THE COMPARISON EFFECT OF *AVENA SATIVA*, *GINGKO BILOBA* AND *WITHANIA SOMNIFERA* ON GERMINATION OF BARLEY SEEDS (*HORDEOM VULGARE*)

By

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I,Zanele Hadebe declare that this dissertation represents my own work in Conception and execution.

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ABSTRACT

Background

Agriculture is one of the main contributors to the South african economy. Feeding a growing population, availability of skilled farmers and sustaining an environment are amongst the three crucial issues agriculture faces. Fertilisers are one of the main solutions for growth of plants in agriculture, however, these fertilisers are often used at the cost of the environment.This has stirred a universal hunt for economic friendly alternatives like biofertiliser.

Aim of the study

This research study aims to determine the effects of three homoeopathic decimal dilutions (3X) of *Avena sativa*, *Gingko biloba* and *Withania somnifera* on barley seeds (*Hordeum vulgare*). The homoeopathic decimal dilutions of *Avena sativa*, *Gingko biloba* and *Withania somnifera* were selected as they possess phytochemicals that could be beneficial to increase plant crop yield.

Methodology

Benyunes(2005) German Homoeopathic Pharmacopoeia (GHP) 4a specifications were followed for creating the homoeopathically manufactured (3X) decimal dilutions of *Withania somnifera, Avena sativa* and *Gingko biloba* for the investigation. A randomised complete block was used in the experimental setup at the Durban University of Technology's Horticulture Department nursery. Two experiments were conducted simultaneously: Experiment A (treatments made with deionised water) and Experiment B (treatments made with 30% ethanol).

Experiment A

A total of 600 barley seeds were germinated in three trays labelled 1 to 4 with tags separating the seedling trays into four categories, namely: *Avena sativa 3X, Gingko biloba 3X, Withanania somnifera 3X* and control (deionised water). Each tray consisted of 200 barley seeds, 200/4 = 50 seeds in each category. Thus, the experiment was replicated three times.

Experiment B

A total of 600 barley seedswere germinated in three trays labelled 4 to 6 with tags separating the seedling trays into four categories, namely: *Avena sativa 3X, Gingko biloba 3X, Withanania somnifera 3X* and control (30% alcohol). Each tray consisted of 200 barley seeds, 200/4 = 50 seeds in each category. Thus, the experiment was replicated three times.

The study was conducted over a 23-day period. This research utilised a quantitative method to collect data before and post-harvest. The number of seedlings that emerged, stem diameter and shoot height were measured before harvest. Ten seedlings were randomly selected from each replicate (i.e., 30 seedlings per treatment). One-way analysis of variance (ANOVA) was used to compare seed germination, leaf number, stem diameter, shoot height, root length, shoot weight and root weight, followed by the Tukey HSD test (a = 0.05). The paired sample test was used to compare the difference between treatments that used water as a vehicle and those that used ethanol as a vehicle (P = 0.05). The data collected were analysed using software (IBM SPSS Statistics v27; IBM Corp).

Results

The results indicated that there were significant differences in the germination percentage, leaf number, shoot height and shoot weight in the seedlings grown in deionised water as a vehicle. It was found that with deionised water as vehicle, *Avena sativa* had the highest germination percentage and highest root weight. Control (deionised water) had the highest leaf numbers and the tallest shoots. *Gingko biloba* had highest shoot weight for the samples grown using deionised water as the vehicle. Furthermore, there were no significant differences in most of the parameters tested for the seedlings grown in ethanol except for except germination percentage between *Gingko biloba* and control and another significance of difference in Gingko *biloba,Withania somnifera* and *Avena sativa* on germination percentage as parameter as well as significance of difference between control (ethanol),*Gingko biloba* and *Withania somnifera* on stem diameter as parameter . It was found that deionised water

appeared to be the best growth vehicle for *Avena sativa* and *Gingko biloba* extracts while *Withania somnifera* was the best when ethanol was used as a vehicle.

Conclusion

Based on the results, it can be concluded that the overall best treatment that can benefit farmers was *Avena sativa* with deionizide water as a vehicle as it had the highest germination percentage and root weight. Ethanol had an inhibitory effect when used with homoeopathic remedies, so deionised water was the best growth vehicle. The results indicated that there is a potential for homoeopathy in agriculture, and more studies are required. Farmers can then produce good crops while sustaining the environment.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

DEFINITIONS

Abscidic acid- a plant hormone that controls various aspects of plant growth and development (Finkelstein 2013).

Agrohomoeopathy- a unique area of practice used to treat plants, mainly because it is non-toxic, often antimicrobial, insecticidal, antifungal, as well as being able to increase crop productivity rate (Sen *et al.* 2018).

Allelopathy- a phenomenon in which a plant's (including microorganisms') direct or indirect, positive or negative effects on another plant through the release of chemicals in the environment (Li *et al.* 2010).

Allopurinol - a xanthine oxidase (XOD) inhibitor that lowers blood uric acid levels by reducing purine synthesis and preventing the production of uric acid (Hou *et al*. 2015).

Ascorbic acid- also called vitamin C is a water-soluble ketolactone containing two ionisable hydroxyl groups (Du, Cullen and Buettner 2012).

Avenalumic acid- are long-chained phenylalkenoic acids made by oats that, like hydroxycinnamic acids, can have extra hydroxy and methoxy substituents (de Bruijn *et al.* 2019).

Avenanthramides- are phenolic compounds that reduce inflammation and trigger apoptosis (Pridal, Böttger and Ross 2018).

Beta-carotene- is an antioxidant that prevents activated oxygen molecules from damaging cells by inhibiting their activity (Johnson and Russell 2010).

Broomrapes- belong to the genera Orobanche and Phelipanche in the family Orobanchaceae, are obligate plant-parasitic weeds that are among the hardest to control of all biotic restrictions that affect crops (Fernández-Aparicio *et al.* 2016).

Catechin- a common secondary metabolite generated from plants that is a member of the flavonol family (Ganeshpurkar and Saluja 2020).

Citrobacter freundii`- a member of the genus Citrobacter and family Enterobacteriaceae regarded as a commensal inhabitant of both human and animal

digestive systems which it virulence features have caused humans infections and diarrhoea (Bai *et al.* 2012).

Costunolides- a well-known sesquiterpene lactone belonging to the germacranolides series which is a colourless, crystalline powder with the chemical formula C15H20O2 and the molecular weight of 232.318 g/mol(Kim and Choi 2019).

Deionised water- an aqueous solution with a resistivity that varies depending on its purity from 0.1 to 10 M cm (Nguyen, Rahman and San Wong 2012).

Fertiliser- describe the chemical make-up of several important minerals and elements intended for the normal as well as expedited growth and feeding of all plants, fertilisers can be categorised as Organic or Inorganic (chemical) (Hazra 2016).

Flavonoids- a class of naturally occurring compounds with varying phenolic structures, which can be found in fruits, vegetables, cereals, bark, roots, stems, flowers, tea, and wine (Panche, Diwan and Chandra 2016).

Gibberic acid- is a tetracyclic di-terpenoid molecule that acts as a plant hormone by promoting the growth and development of plants (Gupta and Chakrabarty 2013).

Ginkgolide- a highly effective platelet activating factor antagonist cage molecule that is isolated from Ginkgo biloba L. leaves which triggers inflammation (Sarkar *et al*. 2020).

Glycowithanolide- are withanolide glycosides, which areacylated to produce sitoindosides, which has a glucose molecule at carbon 27 that possesses properties of withania somnifera (Ahuja *et al.* 2009).

Hydroponic- uses artificial media such as sand, gravel, vermiculite, rockwool, perlite, peatmoss, coir, or sawdust to assist the mechanical growth of plants in nutrient solutions (water containing fertilisers)(Jensen 1997).

Inorganic fertiliser- is either industrially produced via chemical processes or mined from mineral deposits with minor processing (such as lime, potash, or phosphate rock (Gupta and Hussain 2014).

Molyboenum- a trace element that is necessary for all living things, including bacteria, plants, and animals(Novotny and Peterson 2018).

Mother tincture- are liquid preparation resulting from the extraction of suitable source material with alcohol/water mixtures, which form the starting point for production of most homoeopathic medicine (Kayne 2006).

Organic fertiliser- are made from biological or living substances i.e manure, organic waste from agriculture that has decomposed (Sharma and Chetani 2017).

Parthenium hysterophorus- also known as parthenium weed, is a short-lived perennial or annual herbaceous plant that damages natural ecosystems, infests damaged locations, and triggers severe allergic reactions in humans and domestic animals (Adkins and Shabbir 2014).

Parthenolides- is a powerful anti-inflammatory and anticancer sesquiterpene lactone (SL) that was first isolated from the shoots of feverfew (Tanacetum parthenium)(Ghantous *et al.* 2013).

p-hydroxybenzoic acid- is a phenolic substance found in a wide range of agricultural and industrial wastewaters (olive oil and table olive industries, distilleries) (De Heredia *et al.* 2001).

Phytotherapy- The study of employing herbal medicines to treat illness (Weiss 1991).

Polyphenols- are the group of biologically active substances found in plant-based diets (Abbas *et al.* 2017).

Potentisation- the process of transmitting the pharmacological message of the original substance through trituration or succussion, as well as serial dilutions. It is the mechanical and mathematico-physical process of modifying medicines (Gaier 1991).

Remedies- homoeopathic medication given in accordance with the principles of homoeopathic prescription and manufactured in accordance with pharmacopoeial directions (Kent 1995).

Saponins- are highly abundant triterpenoids and steroid physically complicated amphiphatic glycosides that plants generate (Faizal and Geelen 2013).

Sesquiterpene lactone- are terpenes produced using a biosynthetic process that involves three cyclical isoprene units, a fused -methylene-lactone ring, and a basic structure with 15 carbons (thus the prefix sesqui-) (Moujir *et al.* 2020).

Sterol- are tetracyclic triterpenoid lipids needed by all eukaryotes for vital cellular processes, including as phagocytosis, stress tolerance, and cell signalling (Wei, Yin and Welander 2016).

Succussion- the act of repeatedly striking a homoeopathic medicine with force against a hard, elastic surface after each dilution (Hogeland and Schriebman 2008).

Tannins – are metabolites present in the majority of plant species, serve as a defence against predators and may also aid to control plant growth (Das *et al.* 2020).

Tritration- the method of using a mortar and pestle and a neutral diluent to crush raw ingredients. This process extracts their therapeutic properties and makes them soluble (Hogeland and Schriebman 2008).

Vehicle- an inert substance with no therapeutic value that is used in the preparation of mother tinctures, drug potencies and used in dispensing of medicine, Saccharum lactis, cane sugar (globules/pollutes), water, and alcohol are the vehicles used in homoeopathy (Jayasuriya 2002).

Vermiculite- a hydroponic substratum with water-physical characteristics which support plant growth (Kremenetskaya *et al.* 2022).

Vigour seed/seed vigour- refers to the seed characteristics that determine the potential for quick, uniform emergence and development of seedlings under a wide range of field circumstances (Egli and Rucker 2012).

Withaferin - the most bioactive withanolide responsible for the range of Ashwagandha's health-promoting properties (Hassannia *et al.* 2020).

Withanolides - A class of naturally occurring C28 steroids known as withanolides are based on the so-called "withanolide skeleton," which is an ergostane skeleton functionalised at carbons 1, 22, and 26.

ABBREVIATIONS

ANOVA-Analysis of Variance

B-Boron

Ca-calcium

CAT-catalase

CH-1:100 dilution ratio

Cl-Chlorine

CM-Cattle manure

Cu-Copper

DH-1:10 dilution ratio

EFSA-European Food safety

Fe-Iron

GbLE-Gingko biloba leaf extract

GM-genetically modified

GMP-guanosine monophosphate

GT-Goat manure

HGA3- Homoeopathic gibberic acid

HSD test -honesty significant difference

IL-8- Interleukin

K2O-potassuim

K-Potassium

M potency- ratio 1:1000 dilutions

M.T-mother tincture

Mg-Magnesium

mm-millimetres

NF-B-Nuclear factor Kappa light chain enhancer of activated B cells

NFT-Nutrient Film Technique

N-nitrogen fertiliser

O.cumana-Orabache cumana

P-phosphorus

P2O5-Phosphate

PA-Phenylanoic acid

PH-potential of Hydrogen

PM-poutry manure

P-phosphorus

SD-standard deviation

SE-standard error

Si-Silica

SM-sheep manure

SOD-Suphoxole dismulase

TMV-tobacco mosaic virus

WSG-withania somnifera glycowithenolide

Zn-Zinc

CHAPTER 1: INTRODUCTION

1.1 Introduction

About 40% of the earth's surface is occupied by agriculture involved with the global food system, providing secure and nutritive food to populations that are likely to grow to almost 10 billion by 2050 (Brooks, Deconinck and Giner 2019). The agricultural sector is facing challenges, including feeding a growing population, securing a future for farmers, as well as protecting the environment (Brooks, Deconinck and Giner 2019). Agriculture is an essential industry in any nation, and is a key contributor to South Africa's gross domestic product (Oluwatayo *et al.* 2016). With that being said, the global agricultural sector is currently facing a huge challenge with the production of plant crops and plant health. The challenge arises from the use of synthetic fertilisers to maximise plant growth (Brooks, Deconinck and Giner 2019).

Fertilisers have been documented to cause significant environmental pollution (i.e. soil and water pollution). Hepperly *et al*. (2009) point out that a high volume of fossil fuels are needed to produce the large quantities of energy required for the production of synthetic fertilisers. A process called nitrate leaching occurs as a result of the use of these fertilisers which significantly decreases the amount of carbon and nitrogen (N) in the soil as well as acidification of the soil, which inhibits the growth of future crops on that land (Hepperly *et al*. 2009). Soil biodiversity can be negatively impacted by agricultural management techniques that acidify the soil. This further causes dependence on other chemical inputs such as liming, which increases atmospheric carbon (Herren *et al.* 2020). All the negative effects of synthetic fertilisers have led to a global quest amongst agricultural researchers to identify alternative resources that can potentially replace or at least supplement synthetic fertilisers.

Agrohomoeopathy (the application of homoeopathy in agriculture) is a unique area of plant treatment, mainly because it is non-toxic, often antimicrobial, insecticidal, antifungal, and can increase crop productivity rate (Sen *et al.* 2018). Economic savings and environmental protection are two significant advantages of agrohomoeopathy (Moreno 2008). Agrohomoeopathy enhances the plant's own life energy, balances the

soil, and uses a systemic technique to permanently solve the illnesses, with no side effects (Tichavsky 2008). Agrohomoeopathy is an avenue for demonstrating the validation of homoeopathy since it gives objective proof of its effectiveness, refuting the idea that it is only a placebo (Tichavsky 2008). Homoeopathic preparations have been successfully utilised to increase active ingredients in medicinal plants, plant detoxification from metals like aluminium and copper, and improve plant growth rate and productivity (Singh, Singh and Kumar 2020). Agrohomoeopathy is a method for re-establishing homeostasis in plant systems within a dynamically balanced agro-ecosystem (Rossi *et al.* 2006). Agrohomoeopathy offers a variety of alternatives for a sustainable agricultural output, such as cost-effective and residue-free therapies for yield improvement and disease and pest management (Kokornaczyk, Dinelli and Betti 2014).

One of the old world's original neolithic crops was barley (*Hordeum vulgare L*.), originating from *Hordeum spontaneum* (K. Koch) Thell, a flowering plant from the Poaceae or Gramineae family that is cultivated in temperate areas between 350 m and 450 m above sea level (El-Hashash and El-Absy 2019). Barley is characterised by three flowered spikelets which alternate on opposite sides. These spikelets have flat rachis on the head, where the two laterals and a central nodes form a triplet of spikelet. Each spikelet is subtended by two glumes and three spikelets that are fertile. The spike is described as six-rowed, two rows form on the spike when only the center spikelet is fertile (Evans 2008).

There are 31 species of *Hordeum*,including cultivated barley (*Hordeum vulgare*).Both cultivated and wild versions of the two subspecies of *H.vulgare* self-pollunates exclusively as cross-pollunation rarely occurs in cultivated barley(Hordeum vulgare) (Evans 2008) There are various conditions that can inhibit barley seed germination, including salinity, drought, heavy metal poisoning and abiotic and non-abiotic stress (Batyrshima *et al.* 2018).

Barley is believed to have been the first crop cultivated and became the primary nutrition source for the earliest farmers. Although it is still a staple diet in many areas, its prim ary uses today is as animal feed and to make beer (Langridge 2018).

Ancient civilisations cultivated barley from as far back as 8000 BC. In East Africa and in China in the Tibet region it is seen as a staple food source. Barley crops in the highlands of eastern Tibet have an important role in the economy (Guedes *et al*. 2015).

Malting barley for the process of brewing is a well-known process in the agricultural world and the beer manufacturing industry, the increase in the use of this process is enhanced due to barley impacting the flavour of beer indirectly with volatile compounds comprising aldehyde, ketones, alcohols and furans which give, provide flavour stability which is an important attribute of quality beer (Dong *et al.* 2015).

