SEED GERMINATION AND SEEDLING DEVELOPMENT OF *JATROPHA ZEYHERI*: AN INDIGENOUS TEA PLANT IN LIMPOPO PROVINCE OF SOUTH AFRICA

BERTHA MOGALEADI LEHLOKOA

A MINI-DISSERTATION SUBMITTED FOR THE DEGREE OF MASTER OF SCIENCE IN HORTICULTURE IN THE DEPARTMENT OF PLANT PRODUCTION, SOIL SCIENCE AND AGRICULTURAL ENGINEERING, SCHOOL OF AGRICULTURE AND ENVIRONMENTAL SCIENCES, FACULTY OF SCIENCE AND AGRICULTURE, UNIVERSITY OF LIMPOPO, SOUTH AFRICA

SUPERVISOR	:	DR M.Y. MAILA
CO-SUPERVISOR	:	DR K.G. SHADUNG

2022

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DECLARATION

I, Bertha Mogaleadi Lehlokoa, declare that the mini-dissertation hereby submitted to the University of Limpopo, for the degree of Master of Science in Horticulture has not been submitted previously by me or anybody for a degree at this or any other University. Also, this is my work in design and in execution, and related materials contained herein had been duly acknowledged.

Candidate: Bertha Mogaleadi Lehlokoa Date

DEDICATION

To my lovely mother Ms S.M Lehlokoa and my beautiful sisters Mmagauta, Lerato and Mantsha.

ACKNOWLEDGEMENTS

First of all, I would like to express my gratitude and appreciation to God Almighty for His love, guidance, courage wisdom and most importantly strength and for seeing me through all the challenging and happy times during the process of completing this research study. I strongly believe in my heart that this is part of His purpose for my life, for me to gather the knowledge that I have gathered and to work with all the amazing and kind people who helped me throughout my research journey.

Secondly, I would like to extend my special appreciation to my supervisory team Dr M.Y. Maila and Dr K.G. Shadung, for providing me with guidance, patience, encouragement and support throughout the duration of my research study until completion. I would also like to send my deepest thanks to the Limpopo Agro-Food Technology Station management and team for allowing me to use their laboratory to conduct a portion of my research as well as the Green Biotechnologies Research Center of Excellence (GBRCE) management and team. Sincere gratitude to Prof P.W. Mashela for giving me the opportunity to use the facility and all the study consumables used in my experiments. Not forgetting Mr N. Mutshekwa for his technical advises, Mrs L.F. Malatjie and Miss M.J. Ramputla for guiding me on how to use all the tools required during the process of data collection. Thanks again to Miss M.K. Sekhukhune and Mr H. Bango for assisting me with the statistical analysis.

My sincere gratitude again is directed to the University of Limpopo for the enrolment of my Master's degree, National Research Foundation (NRF) and Agricultural Sector Education and Training Authority (AgriSETA) for funding various aspects of my study. Thanks to the Faculty of Science and Agriculture for sponsoring my conference trip to Bolivia lodge in 2021 for the presentation of the study findings. Lastly, I would also like to pass my heartfelt gratitude to my family, they have offered their love and support throughout the period of my study and they are the greatest pillar of strength. I pray that we continue to grow and support each other as we and celebrate more achievements together.

