The effect of homoeopathic potencies of abscisic acid on the production of α-amylase in barley seeds (*Hordeum vulgare*) in the presence of gibberellic acid.

by

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Dissertation submitted in partial compliance with the requirements for the Master's Degree in Technology: Homoeopathy in the Department of Homoeopathy at the Technikon Natal, Durban.

I, Ingrid Couchman, do hereby declare that this dissertation represents my own work both in concept and execution.

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DEDICATION

This is dedicated to my husband, for his endless support and enthusiasm to help me to complete this dissertation, and my family for all their patience and motivation.

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ABSTRACT

This study investigated the effect of different homoeopathic potencies of abscisic acid (ABA) in the presence of gibberellic acid (GA_3) on the production of α-amylase in the de-embryonated endosperm half-seeds of barley (*Hordeum vulgare*) in order to investigate whether these potencies were able to produce a biological effect.

The aleurone layer of the barley (*Hordeum vulgare*) grain secretes hydrolases that mobilise endosperm reserves during germination. The synthesis and secretion of these hydrolases (principally α-amylase) is under hormonal regulation. Gibberellic acid (GA) stimulates the synthesis and secretion of α-amylase and abscisic acid (ABA) reverses this effect. It is for this reason that barley aleurone has been used extensively as a model system for the study of signal transduction in response to GA and ABA (Ritchie, McCubbin, Ambrose, Kao and Gilroy, 1999).

For this research five different homoeopathic potencies were used namely – the 4CH, 9CH, 15CH, 30CH and the 200CH. Two methods were used in this experiment namely – spectrophotometry and radial diffusion.

For each method three replications were used. Each replication consisted of: five groups of 20 half-seeds treated with the various potencies (the 4CH, 9CH, 15CH, 30CH and the 200CH) of ABA, with a dilution of GA_3 at $1x\ 10^{-9}$; five groups of 20 half-seeds treated with the of GA_3 at $1x\ 10^{-9}$ (the control) and five groups of 20 half-seeds treated with only the incubation buffer (calcium nitrate).

An α-amylase curve was constructed for both methods using pure α-amylase at 1350 enzyme units/mg.

Each group of half-seeds were first weighed. To each group 5ml of the respective treatment was then added. They were then placed into an incubator for 48 hours. The α-amylase was then extracted by maceration of the seeds. The slurry was then incubated in a shaker bath for 60 minutes. The slurry was then centrifuged and filtered. From the centrate 0.5ml of each group was extracted and set aside for the radial diffusion. For the spectrophotometry, the assay was prepared by adding a dye-labelled Phadebas tablet to each group, which was then incubated for 10 minutes. The reaction was then halted by the addition of 1ml of sodium hydroxide (NaOH).

The gels had wells cut into them using a well cutter. The 0.5ml extracted from each group for the radial diffusion was then syringed into the relevant wells. These petri dishes were then incubated for 24 hours. The reaction was then stopped by the addition of 1ml acetic acid.

There was no physical difference between the control and the various potencies as each group produced α-amylase to some extent. Since ABA inhibits this production it can be stated that the homoeopathic potencies of ABA did not have a significant biological effect.

The data was analysed statistically using the ONEWAY ANOVA (SPSS programme for Windows). The results showed that there was no significant difference between the control and the various potencies of ABA.

This leads to the conclusion that the homoeopathic potencies of ABA had no effect on the production of α-amylase in the endosperm half-seeds of barley. It can also be concluded that more research needs to be undertaken in homoeopathy in the field of plant physiology.

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THE DEFINITION OF TERMS

Arndt-Schultz Law

This law states that minute stimuli encourage life activity, medium to strong stimuli tends to impede it, and very strong stimuli to stop or destroy it. (Gaier, 1991).

Avogadro's Law

According to Avogadro the molecular weight of any material expressed in grams contains 6.023×10^{23}. (Stephenson, 1973).

Homoeopathy

Homoeopathy is a system of therapeutics for treating people and animals on the basis of the simile principle. The word "homoeopathy" is derived from the Greek words *homoios*, meaning like or similar, and *pathos*, meaning suffering. (Boyd, 1989).

Potentisation

In homoeopathy, potentisation is understood as the stepwise dilution of a substance in an indifferent diluent in a ratio of 1:10 which is followed by agitation by sharp manual succussions against a hard but elastic body. (Majerus, 1991).

Potency

The especially produced capability in a medicine to effect a dynamic stimulus (Gaier, 1991)

Succussion

The action of shaking vigorously a liquid dilution of a homoeopathic medicine in its vial/ bottle, where each stroke ends with a jolt. Usually effected by pounding the hand engaged in shaking against the palm of the opposite hand (Kayne, 1997).

Hydrolases

Enzymes which catalyse hydrolytic reactions (Palmer, 1981).

Carotenoid

Any group of pigments, yellow to deep red in colour, chemically consisting of tetraterpene hydrocarbons (Saunders, 1988).

Stomata

Minute pores or orifices on a free surface on the plant (Saunders, 1988).

Amylopectin

The insoluble constituent of starch (Saunders, 1988).

Oligosaccharide

A carbohydrate which on hydrolysis yields a small number (from two to ten) of monosaccharides (Saunders, 1988).

Endogenous

Developing or originating from within (Collins, 1994).

Exogenous

Developing or originating outside the organism (Collins, 1994).

Agronomy

The science of land cultivation, soil management and crop production (Collins, 1994).

Triturate

Rub to a powder (Saunders, 1988).

CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

This research investigated the effect of homoeopathic potencies of abscisic acid on the production of α-amylase in barley seeds in the presence of gibberellic acid.

The seed phase is the most important stage in the higher plant life cycle with respect to survival as a species. The surrounding aleurone layer is important in the regulation of dormancy and germination. (Hilhorst and Toorop, 1997).

The aleurone layer of the barley (*Hordeum vulgare*) grain secretes hydrolases that mobilise endosperm reserves during germination. The synthesis and secretion of these hydrolases (principally α-amylase) is under hormonal regulation. Gibberellic acid (GA) stimulates the synthesis and secretion of α-amylase and abscisic acid (ABA) reverses this effect. It is for this reason that barley aleurone has been used extensively as a model system for the study of signal transduction in response to GA and ABA (Ritchie et al., 1999).

Starch is the major stored reserve found within the cereal endosperm. Not unexpectedly, the enzyme that has been most studied in relation to the control of reserve mobilisation is the one largely responsible for its hydrolysis, i.e. α-amylase. ABA, an antagonist of gibberellin action in many plant systems, is inhibitory to α-amylase production (Black and Bewley, 1994).

Many investigations have been undertaken in order to elucidate the enzymatic mechanism of the breakdown of reserve substances in cereal seeds at the onset of germination. Haberlandt (1890) reported that the aleurone layer of the endosperm tissues secrete the hydrolytic enzymes. Afterwards, numerous investigators, using different types of cereal seeds, reported

that α-amylase activity is localised in the aleurone cells surrounding the endosperm tissue (Okamoto, Kitano and Akazawa, 1980).

The effects of growth regulators on the mobilisation of food reserves in seeds are important for several reasons. Firstly for its intrinsic interest, but also because it exemplifies a system which is clearly "pre-programmed" to respond in a particular way to appropriate internal and external signals; moreover, the systems involved appear to implicate all the known groups of plant growth regulators (Salisbury and Ross. 1978)

Pre-harvest sprouting is a serious problem in cereals. As well as producing shrivelled seeds, pre-harvest sprouting involves an activation and synthesis of enzymes involved in starch breakdown and thus produces grains with partially modified storage carbohydrates. As these processes decrease the baking quality, it has been a central breeding objective to produce varieties with greater resistance to sprouting in the ear. Due to the frequent correlation between high α-amylase activity and pre-harvest sprouting there exists a need for a fast and simple α-amylase assay in breeding programmes. Furthermore, rapid α-amylase determinations are useful in the milling, baking and brewing industries. Thus, radial diffusion into a gel-substrate was developed as a fast, effective way of determining α-amylase activity (Hejgaard and Gibbon, 1979).

Radial diffusion permits the visualisation of α-amylase secretion by monitoring the degradation of starch from the agarose matrix in which they are embedded (Hilmer, Gilroy and Jones, 1992). The degradation is shown by a white halo formed around the barley endosperm in the otherwise blue gel. The diameter of the white halo represents α-amylase activity.

Since the homoeopathic method of preparing medicines is controversial, research into their effectiveness is needed. This research aimed to establish that potentised medicine does have an effect to further the scientific basis for Homoeopathy. Potentisation is one of the most controversial parts of the Homoeopathic science. This is because most of the scientists

believe that any remedy over the 24th potency (24CH) would not have any physiological effect since it is improbable that there are any remaining molecules. (Ullman, 1981).

1.2 AIM OF THE STUDY

The aim of the study was to investigate the effect of different potencies of ABA on the synthesis of α-amylase in barley endosperm half-seeds in the presence of gibberellic acid.

1.3 STATEMENT OF THE OBJECTIVES

1.3.1 THE FIRST OBJECTIVE

The first objective was to determine the effect of different potencies of ABA, using the Hahnemannian method of centesimal dilution, on the synthesis of α-amylase in barley endosperm half-seeds in the presence of gibberellic acid.

1.3.2 THE SECOND OBJECTIVE

The second objective was to compare the analysis of data using spectrophotometry and radial diffusion.

1.4 STATEMENT OF THE HYPOTHESES

All hypotheses are stated in the null form.

1.4.1 THE FIRST HYPOTHESIS

It is hypothesised that the Hahnemannian potencies of ABA will have no effect on the synthesis of α-amylase in barley endosperm half-seeds in the presence of gibberellic acid.

1.4.2 THE SECOND HYPOTHESIS

It is hypothesised that there will be no significant difference between spectrophotometry and radial diffusion in the analysis of the data.

1.5 THE SIGNIFICANCE OF THE STUDY

The results have proved that the Hahnemannian potencies of ABA did not have an effect on the production of α-amylase in barley endosperm half-seeds in the presence of gibberellic acid. This leads to the conclusion that more research needs to be done in the field of ultra high potencies on plant metabolic activities as Steele (1999) did find that ultra high dilutions of gibberellic acid did have a significant effect on the production of α-amylase in barley endosperm half-seeds.

1.6 THE IMPLICATIONS OF THE STUDY

The first implication of this study is that more research needs to be undertaken to verify whether ultra high dilutions of plant hormones do in fact have a significant effect on plant metabolism as this study has proven that there was no significant effect produced.

1.7 THE BENEFITS OF THE STUDY

Since the relative contributions of the plant hormone ABA to biological responses are yet poorly understood (Walker-Simmons, Holappa, Abrams and Abrams. 1997) this study further investigated the effect of ABA on the production of α-amylase in barley endosperm half-seeds. In addition to this, the study showed that there is a need for more homoeopathic research in the field of plant biology as there are conflicting results. Another benefit is that this research has shown that there is no significant difference between using spectrophotometry and radial diffusion and thus either method could be used. Since the radial diffusion method is more cost effective as well as more visual it should be investigated further as a means for analysing α-amylase production.

CHAPTER 2

LITERATURE REVIEW

2.1 BARLEY

Barley is the one of the world's oldest domestic crops. It is the most suitable cereal grain for malting and is thus used in beer production. Ground barley malt is also used in baking as a source of amylases, and malt syrups are used in baking and as ingredients for colouring and flavouring for other foods (Matz, 1991).

There are two categories of uses for barley –food and feed.

Animal feed: The grain has many possible applications as a source of carbohydrate for livestock and poultry. It can be used in blends with other feed materials for all farm animals, and has been especially favoured for feeding bacon hogs, and sheep and lambs for show purposes. (Matz, 1991).