The extracts of barley are one of the reasons why beer is the most consumed alcoholic beverage (Kong *et al.* 2016). Barley is reported to have peptides that remain in the beer after fermentation bringing flavour to the beer (Kong *et al.* 2016). The food industry has faced a lot of pressure to produce barley because of its health benefits; barley is high in dietary fibre and beta-glucan which are known to decrease the risk of heart diseases and type 2 diabetes, as well as lowering cholesterol (Sullivan, Arendt and Gallagher 2013).

Homoeopathic medicines can be used in agriculture, from seed germination to crop production. Most of the preliminary work in this field started with observing the effect of homoeopathic drugs on seed germination of different crop species (Sen *et al.*2018). However, agrohomoeopathy is a new concept so there is not much literature available on the effect of herbs and decimal dilutions on the growth of seedlings and plants (Gardiner *et al*. 2013). Homoeopathy can either enhance or inhibit the growth of plants without damaging the plant and the environment (Moreno 2008). This is because homoeopathy uses a process of dynamisation to prevent toxicity.

Agrohomoeopathy is non-toxic, antimicrobial, insecticidal, antifungal, and can increase crop productivity rate (Sen *et al.* 2018). This practice is gaining more ground all around the world. Nearly 200 years ago, Clemens Boenninghausen noted how remedies discarded in gardens increased the yield of new crops (De Ponte 2013). De Pontes (2013) theorised that homoeopathic remedies somehow activate the plant growth process. Seed germination is the most important preliminary step for crop production, with research indicating that some homoeopathic remedies increase seed germination by positively altering a plant's physiological activities (Sen et *al.* 2018).

The extensive use of synthetic N fertiliser in agriculture is causing environmental problems. Therefore, it is desirable to find alternative solutions to increase plant growth without compromising the quality of food and soil (Mondal 2016). Fertilisers have been documented to cause significant environmental pollution (i.e. soil and water pollution). Hepperly *et al*. (2009) further emphasises that a high amount of fossil fuels are needed to produce the large quantities of energy required for the production of synthetic fertilisers.

A process called nitrate leaching occurs during the use of these fertilisers; this process significantly decreases the amount of carbon and N in the soil, as well as acidifying the soil which inhibits the growth of future crops on that land. Soil biodiversity can be negatively impacted by agricultural management techniques causing acidification which then causes dependence on other chemical inputs such as liming which increases atmospheric carbon (Herren *et al.* 2020). The negative effects of synthetic fertilisers have led to a global quest amongst agricultural researchers to identify alternative practices that can replace or at least supplement chemical fertilisers. Agrohomoeopathy is a unique area of practice used to treat plants, mainly because it is non-toxic, often antimicrobial, insecticidal, antifungal, and can increase crop productivity rate (Sen *et al.* 2018). Homoeopathic medicine is cost-effective as compared to synthetic fertilisers and is required in very small amounts (Evans 2008). For that reason, agrohomoeopathy can be a good alternative to traditional agriculture and pest control methods (Mondal 2016).

Ashwagandha (*Withania somnifera*) is used traditionally as a tonic for exhaustion and nervous debility as well as to boost a weak immune system. *Withania somnifera* has active ingredients called withanolides as well as withaferin; these ingredients have a stimulatory and depressant effect (Laidlaw 2016). *Withania somnifera* also has bactericidal, antiviral, and antitumor effects. *Withania somnifera* has been shown to increase plasma corticosteroid levels n assist patients with hyperglycaemia and glucose intolerance (Laidlaw 2016). *Withania somnifera* inhibits both lipid peroxidation and the protein oxidation change prompted by copper, as well as has antibacterial activity against *Clavibacter michiganesis*, and fungistatic effect against *Aspergillus flavus*, *Fusarium oxysporum* and *Fusarium verticilloides* (Braun and Cohen 2010).

Gingko biloba has numerous medicinal active ingredients including flavonoids, glycosides and trilactones (Zhou *et al.* 2015). These can enhance growth, improve lipid metabolism, increase immunity, and act as an antioxidant. This herb may also act as a circulatory stimulant by increasing blood flow and plays a role in how neurotransmitters in the brain operate by protecting nerve tissue. It is often used to treat mental health conditions and fatigue as it has been proven to improve mental performance and strengthen memory and cognitive function (Belwal *et al.*2018). *Ginkgo biloba* leaf is often taken by mouth for memory and thought problems, anxiety, vision problems, and many other conditions, but there is no good scientific evidence to support most of these uses. In addition Li *et al*. (2018) state that *Gingko biloba* is widely known for prevention against Alzheimer's disease because it has amyloid B-proteins known to affect hormone regulation and maintenance of the integrity of endothelial micro vascularity. It has six other proteins that can reduce oxidative stress and have antiapoptotic effects.

Avena sativa structures contain active ingredients that benefit both plants and animals. Oats (*Avena sativa*) is well known to increase and produce important metabolites such as flavonoids, sterols and other phenols (Soriano *et al. 2004)*. These metabolites help against nematode infestations, so can be beneficial to plants (Soriano *et al.* 2004). Individuals with celiac disease or gluten sensitivity tolerate *Avena sativa*-containing foods. Health-promoting components of oats include dietary fibre (beta-glucan) and phytochemicals with antioxidant activity. Oat carbohydrates are composed of starch and fibre. The type of starch found in oats is slowly digested and therefore has minimal effects on blood sugar level or glycaemic index. About one-third of oat carbohydrate is dietary fibre, an energy source for beneficial gut microbiota (Harland 2014).

1.2 The aim of the study

This study aims to compare the effect of *Withania somnifera, Avena sativa* and *Gingko biloba* extracts on the germination and growth of barley.

1.3 The study objectives

To determine the effect of *Withania somnifera(3X), Avena sativa (3X) and Gingko biloba (3X)* with deionised water or alcohol as vehicle on the germination of barley seeds and seedling growth.

1.4 The hypotheses

It is hypothesised that *Withania somnifera (3X), Avena sativa (3X) and Gingko biloba (3X)* with deionised water as the vehicle will perform better than treatments that used ethanol as a vehicle.

It is hypothesised that *Avena sativa* (3X) with deionised water as vehicle will have the highest barley seed germination percentage.

1.5 Benefits of the study

Little is known about agrohomoeopathy and it is poorly understood, hence this study will add to the literature on agrohomoeopathy. In addition, the study will illustrate the efficacy of *Avena sativa* 3X, *Gingko biloba* 3X and *Withania somnifera* 3X on the germination of barley seeds. The experimental procedure was completed in a period of one month, requiring only rudimentary laboratory skills. Remedies and materials were relatively inexpensive and were easily accessible. The barley industry can grow by using homoeopathy for farming, thus providing more potential for other seeds and crops to be experimented on. There is very little research on the effect of homeopathic dilutions on the germination of seeds and plant growth. Therefore, this research will contribute to the literature on homoeopathy in plant studies/germination of seeds and plant growth.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Agriculture is connected to almost one-third of the world's land usage and makes a significant contribution to the world economy (Newton *et al*. 2020). However, environmental factors have challenged agriculture to transition from an efficiency-driven industrial agriculture to an eco-friendly one (Dong 2021). Shifting to sustainable agriculture, where food and other agricultural products are produced without jeopardising the future environment, food security and general welfare, is a major challenge (Robertson 2015).

This literature review discusses the state of agriculture, food security, strategies used to improve agricultural yield sustainably and healthily, fertilisers, as well as homoeopathy and phytotherapy and their applications within agriculture.

2.2 The impact of agriculture in the world

Agriculture refers to the many ways that domesticated animals and crop plants support the world's population by producing food and other goods (Harris and Fuller 2014). .

Since humans became sedentary and began farming some 12 000 years ago, producing adequate food for an expanding population has always been difficult (Qaim 2020). Though food production has been prevalent throughout the world's hunter-gatherer societies for over 10 000 years, it only supported four million people before agriculture was developed (Parke 2013). The current state of agriculture was severely impacted by the COVID-19 epidemic which affected food security – food insecurity is now on the rise in many nations around the world (Roubík *et al.* 2022).

The necessity of ensuring food security for the expanding human population and reducing the swift loss of precious biological diversity looms large among global concerns today. Both situations call for immediate action: estimates of the current rate of biodiversity loss range from several hundred times the background (i.e. "natural") rates to between 1 000 and 10 000 times the background rate, and there are currently about one billion starving people (Chappell and LaValle 2011).

Although agricultural production has improved significantly over the past 50 years due to greater usage of fertilisers, irrigation water, agricultural equipment, pesticides, and land, it would be overly optimistic to predict that these correlations will continue to be linear in the future (Rehman *et al.* 2016). New strategies are required that integrate biological and ecological processes into food production, reduce the use of non-renewable inputs that are harmful to the environment or to the health of farmers and consumers, make productive use of farmers' knowledge and skills by replacing expensive external inputs with human capital, and make productive use of people's collective capacities to work together to solve common agricultural and resource problems (Pretty 2008).

Strategies that have been used within agriculture to prevent food insecurity include multidisciplinary collaboration to increase crop productivity, enhance agricultural methods (fertilisers), decrease waste, change diets, and expand aquaculture (Sanchez 2015). Development of genetically modified food (GMOs), enhancing agrobiodiversity and precise farming are among the strategies to prevent food insecurity, though these methods come with unintended disadvantaged that need to be minimised or eliminated (Hindwan 2018).

2.3 Challenges with genetically modified food

The World Health Organization (WHO) (2014) describes GMOS as “Plants, animals, or microbes whose genetic makeup (DNA) has undergone a change that does not happen normally as a result of mating or natural recombination”. Further, genetically modified foods (GMF) are described as food made from GM (genetically modified) plants or animals (Zhang, Wohlhueter and Zhang 2016). The potential to improve the global food situation, increase agricultural output, increase the nutritional value of food, and develop pharmaceutical preparations with established clinical importance support genetic modification of plants and animals (Kramkowska, Grzelak and Czyzewska 2013).

The challenges related to the use of genetically modified foods are that these can cause allergic reactions because gene combinations derived from non-food sources because they have the potential to trigger an allergic reaction (Ozkok 2015). Concerns have been raised that GMO organism genes could unintentionally recombine with pathogenic bacteria in the environment causing toxicity (Bakshi 2003). Other

arguments are that GM crops promote antibiotic resistance, increase the risk of cancer in humans, degrade food quality, and pose other hazards (Kamil and Yakup 2011).

2.4 Fertilisers

“Fertiliser” is the word used used to describe the chemical make-up of several important minerals and elements intended for the normal as well as expedited growth and feeding of all plants. Fertilisers can be categorised as organic or inorganic chemicals (Hazra 2016). A little less than half of the world's population is thought to be dependent on fertilisers which boost crop production (Ritchie, Roser and Rosado 2022). Numerous studies have demonstrated the significance of fertiliser use in raising agricultural output in various regions of the world (Mwangi 1996). However, overuse of fertilisers is known to cause harm to the ecosystem by causing nutrient runoff into ecosystems and water systems. Growing food demand due to population growth, economic development, and climate change influence the dependence on fertilisers for agricultural crop production to supply essential plant nutrients such as potassium, phosphorus, and N (Mashamaite *et al.* 2022).

Agricultural output increasingly relies on fertilisers due to land degradation brought on by the loss of soil fertility (Wairiu 2017). This has affected the agricultural chain as profitability depends on soil fertility (Sileshi 2021). At the same time, low fertiliser use is associated with low agricultural output in Africa. It is increasingly evident that the use of agricultural fertilisers boosts agricultural yields but at the same time costs the environment. Thus, advocates have urged reducing the use of inorganic fertilisers (William, Börjesson and Hedlund 2013).

2.4.1 Organic fertilisers

Organic fertilisers are made from biological or living substances, i.e. manure or organic waste from agriculture that has decomposed (Sharma and Chetani 2017). The first systematic use of organic fertiliser has been dated to the Neolithic period around 12 000 years ago in the area of the Fertile Crescent in the Middle East. During this period the human population and culture began to transition from hunters and gatherers to farmers and breeders (Larramendy and Soloneski 2019).

Chen (2006) describes the advantages of organic fertiliser as boosters of the biological activity of the soil, releasing nutrients to the soil and lowering N leaching and

increasing phosphorus fixation. Organic fertiliser further encourage the formation of soil aggregates and safeguard against pests, harmful heavy metals, acidity, alkalinity, and salt (Chen 2006). Disadvantages of organic fertiliser include being pathogenic and having low nutritional supply (Roba 2018).

Fresh or dried plant material, animal manures and litter, agricultural products, and other plant-derived substances make up organic fertilisers (Ochieng 2018). Manure nutrient value can vary seasonally on farms, among farms or broader geographic scale depending on the diet of the animal, the usage and kind of bedding material, manure age and how it was kept (Kapuya 2022). Different types of manure are used as organic fertilisers, including poultry, cattle, sheep, horse and goat manure. Poultry manure is one of the most beneficial manures as it is readily available as most people have chickens on their farms.

2.4.1.1 Poultry manure

Almost all 13 of the basic elements that plants require for growth and development are present in poultry manure (Jordaan 2018), namely:

- copper (Cu)
- zinc (Zn)
- chlorine (Cl)
- boron (B)
- iron (Fe)
- molybdenum
- nitrogen (N)
- phosphorus (P)
- potassium (K)
- calcium (Ca)
- magnesium (Mg)
- sulphur (S)
- manganese (Mn)

Despite having a high quantity of N, poultry manure has a relatively low availability of N to plants. This is because around a third of the nitrogen in poultry manure is in the

form of ammonium, and the remaining two-thirds are present as organic N (Loes 2021).

2.4.1.2 Cattle manure

Cattle manure's nutrients are released more gradually and keep for longer, promoting stronger root growth and larger crop yields. A 453.59 kg cow generates 15 tons of manure annually (Naisam 2020). This amount of manure includes the equivalent of 96.6 kg of N, 87.1 kg of phosphate (P_2O_5), and 121.11 kg of potassium (K_2O), depending on the type of diet the cow was fed. Cow manure is composed of 30% organic content, 70% moisture, and 3% mineral substance. Because of the high carbon-to-nitrogen ratio, it is employed as a bulk material in recycling agricultural leftovers.

2.4.1.3 Goat manure and sheep manure

Goat manure enhances soil texture by increasing plant N uptake and water usage efficiency. Since goat manure is dry, it composts quickly and is relatively odourless, though, in certain circumstances, fresh goat manure contains microorganisms that can cause diseases. Goat manure is significantly higher in N than horse and cattle manures; on average goat manure has 9.98 kg of N per ton whereas cattle manure has 4.5 kg of N per ton (Musagomba 2019).

One of the benefits of using sheep dung as an organic mulch in vegetable gardens, flower beds, seedlings, and other crops is that it naturally has a delayed N release. Low N concentration and high phosphorus and potassium levels in sheep manure make it the perfect fertiliser for plant development (Gana 2021).

2.5 Research on the different types of fertilisers

Strong correlations have been shown between increased population pressure, shorter fallow seasons, and soil nutrient depletion. Since the 1960s over 200 million hectares of farmed land sub-Saharan Africa lost an average of 660 kg of N per hectare, 75 kg of phosphorous per hectare, and 450 kg of potassium per hectare (Sanchez 1997.

Fallowing, establishing new lands, intercropping, and mixed crop-livestock systems are traditional African coping mechanisms that are not able to adapt rapidly enough to rapid population expansion coupled with shrinking farm area and declining soil fertility

(Naisam 2020). Therefore, the only practical option to maintain the soil health required for ongoing agricultural output is to add nutrients back to the soil. Given the high amounts of N and phosphorus content, applying fertiliser is seen to be the logical solution to reverse the loss of soil fertility. This helps to maintain and possibly increase soil organic matter (Naisam 2020).

Inorganic fertiliser has earned a bad reputation in more developed nations due to its well-documented negative effects on the environment and human health, such as N leaching, ammonia volatilisation (related to acid rain), the emission of nitrous oxides, and the eutrophication of aquatic environments brought on by phosphorus run-off (Nguyen 2018).

Some small and resource-constrained farmers blend organic and inorganic fertilisers because of concerns about the nutritional content of organic fertilisers and the greater cost of buying inorganic fertilisers (Naisam 2020).

Another significant problem is the soil's loss of nutrients. In Kenya, only 20% of the land is thought to have a medium to high potential for agriculture (Chapeyama 2018). Farmers are compelled to cultivate less-than-ideal agricultural land (the remaining 80% of land) and to utilise the same plots of land year after year without replenishing the soil via fallowing due to significant population development, particularly in the agriculturally productive areas.

Research indicates that more than 50% of the productivity increases seen in Asia during the Green Revolution can be ascribed to higher fertiliser use rather than merely better seed (Sakrabani 2017). Irrigation's ability to manage the flow of water was a significant factor in fertiliser's contribution to productivity increase in Asia (Kumar, Mittal and Hossain 2008). Fertiliser usage is responsible for one-third of the rise in grain output globally (Stewart and Roberts 2012).