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ABSTRACT

Indigenous teas are receiving much attention and global interest as a result of their nutraceutical and pharmaceutical benefits. Most of these tea plants are still collected from the wild and their domestication has become a necessity as a result of the rapid growth of the tea industry and increased global demands. However, like other indigenous plants, setbacks exist in the successful seed germination of wild tea plants on account of seed parasitism, low seed viability andgermination difficulties A study was undertaken to: (a) determine the effect of pre- treatments of *Jatropha zeyheri* seeds grown under laboratory conditions and (b) to evaluate growth and development of *J. zeyheri* seedlings under controlled conditions on different growth media. In the initial objective, matured seeds sourced from the wild were subjected to five (5) pre-treatments methods namely, clipping, piercing, de-coating, cold and hot water soaking. The sixth (6) untreated dry intact seeds served as reference. Treatments were organised in a completely randomised design (CRD) with 10 replications. Treated and untreated seeds were all placed on moistened filter-papers inside petri-dishes and incubated in a growing chamber at 27°C. At 21 days, data on mean germination time (MGT), germination rate (GR), germination index (GI) and germination percentage (GP) were collected and calculated, prior to analysis of variance (ANOVA). Fisher' Least Significant Difference test was used to separate the means at 5% level of probability. In the subsequent objective, under greenhouse conditions, six (6) treatments namely, sand, sand + loam, loam + compost, loam + Hygromix, sand + compost, sand + Hygromix were used, while sand alone served as the reference medium. The treatments were arranged in a randomised complete block design (RCBD), replicated 10 times. Seeds were de-coated first and each seed was planted in 10 cm plastic pot, filled with the various growing media-mixtures at a depth of 2 cm and irrigated to field capacity. Data collected included seedling performance parameters, namely, MGT, GR, GI and GP as well as plant parameters namely, stem diameter (SD), chlorophyll content (CC), seedling length (SL), number of shoots (NS), dry shoot mass (DSM) and dry root mass (DRM) and were subjected to ANOVA. Fisher' Least Significant Difference test was used to separate the means at 5% level of probability. Germination occurred at the reference and seeds treated with clipping and de-coating methods. Pierced, cold and hot-water soaked seeds failed to germinate. Treatments had significant ($P \leq 0.05$) effect on MGT, GP, GI and GR, contributing 57, 55, 51 and 55% to total treatment variation (TTV) in the respective

variables. When compared with the intact seeds, MGT for the clipped seeds was at 3 days, whereas in the de-coated seeds it was in 7 days. The clipped seeds achieved 11% GP and 0.29 GI at a GR of 0.05. Noticeably, the de-coated seeds achieved highest (16%) GP and 0.3 GI at a GR of 0.08 when compared to the reference seeds. Treatments had significant (P≤0.05) effect on MGT, GP and GR of *J. zeyheri* seedlings, whereas no significant effects were observed on GI. Treatments contributed 25, 21, 9 and 22% to TTV in the respective variables. Relative to the reference medium, MGT for the de-coated *J. zeyheri* seeds sown on sand + compost occurred at 2 days, whereas emergence in sand + loam occurred at 7 days. The longest MGT (26 days) was observed in sand + Hygromix growing media. Germination percentage and GR for seeds sown on sand + compost was 1% and 1.1, whereas for seeds sown on sand + loam was 2% and 3.33E-03 and in seeds sown on sand + Hygromix, it was at 4% and 4.44E-03, respectively, when compared to the reference. In terms of plant parameters, treatments had no significant (P≥0.05) effect on SD, CC, SL, NS, DSM and DRM of *J. zeyheri* plantlets. In conclusion, the de-coating pre- treatment proved to be a better method for improving germination in seeds of *J. zeyheri* tea plant and the same de-coated seeds sown on sand + Hygromix achieved better emergence and development, proving that the growing media used was suitable to provide optimum germination and seedling development.

Keywords: Beverage, bush tea, cultivation, food security, seedling performance, ‘Sefapabadia’

CHAPTER 1
RESEARCH PROBLEM

1.1 Background

In most local rural communities indigenous tea plants are relied upon for medicine, food and beverages preparation (Joubert *et al*., 2011). Many indigenous tea plants are collected as wild species in their natural habitats and are only found in small quantities due to deforestation (Yineger *et al*., 2008). Indigenous tea plant species and medicinal plants as well as species with newly discovered value should be promoted for commercialisation by agri-enterpreneurs as they have great potential for establishing regional business incubators and in so doing, creating wealth, jobs and alleviating poverty in rural households (Van Wyk, 2015). With different species of indigenous tea plants concentrated in the rural, semi-rural and peri- urban environments, this creates an opportunity for exploitation as sources of economic development in such communities (Makunga *et al*., 2008).

Due to the increasing rural population of South Africa, the demand for plants with pharmaceutical and nutraceutical properties is increasing and sustainable harvesting techniques can no longer meet these needs (Mander *et al*., 2007). Consequently, exploitation of popular indigenous plants is increasing which necessitates their domestication. Therefore, suitable domestication techniques are required for species conservation and establishment of large production facilities or nurseries of important tea plants (Leakey, 2019).