Food and beverage ingredients: The major non-feed use of barley is in the production of malt, and by far the largest part of this malt is used for brewing beer. Some malt is used by distillers and a substantial amount is used in ground form as a diastatic enzyme source in yeast-leavened baked products. Barley has been used in snacks, breakfast cereals, baby foods, cookies, breads, and grain yoghurts. The sprouts have been used as a health food. Barley meal and cracked barley are two of several grain components found in multi-grain breads. The grain is high in fibre and so could be considered as a constituent wherever that ingredient is required. (Matz, 1991).

Barley is one of the cereal members of the grass family. Cultivated barleys have been classified into the two groups, *H.vulgare* L., the six-rowed barleys, and *H.distichum* L., the two-rowed types (Matz, 1991).

2.2 SEEDS

The seed phase is the most important stage in the higher plant life cycle with respect to survival as a species. The embryo, containing the full genetic makeup is protected by one or several layers of other tissue, such as endosperm, perisperm, seed coat, and fruit tissues. These structures protect the embryo from physical damage but also play a role in spreading the seeds after abscission. Often, the surrounding layers are important elements in the regulation of dormancy and germination. (Hilhorst and Toorop, 1997).

In cereals, the endosperm is the major source of stored reserves within the mature seed. It's storage capacity is established during the early stage of grain development and depends on the processes of cell division, organelle proliferation and cell enlargement which will determine the metabolic activity and final volume of this tissue (Jones, Bush, Gilroy and Hilmer, 1996).

The aleurone layer of the barley (*Hordeum vulgare*) grain secretes hydrolases that mobilise endosperm reserves during germination. The synthesis and secretion of these hydrolases (principally α-amylase) is under hormonal regulation. Gibberellic acid (GA) stimulates the synthesis and secretion of α-amylase and abscisic acid (ABA) reverses this effect. It is for this reason that barley aleurone has been used extensively as a model system for the study of signal transduction in response to GA and ABA (Ritchie et al., 1999).
(See Appendix A)

Starch is the major stored reserve found within the cereal endosperm. Not unexpectedly, the enzyme that has been most studied in relation to the control of reserve mobilisation is the one

largely responsible for its hydrolysis, i.e. α-amylase. ABA, an antagonist of gibberellin action in many plant systems, is inhibitory to α-amylase production (Black and Bewley, 1994).

2.3 HORMONES

Plant growth and development are strongly influenced by plant growth regulating substances, which are organic compounds made in one part of a plant and transported to another part, where they elicit a response. These growth-regulating substances have traditionally been referred to as hormones. There are five major classes of plant hormones: auxin, gibberellins, cytokinins, abscisic acid and ethylene. These hormones each elicit many responses and interact in complex ways to stimulate or inhibit growth (Moore, Clark and Stern. 1995)

This research concentrated on two of these hormones, primarily on abscisic acid and secondly on gibberellin.

2.3.1 ABSCISIC ACID

The hormone abscisic acid was discovered independently by Addicot at the University of California and Wareing at the University of Wales. Addicot discovered that extracts of ageing cotton bolls and other senescent tissues promoted the abscission, or shedding, of leaves. Wareing extracted a chemical out of dormant winter buds that dramatically slowed stem growth and caused other buds to become dormant. This chemical turned out to be identical to Addicot's. Because Addicot's report was published slightly earlier than Wareing's, the chemical was officially named abscisic acid, now designated as ABA. It is now clear, however that ABA is more closely associated with the induction of dormancy than with abscission (Galston, 1994).

ABA in plants is made from carotenoids. Once synthesised, ABA moves throughout a plant in xylem, phloem and parenchyma (Moore et al, 1995).

ABA has been shown to regulate many physiological and developmental processes. It is known to regulate the accumulation of some storage proteins during seed development. The onset and maintenance of seed dormancy also relies on the synthesis of ABA. In vegetative tissue, ABA appears to be involved in the response and adaptation of plants to environmental stresses, especially in drought and cold conditions. It has been well documented that levels of ABA increase in response to drought. This increase of ABA levels leads to the closure of stomata to avoid water stress and the induction and accumulation of compatible solutes for water stress tolerance (Mambelli and Setter, 1997).

2.3.2 GIBBERELLIC ACID

Japanese scientist, Kurosawa and his colleagues were studying rice (*Oryza sativa*) plants suffering from "foolish seedling disease", which caused the plants to become pale and spindly, when they discovered that these plants were infected by a fungus, *Gibberella fujikuroi.* From this they extracted a compound known as gibberellin ((Moore et al, 1995:).

Gibberellin is made via the mevalonic acid pathway. Gibberellins are abbreviated GA (Moore et al, 1995:).

Gibberellins cause certain dormant seeds to germinate by accelerating the use of reserves stored in the endosperm in the form of starch grains, fat globules, or protein globules. Such reserves must be digested by specific enzymes before they can be utilised for growth. Gibberellin stimulates the production of the required enzymes and in doing so initiates the germination process (Galston, 1994).

(See Appendix B for the chemical composition of both ABA and GA_3.)

2.4 ENZYMES

Most proteins in a living cell are enzymes, which are the catalysts for biochemical reactions. This means that enzymes speed up reactions without being consumed in the process (Moore et al, 1995).

The aleurone layer of cereal grains secretes a wide spectrum of hydrolases into the starchy endosperm. The synthesis and secretion of these hydrolytic enzymes is regulated by calcium and the plant hormones GA_3 and ABA. The α-amylases make up as much as 70% of the newly synthesised proteins in the aleurone cell. (Hillmer et al., 1992)

The amylose and amylopectin in the native starch grain are first hydrolysed by α-amylase, which breaks the α(1→4) glycosidic links between the glucose residues randomly throughout the chains. The released oligosaccharides are then further hydrolysed by α-amylase until glucose and maltose are produced

$$\text{Amylose} \xrightarrow{\alpha\text{- amylase}} \text{Glucose + maltose}$$

Multiple forms of this enzyme occur in germinated seeds of many species. (Black and Bewley, 1994).

The initial production of α-amylase occurs in the scutellum, which surrounds the aleurone layer, and in the few aleurone cells that penetrate the peripheral regions of the scutellum. The α-amylase is then released into the starchy endosperm and diffuses away from the scutellum. Later the enzyme is synthesised within the aleurone layer and is secreted inward to complete the hydrolysis of starch reserves

Although α-amylase from the scutellum may be important at early stages of mobilisation it is likely that later most of the hydrolysis is effected by isoenzymes from the aleurone layer (Black and Bewley, 1994).

2.5 RESEARCH

The barley aleurone cell is well established as a model for studying hormonal regulation. The aleurone of the barley grain is a digestive tissue that secretes hydrolases that mobilise endosperm reserves during germination. The synthesis and secretion of these hydrolases (principally α-amylases) are under hormonal regulation. GA stimulates α-amylase synthesis and secretion, whereas ABA reverses this effect (Gilroy and Jones, 1994).

The relative contributions of ABA to biological responses are as yet poorly understood in cereals (Walker-Simmons et al., 1997).

Paleg (1960) and Yomo (1960) independently showed that GA stimulated the production of α-amylase. Paleg then speculated that an endogenous gibberellin produced by the barley embryo played a role in regulating the production of enzymes by the barley endosperm. Subsequently, scientists have stimulated the production of hydrolytic enzymes from the aleurone layer of the endosperm, and not the embryo, by applying exogenous GA. Many experiments support the view that exogenous GAs play a role in regulating α-amylase production in the aleurone layer of barley (Skadsen, 1993; Zwar and Chandler, 1995). (Bethke Schuurink and Jones, 1997)

The cereal aleurone is also responsive to added ABA (Hetherington and Quantrano, 1991; Chandler and Robertson, 1994). ABA both inhibits many GA-induced responses. There is, however, a limited amount of information on the role of ABA as an endogenous regulator of aleurone function in cereals. ABA is a useful tool for studying hormonal signalling in the aleurone cell (Bethke et al., 1997).

This research investigated further the effect of exogenous ABA on barley endosperm albeit in a Homoeopathic form thus furthering the study of the effects of ABA as well as the effectiveness of the Homoeopathic potencies on barley seeds.

2.6 RADIAL DIFFUSION

Due to the frequent correlation between high α-amylase activity and pre-harvest sprouting, which decreases the quality of cereals, faster and simpler α-amylase assays were developed. One of these was radial diffusion. Radial diffusion is a visual method of determining the production of α-amylase using a gel substrate.

Hejgaard and Gibbons (1979) used a commercially available dye-labelled starch (Phadebas) in a gel substrate to assay α-amylase. The level of α-amylase was determined by measuring the haloes formed in the gel.

Hillmer and Jones (1992) used this technique to measure the secretion of α-amylase from individual barley protoplasts and found that this technique was comparable to that obtained by measuring α-amylase secretion from a population of cells.

The gel substrate used in this research consisted of 150mg agarose, which was dissolved in 15ml boiling 50mM-phosphate buffer, which contained 20mM$CaCl_2$, 20mg blue-starch substrate, (1 Phadebas tablet crushed to a fine powder) with a pH of 6.9. This was vigorously shaken to form a uniform suspension. It was then poured into a petri dish and wells of 5mm diameter were punched out (Hejaard and Gibbons, 1979).

The extracts of the half-seeds were added into the wells and incubated overnight. The reaction was stopped using acetic acid and then rinsed off with distilled water (Hejaard and Gibbons, 1979).

The outer diameters of the transparent zones around the wells were measured to evaluate the α-amylase activity.

2.7 HOMOEOPATHY

Samuel Hahnemann introduced the method of making medicinal preparations by alternating dilution and succussion. This method is known as potentisation. Time and again there has been controversy as to whether these potencies are therapeutically active or not. The effects in patients and healthy subjects are often put down to suggestion. The situation is different when it comes to demonstrating the effects of potencies on plants. In this case there is no possibility of suggestion affecting the results (Pelikan and Unger, 1971).

It is for this reason that this research was chosen to investigate the effect of homoeopathic potencies and thus further the study into the high dilutions and potencies used in Homoeopathy.

2.7.1 POTENCY AND POTENTISATION

Potency is described as the stage of altered remedial activity to which a drug has been taken by means of a measured process of deconcentration, with succussion, or by trituration, of the medicinal substance, which is thus brought to a state of diminutive or infintesimal subdivision. This process, if performed according to the prescribed mathematico-mechanical attenuation procedures for potentisation (dynamization), increases both the physical solubility and the physiological assimilability of the drug, while also changing its therapeutic activity in its use as a homoeopathic remedy. In a broad sense, the altered therapeutic activity of a homoeopathic medicine is brought about by dynamization (the process of potentization) follows a constant rule, known as the "Arndt-Schulz law". The simple summary of this is that minute stimuli encourage life activity, medium to strong stimuli tends to impede it, and very strong stimuli to stop or destroy it. (Gaier, 1991).

Potentisation is described as the imparting (along serial dilutions) the pharmacological message of the original substance (i.e. creating a template of the active principle) by means of trituration or succussion. It describes the process of modification of medicines as invented by Hahnemann. (Gaier, 1991).

Samuel Hahnemann discusses potentisation in the Organon, Aphorism 269, as follows: "For its own special purpose and by its own special procedure, never tried before my time, homoeopathy develops the inner, spirit-like medicinal powers of crude substance to a degree higher hither to unheard of and makes all of them exceedingly, even immeasurably, penetratingly effective and helpful, even those substances that in the crude state do not have the slightest medicinal effect on the human organism." (Isbell and Kayne, 1997).

There is a lack of experimental investigation into the high dilutions of homoeopathic potencies mainly because the action of substances in dilutions beyond $1x10^{-24}$ violates two firmly-held principles of physical chemistry – Avogadro's law and the doctrine of the non-specificity of sub-atomic particles. According to Avogadro the molecular weight of any material expressed in grams contains $6.023x10^{23}$ molecules. Theoretically, therefore, any substance diluted beyond $1x10^{-24}$ will contain no molecules of the original material. Dilutions of this degree of fineness should, then, contain nothing but the liquid vehicle in which the substance was first diluted, and should act in no manner different from it. Nor can the specific action of dilutions greater than $1x10^{-24}$ be attributed to electrons which become separated from the diluted material and remained in solution, for the other principle already mentioned, states that the electrons from one atom differ in no manner from those of any other atom (Stephenson, 1973).