Khumalo (2020) studied the effect of planting density and N application rate on grain quality and yield of three barley *seeds* (*Hordeum vulgare L*) by means of analysis of N application. Findings were that higher N rates typically lowered kernel size. By raising planting density and N fertiliser rate, kernel size was both raised and lowered. The three cultivars significantly influenced the grain's N content and kernel plumpness.

Debutteville (2017) found that a mix of inorganic, organic, and biofertilisers was optimum for cucumber yield and earliness. The researcher tested how the production and economics of cucumbers were affected by such a mix and found that the number of fruits per plant and the weight of each fruit rose, increasing the overall fruit output per hectare. A balanced diet, higher nutrient intake, and enhanced carbohydrate synthesis were found to be the causes of the increased fruit length, fruit cavity, fruit volume, and fruit diameter.

Omidire *et al.* (2015) investigated the impact of inorganic and organic fertiliser on crop performance under a micro irrigation-plastic mulch regime and demonstrated that the yields (lbs/acre) of inorganic fertiliser were greater than those of organic fertiliser Further, the researchers found that the quantity of cucumbers and okra per acre dramatically increased with the use of microorganisms in the inorganic fertiliser. Overall, compared to the organic-based fertiliser, the “Farmer mix”, with or without the addition of microbes, considerably enhanced yields for all crops.

Organic manure distributes nutrients slowly so that the crop has access to them for a longer period, in contrast to inorganic fertilisers which provide nutrients fast and are thus easily exhausted. Research conducted at the University of Alaska Fairbanks revealed that manure loses roughly one-third of its nutrients and organic matter value in three months and the other half in six months or even longer (Naisam 2020). Compared to inorganic fertilisers, manures are administered in greater quantities to provide a substantial residual influence on the development of the next crops. Inorganic fertilisers offer large returns to farmers, but they do not consider their negative effects, such as increased erosion owing to loss of soil structure, which results in tons of soil being lost per hectare (Langyintuo 2020). Utilising manure has led to the development of the more ecologically and consumer-friendly organic farming method, which avoids the use of synthetic and artificial ingredients.

2.6 Agricultural techniques

A technique called hydroponics uses artificial media such as sand, gravel, vermiculite, rockwool, perlite, peat moss, coir, or sawdust to assist the mechanical growth of plants in nutrient solutions (water containing fertilisers) (Jensen 1997).

There are different types of hydroponics systems, the main ones being:

- **Drip system**, in which a pump in the reservoir delivers water or a nutrient solution to each plant or pot, with the amount of water for each plant being regulated by an electronic timer. The water or nutrient solution is gathered and returned to the reservoir in the recovery system, where it is then circulated once more (Lee and Lee 2015).
- **Deep water culture system** which continuously feeds the plant's roots with water containing nutrients. By using this method, you can guarantee that the plant's roots are always immersed in water and oxygen. The benefit of this system is that it is highly oxygenated, consumes less fertiliser, and has fewer maintenance requirements (Saaid *et al.* 2013).
- **Nutrient film technique (NFT)** in which plant roots are contained in plastic channels without solid planting material, allowing a root mat to form in and partially above a thin film of nutritional solution flowing through the channels (Morgan 2011).
- **Aeroponics system** is based on the premise that plants can be grown in closed or semi-closed environments while suspended by spraying their dangling roots with a nutrient-rich, highly aerated fertigation solution (Wang, Dong and Gao 2019).

2.6.1 Inorganic fertiliser

Inorganic fertiliser is either industrially produced via chemical processes or mined from mineral deposits with minor processing (such as lime, potash, or phosphate rock) (Gupta and Hussain 2014). Fritz Haber, a German chemist in the 20th century, was given the 1918 Nobel prize in chemistry for inventing chemical fertiliser by the process of creating ammonia from its constituent parts, which was believed to prevent a global famine by supplying fertilisers with N. However, Haber's initial goal in creating ammonia was to support warfare programmes during World War I (Gal 2015).

Bhatt, Labanya and Joshi (2019) say that inorganic fertiliser has advantages as it increases soil organic matter and crop yield and raises soil fertility. However, over application of inorganic fertiliser causes leaching, pollution of water resources, destruction of beneficial insects and microorganisms, and increases susceptibility of crops to disease and causes soil acidification (Chen 2006).

Chemicals like ammonium nitrate, potassium chloride, urea, and NPK (nitrogen, phosphorus and potassium) which fall into the category of compost fertilizer, are examples of inorganic fertilisers (Sharma and Chetani 2017).

2.6.1.1 Nitrogen fertiliser

Sharma and Chetani (2017) categorise the following fertilisers as high N sources:

- ZA (Zwavelvuur Ammonium): N content of 20.5% to 21%.
- Urea or $CO(NH_2)$: N content 45.5% to 46%.
- Ammonium nitrate, or NH_4NO_3, which has 35% more nitrogen than Chilean saltpetre with 15% N.

Nitrogen is easily lost to the surrounding environment. This process has been linked to the production of smog and tropospheric ozone, the effects of global warming, the loss of stratospheric ozone, and the deterioration of groundwater and surface water quality. This has a significant effect on ecosystem quality, human health, the health of animals and plants, and, ultimately, biodiversity (Schröder 2014).

2.6.1.2 Phosphorus fertiliser

Sharma and Chetani (2017) categorise the following fertilisers as high phosphorus sources:

- Multiple superphosphates (DS=Double superphosphate): P_2O_5 of 30%.
- Triple superphosphate: P_2O_5 of 45%.

Due to the rising demand for phosporus fertilisers, the need to improve and maintain the phosphorus status of agricultural soils in developing nations has increased (Roberts and Johnston 2015). The negative effects of soil phosphorus transfer to surface waters is eutrophication (Roberts and Johnston 2015).

2.6.1.3 Potassium fertiliser

Sharma and Chetani (2017) categorise the following fertiliser as a high sources of potassium:

- Potassium sulphate (ZK=Zwavelvuur time), also known as potassium chloride: 50% K_2O.

Potassium fertilisers are applied at a far lower rate than N and phosphorus fertilisers, and less than half of the potassium lost by crops is replaced. The use of mineral potassium fertilisers increases soils' ability to retain water and improves the structural integrity of sandy soil (Zörb, Senbayram and Peiter 2014).

2.6.2 Vermiculite

Vermiculite is a hydroponic substratum with water-physical characteristics which supports plant growth (Kremenetskaya *et al.* 2022). It is a popular plant growth substrate that is widely used in horticulture due to its capacity to store water, good porosity, significant capacity for cation exchange and base exchange, vermiculite is a popular plant growth substrate that is widely used in horticulture (Tingey *et al.* 1982). This because it has good water holding capacity and good capacity for exchanging cations as well as supply plants with trace amounts of calcium, magnesium, and potassium (Tingey *et al.* 1982)

2.7 Barley

The Middle East, North Africa, northern and eastern Europe (Iran, Morocco, Ethiopia, Finland, England, Denmark, Russia and Poland), as well as Asia, Japan, India, Tibet and Korea, have historically relied heavily on barley as a food source (Baik and Ullrich 2008). Barley has not received as much attention as other crops in attempts to systematically breed and produce variants for food applications (Baik and Ullrich 2008).

After wheat, barley is the most significant minor grain in South Africa, and its primary applications are in the manufacture of malt, animal feed, and pearl barley. Due to the overproduction of maize, barley is mostly grown in South Africa for malting because there is not a substantial demand for it as a feed (Loes 2021). Animal feed is made from the small portion of the barley harvest that is less suited for malting. According to Khumalo (2020), the average annual commercial output of barley in South Africa is approximately 261 000 tons, while the country's annual consumption exceeds 287 000 tons. Barley was only cultivated extensively in the Northern Cape under irrigation until 1997, but now it is mostly grown in the Western Cape under dryland conditions with a minor amount being grown in the Northern Cape.

Barley grain yield and quality relationships are frequently erratic and influenced by cultivar, overall soil fertility, soil water availability, fertiliser N management, and pre- and post-anthesis N absorption patterns (Gana 2021). Given that N is the nutrient that limits crop output the most in the major agricultural regions of the globe, proper N management practices can provide significant economic advantages for farmers (Spiertz 2009). In seasons of excess malting barley supply, the feed market serves as a fallback outlet since malting barley is excellent for animal feed. However, feed barley is typically too rich in N for malting (Newton *et al.* 2011).

2.7.1 Barley grain structure

The barley grain has a body that resembles a spindle, tapering at each end, and a shallow furrow that runs along the ventral side (Briggs 2012).

The hull, or husk (the lemma and palea), surrounds the barley kernel's anatomical structure consisting of the endosperm, endocarp, integuments, aleurone layer, and germ or embryo which make up the caryopsis. Over the entire kernel, the pericarp, which forms from the ovary walls, serves as protection. The seed coat is differentiated from the external cell layers (the integuments). The growing embryo gets its nourishment from the endosperm, a starchy substance that is encased in a protein matrix (Jadhav *et al.* 1998).

If the awn or other apical appendages and basal attachment are destroyed during threshing, the husk at the apex and base of the grain is typically damaged as well. Typically, grain has creases and is pale, yellow, or buff in colour. The dorsal, rounded side of the grain is invested with the lemma, palea inferior, and flowering glume. It’s the ventral surface has five longitudinal surface ridges, which are nerves, under which run vascular bundles. The lemma's thinner edges overlap the palea's thinning edge (palea superior palet). The palea is indented across two ventral furrows and has two nerves.

The rachillar (basal bristle) is a little, less hairy, appendage that can be found at the base. The embryo is covered by two flat, hairy lodicules on the rachilla (Briggs 2012). The outermost layer of the endosperm cells, known as the aleurone, contains protein structures and enzymes involved in the digestion of the endosperm. The embryo is found on the dorsal side of the caryopsis, towards the attachment end. The spindle-

shaped barley kernels are tapered toward each end and thicker in the centre. When characterisation must be done from the threshed grain, some kernel features are particularly helpful for differentiating one cultivar from another. When the grain is threshed, the lemma and palea (husk or hull) of hulled barley remain attached to the caryopsis, whereas in hulless barley the caryopsis threshes free (Jadhav *et al.* 1998).

2.7.2 Barley adaptation

Several morphological and commercial variants of barley have developed over time, including winter, spring to-row, six-row, awned, naked, and hulllness. Barley is probably the most adaptable cereal crop, and can withstand salt, cold, and drought. It is typically productive in semiarid subtropical regions (winter planting) and temperate climates (winter or spring plants). It cannot survive in extremely humid warm areas. Compared to other cereal crops, grain cultivation takes place at higher latitudes, higher altitudes, and farther into deserts (Ullrich 2010).

2.7.3 Barley germination

Germination requires a series of synchronised morphogenetic processes, including energy redistribution, endospermic nutrition absorption, and physiological and metabolic alterations (Tarnawa *et al.* 2023). Regulation of phytohormones like auxin, ethylene, gibberellic acid (GA_3), and abscisic acid (ABA) are essential to promote germination in barley seeds (Han and Yang 2015). Seed germination is primarily influenced by environmental factors controlling the metabolism such as light, temperature, soil water content, and nutrients (Han and Yang 2015). A seed's ability to germinate and the timing of that germination depend on a variety of variables,these include water availability,oxygen, light and favourable temperature. However, it happens frequently that despite the seed meeting all of these requirements, it doesn't germinate(Bewley, J.D and Black 1994.) (

The hormone gibberellin, which is produced by the plant embryo during seed germination, causes the aleurone cells to release amylase, which then causes starch and storage proteins to be hydrolysed and released into the endosperm. The starchy endosperm's breakdown releases sugars that fuel the development of roots and acrospires. The plant hormone abscisic acid, which keeps the seed dormant, inhibits this effect (Jacob and Pescatore 2012).

2.7.4 Barley diseases

One of the barley diseases with the greatest economic impact is powdery mildew. This lowers malting quality, kernel weight and yield (Grando and Macpherson 2005). Other diseases are leaf rust, barley leaf stripe, net blotch, and dry-land root rot. Every year leaf rust develops under irrigated conditions, particularly in places with recently reclaimed soil. Rainfed conditions produce barley leaf stripe. Barley as food

Barley is utilised for food preparation (15%) and animal feed (85%), with the local landraces preferred for food over the modified cultivars (El Felah and Medimagh 2005). Whole grain barley provides good amounts of several bioactive chemicals and minerals, including iron (6 mg/100 g raw material), zinc (3.3 mg/100 g raw material), calcium (50 mg/100 g raw material), dietary fibre (14.8 g/100 g raw material) and barley protein which has advantageous functional characteristics and includes important amino acids which contribute to elasticity, water holding, and emulsifying capacity (Sakellariou and Mylona 2020).

Barley is used as food because of its health benefits as it is high in fibre. Health benefits include lowering the risk of coronary heart disease and lowering the risk of type 2 diabetes and cholesterol (Sullivan, Arendt and Gallagher 2013). Barley is used as an animal feed because it has high carbohydrate content and a comparatively low protein, calcium, and phosphorus content, as well as containing small levels of vitamin B. After being steam rolled or put through a grinding process, the entire barley kernel is used as feed. Malt sprouts and by-products from the brewing process are also utilised in animal feed (Zhou 2009)

2.7.5 Barley for malting

Barley is utilised for malting because the hull provides natural filtration during mashing and safeguards the developing acrospires during the malting process. However, the hull, through the fermentation of microscopic hull particles, can contribute to "off" tastes in beer and tannins, and polyphenols from the hull can also result in the creation of unwelcome haze and lower-quality beer (Meints, Vallejos and Hayes 2021). Other potential or existing uses for barley include as industrial starch to produce paper and fabric, paper pulp, construction-grade fibre board, biofuels, and industrial chemicals like ethanol and methanol (Shewry and Ullrich 2014).

2.8 Laws of homoeopathy

2.8.1 Law of similars

In simple terms, the law of similars is: "like cures like". This is the first principle of homoeopathy, and effectively indicates that any ailment that exhibits symptoms identical to those produced by a pharmaceutical when taken by a healthy human being, can be cured by that same substance (Sankaran 1991).

2.8.2 Law of Individualisation

Each patient should receive an individual treatment plan and the chosen medicine should have properties as close as possible to those of the patient's ailment. "Similimum" is the name for this similar material (Mathie 2015).

2.8.3 Law of Infinitesimal dose

Homoeopathy varies from conventional medicine because of the use of ultra high dilutions. Highly diluted substances (above 12C) contain no trace of original substance because such dilutions surpass Avogadro's constant (6,02X 10^{23} per mol) (Mathie 2015).

This law is founded on the idea that a substance’s medicinal effects increase as they are diluted, but only through the crucial process of potentisation, in which substances are given energy by succussion and trituration (Macquet 2007). Hahnemann demonstrated clearly over two centuries ago that if you treat a patient, or more specifically, their vital force, with minute doses that are appropriately charged, this will initiate a healing process (Macquet 2007).

2.9 Potencies in Homoeopathy

Potentisation is the process of transmitting the pharmacological message of the original substance through trituration or succussion, as well as serial dilutions. It is the mechanical and mathematico-physical process of modifying medicines (Gaier 1991). Trituration is the process of continuously grinding an insoluble substance into tiny pieces in order to release its active components. A succession of dilutions and succussions can now be used to "potentise" the trituration or mother tincture. To infuse energy into a liquid potency and enable the material to completely envelop the liquid,

succussion is the violent shaking of the liquid (water and alcohol mixture) (Pollack 2013).

A number and a letter are used to indicate the homoeopathic potency (for instance, 3X or 3C). The number indicates how many successive dilutions were used to create the medicine from the tincture. The Roman Numeral X denotes a scale of 10, and the Roman Numeral C denotes a scale of 100. The letter also denotes the number of succussions that the vial of solution passes through at each subsequent stage (Sagar 2007).

The two major scales used in homoeopathy are:

- **Centisimal Hahnemann scale-** Introduced by the founder of homoeopathy, Samuel Hahnemann. In this scale, the first potency should have 1/100th the original drug content (Banerjee 2002).
- **Decimal Hahnemann scale** -Decimal dilution is founded on the idea that the homoeopathic base medicine should be present in the first potency in a ratio of one part per ten, and that each following potency should also be one part per ten of the one before it (Williams 2003).

2.10 Phytotherapy

The study of employing herbal medicines to treat illness is known as phytotherapy or herbal medicine (Weiss 1991). The preparations used in phytotherapy are standardised, meaning they are created, gathered, and processed in a way that yields a consistent and reliable quantity of active chemicals. Quality and safety are crucial issues in phytotherapy (Smith 2012).

Unlike many other single biochemical agents, the interaction between the many elements found in the composition of numerous herbs strengthens their therapeutic effect. Depending on the individual's disease and constitution, combined action of these ingredients may have a variety of consequences (Govender 2003).

