	94
4	Medium	1	24	46	92
4	Medium	2	24	47	94
4	Medium	3	24	46	92
4	Low	1	24	43	86
4	Low	2	24	42	84
4	Low	3	24	40	80
5	High	1	24	47	94
5	High	2	24	47	94
5	High	3	24	46	92

5	Medium	1	24	45	90
5	Medium	2	24	43	86
5	Medium	3	24	46	86
5	Low	1	24	44	88
5	Low	2	24	43	86
5	Low	3	24	42	84
1	High	1	48	47	94
1	High	2	48	48	96
1	High	3	48	47	94
1	Medium	1	48	44	88
1	Medium	2	48	41	82
1	Medium	3	48	46	92
1	Low	1	48	44	88
1	Low	2	48	44	88
1	Low	3	48	45	90
2	High	1	48	49	98
2	High	2	48	47	94
2	High	3	48	48	96
2	Medium	1	48	42	84
2	Medium	2	48	46	92
2	Medium	3	48	48	96
2	Low	1	48	42	84
2	Low	2	48	40	80
2	Low	3	48	45	90
3	High	1	48	49	98
3	High	2	48	47	94
3	High	3	48	48	96
3	Medium	1	48	42	84
3	Medium	2	48	45	90
3	Medium	3	48	43	86
3	Low	1	48	43	86
3	Low	2	48	45	90
3	Low	3	48	44	88
4	High	1	48	50	100
4	High	2	48	47	94
4	High	3	48	47	94
4	Medium	1	48	47	94
4	Medium	2	48	47	94
4	Medium	3	48	46	92
4	Low	1	48	43	86
4	Low	2	48	42	84
4	Low	3	48	41	82
5	High	1	48	47	94
5	High	2	48	47	94

5	High	3	48	46	92
5	Medium	1	48	45	90
5	Medium	2	48	43	96
5	Medium	3	48	46	92
5	Low	1	48	46	92
5	Low	2	48	43	86
5	Low	3	48	42	84