This problem has been approached by recent workers by suggesting that the therapeutic properties of the remedy, or original substance, lie in the vehicle (i.e. the solvent). Various techniques have been employed to demonstrate that there are physical differences between potentised dilutions and the vehicle. These studies have included the use of UV spectrophotometry conductivity measurements and IR analysis (Heintz, 1941) surface tension

measurements (Kumar and Jussal, 1979), NMR spectroscopy (Smith and Boericke, 1966; Young, 1975), dielectric strength measurement (Brucato and Stephenson, 1966) and other methods (Scofield, 1984).

2.7.2 HOMOEOPATHIC RESEARCH IN PLANTS

The idea of carrying out biological trials was originated by Rudolf Steiner. Mrs L. Kolisko started experiments along these lines in 1923. She used mainly wheat in her experiments (Pelikan and Unger, 1971).

Kolisko, in 1923, added dilutions of iron sulphate, antimony trioxide and a copper salt up to 10^{-30} to wheat germ seed, and then measured the average weight and length of the leaves, stem and root as compared to control plants (Stephenson, 1973), finding that growth was promoted by the lower dilutions, then inhibited by the higher dilutions and finally stimulated at even higher dilutions. (Kayne, 1991)

This stimulated a detailed study by Pelikan and Unger (1971) of the effect of potentised silver nitrate ($AgNO_3$) on the growth of wheat seedlings. They found that the growth curve rose from $1x10^{8}$ to $1x10^{14}$, falling at $1x10^{16}$ and then rising again (Scofield, 1984).

Jones and Jenkins (1983) compared the potential of yeast and wheat seedlings as models for testing homoeopathic remedies in the laboratory. They found these models to be successful (Scofield, 1984).

Wannamaker as cited by Kayne (1991) conducted experiments over a period of years to investigate the effect of Sulphur and Boron potencies on the growth of onion plants and found significant improvements in weight and dimensions.

Saxena et al. (1987) found that homoeopathic drugs had a significant effect on the incidence of seed-borne fungi, and on the germination of, *Abelmoshcus. esculentus.*

Som et al. (1995) found that Iodum and Neem, two homoeopathic medicines, helped to sustain the vigour and viability of *Vigna radiata*, or mung bean, to a significant extent.

Betti, Brizzi, Nani and Peruzzi (1994) studied the effect of different potencies of *Arsenicum album*, a homoeopathic remedy, in wheat germination. They found a difference in the treatments. They furthered their research in 1997 by testing the effects of high dilutions of *Arsenicum album* on wheat seedlings from seed poisoned with the same substance. The plants showed significant recovery after treatment.

Endler and Pongratz (1991) studied the effects of homoeopathically prepared dilutions of the plant growth substance indole butric acid on the development of roots and new leaves of African violet slips. Plant development was enhanced by the addition of a homoeopathic dilution of indole butric acid.

Steele (1999) conducted a research using homoeopathic potencies of gibberellic acid on the synthesis of α-amylase in de-embryonated halves of barley seed. He found that ultra high dilutions of GA_3 were biologically active. He also suggested that other plant hormones, for example abscisic acid, be used in the same experimental mode.

CHAPTER 3

RESEARCH METHODS AND MATERIALS

The research was conducted at the Seed Physiology Laboratory of the Crop Science discipline in the School of Agricultural Sciences and Agribusiness, at the University of Natal, Pietermaritzburg.

Two methods were used to determine the level of α-amylase activity – namely spectrophotometry and radial diffusion.

3.1 BARLEY SEEDS

Seeds of the variation *H. vulgare* were used in this research. The seeds were obtained from the Caledon Development and Operative, Caledon.

3.2 ABSCISIC ACID AND GIBBERELLIC ACID

3.2.1 PREPARATION OF GIBBERELLIC ACID

Steele (1999) found that a serial dilution (with no succussion) of 9cs (centisemal serial) of Gibberellic Acid (GA_3) initiated α-amylase production. This serial dilution was used rather than a homoeopathic potency of GA_3 so that no discrepancies resulted from using two homoeopathically prepared hormones.

This 9cs of GA_3 was made up as follows:

- 34mg of GA_3 $x10^{-3}$ was dissolved in 100ml of incubation buffer -$Ca(NO_3)_2$.
- 1ml of this was dissolved in 1000ml of $Ca(NO_3)_2$. to give GA_3 $x10^{-6}$.
- 1ml of GA_3 $x10^{-6}$ was dissolved in 100ml of $Ca(NO_3)_2$. to give GA_3 $x10^{-8}$

3.2.2 THE PREPARATION OF HOMOEOPATHIC POTENCIES OF ABSCISIC ACID

Homoeopathic potencies of 4CH, 9CH, 15CH, 30CH and 200CH of ABA were used. This allowed a representation of potencies above and below Avogadro's constant.
(See Appendix C)

The Homoeopathic preparation of the ABA to the different potencies was done by the researcher to ensure that all the potencies were prepared in the same manner.

ABA is insoluble in water, thus according to the German Homoeopharmacopoeia it should be triturated with lactose up to the 4^{th} potency. However, lactose is a sugar that will interfere with the formation of α-amylase.

It is for this reason that ethanol was added in the first three potencies to dissolve the ABA. After the third potency, distilled water was used to remove the chance of interference of ethanol on the production of α-amylase. The necessary potencies, that is to say the 4CH, 9CH, 15CH, 30CH and 200CH, were made up in incubation buffer – which in this case was 20mM calcium nitrate $Ca(NO_3)_2$. The incubation buffer had to be added to the potency to maintain the optimal conditions for the production of α-amylase.

The ABA was made according to method 5a set out in the German Homoeopharmacopea as follows:
Liquid preparations made by 5a are solutions from basic drug materials and a liquid vehicle. 1 part of the basic drug material is dissolved in 99 parts of the liquid vehicle and succussed.

Thus for this research 1 drop of ABA was dissolved in 3.2ml of a 30% ethanol and distilled water mixture for the first three potencies. All other potencies, excluding the 4CH, 9CH, 15CH, 30CH and 200CH, were made by adding 1 drop of the previous potency to 3.2ml of distilled water and

succussed 100 times. The 4CH, 9CH, 15CH, 30CH and 200CH were made by adding 30 drops of the previous potency to 96 ml of incubation buffer containing GA_3 $x10^{-8}$ and succussed 100 times. (See Appendix D)

3.3 PREPARATION OF THE α-AMYLASE STANDARD CURVES

The standard curve was based on the method of used by Steele (1999) using pure α-amylase and Phadebas tablets.

Method:

- The extraction buffer, containing sodium chloride and calcium acetate was made up.
- 10mg of α-amylase was measured out and dissolved in 1000ml of distilled water.
- 10ml of the α-amylase was extracted and made up to 100ml with the extraction buffer (10^{-1}).
- 10ml of 10^{-1} was extracted and made up to 100ml with the extraction buffer (10^{-2}).
- This was continued to the 10^{-5}.
- 10ml was extracted from each dilution and added to test tubes marked 1 to 5.
- A test tube containing 10ml extraction buffer was made up and marked 6 (blank).
- From each dilution, including the blank, 0.5ml was extracted for the radial diffusion standard curve.
- To each test tube a Phadebas tablet was added and shaken vigorously for 15 seconds.
- The test tubes were placed in a shaker bath at 50°C for 10 minutes.
- The reaction was stopped by adding 1ml sodium hydroxide (NaOH).
- The contents of each tube were then filtered and made up to 10ml with extraction buffer.
- The spectrophotometry readings were then taken with the blank used as a reference.
- The radial diffusion standard curve was made by syringing the 0.5ml of each dilution and the blank into the wells in the gel substrate, incubating them at 30°C for 24 hours, then stopping the reaction using 1ml acetic acid, and measuring the diameter of each halo around the well.

3.4 PREPARATION OF THE SEEDS

600 seeds were selected for each series. Only those that had not sprouted were considered for this research. No damaged seeds were selected.

The seeds were then cut in half and the endosperm retained whereas the embryo half was disregarded.

The endosperm halves were divided into 30 groups of 20 half-seeds each.

Each group was tested with a specific potency of ABA. There was in each group a control group, that is, one set that was incubated only in the incubation buffer ($Ca(NO_3)_2$). For each potency tested, there were five replications to validate the results.

3.5 INCUBATION

Each of the petri dishes was lined with no. 1 Whatman paper to absorb any moisture. Each petri dish was marked with the number of the repetition and the number of treatment solution.

20 half-seeds were soaked in each petri dish with 5ml of the relevant solution of ABA.

Each petri dish was placed in a draw string plastic bag containing 2 ml of distilled water to minimise evaporation, and the bag was sealed.

The petri dishes were placed into the growth chamber set at a constant temperature of 15°C and incubated for 48 hours in the dark.

After 48 hours, the half-seeds were removed from the growth chamber and placed in the freezer.

3.6 ENZYME EXTRACTION

The half- seeds were thawed by leaving them at room temperature for 5 minutes.

The half-seeds were then macerated with a mortar and pestle with 10ml of extraction buffer, which in this case was sodium chloride and calcium acetate.

The slurry was then decanted into test tubes and extracted for 60 minutes in a shaker bath at 30°C.

The slurry was then centrifuged at temperature 4°C, speed 20000 rpm, time 10 minutes.

The centrate was then filtered, 0.5ml syringed out (for the radial diffusion) and made up to 10ml with extraction buffer.

3.7 ENZYME ASSAY

Phadebas tablets were then added to each test tube, shaken vigorously for 15 seconds, and then incubated in the shaker bath for 10 minutes at 50°C.
The reaction was terminated by adding 1ml of NaOH (sodium hydroxide) and mixing well.
The homogenate was then filtered again and made up to 10ml with extraction buffer.
The homogenate was then assayed using a blank as reference. The spectrophotometer was set at 620nm and the absorbance of each sample was recorded.

3.8 RADIAL DIFFUSION

Radial diffusion into a substrate-containing gel slab is a rapid technique for the determination of enzymes. Starch was used as a substrate for the assay of ∂-amylase (Hejgaard and Gibbons, 1979).

The starch used in this research was Phadebas tablets obtained from the University Of Pietermaritzburg.

3.8.1 PREPARATION OF THE GEL

The solution was made in the following manner:

150mg agarose was dissolved in 15ml boiling 50mM-phosphate buffer, pH 6.9, containing 20mM-$CaCl_2$ and 20mg blue-starch substrate (1 Phadebas tablet crushed to a fine powder). This was vigorously stirred to cause uniform suspension. The gel was poured into a petri dish forming a 1.5mm gel layer. Following solidification of the gel (5-min), wells of 2.5mm diameter were cut into the gel with a gel puncher.

The homogenate was applied into the wells by using constriction pipettes and incubated overnight at 25°C in a moist chamber.

3.8.2 ENZYME ASSAY

After incubation, the plate were immersed for 5 minutes in acetic acid to stop the reaction and then washed off with distilled water for 5 minutes.

The outer diameters of the transparent zones around each endosperm were measured half twice at right angles, and the average used to evaluate α-amylase activity.

3.9 STATISTICAL METHOD OF DATA ANALYSIS.

There was one treatment substance in this experiment.

There were 6 potency levels (1 control and 5 potency levels, namely 4CH, 9CH, 15CH, 30CH and 200CH) in the experiment.

There were five replications per potency; each repetition contained 20 half-seeds.
The sixth replication was a control.

The unit of measurement is the rate of synthesis of each group of the half-seeds. The ONEWAY ANOVA was used in this research.

CHAPTER 4

RESULTS

4.1 THE CRITERIA GOVERNING THE ADMISSIBILITY OF DATA

Two types of data were utilised in this research namely – optical density (spectrophotometry) and halo size (radial diffusion). No data was excluded from statistical analysis.