Tolentino *et al.* (2019) states that there are numerous ways to prepare a certain herbal plant to treat various ailments,including-:.

a) Infusion method where water is boiled before being poured over the herb then in ten to fifteen minutes, lid is closed and left to sit or steep.

b) Decoction are typically the preferred technique when working with tougher and more fibrous plants,barks,and roots.Instead of just steeping it in hot water,the plant material is cooked for 20 minute,allowing the tougher part to soften and realease its active component
c) Tinctures are alcohol and water extracts used so that the prepared product can have a longer shelf life or when a plant's active compounds are not soluble in water.

2.11 Mother tinctures

Mother tinctures are liquid preparations resulting from the extraction of suitable source material using alcohol/water mixtures, which form the starting point for the production of most homoeopathic medicine (Kayne 2006). This research utilised different types of classes or categories of mother tinctures that are described in Appendix E. The mother tinctures are made from different extractions.

2.11.1 Extractions

Tinctures, liquid extracts, and solid extracts are produced through extraction. Extraction is the process of physically or chemically removing the desired substance from a plant with the use of a solvent (alcohol or water) (Murray 1995).

Murray (1995) further described extracts' strengths in terms of active principles content, with tinctures generally being made at a 1:5 concentration. This means that one part herb (in grams) is soaked in five parts of liquid (ml) of volume. This means that there is five times the amount of solvent (alcohol/water) in a tincture as there is herbal material. A tincture is typically a 1:10 or 1:5 concentration, whereas the fluid extracts are usually 1:1. A solid extract is typically at least four times as potent when compared to an equal amount of fluid extract and 40 times as potent as a tincture if they are produced from the same quality herb.

2.11.1.1 Alcohol-based extraction

2.11.1.2 Only pure grain alcohol that has been redistilled,completely removing the fusel oil from it,should be utilised in a pharmacy that specializes on homoeopathy(Blackwood 2016)

A solvent extract with a minimum 1:5 dilution is what is commonly referred to as a tincture, while a fluid extract is 1:1, 1:2, or even stronger. A fluid extract should be administered in lower doses because it is around 5–10 times more powerful than a tincture (Rotblatt *et al.* 2002).

The medicine is made from the liquid that is extracted from the plant material, with the remaining herb being thrown away. Since alcohol may extract components that are water-insoluble, alcohol-based solvents or extracts are often stronger than infusions or decoctions. Additionally, alcohol is a powerful natural preservative. The tincture must be at least 25% alcohol to assure sterility. It is a very efficient approach to prescribe herbal substances since a tincture is easily absorbed by the body (Singh 2004).

2.11.1.3 Water-based extraction

The herb's plant material (either the root, leaves, stems, or bark) is weighed into a glass jar before being extracted. 1-part plant material is combined with 10 parts heated distilled water in a container after being crushed, then rhythmic movement and light treatments are applied to the essences, and the combination is exposed to sunlight at various intervals. After one week is the infusion is pressed through a cotton cloth and filtered through Whatman filter paper No. 1 (Naidoo 2004).

Due to bacterial contamination, water extracts have a limited shelf life and must be refrigerated and thrown away after a few days. They are extremely challenging to standardise and can have a disagreeable or bitter flavour (Singh 2004).

2.12 Vehicles

A vehicle is an inert substance with no therapeutic value that is used in the preparation of mother tinctures, drug potencies and used in dispensing of medicine. Saccharum lactis, cane sugar (globules/pollutes), water, and alcohol are the vehicles used in homoeopathy (Jayasuriya 2002). Van Schalkwyk (1998) proposed that the influence of the potencies can be traced to the transmission of a micro-configuration between the various molecules of the vehicle.

2.12.1 Water and alcohol vehicles

Homoeopathy employs water for chemical operations that necessitate the purification of many primitive substances, preparation of some attenuations, and administration of medicine in the form of a watery solution (Jahr 1842).

Purified water used as a vehicle goes through a deionisation process, which is prepared by an electrically operated automatic deioniser that uses distillation by collecting steamed water through a condensation process to ensure purity and water free of acids, alkalis, and solid minerals (Mazumdar 2001).

Homoeopathy claims an efficacy of a medicine despite the absence of the substances originally introduced into the preparation, by assuming that water retains a memory of the solutes even after all trace has vanished (Aversa *et al.* 2016). The 'vital force' is released through the process of 'succussion' to the 'vehicle' that now functions as the medicine. The medicinal properties of the drug are assumed to be transferred to and apparently retained by the 'vehicle' although this cannot be scientifically explained, despite sporadic positive efforts to understand how water could display some sort of memory, and how biological activity could be displayed in the absence of the original molecule linked with this activity (Khuda-Bukhsh 2003).

Banerjee (2002) states that alcohol is safe as a vehicle because it is imbibed in modest quantities and is soluble in water at all concentrations. Ethanol can extract therapeutic qualities from both plant and animal material while preserving both. It is possible to store items in an alcoholic solution for a long time.

2.13 Studies showing effect of alcohol on plant growth

The most common vehicle used in the preparation of homoeopathic medicine is alcohol (propyl, isopropyl, and amyl) which is commonly used for extraction of the drug essence from drug material and also of preserving the final product (Banerjea 2003).

The amount of alcohol in a homoeopathic remedy is adjusted according to the various homoeopathic pharmacopoeias but it is rarely less than 30% v/v. Alcohol may cause some concern when remedies are tested in vitro or in laboratory animals because of the possible toxicity of ethanol on cell cultures and organisms (Chirumbolo and Bjørklund 2018).

The effect of alcohol on seed germination has been investigated. Chen *et al.* (2020) conducted a study on the effect of ethanol on tomato seeds and found stacked seeds germinated less than spread out seeds, indicating that ethanol inhibits germination. These findings show that ethylene sensing and ethanol interact in plants.

Salehi, Ashiri and Salehi*(* 2008) found that exposure to alcohol for longer than four hours decreased germination. The researchers also discovered that some species were more susceptible to ethanol than others. Further Miyoshi and Sato (1997) found that ethanol has either inhibition, no effect or minimal stimulation of seeds on germination of *Japonica* and rice.

Taylorson and Hendricks (1979) found that ethanol has the ability to break the dormancy in some seeds, but germination under dark conditions was not improved using tiny amounts of ethanol. Contrary to other studies, Afzal *et al. (*2013) found that low doses of ethanol (2% and 4%), when used as a priming agent, increased seed germination and seedling vigour, while priming with 6% ethanol did not. Thus, the concentration of ethanol is important when investigating the effect of herbal therapies on seed germination.

2.14 Studies on the effect of water in plant growth

Water is a crucial component for plant seed germination (Khodakovskaya *et al.* 2009); if a plant undergoes water stress it will not grow (Forni *et al.* 2017). Water stress is related to numerous factors, such as water absorption of plants, soil water availability and water loss by plant soil (Kramer 1963). Silber *et al.* (2003) state that nutrient insufficiency, rather than water scarcity, may lead to a decrease in crop production and high irrigation may lead to nutritional deficit. The salinity (salt content of water) may have an effect on the number of leaves produced (Silber *et al.* 2003). Stress from drought significantly reduces height, basal diameter, number of leaves, leaf area, root length, and biomass (Wu *et al*. 2008).

2.15 Overview on homoeopathy in agriculture

In homoeopathy, illnesses are treated with drugs (in extremely diluted form) that, when administered to healthy people in undiluted form, cause the same symptoms as the disease being treated (Bonjeer 2018). In homoeopathy, the entire organism is treated to boost the body's degree of resistance and encourage its capacity to defend against

sickness. hus, it complements the holistic ideas of biological agriculture. Due to the high dilution of the cures, they are often inexpensive, have negligible to no negative impacts on the environment, and are risk-free (Norton 2021).

Homoeopathy may be useful in treating and preventing illnesses in crops, according to some of the experimental work that has previously been done in this area. Agricultural homoeopathy is the use of homoeopathy in agriculture; it provides an environmentally and economically sound approach with the potential to lessen the use of agrochemicals in global agriculture (Patel 2020). Homoeopathy helps to enhance internal functions that optimise growth and development (Naisam 2020). Some homoeopathic remedies have demonstrated their potential to alter the plant's physiological response, the amount of foliage, and the quantity of fruit. Homoeopathic remedies such as *Calcarea carbonica, Carbo vegetabilis*, and *Magnesia carbonica*, are advised for use because their active ingredients can elicit favourable responses in plants. Recently, the homoeopathic medicines *Sulphur*, *Silicea terra*, and *Nux vomica* have been evaluated on various plants of commercial interest, including corn (Naisam 2020).

2.15.1 History of agrohomoeopathy

About 200 years ago Baron von Boenninghausen, the son-in-law of Hahnemann (the creator of homoeopathy), made the first reference to using homoeopathy on plants. Boenninghausen observed that the extra or unused medications he dumped into his plant pots had an impact on the plants (Lambert, Hopkins and Hawks 2020). In order to hasten or slow development, the homoeopathic approach to agriculture stimulates biological plant processes. When homoeopathic dynamisations are used, there is no risk of environmental or plant toxicity, and in certain situations, the cure may even help to cleanse the affected ecosystem (Donkor and Owusu 2019).

Economically speaking, such an approach results in significant cost savings because homoeopathic remedies are far less expensive than agrichemicals and insecticides. Homoeopathic plant research has become a popular paradigm in homoeopathic research circles because it disproves common objections to homoeopathy and the placebo effect related to human scientific trials. Plants are immune to the placebo effect and ethical dilemmas since they have a lesser level of consciousness than humans do. Majewsky *et al.* (2009) concluded that using healthy plant models is a

good way to investigate fundamental problems concerning the specificity of homoeopathic remedies. Betti *et al*. (2009) identified 44 studies in which field trials and in vitro and in planta phytopathological model systems were utilised to conduct experimental homoeopathic treatments. With decimal and centesimal potencies, as well as dilution levels above Avogadro's number, Betti *et al.* (2009) discovered substantial and repeatable effects. The authors nevertheless state that a lot more research is required, particularly at the field level and on potentisation procedures, effective potency levels, and repeatability circumstances.

Homoeopathic potencies such as decimal, centesimal, and fifty-millimetre have been found to have a distinctive impact, despite being dilution levels that are beyond the Avogadro number (Majewsky *et al.* in 2009). Schofield (1984) examined the application of homoeopathy in farming and discovered that both low and high dilutions encouraged germination rates and plant growth (stem length and root growth) of wheat. Brizzi and Betti (2010) investigated *Arsenicum album* (arsenic trioxide) with potencies ranging from 5DH to 45DH compared to potentised distilled water in the germination of wheat plants, and found that *Arsenicum album* 45DH significantly stimulated the germination and development of wheat plants. Furthermore, they found that 4DH was effective in the treatment of tobacco mosaic virus. However, dynamised water also significantly reduced tobacco mosaic virus lesions, indicating that solvent dynamisation alone can have effects that are comparable to but less potent than those of homoeopathic *Arsenicum album.*

Field studies were carried out in Brazil by Boff *et al*. (2008) in response to concerns about increased pesticide usage and chemical residues in food. They collaborated with potato growers who were striving to transition from conventional, industrial farming practices to more natural and holistic anti-pest practices. *Phytophthora infestans* 60CH, *Chamomilla* 60CH, *Silicea* 60CH, *Thuja* 60CH, *Kali* 60CH, and homemade preparations of Bordeaux mixture at 0.3% and propolis extract at 0.5% were blended with water at a rate of 12 ml per litre and sprayed through a foliar spray every two weeks. The plots that were not sprayed made up the control group. Four replicates and randomised blocks made up the statistical design. The researchers discovered that there were no appreciable variations in yield, disease, or pest severity amongst the various homoeopathic formulations, although they did increase yield when compared to untreated plots. They came to the conclusion that homoeopathic

remedies are just as effective as the Bordeaux combination, a typical spray frequently applied to organic agriculture systems. Sadly, this study did not state which Kali salt was used homoeopathically.

Bonato and Silva (2003) examined the effects of homoeopathic dilutions of *Sulphur* (5CH up to M) on the development and yield of radish cultivated in 3-litre vases (pots). The dilutions of sulfur were administered at a rate of 20 drops (1.5ml) per litre of water. Every seven days, 100ml of the solution was poured into each vase. When compared to a control, the application of sulfur enhanced the general conditions of the plants in almost all the evaluated variables. The mass of the substance in the shoot increased more in the radish plants that received *Sulphur* at potencies of 5CH and 12CH. When compared to the control, the plants treated with *Sulphur* 5CH displayed more than double the mass of the dry matter of the shoot.

Biplantol SOS®, a homoeopathic complex, was researched by Shah-Rossi, Heusser, and Baumgartner (2009) for its ability to prevent *Pseudomonas syringae* infection in *Arabidopsis thaliana* plants. Homoeopathic preparation and the control solution, The plants were totally submerged upside down for 30 seconds in 20 ml of either the homoeopathic preparation or the control solution. The irrigation water was then infused with the leftover solution. The size of the therapeutic effect of Biplantol was around 50% more than that of Bion®, a popular SAR inducer utilised as positive control. After additional adjustment, the researchers concluded that homoeopathic formulations would be useful for treating plant diseases.

The effectiveness of homoeopathic *Cina MT* and *Cina 200CH* as a foliar spray against the worm *Meloidogyne incognita* (commonly known as root-knot) on Mulberry trees was examined by Datta (2006). In terms of the quantity of root galls and the nematode population in roots, nematode infection considerably decreased in all treatment groups. Fresh biomass of the shoot and root, shoot and root length, number of leaves, leaf surface area, and root and leaf-mass were other growth characteristics that improved. In addition to being less nematode affected, the inoculation and treated plants grew better than the untreated control.

The effectiveness of homoeopathically generated (HGA_3) on the germination of barley (*Hordeum vulgare L*.) seeds was investigated by Hamman, Koning, and Lok (2003). HGA_3 (4CH, 30CH, 200CH) was compared to control and HGA_3 at a concentration of

0.5g L-1 for its impact on seed germination rate and seedling development (distilled water). The homoeopathically treated seeds consistently produced bigger seedlings from a statistical perspective. The roots of medium-vigour seed lots treated with HGA_3 15CH had longer roots than high-vigour seed lots treated with HGA_3 4CH, 30CH, and 200CH. The capacity of HGA_3 to trigger a biological response was effectively proven using developing barley seeds as a plant model. Hopkins (1998) used a germination index to examine the impact of homoeopathic dilutions (*Sulphur, Nitric acid,* and *Camphor*) on lettuce seed germination in various potencies. The outcomes showed that homoeopathic drugs have biological impacts on seed germination. Hopkins (1998) suggested testing homoeopathically synthesised plant growth regulators and assessing potencies over 30CH. According to Jones and Jenkins (1983), *Pulsatilla* was used to promote the development of wheat seedlings in several dilutions up to 13CH.

2.16 The herbs utilized in this study

2.16.1 *Gingko biloba*

Active ingredients of *Gingko biloba* include flavonol and flavone glycosides, diterpene lactones, ginkgolides, sesquiterpenes, iron-based superoxide dismutase, p-hydroxybenzoic acid, ascorbic acid (vitamin C), and catechins (Jacobs and Browner 2000). Flavonoids and terpene trilactones are thought to be the primary bioactive components, and terpenoids, polyphenols, allyl phenols, organic acids, carbohydrates, fatty acids and lipids, inorganic salts, and amino acids the secondary metabolites (Singh *et al.* 2008). Sierpina,Wollschlaeger and Blumenthal (2003) concluded that *Gingko biloba* has antioxidant and free-radical scavenger properties.

Çavuşoğlu and Karaferyeli (2015) found that *Gingko biloba* increased the number, width, and length of stomata on the upper surface and the length of epidermal cells on the lower surface, as well as the width of epidermal cells, and the stomatal index on both surfaces of barley seeds in which the distance between vascular bundles and stomata breath and length were increased all under saline conditions. Zhao *et al.* (2014) investigated *Ginkgo biloba* exocarp's effects on cucumber and radish seed germination and seedling growth and found that the extracts had an inhibiting influence on the seeds' germination and growth. The extracts strongly suppressed the growth of

the radish stem and root and significantly inhibited cucumber seed germination and root growth.

Çavuşoğlu, Tabur and Çavuşoğlu (2016) investigated germination of *Allium cepa* seeds in a medium with *Gingko biloba* leaf extract and found that they had longer radicles than control seeds that germinated in the media with distilled water, but their fresh weight was lower. The root tip meristems of *A. cepa* seeds germinated in the medium with *Gingko biloba* leaf extract showed a significant increase in the mitotic index.

When *candida albicans* and *staphylococcus aureus* were used as elicitors in cell cultures, more bilobalide (BB), ginkgolide A (GA), and ginkgolide B (GB) were produced, along with a minor growth inhibition (Kang *et al.* 2009).