The establishment of wild tea plants can be either through seeds or vegetative propagation in the form of cuttings (Hartmann *et al*., 1990). Seed germination and seedling establishment are the most significant stages to be taken into consideration, as they affect both quality and quantity of crop yields. The most important measure in the establishment of tea plants from seeds is their seed germinability. The latter poses serious challenges in the proper domestication and seedling establishment of indigenous tea plants, consequently many indigenous tea plant species go extinct because of poor germination (Hamilton, 2004). Seed germination is a complicated processbecause it consists of numerous biochemicals, physiological and morphological changes within a seed (Graeber *et al*., 2014). Seedling development takes place from continued cell division in different growing points of the embryo,

which is subsequently followed by the expansion of the seedling structures (Linkies *et al*., 2010).

1.1.1 Description of the research problem

In order for germination to take place, seeds must be viable, but viable seeds may not germinate (Philippi, 1993), it is suggested that the poor germination of indigenous plants could be a result of seed dormancy (Bareke, 2018). Seeds of many indigenous plants often appear to germinate poorly. The failure to break seed dormancy successfully can result in conclusions that seeds of indigenous plants are inherently poor germinators (Kildisheva *et al*., 2019).

1.1.2 Impact of the research problem

Many plants express seed dormancy as a form of survival strategy, which allows them to survive in harsh or extreme climates. The term dormancy is used to describe a seed that fails to germinate under favourable conditions at a specific time. Dormancy in indigenous seeds serves as a benefit for seed dispersal as a result ofseasonal changes in different parts of the world (Baskin and Baskin, 1998). Accessing the part of the seed from which dormancy can be classified, exogenous and endogenous dormancy are the two types of dormancy recognised. Exogenous dormancy is a result of the seed-coat on the matured embryo and it is caused when the seed-coat is extremely hard, resulting in restrictions in the free expansion of the embryo for protrusion of the plumule and radicle during germination. Impermeable seed-coats and other layers prevent seeds from water uptake and gaseous absorption consequently preventing seed germination (Li and Foley, 1997). High concentration of growth inhibitors such as abscisic acid on the seed coat and its surrounding layers can retard embryo growth. Endogenous dormancy is also known as embryo dormancy, which result from some conditions embedded in the embryo such as the presence of growth inhibitors and absence of growth promoters. The dormancy will prevent embryo growth and seed germination until chemical change takes place (Finch-Savage and Leubner-Metzger, 2006).

1.1.3 Possible causes of the research problem

The problem with poor seed germination and seedling establishment may be a result of the inability of the seed to germinate even when conditions are favourable for the

germination process (Buijs, 2020). Dormancy in indigenous plants can be imposed by physical or physiological mechanisms such as fruit, impermeable seed-coat or embryo dormancy, and in some cases it can be a combination of both (Baskin and Baskin, 2004). Several studies have shown that seed predators, particularly herbivores and granivores have the potential to significantly reduce seed germination (Bricker *et al*., 2010). Problems with poor seedling emergence and establishment may also be a result of allopathic effects from neighbouring plants, physical hazard like desiccation and herbivory (Terborgh *et al*., 2006).

1.1.4 Possible solutions of the research problem

In order to overcome the challenges of poor germination when propagating indigenous plant species from seeds, and alleviate unpredictable and erratic nature of indigenous seed germination, which is undesirable for commercial growers (Basey *et al*., 2015), different seed treatment methods can be applied on seeds with poor germination (Goussous *et al*., 2010). Treatments such as hot water soak and cracking of the seed coat have been performed to improve the germination process and enhance seedling establishment and development of many crops more especially wild species (Taylor *et al*., 1998). Suitable growing media with suitable conditions such as temperature, moisture retention, air movement and absence of soil predators can also be determined for different crops as different seeds of various crops require different growing media for their seeds to germinate and for seedlings to develop well.

1.1.5 General focus of the study

The study was conducted to determine the suitable seed treatment technique(s) and growing media that will enhance seed germination and promote germination rate and seedling development of *Jatropha zeyheri* seeds.

1.2 Problem statement

Indigenous teas have recently received attention and had attained a global interest as a result of their nutritional and medicinal benefits. Continuous deforestation/depletion of woodlands/forests is threatening the availability and supply of these teas. (Hishe *et al*., 2016). However, seed propagation of many indigenous tea plants is hampered by various factors such as seed parasitism, low seed viability, low germination rate and

failure to produce true-to-type progeny bearing identical characteristics of the parent seed bearer.