4.2 PHYSICAL OBSERVATIONS

No physical differences were noted between the different potencies and the control in both the spectrophotometry and the radial diffusion.

(See Plates 4.2.1 to 4.2.12)

Plate 4.2.1 The uncut barley seeds (*Hordeum vulgare*)

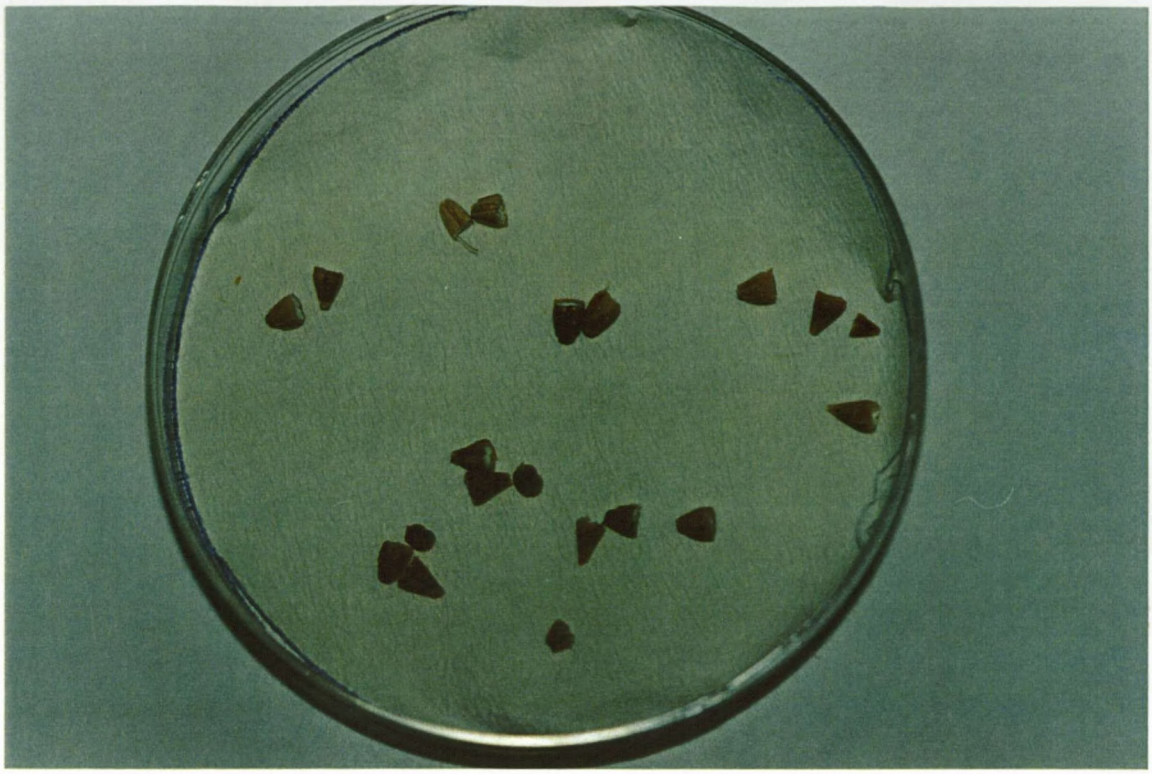

Plate 4.2.2 The de-embryonated endosperm half-seeds before incubation

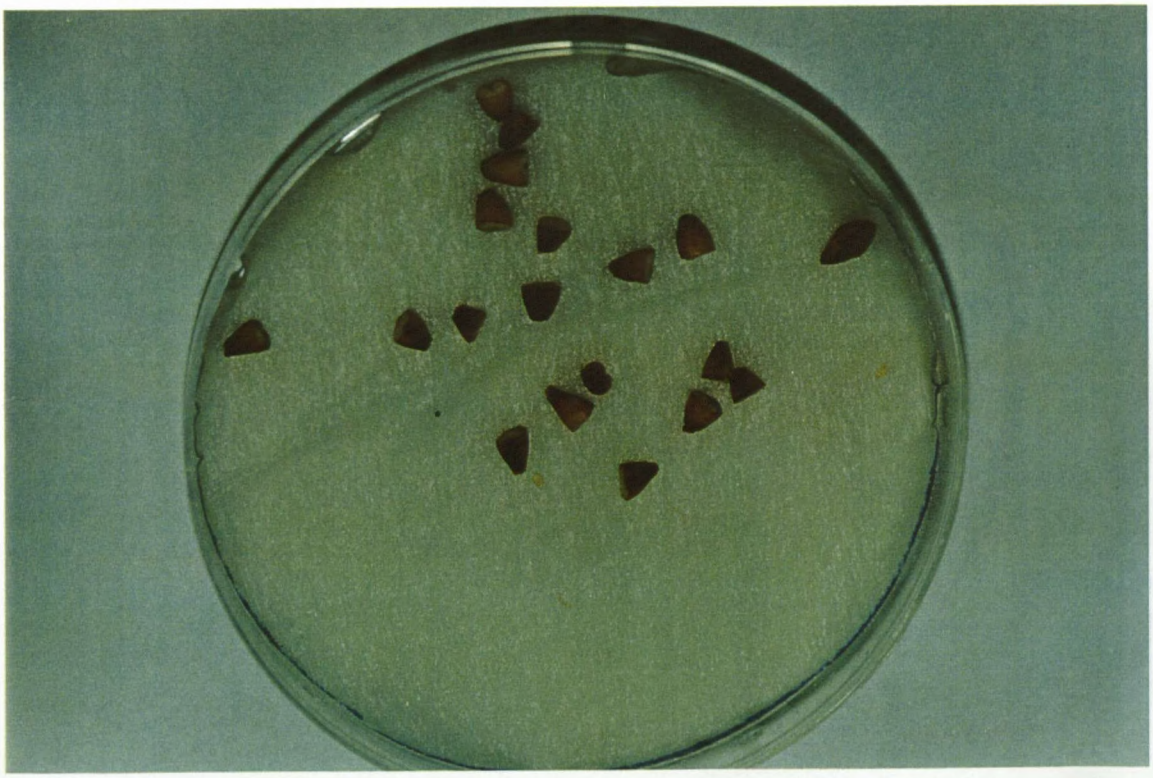

Plate 4.2.3 The half-seeds after the initial 48 hour incubation at 15°C

Plate 4.2.4 The half-seeds after maceration

Plate 4.2.5 The filtered solutions after incubation with the Phadebas tablets prior to the determination of the optical density

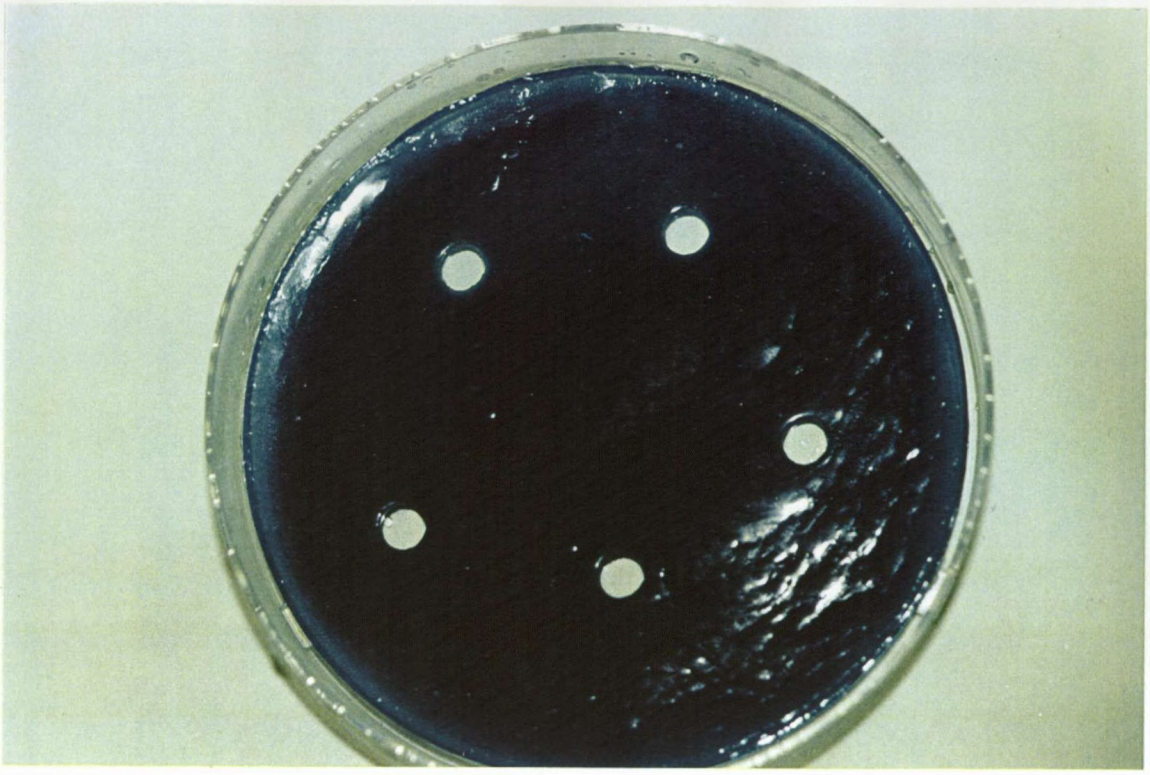

Plate 4.2.6 The radial diffusion plate of ground Phadebas tablets in 10% agar of the extraction buffer

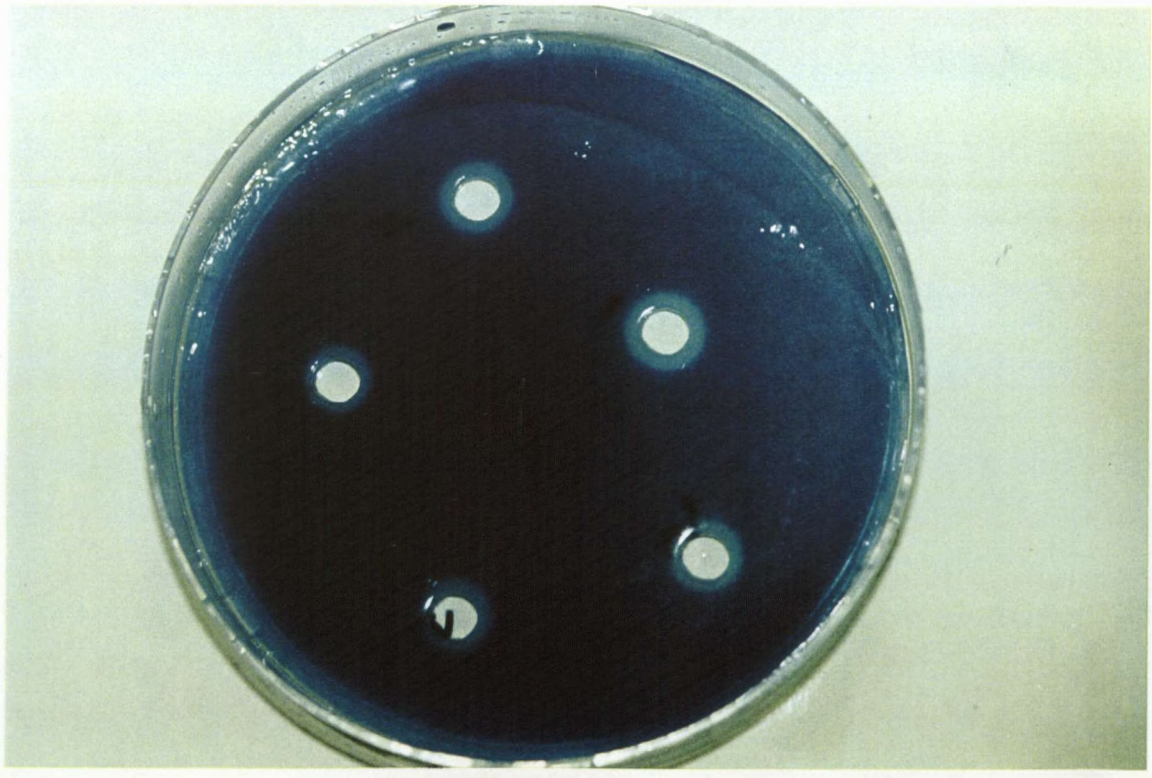

Plate 4.2.7 The radial diffusion plate of ground Phadebas tablets in 10% agar of the control