2.16.2 *Avena sativa*

Avena sativa is known to increase and produce important metabolites such as flavonoids, sterols and other phenols and these metabolites help against nematode infestations, which could be beneficial to plants (Soriano *et al.* 2004). *Avena sativa* is a unique source of avenanthramides and avenalumic acids, as well as several naturally occurring antioxidants such tocopherols, alk(en)ylresorcinols, and phenolic acids and their derivatives (ethylenic homologues of cinnamic acids) (Ahmad *et al.* 2014). Avenanthramides have been found to display fungal germination inhibition and to contribute to oat disease resistance (de Brujin *et al.* 2019). Species of *Avena sativa* also include triterpenoid saponinstannins,. *Avena sativa* L. which are higher in antioxidants and amino acids (beta-carotene, polyphenols, chlorophyll, and flavonoids) (Kim *et al.* 2021).

Avena sativa is a great source of saponins and phenolic antioxidants which are strong antioxidants and anti-inflammatory substances, and avenanthramides which contain nitrogen-phenolic compounds that have demonstrated suppression of NF-B and IL-8 release (Reynertson et al. 2015). Saponins are glycosylated compounds that protect *Avena sativa* plants from diseases and the avenalumins inhibit plant diseases such as rust and fungi (Mayama *et al.* 1982). Other ingredients of *Avena sativa* include beta-D glucan, flavonoidssaponins, protein, copper, zinc, silicon, selenium, potassium,

magnesium, iron, and various vitamins and lipids (Baba *et al.* 2016). The grains and leaves are a significant source of carbohydrates and carotene (Kanwal *et al.* 2022).

2.16.3 *Withania somnifera*

There are not many studies of *Withania somnifera* regarding germination or plant growth and so further research needs to be conducted, but phytochemicals of *Withania somnifera* are relevant in relation to plant growth.

Withanine is the primary component of the different alkaloids. The additional alkaloids are choline, cuscohygrine, isopelletierine, anaferine, and anahydrine, somniferine, somnine, somniferinine, withananine, pseudo-withanine, tropine, and pseudo-tropine (Singh *et al.* 2010). *Withania somnifera* has anti-inflammatory, antioxidant, anxiolytic, and antibacterial activity (Gupta and Rana 2007). These could all be beneficial to plant growth.

The extract of *Withania somnifera* is a complex mixture of many phytochemicals, such as flavonoids, phenolic compounds and withanolides which is one of nature's largest sources of steroidal lactones (Dhanani *et al.* 2017). Abdelgaleil and Hashinaga (2007) tested sesquiterpene lactones(costunolide and parthenolide) on wheat and radish seeds and found that root length, shoot growth and seed germination was significantly inhibited by both sesquiterpenes.

Lactones which are produced by withanolides which was found to inhibit plant growth by Raupp and Spring (2013). They found that above 1M concentrations of costunolide and dehydrocostus lactone decreased the activity of sunflower (*O. cumana*) while 100M of the lactones application irreversibly stopped germination. Andolfi *et al*. (2013) found that germination rates of plants from other families (*Solanaceae, Solanum lycopers icum*) were decreased by any sesquiterpene lactones from sunflowers. Chadwick *et al*. (2013) stated that sesquiterpene lactones have been shown to impede growth in a wide range of plants.

Javaid, Shafique and Shafique (2011) tested *Withania somnifera* (L.) Dunal on its ability to control the weed *Parthenium hysterophorus* and found *Parthenium*'s germination, root and shoot growth were severely inhibited by aqueous and methanol extracts. The germination of seeds was severely affected as it decreased in all soil amendment treatments. Dar, Hamid and Ahmad (2015) found that a methanolic leaf

extract of *Withania somnifera* demonstrated significant anti-bacterial activity against Gram-positive clinical isolates of methicillin-resistant *Staphylococcus aureus* and *Enterococcus spp.* Gram-negative species were all successfully eradicated by *Withania somnifera's* powerful anti-microbial capabilities.

Waris et al. 2016 found on the other hand that, methanol extracts of Camellia sinensis entirely prevented seed germination of Triticum aestivum L. and Zea mays. Fatima *et al*. (2009) found that the methanol extracts of the leaf and stem decreased radish seed germination as well as root length. Further alkaloids, saponins, anthraquinones, and tannins which are also constituents of *Withania somnifera* were present in the *Rumex dentatus* methanol extractThese are all phytochemicals that are also present in *Withania somnifera*.

2.17 General research on plants and homoeopathy

De Pontes (2013), used a hydroponic closed system and compared different potencies of *Ozomum* on barley germination and found that *Ozonum* 30CH had a tendency to have a lower tip-burn percentage, but this was not statistically different from *Ozonum* 15CH or the control. De Pontes (2013) recommended that the study be repeated in November, December, and January.

Kleingeld (2016) compared different potencies of radionically prepared GA_3 and homoeopathically prepared GA_3 (HGA_3) on germination of seeds against those with distilled water as control and the results showed the remedy treatment having suppressive effects on seed growth and development The control group(distilled water) had greater seedling development with discernible roots than all the remedy treatment groups. The researcher recommended the use of other plant-based biological testbeds (e.g. wheat) to determine if similar results could be produced (Kleingeld 2016).

Evans (2008) compared the effectiveness of homoeopathically prepared acids such as ABA, molybdenum and allopurinol on inhibiting or promoting the germination of barley seeds (*Hordeum vulgare*). Findings revealed a statistically significant interaction between the treatments and potencies. The results demonstrated that homoeopathic dilutions of allopurinol were the most successful in promoting germination. The use of ABA in homoeopathic dilutions was the most successful

method of preventing germination. The researcher recommended that other plant types that show a sensitivity to ABA should be utilised to ensure the reliability of the results.

Couchman (2001) tested the effect of different homoeopathic potencies of ABA and GA_3 on the de-embryonated half-seeds of barley seeds (*Hordeum vulgare*) and found homoeopathic ABA did not influence the production of the α-amylase enzyme. Further, Couchman (2001) recommended the use of whole seed and testing whether germination occurs.

Pieterse (2002) investigated the effects of light and heat on homoeopathic dilutions of gibberellic acid ($cHGA_3$) as measured on *Hordeum vulgare* (barley) seed germination showed that the only significant variation in root length was between water and 4CH. When comparing seeds treated with $cHGA_3$ 4cH to seeds not treated with 4CH and exposed to 60°C, statistically significant differences in root length were found. The activity of $cHGA_3$ in relation to root length may have been affected by 60°C for a variety of reasons that were proposed. According to one theory, heat might make water molecules oscillate less coherently, which would lead to the loss of the initial solute.

2.18 Research output

This research will increase knowledge of the role of homoeopathy in agriculture. The study will provide a potential foundation for further studies.

CHAPTER 3: METHODS AND MATERIALS

3.1 Study design

The research was conducted using homoeopathically made (3X) decimal dilutions that followed the manufacturing guideline according to the German Homoeopathic Pharmacopoeia (GHP) 4a specification (Benyunes 2005). The experimental design was a randomised complete block design with four treatments (including control). This study aimed to compare the effect of *Withania somnifera 3x, Avena sativa 3x* and *Gingko biloba 3x* made in either deionised water or 30% ethanol (vehicle) on the germination and growth of barley seeds.

3.2 Treatment groups

The study utilised deionised water and 30% ethanol to manufacture the treatments given that ethanol has either inhibition, no effect or minimal stimulation on seeds (Miyoshi and Sato 1997). Hamman, Koning and Him Lock (2001) and Kleingeld (2016) also used deionised water to manufacture the treatments stating that alcohol had a stimulatory or inhibitory effect on seed germination performance. Both vehicles (deionised water and 30% ethanol) were used to manufacture the treatments in this study. Two experiments were conducted: Experiment A (treatment manufactured using deionised water) and Experiment B (treatments manufactured using 30% ethanol). The experiments were conducted at the same time and at the same place at the Durban University of Technology's (DUT's) Horticulture Department Nursery.

Experiment A: Water based extract

A total of 600 barley seeds were germinated in three trays labelled 1 to 3 with tags separating the seedling trays into four categories, i.e., *Avena sativa 3X, Gingko biloba 3X, Withanania somnifera 3X* and control (deionised water). Thus, the experiment was replicated three times. Each tray consisted of 200 barley seeds, 200/4 = 50 seeds in each category.

Experiment B: Ethanol based extract

A total of 600 barley seeds weregerminated in three trays labelled4 to 6 with tags separated the seedling trays into four categories, namely *Avena sativa 3X, Gingko biloba 3X, Withanania somnifera 3X* and control (30% alcohol). Each tray consisted of 200 barley seeds, 200/4 = 50 seeds in each category. Thus, the experiment was replicated three times. In total, 1200 barley seeds were germinated in six seedling trays.

The planting procedure was the same for Experiments A and B:

1. Vermiculite (Grovida Horticultural Products,Durban,RSA) was used as a growing media and was dispersed equally between the cell trays filling each cell to 75% of capacity (Figure 3.1).
2. Small indentations were made in each cell, and one seed of barley (*Hordeum vulgare*) was sown by hand in each cell in the tray (200 cells per tray) (Figure 3.2).
3. There was 70 ml of each treatment and three treatments plus controls (200 seeds per trial treatment). On the first day of the experiment, 0.5 ml of treatment (*Avena sativa, Gingko biloba and Withania somnifera, ethanol and* deionised water (control) were administered directly to the seeds on vermiculite using a micropipette (Figure 3.3). The trays were taken to the greenhouse (Figure 3.4).
4. Thereafter 5 ml of deionised water (Experiment A) or 30% alcohol (Experiment B) was then poured into each cell twice a week on Tuesdays and Fridays between 8 am and 10 am to prevent the seeds from drying out.
5. Daily observation was carried out and a logbook was kept for recording the number of emerging seedlings (seed germination). Photographic evidence was taken to monitor the growth rate.
6. The experiments ran for 23 days.

3.3 Harvesting and population sample

1. Harvesting was done on the 23rd day at 8 am, 12 pm, 15 pm and 17 pm at the work lab (Figure 3.5).
2. Two brown bags were used as dividers to avoid cross-contamination and seedlings were harvested from the middle of each division to avoid the edge effect.(Figure 3.6)

3. Ten seedlings were harvested from each replicate due to difficulties on number of seedling for seeds utilizing Withania somnifera as treatment with ethanol as vehicle .
4. The data collected were the number of leaves per seedling, stem diameter (10 mm above root collar) (Figure 3.7), shoot length (Figure 3.8), root length measured using ruler (Figure 3.10), shoot weight, and root weight (Figure 3.14). The seedling roots and shoots were separated after harvesting (Figure 3.11) and washed so as to clear the vermiculite (Figure 3.9) and put in small brown bags tothe oven for 48 hours at 60℃ (Figure 3.13). The weight of the shoot and roots was measured (Figure 3.14 using a balance scale at the Department of Horticulture.

Figure 3.1: Vermiculite evenly poured into trays

Figure 3.2: Barley seeds (*Hordeolum vulgare*) sowed by hand

Figure 3.3: Decimal dilutions of *Avena sativa* 3X, *Gingko biloba* 3x and *Withania somnifera* 3x and 30% of alcohol as control are administered directly to seeds planted in vermuculite

Figure 3.4: The trays placed in the greenhouse

Figure 3.5: Trays were taken to the work lab for data collection

Figure 3.6: Two brown bags were used as dividers to avoid cross-contamination and seedling were harvested in the middle to avoid the edge effect

Figure 3.7: Stem diameter was measured using an electronic vernier calliper while the seedlings were still in the tray 10 mm above the ground

Figure 3.8: Shoot length (from the media to the tip of the plant) measured by a ruler

Figure 3.9: Seedling suspended in water and vermiculite moves away from the seeds

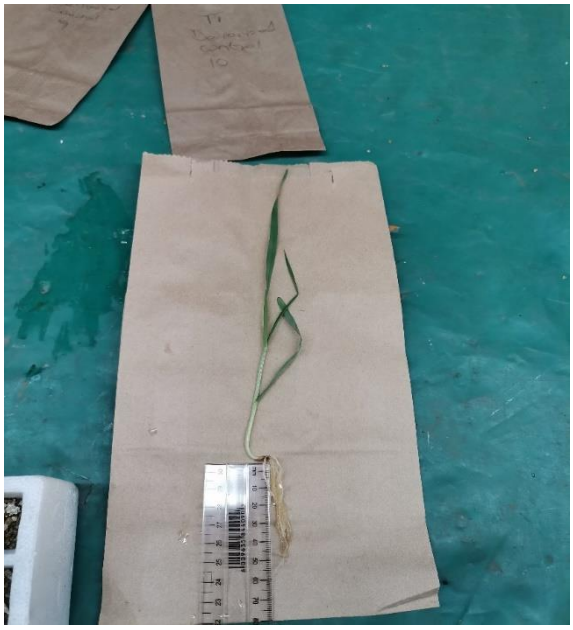

Figure 3.10: Roots were measured using a ruler from the root collar to the longest root strand

Figure 3.11: Roots and shoot were separated using scissors to cut them at the collar of the stem

Figure 3.12: The shoot and roots were placed in labelled brown bags

Figure 3.13. Small brown bags placed into the oven for 48 hours at 60℃

Figure 3.14: Weight of shoot and roots measured by balance scale at the Horticulture Department

3.4 Materials

3.4.1 Seeds

The barley seeds (*Hordeum vulgare*) utilised in the experiment were supplied by Organic Matter garden supplier (Garsfontein, Pretoria, South Africa). The seeds were untreatmented.

3.4.2 Aqua purificata

The deionised water was obtained from the Homoeopathic Department, DUT, and was prepared by an Aqua purificator in a 4-step reverse osmosis process on the 27th June 2022.

3.4.3 Ethanol

Absolute 99% ethanol supplied by Glassworld & Chemical Supplies cc was obtained from Department of Chemistry, DUT, and converted to 30% ethanol.

Aim: To prepare ethanol 30% from 99% ethanol

XVol x Xroh percentage=Yvol x Yroh percentage

300 ml x 30% = Yvol x 99%

300ml x 30%= Yvol

99%

= Needed 90.9ml of 99% ROH

Therefore 300ml-90.9ml = 209.1 ml of deionised water was added

Water was poured into 500 ml glass bottle by means of a 100 ml measuring cylinder to make 30% ethanol

3.4.4 Treatments

All treatments were made using the homoeopathic guideline as per Appendix C.

3.4.5 Potencies

Potentisation, also known as dynamisation, is "the remarkable transformation of the natural bodies through the mechanical action of trituration and succussion on their tiniest particles (while these particles are diffused in an inert manner on liquid substance), developing the latent dynamic powers previously imperceptible and as it were lying hidden asleep in them"(Hahneman 1921 cited in Van Schalkwyk 1998).

The German Homoeopathic Pharmacopoeia (GHP) 4a specification was used to produce the homoeopathic treatments *Avena sativa 3x, Gingko biloba 3x*,*Withania 3x* and control per vehicle used (Bunyunes 2005) as noted in Appendix C.

Test sample 1

- ***Avena sativa*** 3X (deionised water as vehicle) on barley seeds

Test sample 2

- ***Withania somnifera*** 3X (deionised water as vehicle) on barley seeds

Test sample 3

- ***Gingko biloba*** 3X (deionised water as vehicle) on barley seeds

Test sample 4

- Deionised water as control on barley seeds

Test sample 5

- ***Avena sativa*** 3X (30% ethanol as vehicle) on barley seeds

Test sample 6

- ***Gingko biloba*** 3x (30% ethanol as vehicle) on barley seeds

Test sample 7

- ***Withania somnifera*** 3x (30% ethanol as vehicle) on barley seeds

Test sample 8

- 30% ethanol as control on barley seeds

3.4.6 Instruments utilised to collect and record data

3.4.6.1 Digital vernier calliper

The bisector vernier calliper is a precision measuring tool which was used to determine the specimen's length, depth, and diameter (Alshamali 2021). For the purpose of this research the researcher used the digital vernier calliper instrument to measure stem diameter, and this was done at the point where the stem emerged from the ground .

3.4.6.2 Ruler

A ruler was used to measure (in mm) the height of the stem from above the root collar to the tip of the tallest leaf, and root length from the root collar to the longest root strand.

3.4.6.3 Brown bags and oven

Brown bags were used to package each harvested plant. The bags were placed in an oven for 48 hours at 60°C. Measurement of oven-dried biomass is the standard procedure for determining the biomass of individual plants (Tackenberg 2007). The dry oven method, which involved exposing the seeds to 60°C for 48 hours, then the

dry seedling shoot and root weight were measured on mass scale as previously done by Hamman, Koning and Lok (2001), De Pontes (2013) and Kleingeld (2016)

3.4.6.4 Camera

A camera was use to provide photographic evidence of each state. This provided further evidence of the growth of the seedlings.

3.4.6.5 Electronic scale

An electronic scale was used to measure shoot and root weight of the plants. After 48hours in the oven the shoots and roots were weighed by an electronic scale as in Kleingeld (2016).

3.4.6.6 Excel spread sheet

Data was captured into Excel from Microsoft Office® 2019 (Appendix D).