In its natural habitat *J. zeyheri* grows suitably in sandy soils and in wooded grasslands and scattered shrubs. Also, *J. zeyheri* plant is documented to germinate from mature seed, but freshly harvested seeds express dormancy challenges and complete ripening is essential before sowing. *Jatropha zeyheri* seeds are recognised by low germination rates and viability (Devappa *et al*., 2011). The main problem in the cultivation of *Jatropha* is poor germination as a result of water impermeable seed coats (Islam *et al*., 2009). Germination in such cases depends upon rotting or abrasion of the seed coat in the soil to weaken the seed coat.

1.3 Rationale or motivation

Jatropha zeyheri is an indigenous tea plant used by local rural communities in some parts of Limpopo province for medicine, food and for making beverages. Medicinal plants have been a basic source of antibiotics against a wide range of illnesses over the years in most communities. South African medicinal plant extracts have been screened for antimicrobial activities (Samie *et al*., 2010). Pharmacologically, extractions from roots and leaves of *J. zeyheri* significantly containsecondary metabolites which have antimicrobial, anti-inflammatory and mutagenic properties (Mongalo *et al*., 2019). The plant has been found to exhibit anti-bacterial activity against 11 human pathogenic bacterial strains (Mongalo, 2014).

The roots of *J. zeyheri* plant are used by traditional practitioners for the treatment of sexually transmitted and urinary tract infections. In women's healthcare, the plant is used as a blood purifier to promote fertility and ensure a strong foetus during pregnancy. The rhizomes are also used to heal wounds and boils (Van Wyk and Gericke, 2007). Furthermore, the plant is used by small-scale farmers for the treatment of wounds and retained placenta in livestock. The roots are pounded together with those of *Pterocarpus angolensis* (kiaat), soaked in water and then administered to the animal (Hoveka, 2016). Therefore, finding the effective dormancy breaking mechanism to stimulate the germination of *J. zeyheri* seeds as well as the best growing media for seedling development will help growers and horticulturists to domesticate and grow the tea plant successfully. Findings will restore the availability of this useful

plant and limit the destruction and unsustainable wild collection of tea plants as well as increasing domestication and eliminating their extinction. Besides, it will allow the possibility for selection and certification of high-quality seeds in order to make accessibility of seeds with vigorous seedling establishment and development for growers in South Africa. As a result, crop commercialisation, economic development and job creation within rural South Africa will be enhanced.

1.4 Purpose of the study

1.4.1 Aim

The study is aimed at the domestication of *J. zeyheri* indigenous tea plant through minimising the challenges ofseed germination and seedling development for future cultivation and species conservation.

1.4.2 Objectives

The objectives of the study are:

a. to evaluate different seed treatments on *J. zeyheri* seeds under controlled conditions.

b. to evaluate growth and development of *J. zeyheri* seedlings under controlled conditions on different growth media.

1.4.3 Hypotheses

a. Germination of *J. zeyheri* seeds under controlled conditions cannot be stimulated though dormancy breaking mechanisms.

b. Growth and development of *J. zeyheri* seedlings under controlled conditions cannot be achieved on different growth media.

1.5 Reliability, validity and objectivity

The reliability of data was based on statistical analysis of data at the probability level of 5%, objectivity was achieved by ensuring that the findings were discussed on the basis of empirical evidence, in order to eliminate all forms of subjectivity (Leedy and Ormrod, 2005).

1.6 Bias

Bias is defined as any influence, conditions or set of conditions that singly or altogether distort the data (Leedy and Ormrod, 2005). In this study, bias was decreased by certifying that the experimental error in each experiment was minimised through increased replication and randomisation (Little and Hills, 1981).

1.7 Scientific significance of the study

The findings obtained from this study will assist local tea consumers, growers, and producers around South Africa with scientific information on effective dormancy breaking mechanisms and suitable growing media mixtures for proper seed germination and seedling development of *J. zeyheri*. The success of the study will result in a greater and improved production of tea plant without limitations such as poor germination and seedling development. Furthermore, data from this study will provide empirical information that will support the tea industry in the promotion of cultivation of *J. zeyheri* indigenous tea.