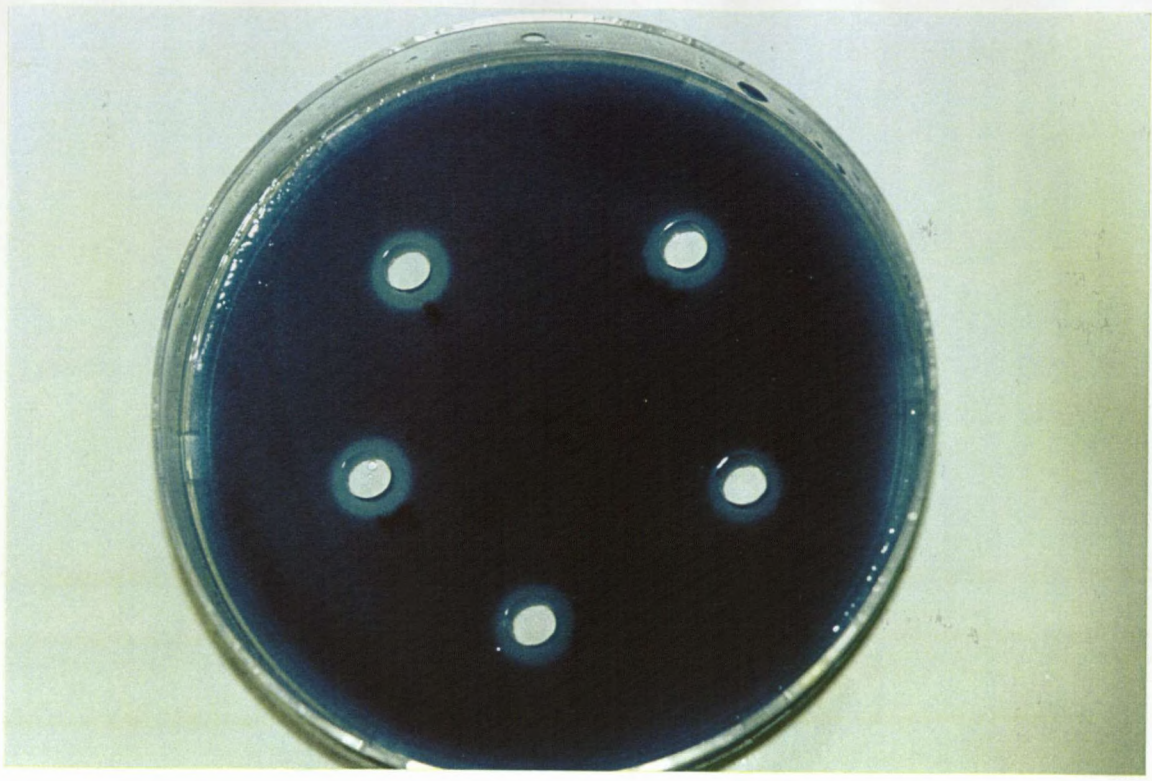

Plate 4.2.8 The radial diffusion plate of ground Phadebas tablets in 10% agar of the 4CH potency

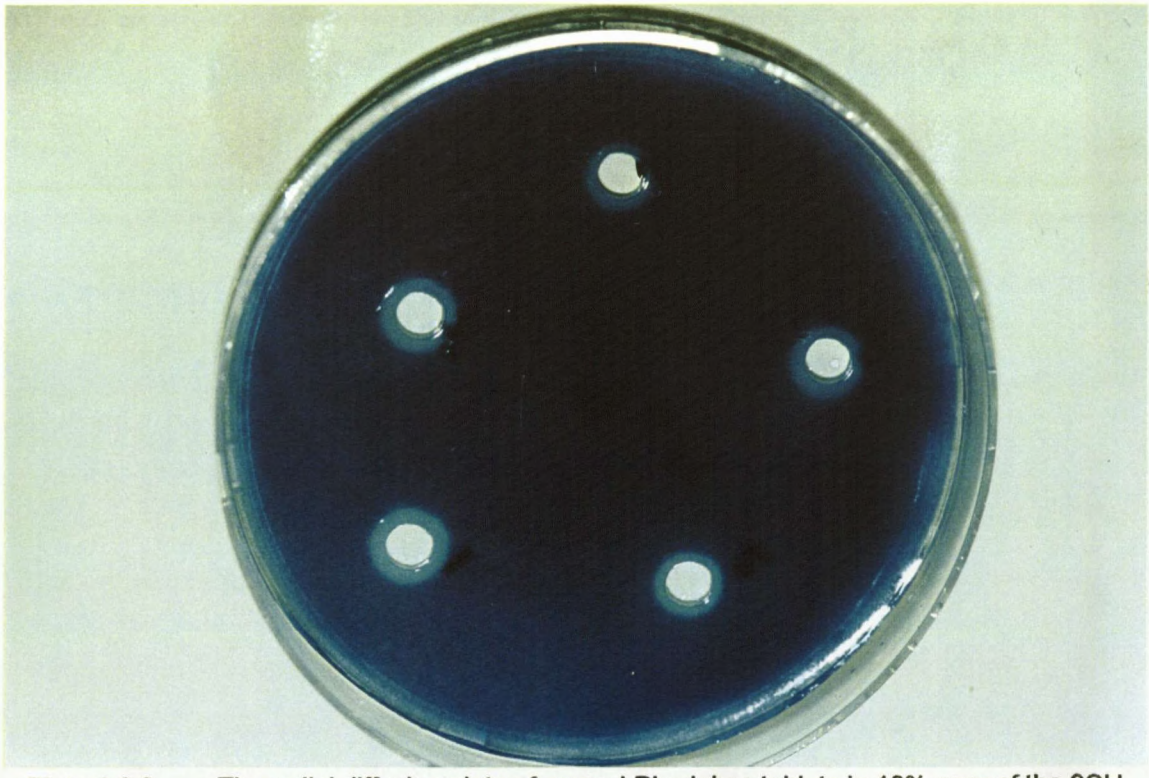

Plate 4.2.9 The radial diffusion plate of ground Phadebas tablets in 10% agar of the 9CH potency

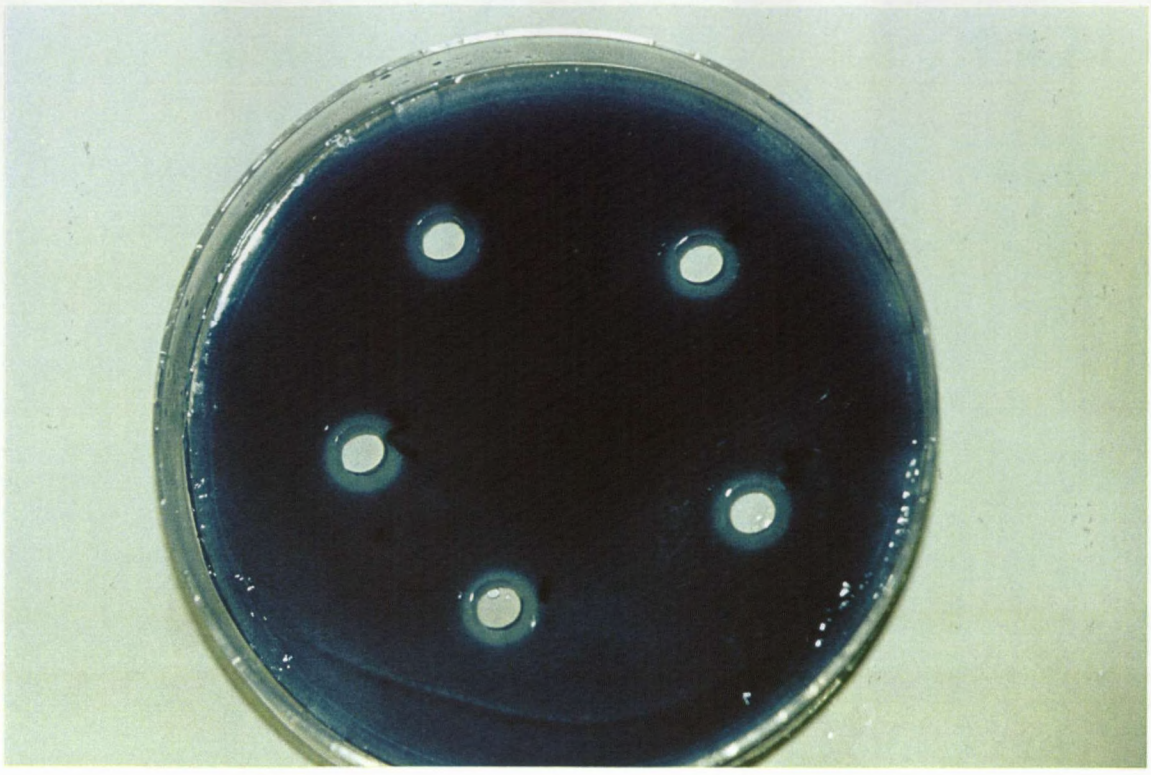

Plate 4.2.10 The radial diffusion plate of ground Phadebas tablets in 10% agar of the 15CH potency

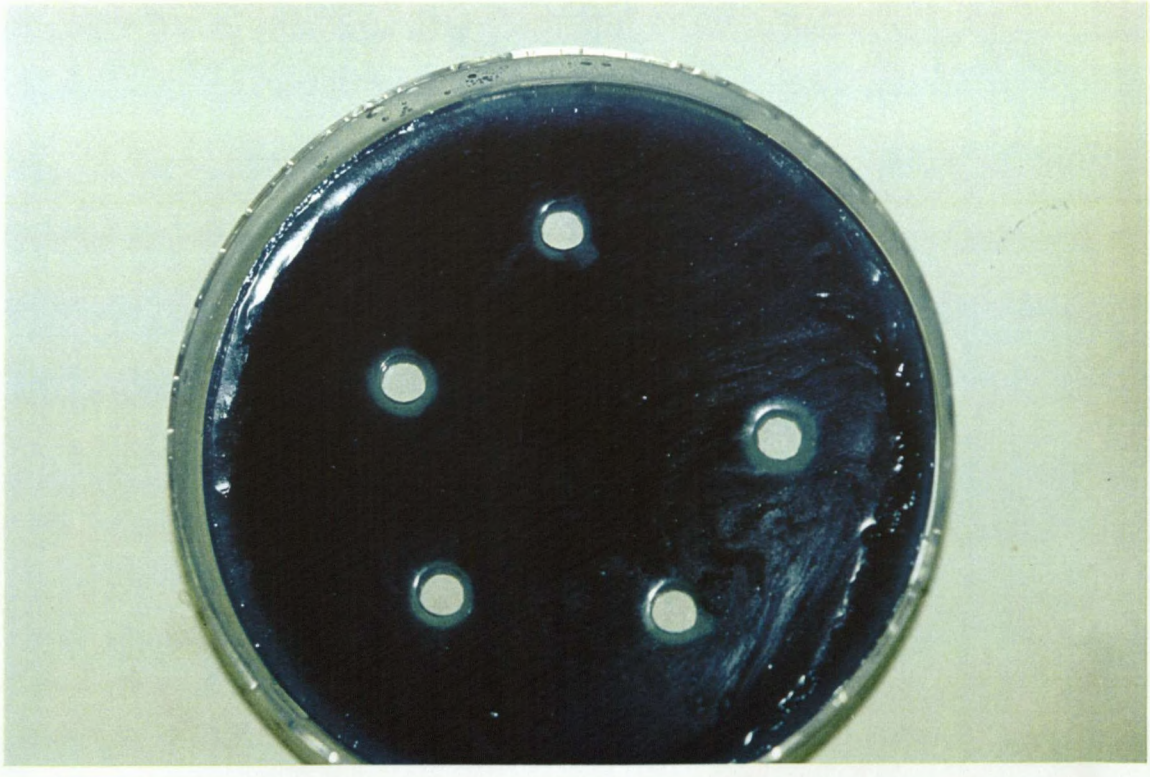

Plate 4.2.11 The radial diffusion plate of ground Phadebas tablets in 10% agar of the 30CH potency