3.5 Statistical analyses

Data were analysed using IBM SPSS Statistics v27 (IBM Corp) software. One-way analysis of variance (ANOVA) was used to compare the germination, leaf number, stem diameter, shoot height, root length, shoot weight and root weight followed by the Tukey HSD test (P = 0.05). A paired sample test was used to compare the mean difference between those planted using water as a vehicle and those planted using ethanol (P = 0.05).

Descriptive and inferential statistics were used to analyse and present the data. Univariate and bivariate analysis is most appropriate for descriptive statistics (Field 2009). Bar graphs and tables were used to present the data (Kleingeld 2016). In terms of inferential statistical analysis, the analysis of variance (ANOVA) test at a significant level of 0.05 was used to analyse the differences in the mean growth. Leedy and Ormrod (2015) explain that ANOVA is a statistical procedure used to calculate difference within a set of data to assess how the difference translates into variations across the groups.

Although ANOVA is used in analysing the differences between two or more sample groups, McHugh (2011) points out that ANOVA output does not provide any analysis of pairwise differences. To compare the difference between each of the three decimal

dilutions treatments and control (distilled water for Experiment A) and ethanol for experiment Band multivariate statistics (multiple comparisons) using Tukey honest significant difference and or Bonferroni test was used to examine pairwise differences in the growth rate in all the sample groups (P = 0.05). According to McHugh (2011), both the Tukey honest significant differences and the Bonferroni test are among the key tests of pairwise differences between sample groups. In addition, the growth rate for each sample on each respective day was compared using a repeated measure test. Simpson (2015) states that repeated measures are suitable for analysing whether the means of three or more measures from the same group of participants are different.

CHAPTER 4: RESULTS

4.1 Introduction

This chapter presents the experimental data collected on the effect of *Withania somnifera, Avena sativa* and *Gingko biloba* on the germination of barley seeds (*Hordeum vulgare*). Experiment A used deionised water as the vehicle for the treatments, and Experiment B used ethanol as the vehicle for treatments. Excel from Microsoft Office® 2019 was utilised to compute the germination percentage as (no. of germinated seedlings/total seeds planted [50 seeds] x100%).

4.2 Comparison of barley seed germination using water as a vehicle

This section compares the effect of *Withania somnifera, Avena sativa* and *Gingko biloba* with deionised water as a vehicle and control on the germination of barley seeds (*Hordeum vulgare*).

4.2.1 Germination percentage

The barley seeds treated with *Avena sativa* had the highest germination percentage (74% ± 12.54%), while *Withania somnifera* had the lowest germination percentage values (50.67% ± 18.37%). There were significant differences across the treatments P = 0.000 (Table 4.1). There were significant differences between the control and *Withania somnifera* treatment (P = 0.007), and *Avena sativa* (P = 0.001). There were significant differences between *Gingko biloba* and seeds treated with *Withania somnifera* (P = 0.004), and *Avena sativa* (P = 0.002). There were significant differences between *Withania somnifera* and *Avena sativa* (P = 0.001) (Table 4.2). No significant difference was found between the control and *Gingko biloba* treatment (P > 0.05).

Table 4.1: ANOVA results for germination rate using water as vehicle

Treatments	N	Mean	Std. Deviation (±SD)	Std. Error (±SE)	95% CI		P value	Significance
					Lower Bound	Upper Bound		

Control	30	61.33	8.523	1.556	58.15	64.52	0.000	Significant
Gingko	30	62.00	7.611	1.390	59.16	64.84		
Withania	30	50.67	18.370	3.354	43.81	57.53		
Avena	30	74.00	12.540	2.289	69.32	78.68		

Table 4.2: Tukey HSD multiple comparison test for germination percentage water as a vehicle

Treatments		Tukey HSD	
(I) HT	(J) HT	P value	Significance
Control	*G.biloba*	0.997	Not significant
	W.somnifera	0.007*	Significant
	A.sativa	0.001*	Significant
G.biloba	*W.somnifera*	0.004*	Significant
	A.sativa	0.002*	Significant
W.somnifera	*A.sativa*	0.000*	Significant

The mean difference is significant at the 0.05 level

4.2.2 Leaf number

The barley seeds treated with control and *Gingko biloba* each had the highest leaf number (av. 3), while *Withania somnifera* had the lowest leaf number (av. 2.6). There was a significant difference across the treatments (P = 0.000, Table 4.3). There was a statistically significant difference between the control and those treated with *Withania somnifera* (P < 0.001). There was a significant difference between *Gingko biloba* and *Withania somnifera* treatment (P < 0.001). No significant differences were found between *Gingko biloba* and *Avena sativa* (P > .05), and *Withania somnifera and Avena sativa* (P > 0.05). No significant differences were found between the control, *Gingko biloba*, and *Avena sativa* treatments (P > 0.05) (Table 4.4).

Table 4.3: ANOVA results for leaf number using water as a vehicle

Treatments	N	Mean	Std. Deviation (±SD)	Std. Error (±SE)	95% CI		P Value	Significance
					Lower Bound	Upper Bound		
Control	30	3.00	.000	.000	3.00	3.00	0.000	Significant
Gingko	29	3.00	.000	.000	3.00	3.00		
Withania	30	2.60	.498	.091	2.41	2.79		
Avena	30	2.80	0.407	0.074	2.65	2.95		

Table 4.4: Tukey HSD multiple comparison test for leaf number using water as a vehicle

Treatments		Tukey HSD	
(I) HT	(J) HT	P value	Significance
Control	*G.biloba*	1.000	Not significant
	W.somnifera	0.000*	Significant
	A.sativa	0.083	Not Significant
G.biloba	*W.somnifea*	0.000*	Significant
	A.sativa	0.087	Not Significant
W.somnifera	*A.sativa*	0.083	Not Significant

* The mean difference is significant at the 0.05 level

4.2.3 Stem diameter

The barley seeds treated with *Avena sativa* had the highest stem diameter (1.932727 mm), while *Withania somnifera* had the lowest stem diameter (1.7570 mm) (Table 4.5). No significant differences were found in the stem diameter between the control and those of *Gingko biloba* and *Avena sativa* (P > 0.05).

Table 4.5: ANOVA results for stem diameter using water as a vehicle

Treatments	N	Mean	Std. Deviation (±SD)	Std. Error (±SD)	95% CI		P value	Significance
					Lower Bound	Upper Bound		
Control	30	14.9013	49.68624	9.07142	-3.6518	33.4545	0.108	Not significant
Gingko	30	1.8353	.12492	.02281	1.7887	1.8820		
Withania	30	1.7570	.15519	.02833	1.6991	1.8149		
Avena	30	1.9327	.15434	.02818	1.8750	1.9903		

4.2.4 Shoot height

The barley seeds treated with control had the tallest shoots (221.67 mm ± 25.152 mm), whereas *Withania somnifera* had the shortest shoots (174.97 mm ± 51.168 mm). There were significant differences found across treatments (P = 0.000) (Table 4.6). There were statistically significant differences between the control and those treated with *Withania somnifera* (P < 0.001). There was a significant difference between *Gingko biloba* and those treated with *Withania somnifera* (P < 0.001) (Table 4.1). No significant difference was found between *Gingko biloba* and *Avena sativa* (P > 0.05). There was asignificant difference between *Withania somnifera* and *Avena sativa*

(P < 0.001). No significant differences were found between the control, *Gingko biloba* and *Avena sativa* treatments (P > 0.05) (Table 4.7).

Table 4.6: ANOVA results for shoot height using water as vehicle

Treatments	N	Mean	Std. Deviation (±SD)	Std. Error (±SE)	95% CI		P value	Significance
					Lower Bound	Upper Bound		
Control	30	221.67	25.152	4.592	212.27	231.06	0.000	Significant
Gingko	30	220.77	24.486	4.471	211.62	229.91		
Withania	30	174.97	51.168	9.342	155.86	194.07		
Avena	30	217.73	22.850	4.172	209.20	226.27		

Table 4.7:Tukey HSD multiple comparison test for shoot height for barley seeds with different treatments using water as vehicle

Treatments		Tukey HSD	
(I) HT	(J) HT	P value	Significance
Control	*G.biloba*	1.000	Not significant
	W.somnifera	0.000*	Significant
	A.sativa	0.967	Not Significant
G.biloba	*W.somnifera*	0.000*	Significant
	A.sativa	0.985	Not Significant
W.somnifera	*A.sativa*	0.000*	Significant

* The mean difference is significant at the 0.05 level

4.2.5 Root length

The barley seeds treated with *Gingko biloba, Withania somnifera, Avena sativa* and control had no statistical difference in root length (P > 0.05) (Table 4.8).

Table 4.8: ANOVA reslts for root length using water as vehicle

Test groups	N	Mean (mm)	Std. Deviation (±SD)	Std. Error (±SE)	95% CI		P value	Significance
					Lower Bound	Upper Bound		
Control	30	66.80	9.946	1.816	63.09	70.51	0.829	Not significant
G.biloba	30	65.43	11.527	2.105	61.13	69.74		
W.somnifera	30	63.63	21.985	4.014	55.42	71.84		
A.sativa	30	66.40	9.722	1.775	62.77	70.03		
Total	120	65.57	14.100	1.287	63.02	68.12		

4.2.6 Shoot weight

The barley seeds treated with *Gingko biloba* had the highest shoot weight (0.0558 g ± 0.016 g), whereas *Withania somnifera* had the lowest shoot weight (0.332 g ± 0.012 g). There were significant differences across the treatments (P = 0.000, Table 4.9). No significant differences were found between the control, *Gingko biloba* and *Avena* sativa (P > 0.05). There was a significant difference between the control and those treated with *Withania somnifera* (P < 0.001). There was a significant difference between *Gingko biloba* and *Withania somnifera* (P < 0.001). No significant difference was found between *Gingko biloba* and *Avena* sativa (P > 0.05). There was a significant difference between *Withania somnifera* and *Avena sativa* (P = 0.002) (Table 4.10).

Table 4.9: ANOVA results for shoot height using water as vehicle

Treatments	N	Mean (g)	Std. Deviation (±SD)	Std. Error (±SE)	95% CI		P value	Significance
					Lower Bound	Upper Bound		
Control	30	0.054637	0.0167219	0.0030530	0.048393	0.060881		
G.biloba	30	0.055807	0.0162764	0.0029717	0.049729	0.061884	0.000	Significant
W.somnifera	30	0.033253	0.0121209	0.0022130	0.028727	0.037779		
A.sativa	30	0.047830	0.0152262	0.0027799	0.042144	0.053516		

Table 4.10: Tukey HSD multiple comparison test for shoot weight for barley seeds with different test groups using water as vehicle

Treatment		Tukey HSD	
(I) HT	(J) HT	P value	Significance
Control	*G.biloba*	0.991	Not significant
	W.somnifera	0.000*	Significant
	A.sativa	0.310	Not Significant
G.biloba	W.somnifera	0.000*	Significant
	A.sativa	0.182	Not Significant
W.somnifera	A.sativa	0.002*	Significant

* The mean difference is significant at the 0.05 level

4.2.7 Root weight

The barley seeds treated with *Avena sativa* had the highest root weight (0.027 g ± 0.009 g), while *Withania somnifera* had the lowest root weight (0.020 g ± 0.010 g).

There were significant differences across treatments (P = 0.009, Table 4.11). There was a significant difference between *Withania somnifera* and *Avena sativa* treatments (P = 0.005, Table 4.12). No significant differences were found between the control and *Gingko biloba, Avena sativa* and *Withania somnifera* treatments (P > 0.05). No significant differences were found between *Gingko biloba* and *Avena sativa* and *Withania somnifera* treatments (P > 0.05).

Table 4.11: ANOVA results for root weight using water as vehicle

Treatments	N	Mean	Std. Deviation (±SD)	Std. Error (±SD)	95% CI		P value	Significance
					Lower Bound	Upper Bound		
Control	30	0.0247433	0.00738959	0.0013491 5	0.0219840	0.0275027		
G.biloba	30	0.0234533	0.00817442	0.0014924 4	0.0204010	0.0265057	0.009	Significant
W.somnifera	30	0.0196933	0.00986946	0.0018019 1	0.0160080	0.0233786		
A.sativa	30	0.0272367	0.00879339	0.0016054 5	0.0239532	0.0305202		

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Table 4.12: Tukey HSD multiple comparison test for root weight for barley seeds with different treatments using water as vehicle

Treatments		Tukey HSD	
(I) HT	(J) HT	P value	Significance
Control	*G.biloba*	0.938	Not significant
	W.somnifera	0.110	Not Significant
	A.sativa	0.677	Not Significant
G.biloba	*W.somnifera*	0.332	Not Significant
	A.sativa	0.327	Not Significant
W.somnifera	*A.sativa*	0.005*	Significant

* The mean difference is significant at the 0.05 level

4.3 Comparison of barley seed germination using ethanol as a vehicle

This section compares the effect of *Withania somnifera, Avena sativa* and *Gingko biloba* with ethanol as a vehicle and control on the germination of barley seeds (*Hordeum vulgare).*

4.3.1 Germination percentage

The barley seeds treated with *Gingko biloba* had the highest germination percentage (60.27% ± 6.70%), while the control had the lowest germination percentage (47.33% ± 4.18%). There were significant differences across treatments (P = 0.000) (Table 4.13). There was a significant difference between the control and *Gingko biloba* (P < 0.001). There were significant differences between the *Gingko biloba* and *Withania somnifera* (P = 0.002), and *Avena sativa* (P = 0.026). No significant differences were found between the control and *Withania somnifera* and *Avena sativa* (P > 0.05). No significant difference was found between *Withania somnifera* and *Avena sativa* (P > 0.05, Table 4.14).

Table 4.13: ANOVA results for germination percentage using ethanol as vehicle

Treatments	N	Mean %	Std. Deviation (±SD)	Std. Error (±SE)	95% CI		P value	Significance
					Lower Bound	Upper Bound		
Control	30	47.33	4.180	0.763	45.77	48.89	0.000	Significant
G.biloba	30	60.27	6.700	1.223	57.76	62.77		
W.somnifera	30	48.67	16.386	2.992	42.55	54.79		
A.sativa	30	51.33	15.960	2.914	45.37	57.29		

Table 4.14: Tukey HSD multiple comparison test for germination percentage using ethanol as vehicle

Treatments		Tukey HSD	
(I) HT	(J) HT	P value	Significance
Control	*G.biloba*	0.000*	Significant
	W.somnifera	0.974	Not Significant
	A.sativa	0.577	Not Significant
G.biloba	*W.somnifera*	0.002*	Significant
	A.sativa	0.026*	Significant
W.somnifera	*A.sativa*	0.829	Not significant

* The mean difference is significant at the 0.05 level

4.3.2 Leaf number

The barley seeds treated with control had the highest leaf number (av. 2.97), with *Withania somnifera* having the lowest leaf number (av. 2.73) (Table 4.15). No significant differences were found across the treatments.

Table 4.15: ANOVA results for leaf number using ethanol as vehicle

Treatments	N	Mean	Std. Deviation (±SD)	Std. Error (±SE)	95% CI		P value	Significance
					Lower Bound	Upper Bound		
Control	30	2.97	0.414	0.076	2.81	3.12	0.150	Not significant
G.biloba	30	2.87	0.346	0.063	2.74	3.00		
W.somnifera	30	2.73	0.450	0.082	2.57	2.90		
A.sativa	30	2.80	0.407	0.074	2.65	2.95		

4.3.3 Stem diameter

The barley seeds treated with control had the highest stem diameter (av. 1.9990 mm), whereas *Withania somnifera* had the lowest stem diameter (av. 1.7230). There were significant differences across treatments ($P < 0.001$, Table 4.16). There were significant differences between the control and *Gingko biloba* ($P < 0.001$), and *Withania somnifera* ($P < 0.001$). No significant differences were found between the control and *Withania somnifera* and *Avena sativa* ($P > 0.05$). No significant difference was found between *Withania somnifera* and *Avena sativa* ($P > 0.05$, Table 4.17).

Table 4.16: ANOVA results for stem diameter using ethanol as a vehicle

Treatments	N	Mean (mm)	Std. Deviation (±SD)	Std. Error (±SE)	95% CI		P value	Significance
					Lower Bound	Upper Bound		
Control	30	1.9990	.27273	.04979	1.8972	2.1008	< 0.001	Significant
G.biloba	30	1.7443	.19452	.03551	1.6717	1.8170		
W.somnifer	30	1.7230	.23177	.04232	1.6365	1.8095		
A.sativa	30	1.8480	.20568	.03755	1.7712	1.9248		
Total	120	1.8286	.25053	.02287	1.7833	1.8739		

Table 4.17: Tukey HSD multiple comparison test for stem diameter for the barley seeds with different test groups using ethanol as a vehicle

Treatments		Tukey HSD	
(I) HT	(J) HT	P value	Significance
Control	Gingko	< .001*	Significant
	Withania	< .001*	Significant
	Avena	0.070	Not Significant
Gingko	Withania	1.000	Not Significant
	Avena	0.487	Not Significant
Withania	Avena	0.216	Not significant

*. The mean difference is significant at the 0.05 level.