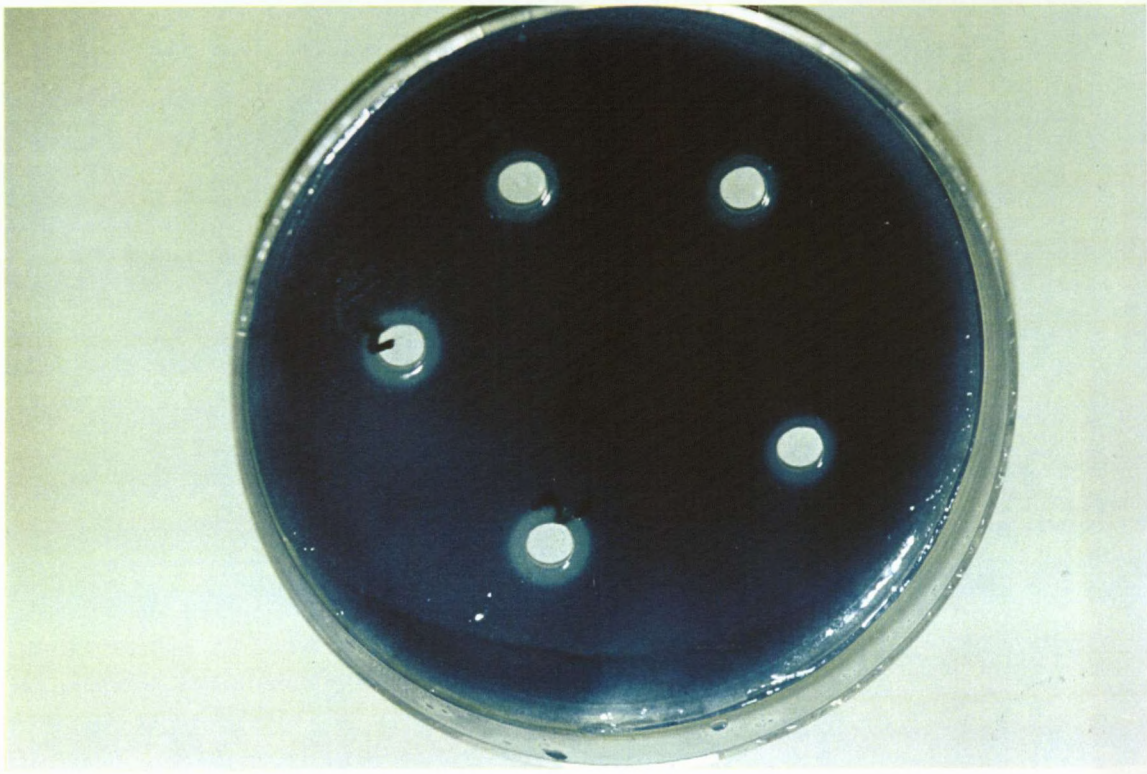

Plate 4.2.12 The radial diffusion plate of ground Phadebas tablets in 10% agar of the 200CH potency

4.3 THE α-AMYLASE STANDARD CURVE FOR SPECTROPHOTOMETRY

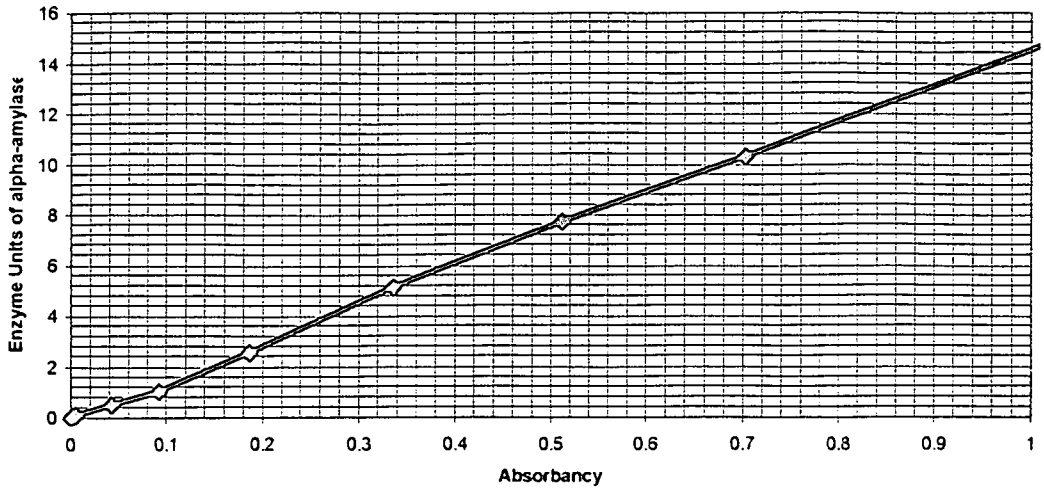

Figure 4.3.1 Absorbance vs. Enzyme units of α-amylase

4.4 THE α-AMYLASE STANDARD CURVE FOR RADIAL DIFFUSSION

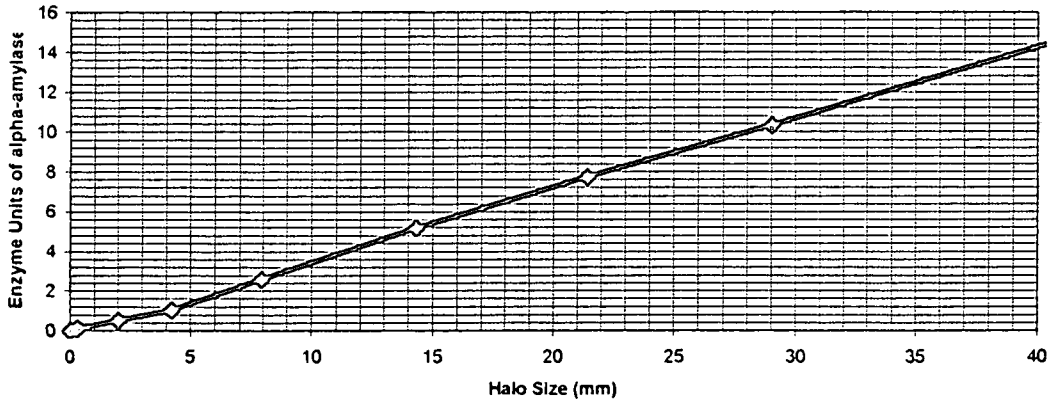

Figure 4.4.1 Halo Size vs. Enzyme units of α-amylase

4.5 STATISTICAL ANALYSIS OF DATA

H_0: null hypothesis

H_1: alternative hypothesis

Decision rule:

At the $\alpha = 0.05$ level of significance,

Reject H_0 if $p < \alpha$

Accept H_0 if $p \geq \alpha$

In this research the null hypothesis is accepted if the p-value is greater than 0.05 for both the radial diffusion and the spectrophotometry readings respectively.

4.6 RADIAL DIFFUSION RESULTS

Table 4.6.1 The amount of α-amylase, expressed as enzyme units, per gram of dry barley endosperm halves as per radial diffusion method

Table 4.6.1 depicts the radial diffusion results. These results were obtained by measuring the halo size around each well (in mm). These measurements were then interpolated from the α-amylase standard curve for radial diffusion and divided by the dry mass of the respective group.

	Replications			
	1	2	3	Average
Control	5.893	9.121	4.597	6.537
4CH	6.177	6.184	6.378	6.246
9CH	5.631	6.609	5.700	5.980
15CH	5.540	6.038	5.555	5.711
30CH	5.988	6.770	5.114	5.957
200CH	5.930	8.117	5.338	6.461

The above table shows that all the groups had a significant amount of α-amylase produced.

4.7 ONEWAY ANOVA FOR RADIAL DIFFUSION

The ONEWAY ANOVA for radial diffusion shows the following:

1) The null hypothesis would be accepted if the significance value was >0.5.
2) At a 95% confidence level, there was no significant difference between the control and the different potencies of ABA. (the significance was 0.489)
3) From this ANOVA it can be concluded that the first hypothesis is true i.e. the Hahnemannian potencies of ABA did not have an effect on the synthesis of α-amylase in barley endosperm half-seeds in the presence of gibberellic acid.

TABLE 4.7.1 ONEWAY ANOVA for radial diffusion

Table 4.7.1 shows the statistical analysis of the data for radial diffusion. This includes the mean, the standard deviation and the confidence levels for the control and for each potency.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Control	15	6.537	2.195	0.567	5.321	7.752	3.83	11.62
4CH	15	6.246	0.760	0.196	5.826	6.667	5.07	7.66
9CH	15	5.980	0.821	0.212	5.526	6.435	4.63	7.33
15CH	15	5.711	0.882	0.228	5.223	6.200	4.27	7.20
30CH	15	5.957	1.093	0.282	5.352	6.563	4.44	7.97
200CH	15	6.461	1.521	0.393	5.619	7.304	4.57	10.34
Total	90	6.149	1.310	0.138	5.875	6.423	3.83	11.62

Table 4.7.2 The significance of the results for radial diffusion

Table 4.7.2 shows the p value (significance) between the groups for radial diffusion.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7.715	5	1.543	0.894	0.489
Within Groups	144.967	84	1.726		
Total	152.683	89			

4.8. GRAPHICAL REPRESENTATION FOR RADIAL DIFFUSION

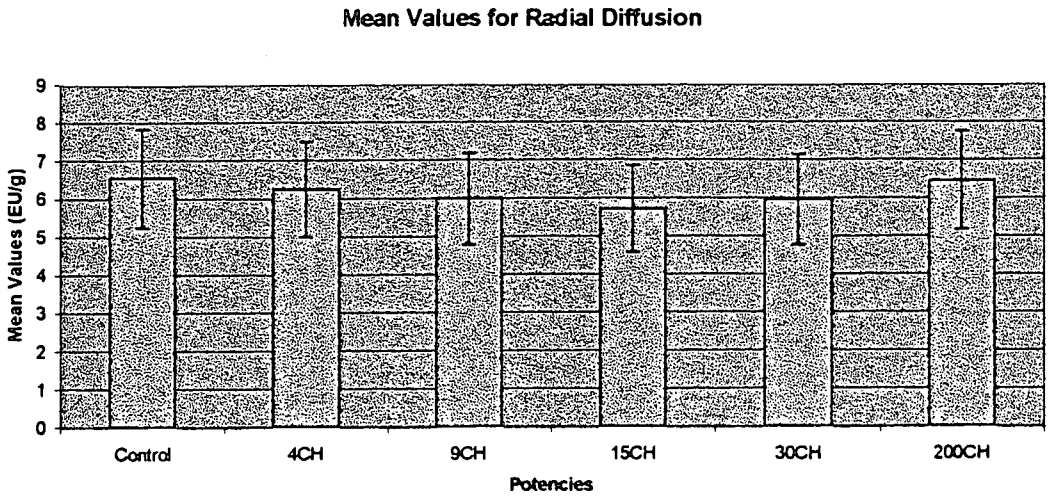

Fig. 4.8 The mean values for the various potencies as enzyme units/gram of dry barley endosperm halves.

The vertical bars in each column represent the standard deviation in each group. Since these lines overlap, it can be stated that there is no significant difference between the groups. The standard deviation is taken from the ONEWAY ANOVA (see table 4.7.1)

4.9 SPECTROPHOTOMETRY RESULTS

Table 4.9 The amount of α-amylase, expressed as enzyme units, per gram of dry barley endosperm halves as per spectrophotometry.

Table 4.9 depicts the spectrophotometry results. These results were obtained by measuring the absorbance of each group. These measurements were then interpolated from the α-amylase standard curve for spectrophotometry and divided by the dry mass of the respective group.

	Replications			
	1	2	3	Average
Control	4.734	10.101	5.014	6.617
4CH	6.998	3.314	6.401	5.571
9CH	5.520	8.403	6.063	6.662
15CH	4.354	5.769	5.605	5.243
30CH	4.792	5.687	3.908	4.796
200CH	6.183	5.921	6.920	6.341

The above table shows that all the groups had a significant amount of α-amylase produced.

4.10 ONEWAY ANOVA FOR SPECTROPHOTOMETRY

The ONEWAY ANOVA for spectrophotometry shows the following:

1) The null hypothesis would be accepted if the significance value was >0.5.
2) At a 95% confidence level, there was no significant difference between the control and the different potencies of ABA. (the significance was 0.358)
3) From this ANOVA it can be concluded that the first hypothesis is true i.e. the Hahnemannian potencies of ABA did not have an effect on the synthesis of α-amylase in barley endosperm half-seeds in the presence of gibberellic acid.

TABLE 4.10.1 ONEWAY ANOVA for spectrophotometry

Table 4.10.1 shows the statistical analysis of the data for spectrophotometry. This includes the mean, the standard deviation and the confidence levels for the control and for each potency.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Control	15	6.617	2.898	0.748	5.012	8.222	2.84	11.40
4CH	15	5.571	3.090	0.798	3.860	7.282	2.44	11.63
9CH	15	6.662	3.065	0.791	4.965	8.360	2.48	12.60
15CH	15	5.243	2.830	0.731	3.676	6.810	2.42	12.12
30CH	15	4.796	2.631	0.679	3.339	6.253	2.13	10.98
200CH	15	6.341	2.618	0.676	4.892	7.791	3.21	11.83
Total	90	5.872	2.871	0.303	5.270	6.473	2.13	12.60

Table 4.10.2 The significance of the results for spectrophotometry

Table 4.10.2 shows the p value (significance) between the groups for spectrophotometry.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	45.662	5	9.132	1.115	0.358
Within Groups	687.706	84	8.187		
Total	733.368	89			

4.11. GRAPHICAL REPRESENTATION FOR SPECTROPHOTOMETRY

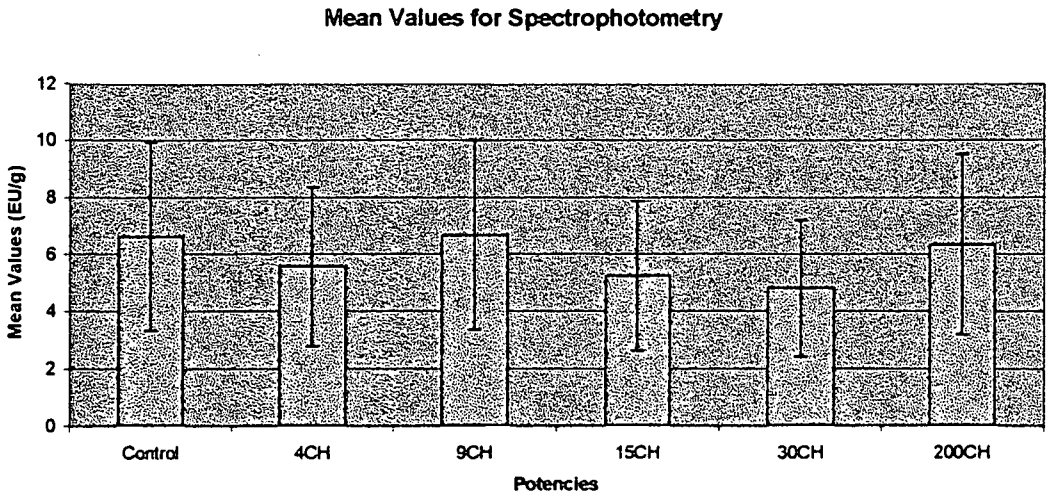

Fig. 4.11 The mean values for the various potencies as enzyme units/gram of dry barley endosperm halves.

The vertical bars in each column represent the standard deviation in each group. Since these lines overlap, it can be stated that there is no significant difference between the groups. The standard deviation is taken from the ONEWAY ANOVA (see table 4.10.1)

CHAPTER 5

DISCUSSION OF THE RESULTS

5.1 INTRODUCTION

The results of this research have shown that homoeopathic potencies of abscisic acid did not have an effect on the production of α-amylase in the presence of gibberellic acid. This can be concluded since in each of the samples α-amylase was produced to some extent. It is known that gibberellic acid initiates the production of α-amylase whereas abscisic acid inhibits this production. In this research it is possible that the gibberellic acid used may have been too strong for the homoeopathic potencies of abscisic acid to compete with.

On physical observation there was no physical difference between the control and the various potencies. After incubation all the samples showed evidence of α-amylase production. Steele (1999) noted that if no α-amylase was produced the half-seeds produced a jelly-like exudate from their cut surface. No evidence of this exudate was found in this research. All the endosperm half-seeds presented with clean-cut surfaces besides the extraction samples, which contained no abscisic acid or gibberellic acid.

There were no physical differences observed during the enzyme assay for the spectrophotometry between the control and the various potencies. All the groups, excluding the extraction, showed a slight blue discolouration with the addition of the Phadebas tablets. The blue discolouration of the samples was due to the hydrolysis of the Phadebas tablets by α-amylase. The samples containing only extraction buffer remained clear.

There were no physical differences observed during the enzyme assay for the radial diffusion between the control and the various potencies. All the groups, excluding the extraction, showed a slight discolouration in the blue gel substrate containing the Phadebas tablets.
The discolouration of the samples was due to the hydrolysis of the starch in the gel substrate by α-amylase. The samples containing only extraction buffer remained blue around each well containing the sample.

The absorbance reading for the spectrophotometry showed no significant difference between the control and the various potencies.

The halo size (mm) for the radial diffusion showed no significant difference between the control and the various potencies.

5.2 AVOGADRO'S LAW

According to Avogadro the molecular weight of any material expressed in grams contains 6.023×10^{23} molecules (Stephenson, 1973). This means that any substance diluted beyond 1×10^{-24} contains no molecule of the original substance. In Homoeopathy this correlates to 12CH (which is a dilution of 1×10^{-24} which has then been succussed). In this research two potencies were below Avogadro's number (4CH and 9CH) and three potencies were above Avogadro's number (15CH, 30CH and 200CH). Steele (1999) found that homoeopathic potencies of gibberellic acid, both below and above Avogadro's constant, did have an effect on the production of α-amylase. However, none of the potencies had any significant effect on the production of α-amylase, so no conclusion regarding whether homoeopathy challenges this theory can be made from this research.

5.3 THE CORRELATION BETWEEN SPECTROPHOTOMETRY AND RADIAL DIFFUSION.

Table 5.3 shows the correlation between radial diffusion and spectrophotometry. The averages of each group for each method were taken and correlated with each other to get a percentage correlation. As can be seen there is a very high correlation between the two methods for each group.

Table 5.3 The correlation between radial diffusion and spectrophotometry

Groups	Average radial diffusion	Average spectrophotometry	Correlation
Control	6.54	6.62	89.37%
4CH	6.25	5.57	75.34%
9CH	5.98	6.66	94.07%
15CH	5.71	5.24	86.72%
30CH	5.96	4.80	85.00%
200CH	6.46	6.34	80.91%

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

This research investigated the effect of the various homoeopathic potencies of abscisic acid on the production of α-amylase in barley endosperm half seeds. In order to investigate these effects, gibberellic acid had to be added to initiate the process. The results of this experiment showed that the various homoeopathic potencies of ABA did not have a significant effect on the production of α-amylase. This could have been due to numerous reasons that warrant some discussion.

Some plant hormones, such as gibberellic acid, have stimulatory effects on growth processes whereas others, such as ABA, are inhibitory to these processes. The importance of the GA-ABA balance for the production of α-amylase has been recognized for some time.

GA has a stimulatory effect on the production of α-amylase even at dilutions of $10^{-6}{}_{M}$ (Ritchie et. al., 1999). In 1999, Steele found that homoeopathic preparations of GA had a stimulatory effect on the production of α-amylase up to a dilution of 10^{-400}.

The investigation of the effect of ultra-high dilutions of an inhibitor substance such as ABA is more complicated in that it is presumed that at some stage in the dilution series the inhibitory substance becomes stimulatory. This combined with the stimulatory effect of the GA might well have confounded the results. This together with the fact that the GA concentrations may have over-ridden any possible interaction with the lower concentration of ABA may have masked any possible homoeopathic effect.

Stebbing (1982) presented numerous examples of growth stimulation in a wide variety of organisms by substances which are toxic to these organisms in higher levels. He argued that

at high levels the toxicant would inhibit growth. At low levels, he suggested that the toxicant may actually cause a stimulatory effect (Scofield, 1987).

A good example of this is with antibiotics. If a high dose of antibiotics is given, it will kill the bacteria. Medium doses of the same antibiotic would just impede the growth of more bacteria and low doses of the antibiotic would cause mutations in any remaining bacteria. That is why the course of antibiotics must always be finished so that any chance of these mutations occurring is minimised.

In regards to the homoeopathic perspective, the results showed that the potencies did not have any significant difference. According to the Arndt-Schultz Law the low potencies (namely the 4CH and the 9CH) should have caused an inhibitory effect on the production of α-amylase whilst the medium potencies (namely the 15CH and the 30CH) should have had a stabilising effect whereas the high potency (namely the 200CH) should have had a stimulatory effect.

The various homoeopathic potencies of ABA also varied vastly in their concentrations. Only five potencies were used in this research. This research has established that further investigation needs to be undertaken as to whether any other potency would have an effect.

Another conclusion that can be drawn is that radial diffusion and spectrophotometry are both viable methods of measuring the amount of α-amylase produced in barley seeds as the results obtained from the one correlated with the other. As not much research has been conducted using radial diffusion, this research has furthered the information available on this type of assaying.

The importance of this is that assaying depends on numerous circumstances. If two assays are conducted in an experiment the results would be more reliable. Radial diffusion proved to be a more visual and cheaper way to assay α-amylase production.

This research has also shown that, despite there being no significant difference, the endosperm halves of barley seeds is a useful system to examine the effect of homoeopathic medicine.

6.2 PROBLEM FACTORS

Numerous factors may have influenced the results which should be investigated in further research.

1. The extraction buffer may have influenced the effectiveness of the homoeopathic potencies since the potencies were made up in the extraction buffer. Another method of adding the extraction buffer and the remedies is recommended.

2. The dilution of gibberellic acid may need to be re-investigated. Although prior to the experiment being carried out tests had been conducted to evaluate which dilution of gibberellic acid would be strong enough to initiate α-amylase production but not too strong to overpower the effect of the abscisic acid, this might have occurred.

3. The endosperm half-seeds were frozen after incubation to stop the production of α-amylase. However, homoeopathic potencies are sensitive to temperature variations which might also have had an effect.

6.3 RECOMMENDATIONS

Repeat the research to validate the results found.

Find another way of preparing the medicine and introducing the extraction buffer.

Use another way of ending the incubation period other than freezing.

Test other potencies to investigate whether they may have an effect.

Test succussed versus unsuccussed of each potency to investigate whether there is a difference.

Vary the incubation period.

Vary the incubation temperature.