4.3.4 Shoot height

The barley seeds treated with *Avena sativa* had the tallest shoots (av. 203.23 mm), while *Withania somnifera* had the shortest shoots (av. 176.07 mm, Table 4.18). No significant differences were found across the treatments.

Table 4.18: ANOVA results for shoot height using ethanol as a vehicle

Treatments	N	Mean (mm)	Std. Deviation (±SD)	Std. Error (±SE)	95% CI		P value	Significance
					Lower Bound	Upper Bound		
Control	30	191.87	37.505	6.847	177.86	205.87	0.108	Not significant
G.biloba	30	187.13	37.950	6.929	172.96	201.30		
W.somnifera	30	176.07	54.950	10.032	155.55	196.59		
A.sativa	30	203.23	38.453	7.021	188.87	217.59		

4.3.5 Root length

The barley seeds treated with *Gingko biloba* had the longest roots (av. 75.47 mm), while *Withania somnifera* had the shortest roots (av. 65.43 mm, Table 4.19). No significant differences were found across treatments ($P > 0.05$).

Table 4.19: ANOVA results for root length using ethanol as a vehicle

Treatments	N	Mean (mm)	Std. Deviation (±SD)	Std. Error (±SE)	95% CI		P value	Significance
					Lower Bound	Upper Bound		
Control	30	73.13	16.162	2.951	67.10	79.17	0.096	Not significant
G.biloba	30	75.47	25.880	4.725	65.80	85.13		
W.somnifera	30	65.43	22.557	4.118	57.01	73.86		
A.sativa	30	79.23	20.969	3.828	71.40	87.06		

4.3.6 Shoot weight

The barley seeds treated with control had the highest shoot weight (av. 0.0545657 g), whereas *Withania somnifera* had the lowest shoot weight (av. 0.036560 g, Table 4.20). No significant differences were found across treatments.

Table 4.20: ANOVA results for shoot weight using ethanol as a vehicle

Treatments	N	Mean (g)	Std. Deviation (±SD)	Std. Error (±SE)	95% CI		P value	Significance
					Lower Bound	Upper Bound		
Control	30	0.045657	0.0181067	0.0033058	0.038896	0.052418	0.053	Not significant
Gingko	30	0.039410	0.0156953	0.0028656	0.033549	0.045271		
Withania	30	0.036560	0.0167333	0.0030551	0.030312	0.042808		
Avena	30	0.046357	0.0137649	0.0025131	0.041217	0.051497		

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4.3.7 Root weight

The barley seeds treated with *Avena sativa* had the highest root weight (av. 0.0346400 g), while *Gingko biloba* had the lowest root weight (av. 0.0274170 g, Table 4.21). No significant differences were found across treatments.

Table 4.21: ANOVA results for root weight using ethanol as a vehicle

Treatments	N	Mean (g)	Std. Deviation (±SD)	Std. Error (±SD)	95% CI		P value	Significance
					Lower Bound	Upper Bound		
Control	30	0.0297133	0.00976206	0.00178230	0.0260681	0.0333585	0.079	Not significant
G.biloba	30	0.0274170	0.01023206	0.00186811	0.0235963	0.0312377		
W.somnifera	30	0.0284767	0.01183635	0.00216101	0.0240569	0.0328964		
A.sativa	30	0.0346400	0.01368010	0.00249763	0.0295318	0.0397482		

4.4 Comparison between water and ethanol

This section compares the differences measured between ethanol and water as a vehicle and the treatment parameters (germination percentage, leaf number, stem diameter, shoot height, shoot weight, root weight and root length) for each of the treatment groups.

4.4.1 Comparison between water and ethanol in the control treatment

The paired sample test shown in Table 4.22 depicts the differences between the parameters measured using water as a vehicle and ethanol in the control group. There were significant differences in the germination percentage ($P < 0.001$), shoot height ($P = 0.002$), stem diameter ($P = 0.006$), root length ($P = 0.031$), and root weight ($P = 0.032$). The germination percentage for water as a vehicle (av. 61.3% ± 8.52%) was found to be significantly higher than for ethanol (av. 47.3% ± 4.18%). The stem

diameter measured for ethanol (av. 2 ± 3.27 mm) was significantly thicker than for water as a vehicle (av. 1.87 mm ±.16 mm). The shoot height with water as a vehicle (av. 221.67 mm ± 25.15 mm) was significantly greater than ethanol (av. 191.87 mm ± 37.50 mm). The root weight with ethanol as a vehicle (av. 0.0297 g ± 0.009 g) was significantly higher than water as vehicle (av. 0.0247 g ± 0.007 g). No significant differences were found for leaf number and shoot weight at P > 0.05.

Table 4.22: Comparison between water and ethanol control treatments

Control		Vehicle	Mean	N	Std. Deviation	Std. Error Mean	P value
Pair 1	Gemination P	Water	61.3333	30	8.52313	1.55610	0.000*
	Germination P	Ethanol	47.3333	30	4.17986	.76314	
Pair 2	Leaf number	Water	3.0000	30	.00000	.00000	0.662
	Leaf number	Ethanol	2.9667	30	.41384	.07556	
Pair 3	Stem diameter	Water	1.8655	30	.16446	.03054	0.006*
	Stem diameter	Ethanol	2.0052	30	.26714	.04961	
Pair 4	Shoot height	Water	221.6667	30	25.15241	4.59218	0.002*
	Shoot height	Ethanol	191.8667	30	37.50470	6.84739	
Pair 5	Root length	Water	66.8000	30	9.94606	1.81589	0.031*
	Root length	Ethanol	73.1333	30	16.16239	2.95084	
Pair 6	Shoot weight	Water	.0546	30	.01672	.00305	0.064
	Shoot weight	Ethanol	.0457	30	.01811	.00331	
Pair 7	Root weight	Water	.0247	30	.00739	.00135	0.032*
	Root weight	Ethanol	.0297	30	.00976	.00178	

4.4.2 Comparison between water and ethanol in the *Gingko biloba* treatment

The paired sample test in Table 4.23 depicts the differences between the parameters measured using water as a vehicle and ethanol in the *Gingko biloba* treatment. There were significant differences in the germination percentage (P < 0.001), leaf number (P = 0.012), shoot height (P = 0.043), root length (P = 0.003), and root weight (P = 0.002), shoot weight (P = 0.033). The germination percentage for water as a vehicle (av. 62.00% ± 7.61%) was found to be significantly higher than for ethanol (av. 51.3% ± 15.33%). The leaf number measured for water treatment (3 ± 0.0) was significantly higher than for ethanol (2.8 ± 0.41). The shoot height measured for water as a vehicle (av. 220.77 mm ± 24.49 mm) was significantly higher than for ethanol (av. 203.23 mm ± 38.45 mm). The root length measured for ethanol (av. 79.23 mm ± 20.97 mm) was

significantly higher than for water (av. 65.43 mm ± 11.53 mm). The shoot weight measured for water as a vehicle (av. 0.0558 g ±0.016 g) was significantly higher than for ethanol (av. 0.0464 g ± 0.014g). The root weight measured for ethanol (av. 0.0346 g ± 0.014 g as a vehicle was significantly higher than for water (av. 0.0235 g ± 0.008g). Stem diameter had no significant difference between the treatments.

Table 4.23: Difference between water and ethanol in *Gingko biloba* treatment

Gingko		Vehicle	Mean	N	Std. Deviation	Std. Error Mean	P value
Pair 1	GeminationP	Water	62.0000	30	7.61124	1.38962	0.000*
	GerminationP	Ethanol	51.3333	30	15.95972	2.91383	
Pair 2	Leaf number	Water	3.0000	29	0.00000	0.00000	0.012*
	Leaf number	Ethanol	2.7931	29	0.41225	0.07655	
Pair 3	Stem diameter	Water	1.8353	30	0.12492	0.02281	0.394
	Stem diameter	Ethanol	1.8480	30	0.20568	0.03755	
Pair 4	Shoot height	Water	220.7667	30	24.48600	4.47051	0.043*
	Shoot height	Ethanol	203.2333	30	38.45345	7.02061	
Pair 5	Root length	Water	65.4333	30	11.52713	2.10456	0.003*
	Root length	Ethanol	79.2333	30	20.96908	3.82841	
Pair 6	Shoot weight	Water	.0558	30	0.01628	0.00297	0.033*
	Shoot weight	Ethanol	.0464	30	0.01376	0.00251	
Pair 7	Root weights	Water	.0235	30	0.00817	0.00149	0.002*
	Root weights	Ethanol	.0346	30	0.01368	0.00250	

4.4.3 Comparison between water and ethanol in the *Withania somnifera* treatment

The paired sample test shown in Table 4.24 depicts the differences between the parameters measured using water as a vehicle and ethanol in the *Withania somnifera* treatment. There were significant differences in the germination percentage (P = 0.03), leaf number (P = 0.03), root length (P = 0.031) and root weight (P = 0.001). It was found that the germination percentage for ethanol as a vehicle (av. 60.27% ± 6.70%) was significantly higher than the water as a vehicle (av. 50.67% ± 18.37%). The leaf number measured for ethanol (av. 2.87 ± 0.35) was significantly higher than for water as a vehicle (2.60 ± 0.49). The root length measured for ethanol as a vehicle (av. 75.47 mm ± 25.88 mm) was significantly higher than for for water (av. 63.63 mm ± 21.99 mm). The root weight measured for ethanol (av. 0.0274 g ± 0.01 g) as a vehicle was significantly higher than for water as a vehicle (av. 0.0197 g ± 0.009 g). No

significant differences were found for the stem diameter, shoot height, and shoot weight (P > 0.05).

Table 4.24: Difference between water and ethanol as vehicle of the *Withania somnifera* treatment

Withania		Vehicle	Mean	N	Std. Deviation	Std. Error Mean	P value
Pair 1	GeminationP	Water	50.6667	30	18.37039	3.35396	0.030*
	GerminationP	Ethanol	60.2667	30	6.70015	1.22327	
Pair 2	Leaf number	Water	2.6000	30	0.49827	0.09097	0.030*
	Leaf number	Ethanol	2.8667	30	0.34575	0.06312	
Pair 3	Stem diameter	Water	1.7570	30	0.15519	0.02833	0.780
	Stem diameter	Ethanol	1.7443	30	0.19452	0.03551	
Pair 4	Shoot height	Water	174.9667	30	51.16807	9.34197	0.313
	Shoot height	Ethanol	187.1333	30	37.94982	6.92866	
Pair 5	Root length	Water	63.6333	30	21.98508	4.01391	0.031*
	Root length	Ethanol	75.4667	30	25.88001	4.72502	
Pair 6	Shoot weight	Water	0.0333	30	0.01212	0.00221	0.093
	Shoot weight	Ethanol	0.0394	30	0.01570	0.00287	
Pair 7	Root weights	Water	0.0197	30	0.00987	0.00180	0.001*
	Root weights	Ethanol	0.0274	30	0.01023	0.00187	

4.4.4 Comparison between water and ethanol in the *Avena sativa* treatment

The paired sample test shown in Table 4.25 depicts the differences between the parameters measured using water as a vehicle and ethanol as a vehicle in the *Avena sativa* group. There were statistically significant differences in the germination percentage (P < 0.001), stem diameter (P = 0.001), shoot height (P = 0.001), and shoot weight (P = 0.017). It was found that the germination rate for water as a vehicle (av. 74.00% ± 12.54%) was significantly higher than for ethanol (av.48.6% ± 16.39%). The stem diameter for water as a vehicle (av 1.93 mm ± 0.15 mm) was significantly higher than for ethanol (1.72 mm ± 0.23 mm). The root length for ethanol (av. 75.47 mm ± 25.88 mm) was significantly higher than for water (63.63 mm ± 21.99 mm). The shoot height for water (217.73 mm ± 22.85 mm) as a vehicle was significantly higher than for ethanol (176.07 mm ± 54.95 mm). The shoot weight (0.0478 g ± 0.02 g) for water as a vehicle was significant for the ethanol (0.0366 g ± 0.02 g). No significant differences were found for the leaf number, root length and root weight (P > 0.05).

Table 4.25: Difference between water and ethanol vehicle in *Avena sativa* treatment

Avena		Vehicle	Mean	N	Std. Deviation	Std. Error Mean	P value
Pair 1	Gemination P	Water	74.0000	30	12.53959	2.28941	0.000*
	Germination P	Ethanol	48.6667	30	16.38614	2.99169	
Pair 2	Leaf number	Water	2.8000	30	0.40684	0.07428	0.601
	Leaf number	Ethanol	2.7333	30	0.44978	0.08212	
Pair 3	Stem diameter	Water	1.9327	30	0.15434	0.02818	0.001*
	Stem diameter	Ethanol	1.7230	30	0.23177	0.04232	
Pair 4	Shoot height	Water	217.7333	30	22.85024	4.17186	0.001*
	Shoot height	Ethanol	176.0667	30	54.95009	10.03247	
Pair 5	Root length	Water	66.4000	30	9.72235	1.77505	0.837
	Root length	Ethanol	65.4333	30	22.55672	4.11828	
Pair 6	Shoot weight	Water	0.0478	30	0.01523	0.00278	0.017*
	Shoot weight	Ethanol	.0366	30	.01673	.00306	
Pair 7	Root weights	Water	.0272	30	.00879	.00161	0.649
	Root weights	Ethanol	.0285	30	.01184	.00216	

The next section discusses the results in relation to the relevant literature.

CHAPTER 5: DISCUSSION

This study was conducted to compare the effects of *Avena sativa*, *Gingko biloba* and *Withania somnifera* on the germination and growth of barley seeds (*Horduem vulgare*).

The ANOVA test indicated that *Avena sativa*, *Gingko biloba* and *Withania somnifera* have a significant effect on germination rate, leaf number, shoot height and shoot weight in the samples grown in water as a vehicle. There was a significant difference between the control and *Gingko biloba* as well as significant differences between the *Gingko biloba* and *Withania somnifera*, and *Avena sativa on germination percentage as parameter using ethanol as vehicle*.There was also significant differences between control ,*Gingko biloba* and *Withania somnifera* on stem diameter as parameter with ethanol as vehicle.

The objective was to determine the effect of *Withania somnifera(3X), Avena sativa (3X) and Gingko biloba (3X)* with deionised water as vehicle on the germination of barley seeds and seedling growth under the following parameters:

5.1 Seed germination

Results showed that *Avena sativa* had the highest effect on germination rate. There was a significant differe nce between *Avena sativa*, *Gingko biloba* and *Withania somnifera* relative to each other, indicating that *Avena sativa* and *Gingko biloba* have a significant positive effect on seed germination.

The effect of *Avena sativa* on germination of seeds or on plant growth has not been studied, hence there is no literature with which to compare the results of these research findings. However, avenalumins have been found to inhibit the plant disease called rust (Mayama *et al*. 1982). Grando and Macpherson (2008) state that leaf rust lowers kernel weight and yield, so this could be a possible explanation for the higher germination percentage found in the current study Thus, this phenomenon needs to be further investigated to confirm the current finding, and to assess the active ingredients within *Avena sativa* which may be responsible for this increase in the rate of germination.

Gingko biloba also had a poitive effect on germination, which was contrary to the findings of Zhao *et al.* (2014) that *Gingko biloba* strongly suppressed the growth of the radish stem and root as well as significantly inhibited cucumber seed germination. This finding suggests further research needs to be conducted.

Withania somnifera appeared to have the least effect on seed germination. Dhanani *et al.* (2017) explained that *Withania somnifera* comprises mainly steroidal lactones. These lactones have been been found to demonstrate an inhibitory effect on seed germination (Abdelgeil and Hashinaga 2007), which could explain the decrease in seed germination found in the current study. Chadwick *et al*. (2013) confirm that sesquiterpene lactones show an inhibition of growth in a vast number of plants. Spring (2013) demonstrated that *Withania somnifera* has an inhibitory effect on plant growth.

5.2 Number of leaves

Withania somnifera appeared to have the least number of leaves. No literature related to *Withania somnifera* and leaf numbers was found in the literature seasrch. *Gingko biloba*-treated barley seeds had the highest leaf number. However no literature is avaible to substantiate the finding. More literature is needed to support the effect of *Gingko biloba* on leaves. *Avena sativa* showed no discernible modification on leaf number. No literature on the effect of these three herbs on leaf number was found, thus more research is warranted.