Do more repetitions per sample to eliminate irregularities.

Apply the abscisic acid only after incubation.

Use other seeds in the same experiment to see whteher any effect is noted in other cereal grains.

Compare the centessimal potency (CH) with the decimal potency (DH) using the same experiment model.

Use the whole seed and check whether any effect occurs on germination.

Test the method of radial diffusion to validate the results.

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APPENDICES

LIST OF APPENDICES

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Appendix D The Hahnemannian method of preparing medicines

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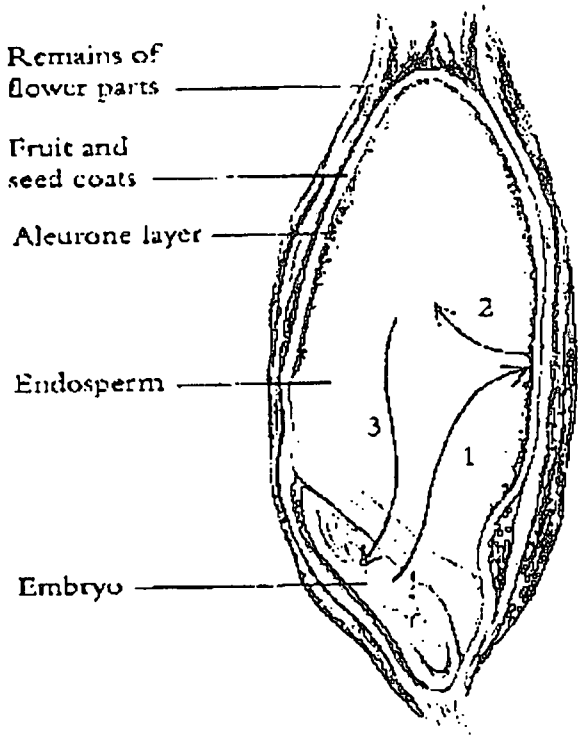
Appendix F The results for each group for radial diffusion

Appendix G The results for each group for spectrophotometry

APPENDIX A

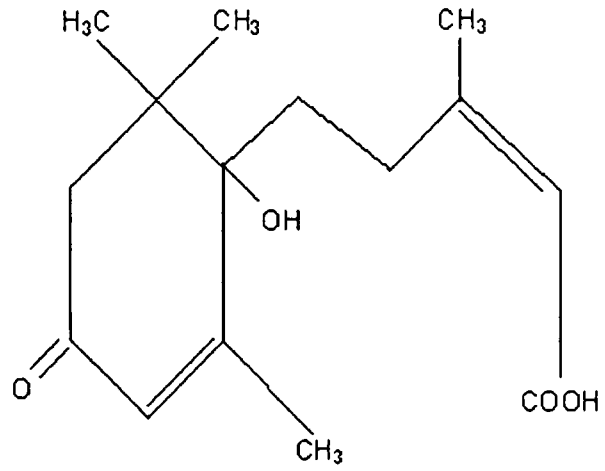
THE SEED



Gibberellin secreted from the embryo (1) stimulates the aleurone to form amylase, which is secreted into the endosperm (2), where it makes possible the digestion of stored starch. The sugars produced by starch digestion are then absorbed by the embryo (3) and used for growth. (Galston, Life Processes of Plants. 1994 p 99)

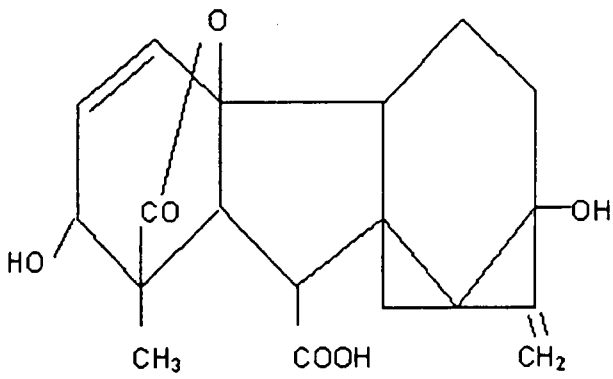
APPENDIX B

CHEMICAL COMPOSITIONS OF ABSCISIC ACID



Abscisic Acid (Moore 1995:431)

CHEMICAL COMPOSITIONS OF GIBBERELLIC ACID



Gibberellic Acid (Moore 1995: 423)

APPENDIX C

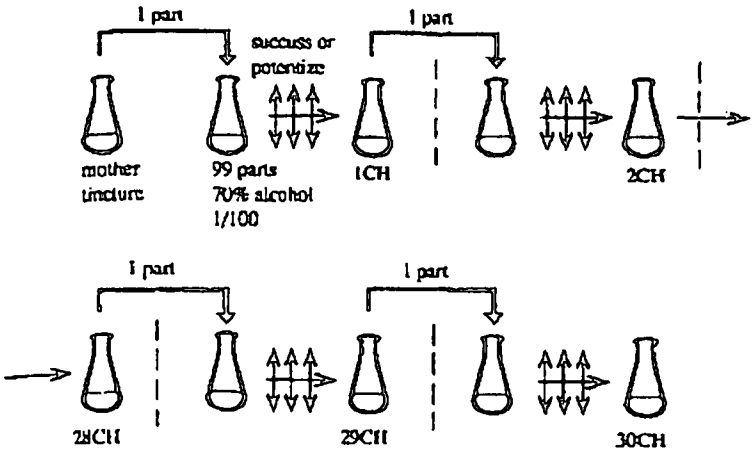
COMPARISON DETWEEN DILUTION, CONCENTRATION AND HOMOEOPATHIC CENTISEMAL POTENCY

Dilution	Concentration	Centisemal Potency
1:100	10^{-2}	1 CH
1:10 000	10^{-4}	2 CH
1:1 000 000	10^{-6}	3 CH
$1:10^{12}$	10^{-12}	6 CH
$1:10^{30}$	10^{-30}	15 CH
$1:10^{60}$	10^{-60}	30 CH
$1:10^{400}$	10^{-400}	200 CH
$1:100^{2\,000}$	$10^{-2\,000}$	1 000 CH or M
$1:10^{20\,000}$	$10^{-20\,000}$	10 000 CH or 10 M

Homoeopathic centisemal potency (Kayne 1997:50)

APPENDIX D

PREPARATION OF CENTISEMAL POTENCIES



Hahnemann's multi-flask method. Preparation of centisemal potencies. (Modification of Abecassis et al 1984)

(Majerus, M. The Berlin Journal on Research in Homoeopathy. Vol.1, No. 4/5, Sept./Dec. 1991)

APPENDIX E

DRY MASS OF HALF-SEEDS

The seeds were weighed before the experiment and the following tables show the masses for each group in each replication.

CONTROL (Mass of endosperm half-seeds in grams)		
Replication 1	Replication 2	Replication 3
0.457	0.493	0.534
0.626	0.549	0.501
0.414	0.493	0.439
0.532	0.482	0.535
0.522	0.576	0.410

4CH (Mass of endosperm half-seeds in grams)		
Replication 1	Replication 2	Replication 3
0.418	0.583	0.451
0.481	0.505	0.471
0.475	0.517	0.482
0.478	0.534	0.506
0.449	0.457	0.493

9CH (Mass of endosperm half-seeds in grams)		
Replication 1	Replication 2	Replication 3
0.466	0.600	0.505
0.484	0.538	0.487
0.518	0.488	0.467
0.486	0.519	0.484
0.507	0.527	0.462

15CH (Mass of endosperm half-seeds in grams)		
Replication 1	Replication 2	Replication 3
0.484	0.537	0.469
0.464	0.601	0.466
0.539	0.550	0.500
0.537	0.530	0.500
0.463	0.471	0.425

30CH (Mass of endosperm half-seeds in grams)		
Replication 1	Replication 2	Replication 3
0.541	0.563	0.501
0.540	0.453	0.395
0.548	0.532	0.493
0.530	0.535	0.500
0.551	0.500	0.499

200CH (Mass of endosperm half-seeds in grams)		
Replication 1	Replication 2	Replication 3
0.529	0.535	0.520
0.538	0.476	0.411
0.529	0.568	0.536
0.525	0.437	0.485
0.458	0.474	0.537

APPENDIX F

RESULTS FOR RADIAL DIFFUSION

Results obtained from the diameter of the halo, interpolated from the α-amyalse standard curve for radial diffusion, and then divided by the weight in grams of the barley half-seeds.

	CONTROL (Enzyme Units / Dry Mass)		
	Replication 1	Replication 2	Replication 3
	6.937	8.052	4.814
	5.829	8.383	3.834
	5.680	11.617	5.797
	5.809	8.037	4.135
	5.208	9.512	4.406
Average	5.893	9.121	4.597

	4CH (Enzyme Units / Dry Mass)		
	Replication 1	Replication 2	Replication 3
	5.866	7.539	7.656
	6.160	6.157	5.821
	6.615	6.224	6.737
	5.243	5.929	5.439
	7.002	5.071	6.236
Average	6.177	6.184	6.378

	9CH (Enzyme Units / Dry Mass)		
	Replication 1	Replication 2	Replication 3
	5.333	7.327	5.150
	5.483	6.674	6.198
	6.250	6.424	4.633
	5.395	5.372	6.996
	5.693	7.251	5.523
Average	5.631	6.609	5.700

	15 CH (Enzyme Units / Dry Mass)		
	Replication 1	Replication 2	Replication 3
	5.587	6.183	5.165
	4.992	5.150	6.681
	5.091	4.600	4.267
	5.660	7.200	5.400
	6.369	7.059	6.263
Average	5.540	6.038	5.555

	30CH (Enzyme Units / Dry Mass)		
	Replication 1	Replication 2	Replication 3
	5.861	6.387	4.806
	7.550	7.975	5.000
	6.297	5.274	5.383
	5.794	7.400	5.660
	4.436	6.814	4.719
Average	5.988	6.770	5.114

	200CH (Enzyme Units / Dry Mass)		
	Replication 1	Replication 2	Replication 3
	5.607	7.019	5.577
	5.882	10.341	5.948
	4.754	8.022	4.915
	6.865	7.938	4.571
	6.540	7.263	5.677
Average	5.930	8.117	5.338

APPENDIX G

RESULTS FOR SPECTROPHOTOMETRY

Results obtained from the absorbance, interpolated from the α-amyalse standard curve for spectrophotometry, and then divided by the weight in grams of the barley half-seeds.

	CONTROL (Enzyme Units / Dry Mass)		
	3.692	9.919	2.880
	2.840	9.249	6.389
	6.410	10.649	6.389
	6.673	11.400	3.550
	4.057	9.290	5.862
Average	4.734	10.101	5.014

	4CH (Enzyme Units / Dry Mass)		
	5.798	3.516	8.748
	8.405	2.436	2.676
	2.487	3.070	11.630
	9.914	5.009	3.516
	8.388	2.539	5.437
Average	6.998	3.314	6.401

	9CH (Enzyme Units / Dry Mass)		
	3.867	11.550	2.483
	4.083	12.600	8.833
	8.200	8.150	3.383
	6.000	4.383	9.917
	5.450	5.333	5.700
Average	5.520	8.403	6.063

	15 CH (Enzyme Units / Dry Mass)		
	2.868	3.259	4.935
	3.538	2.421	8.752
	4.469	2.831	3.352
	7.318	12.123	3.780
	3.575	8.212	7.207
Average	4.354	5.769	5.605

	30CH (Enzyme Units / Dry Mass)		
	3.677	4.405	2.149
	4.352	8.703	4.387
	10.977	2.735	3.410
	2.824	7.673	6.945
	2.131	4.920	2.647
Average	4.792	5.687	3.908

	200CH (Enzyme Units / Dry Mass)		
	3.215	10.972	8.037
	11.832	5.402	5.178
	5.308	3.364	8.953
	6.355	4.916	4.766
	4.206	4.953	7.664
Average	6.183	5.921	6.920