5.3 Root length

Withania somnifera with ethanol as vehicle had the greatest root length. This is in contrast to research by Abdelgaleil and Hashinaga (2007) who found that lactones within *Withania somnifera* inhibit root length. Research by Fatima *et al.* (2009) confirmed that the methanol extracts of *Withania somnifera* decreased root length. In the current study, the greatest root lengths were found in the barley seeds treated with *Gingko biloba.* Çavuşoğlu and Karaferyeli (2015) found that *Gingko biloba* increased stomata on the upper surface and epidermal cells on the lower surface of barley seedling under saline circumstances, though there were no discernible variations between the various treatment. This suggests that *Gingko biloba* has the potential to grow the length of barley seeds. *Avena sativa* appeared to have no effect on root

length, but there was no literature available for *Avena sativa* on growth of rootlengh on barley seeds to compare this finding to.

5.4 Shoot height

Results indicated that *Withania somnifera* had the shortest shoot height with deionised water as a vehicle. Deionised water as control had greatest shoot height. Javaid, Shafique and Shafique (2011) tested *Withania somnifera's* ability to control the weed *Parthenium* and found that it inhibited shoot growth and root growth. *Gingko biloba* and *Avena sativa* did not exhibit any significant variation in height compared to the controls, and there is no literature to compare these results to.

5.5 Root weight

Avena sativa had the highest root weight for with both deionised water ethanol as a vehicle. No literature is available for *Avena sativa* on root weight. *Withania somnifera* had the lowest weight. No literature is available for the effect of *Withania somnifera* on the growth of barley .*Gingko biloba* had no effect on the root weight, and there is not literature to compare this result with.

5.6 Shoot weight

Gingko biloba had the thickest shoot and highest shoot weight using water as the vehicle. No literature of *Gingko biloba* was available on shoot weight. The lowest shoot weight was seen with *Withania somnifera*. Literature indicates that sesquiterpene lactones, costunolide and parthenolide within *Withania somnifera* significantly reduced shoot growth in wheat and radish plants (Abdelgaleil and Hashinaga 2007).

5.7 The comparison effect of *Avena sativa*, *Gingko biloba* and *Withania somnifera* with alcohol as vehicle verses deionised water as vehicle

5.7.1 Comparison between water and ethanol in the control treatment

Deionised water as a vehicle had a substantially higher germination percentage and shoot height than ethanol, while ethanol as a vehicle had a substantially greater stem diameter and root weight than water.There were no discernible variations for leaf number and shoot weight.

Chen *et al.* 2020 confirmed inhibition of plant growth by ethanol in terms of its effect on tomato seeds and found that stacked seeds germinated less than spread out seeds. Salehi, Ashiri and Salehi (2008) confirmed that exposure to alcohol for longer than 4 hours decreased germination. However, Afzal *et al. (*2013) demonstrated that doses of alcohol at 2% and 4% increased seed germination, contrary to the other findings. Neither of these studies investigated the effect of alcohol on plants.

Using distilled water as a control, Kleingeld (2016) examined the effects of varying potencies of radionically prepared GA_3 and homoeopathically prepared GA_3 on seed germination. The results revealed a suppressive effect on seed growth and development. Compared to all remedy treatment groups, the control group exhibited more seedling growth and visible roots.

This shows a need for a conceptualised understanding of using alcohol in seed germination, that alcohol does interact with the results on germination of seeds. No literature was available for the effect of the treatments using alcohol or water as vehicle on the effect of shoot height, stem diameter, root weight, and leaf number.

5.7.2 Comparison between water and ethanol in the *Gingko biloba* treatment

Water as a vehicle had a much higher germination percentage, leaf number, shoot height, shoot weight, and root weight than ethanol. There was no significant variation in stem diameter across treatments. No literature was found to compare to these findings on the effects of different vehicles for *Gingko biloba* in the treatment of barley seeds.

5.7.3 Comparison between water and ethanol in the *Withania somnifera* treatment

The measurements of the germination percentage, leaf number, root length, and root weight for ethanol as a vehicle were found to be much higher than those for water. The stem diameter, shoot height, and shoot weight did not show any appreciable variations. No literature was found to compare these findings on the effects of different vehicles for *Withania somnifera* in the treatment of barley seeds .

5.7.4 Comparison between water and ethanol in the *Avena sativa* treatment

In comparison to ethanol, it was discovered that water had much better germination rates, stem diameters, shoot weights, and shoot heights. In comparison to water, ethanol's root length was noticeably longer. The number of leaves, root length, and root weight showed no discernible changes. No literature was found to support findings on the comparison of the two vehicles and their effect on various parameters of barley seeds and plants

5.8 The two hypotheses of this research

The first hypothesis stated that *Withania somnifera (3X), Avena sativa (3X) and Gingko biloba (3X)* with deionised water as vehicle will perform better than treatment that used ethanol as vehicle. This hypothesis is accepted as there were significant difference in Avena sativa,Gingko biloba and Withania somnifers on the number of leaves,germination rate,shoot height and weighton samples grown on deionised water as vehicle.

The second hypothesis was that *Avena sativa (3X) with deionised water as vehicle will have the highest barley seed germination percentage*. This hypothesis has been accepted as *Avena sativa* in deionised water did increase the rate of germination of barley seeds.

The conclusion drawn from this research is that there were no significant differences in most of the parameters tested for the samples grown in ethanol as vehicle except germination percentage between *Gingko biloba* and control, as well as *Gingko biloba*, *Withania somnifera* and *Avena sativa* with stem diameter as parameter . Seed germination appeared to be the most studied parameter in literature amongst all treatments and *Avenas sativa* appeared to be the least studied in literature regarding plant growth. *Withania somnifera* phytochemicals appeared to be more reactive than the other treatments. There is a potential for *Avena sativa* and *Gingko biloba* in the germination of barley seeds.

5.9 Limitations of the study

- Due to the inhibitory nature of alcohol, two vehicles were utilised, namely deionised water and ethanol.

- This study did not include treatments prepared in saline water or rain water.
- The sample size of the seedlings from a testing point of view was limited to 10 per treatment due to difficulties with seed germination utilising the *Withana somnifera* in alcohol.
- This research only utilised vermiculite as a growing medium. A further study could be repeated using the hydroponic method of propogation.
- The growth was measured for only for 23 days.
- Other variable such as variances in sunlight, ambient temperature and humidity could not be controlled and may have had an effect on the subsequent results.

5.10 Conclusion

Based on the germination percentage, leaf number, stem diameter, shoot height and weight, root weight and total diameter results, it can be concluded that the overall best treatment that can benefit farmers was *Avena sativa* using deionizide water as a vehicle as it had highest germination percentage and highest root weight. Ethanol had an inhibitory effect when used as a vehicle for homoeopathic remedies so deionised water was the best growth vehicle. The results indicated that there is a potential for homoeopathy in agriculture, and more studies need to be conducted. Farmers can then produce higher, healthier plants while sustaining the environment.

5.11 Recommendations

- The research should be repeated in different potencies to investigate the effect of germination of seeds.
- Explore more agrohomoeopathy options using different potencies to increase the literature in this field.
- Future studies should consider using deionised water as the vehicle instead of comparing water and ethanol since ethanol on its own has an effect on the germination of seeds.
- The reseach can compare the use of saline water versus deionised water regarding the germination of seeds.

- Future studies should consider using grown plants (more than 28 weeks old) instead of planting the seeds from scratch; these will also be easier to work with compared to barley seedlings which tend to bend as they get taller.

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APPENDICES

Appendix A: Gate keeper permission letter

02/03/2022

21501569

Request for Permission to Conduct Research

Dear Professor Ross

My name is Zanele Hadebe, a Masters in homoeopathy student at the Durban University of Technology. The research I wish to conduct for Masters Dissertation; involves [The comparison of effect of *Withania somnifera*, *Avena sativa* and *Ginkgo biloba* on the germination of barley seeds.

I hereby seek your consent to conduct my experiment using facilities of your department. This include the homoeopathic laboratory in preparation of the treatment (3X decimal dilutions).

I have provided you with a copy of my proposal which will have information on what exactly I will be doing in the facility.

If you require any further information, please do not hesitate to contact me at 081457 2923 and email zaneletshegofatsho@gmail.com.

Thank you for your time and consideration in this matter.

Yours sincerely

Zanele Hadebe
Durban University of Technology

Appendix B: Gate keeper permission letter

02/03/2022
21501569

Request for Permission to Conduct Research

Dear Dr Matimati

My name is Zanele Hadebe, a Masters in homoeopathy student at the Durban University of Technology. The research I wish to conduct for Masters Dissertation; involves [The comparison of effect of *Withania somnifera*, *Avena sativa* and *Ginkgo biloba* on the germination of barley seeds.

I hereby seek your consent to conduct my experiment using facilities of your department. This include conducting the experiment on your premises (nursery) and using the department materials such as trays, cylinders and spatula.

I have provided you with a copy of my proposal which has further details of what my research is about and how will I be conducting this research.

If you require any further information, please do not hesitate to contact me at 081457 2923 and email zaneletshegofatsho@gmail.com.

Thank you for your time and consideration in this matter.

Yours sincerely

Zanele Hadebe
Durban University of Technology

Co-supervisor
Dr Lutendo Mugwedi

Appendix C: Remedy production

The treatments were made at the Durban University of Technology in the homoeopathic lab on lamina flow clean lab, prepared and supervised by the laboratory technician. The researcher had sanitised hands before entering the lab and had a hair net to prevent any contamination. The German Homoeopathic Pharmacopoeia (GHP) 4a specification was used to produce the homoeopathic treatments Avena sativa 3x,Gingko biloba 3x,Withania 3x and control per vehicle used (Bunyunes 2005) noted in appendix C.The research given the fact

Apparatus

1. ***Avena sativa*** HAB 1A Batch K21035
2. ***Gingko biloba*** HAB 3A Batch C19031
3. ***Withania somnifera*** 2:1 Batch 28151
4. Aqua purificata prepared in 4 step reverse osmosis on the 27th June 2022 (de-ionised water)
5. ROH 99%bsolute ethanol, manufactured Batch:EE757_2019/11 taken at the department of Chemistry at Durban university of Technology.
6. 30ml amber glass bottles
7. 100ml amber glass bottles
8. 10ml pipette
9. 50ml mc
10. Micropipette and tips
11. Measuring cylinder
12. 500ml amber glass bottle

3.2.1 Aim: To prepare *Avena sativa* 3x from mother tincture

Method

- Wash, clean and flame all surfaces and equipment as necessary.
- *Avena sativa* mother tincture is in a 1:10 ratio therefore it is considered as 1X.

1. To make *Avena sativa* 2X:

1/10 x 20ml = 2ml of *Avena sativa* 1X (By means of micro pipette)

20ml – 2ml = 18ml Aqua purificata (By means of 10ml pip)

In a 30ml amber glass bottle add 2ml of *Avena sativa* 1X and 18ml of Aqua purificata

Cap, seal, and success 10 times.

Label

2. To make *Avena sativa* 3X:

In Aqua purificata
1/10 x 70 = 7ml (10ml pip)
70 – 7 = 63ml aqua purificata (50ml M.C and 10ml pip)
In a 100ml amber glass bottle add the above
Cap, seal and success 10 times
Label

3.2.2To prepare *Avena sativa* 2x(with aqua purificata) to Avena sativa 3x (with 30%)

In 30% ROH:
1/10 x 70 = 7ml (10ml pip)
70 – 7 = 63ml aqua purificata (50ml M.C and 10ml pip)
In a 100ml amber glass bottle add the above
Cap, seal and success 10 times
Label

3.2.3To make *Gingko biloba* 2X:

Method

- Wash, clean and flame all surfaces and equipment as necessary.
- *Gingko biloba is a* mother tincture is in a 1:10 ratio therefore it is considered as 1X.

1. 1/10 x 20ml = 2ml of GB 1X (By means of micro pipette)

20ml – 2ml = 18ml Aqua purificata (By means of 10ml pip)

In a 30ml amber glass bottle add 2ml of *Gingko biloba* 1X and 18ml of Aqua purificata.

Cap, seal and success 10 times.

Label

2. To make *Gingko biloba* 3X:
In Aqua purificata
1/10 x 70 = 7ml (10ml pipette) of *Gingko biloba* 2X
70 – 7 = 63ml aqua purificata (50ml measuring cylinder and 10ml pipette)
In a 100ml amber glass bottle add the above
Cap, seal and success 10 times
Labelled

3.2.4 To make Gingko biloba 3X(30%) from Gingko biloba 2x(with aqua purificata)

1. In 30% ROH:
1/10 x 70 = 7ml (10ml pipette)
70 – 7 = 63ml aqua purificata (50ml measuring cylinder and 10ml pipette)
In a 100ml amber glass bottle add the above
Cap, seal and success 10 times
Label

3.2.5 To make *Withania somnifera* 1:1: from *Withania somnifera* 2:1

Method
1.Add 10 ml of aqua pur to 10ml of *Withania somnifera* in a 30ml AGB. DO NOT SUCCUSSED

3.2.6 To make *Withania somnifera* 1X

1/10 x 20ml = 2ml of ***Withania somnifera*** 1:1 (By means of micro pipette)

20ml – 2ml = 18ml Aqua purificata (By means of 10ml pip)

In a 30ml amber glass bottle add 2ml of ***Withania somnifera*** 1X and 18ml of Aqua purificata.

Cap, seal, and success 10 times.

Label

3. **To make *Withania somnifera* 2X**:

1/10 x 20ml = 2ml of ***Withania somnifera*** 1X (By means of micro pipette)

20ml – 2ml = 18ml Aqua purificata (By means of 10ml pip)

In a 30ml amber glass bottle add 2ml of *Withania somnifera* 1X and 18ml of Aqua purificata.

Cap, seal and success 10 times.

Label

4. **To make <u>Withania somnifera</u> 3X**:

In Aqua purificata
1/10 x 70 = 7ml (10ml pippete)of Withania somnifera 2X
70 – 7 = 63ml aqua purificata (50ml M.C and 10ml pip)
In a 100ml amber glass bottle add the above
Cap, seal and success 10 times
Label

<u>To prepare withania somnifera 3x(30%) using *Withania somifera* 2x (aqua purificata)</u>

Method

1. In 30% ROH:
1/10 x 70 = 7ml (10ml pipette)
70 – 7 = 63ml aqua purificata (50ml measuring cylinder and 10ml pipette)
In a 100ml amber glass bottle add the above
Cap, seal and success 10 times
Labelled

The tictures to prepare decimal dilutions were bought from the Department of Homoeopathy which was supplied by Fusion.The mother tinctures were in different classes and strengths mainly-:

1. ***Avena sativa*** HAB 1A Batch K21035(Class 1A)
2. ***Gingko biloba*** HAB 3A Batch C19031(Class 3A)
3. ***Withania somnifera*** 2:1 Batch 28151

Appendix D: Excel spreadsheet of data collection

Appendix E: How classes or remedies are prepared

How classes are prepared

Mother tinctures depend on how much water a plant contains, Hahnemann divided plants into various groups. Classification of plants into four categories: class one includes plants with the highest water content, class two includes plants that are mediumly juicy, class three includes plants with the lowest water content, and class four includes dry plants, herbs, and dried or fresh animal products (Hahnemann 2002).

In order to extract the excess water content or juice from the fresh plant material, the plant is broken up and covered in a piece of fabric. A precise amount of this juice is combined with an equal amount of alcohol before being bottled. After standing for eight days in a cold, dark environment, this combination is filtered (Banerjee 2002).

For class three plants, tinctures are created by weighing out one part plant material to two parts alcohol (Kumar 2014). Further Kumar (2014) says that the plant is weighted after being finely pulped, two parts of alcohol that were weighed are used to mix and wet the pulp before the remaining two parts of alcohol are added. For eight days, the mixture must stand in a cold, dark place after being mixed and bottled. The tincture or combination is then filtered and strained after this.

One part plant material to five parts alcohol, measured by weight, is how class four plant-sourced mother tinctures are made. Five parts of alcohol are measured out and placed on top of the crushed plant material after one part of the plant material is weighed. After that, this plant and alcohol mixture is let to sit in a glass bottle for eight days in a dark place with room temperature. The mixture is shaken twice a day for the duration of this time. The combination is filtered and strained at the end of the eight-day period (Banerjee 2002).

Appendix F: Editing certificate

DR RICHARD STEELE
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EDITING CERTIFICATE

Re: **Zanele Hadebe**
Master's dissertation DUT: **THE COMPARISON EFFECT OF *AVENA SATIVA*, *GINGKO BILOBA* AND *WITHANIA SOMNIFERA* ON GERMINATION OF BARLEY SEEDS (*HORDEOM VULGARE*)**

I confirm that I have edited this dissertation and the references for clarity, language and layout. I returned the document to the author with track changes so correct implementation of the changes and clarifications requested in the text and references is the responsibility of the author. The intellectual content of the document is the responsibility of the author. I am a freelance editor specialising in proofreading and editing academic documents. My original tertiary degree which I obtained at the University of Cape Town was a B.A. with English as a major and I went on to complete an H.D.E. (P.G.) Sec. with English as my teaching subject. I was a part-time lecturer in the Department of Homoeopathy at the Durban University of Technology for 13 years and supervised many master's degree dissertations during that period.

Dr Richard Steele
22 June 2023
per email