1.8 Structure of the mini-dissertation

Following the description and thorough outlining of the research problem (Chapter 1), work done and the related gaps on the research problem were reviewed (Chapter 2). Each of the two objectives would constitute separate chapters (Chapter 3 and 4). In the final chapter (Chapter 5), findings from all chapters would be summarised and combined to give the significance of the findings and recommendations regarding future research and then close with in an overall conclusion of the study. The citation and references followed the Harvard style of author-alphabet as approved by the Senate of the University of Limpopo.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Seed propagation of many indigenous tea plants is hampered by various factors such as mechanical damage, seed parasitism, failure to produce true-to-type progeny bearing identical characteristics of the parent seed bearer, low seed viability and germination rates (Jose, 2011). Seeds of most indigenous tea plants germinate poorly due to seed dormancy and the knowledge on their dormancy-breaking and germination requirement in the literature is limited (Baskin *et al*., 2004). When studying the germination of indigenous tea plants, it is significant to reflect on the timing of germination in nature which includes dormancy-breaking and germination requirements of indigenous seeds (Tahaei *et al*., 2016). Indigenous tea seeds such as the honeybush (*Cyclopia vent*) exhibit a form of physical dormancy, thus necessitating scarification measures to improve germination (Motsa *et al*., 2017a). Understanding seed dormancy in indigenous tea plants will enablethe implementation of measures to improve germination and subsequent cultivation, and permit rehabilitation of degraded areas where indigenous tea plants occur in the wild (Motsa *et al*., 2017b).

Jatropha zeyheri is an indigenous plant in the Euphorbiaceae family. It is a perennial herb, characterised by its dense and hairy appearance, which grows up to 300 mm in height and has a simple branched stem and thick rootstock (Mathibela, 2013). *Jatropha zeyheri* plant is used in women's health care. Fresh tubers are pounded and mixed with porridge to regulate menstrual cycles, ease uterine pain and treat urinary tract infections. The plant is also used as a general blood purifier to promote fertility and ensure a strong foetus during pregnancy (Van Wyk and Gericke, 2007). The rhizomes are also used to heal wounds and boils. However, seed dormancy and germination are serious challenges in *J. zeyheri* since it is an indigenous plant. Most indigenous plant species possess germination inabilities unless certain treatments are applied to the dormant seeds to release seeds from dormancy (Płażek *et al*., 2018).

2.2 Work done on the research problem

2.2.1. Indigenous tea plants

A great number of various plants that are indigenous to South Africa are famous traditionally for making tea beverages (Van Wyk and Gorelik, 2017). Rooibos

(*Aspalathus linearis*) is the most renowned (Joubert *et al*., 2008), honeybush tea (*Cyclopia* species) has also gained popularity in recent years (Joubert *et al*., 2011) and bush tea (*Athrixia pylicoides*) boasts an equally impressive history of use by indigenous people of South Africa (Araya, 2007). Other indigenous tea plants include buchu (*Agathosma beulina* and *A. crenata*), hoodia (*Hoodia gordonii*) (Van Wyk, 2011), Sutherlandia or cancer bush (*Lessertia frutescens*) and sceletium (*Mesembryanthemum tortuosum*) (Van Wyk, 2015). The regionalisation and globalisation of natural products and the introduction of new drinks such as African teas to other countries and even regions in Africa can make new indigenous teas available to a wide range of new consumers (Juliani *et al*., 2009).

2.2.2 Medicinal benefits of indigenous tea plants

The traditional uses of indigenous herbal infusions in tropical Africa are recorded in various literatures (Madikizela and McGaw, 2017, Maroyi, 2013, Hayat *et al*., 2015). The use of these herbal infusions was not only limited to their use as tea but also as health beverages based on their health and medicinal properties (Erhabor *et al*., 2020). Indigenous tea plants have been used for centuries because of their nutritional and medicinal properties (Erukainure *et al*., 2020). Indigenous teas contain polyphenols which have antioxidant activities and prevent oxidation of low-density lipoprotein that may lead to high cholesterol levels (Liu *et al*., 2000). Other medicinal benefits linked to tea are their potential effects against oxidative stress. Oxidative stress has been implicated in diseases such as cancer, diabetes and Alzheimer's (Darvesh *et al*., 2010). Antioxidants in plants are good defence mechanisms for scavenging carcinogenic free radicals (Oyebode *et al*., 2019). Indigenous teas also treat colds, cough, fever, wounds, diarrhoea, chest pains, asthma, headaches, body itches and pains, swollen feet and legs, high blood pressure, tuberculosis and they are also used as anti-inflammatory, anti-malaria and anti-bacterial medicine (Jonville *et al*., 2011, Freese *et al.,* 2015, Hayat *et al*., 2015,).

Generally, tea contains polyphenols, alkaloids, amino acids, proteins, volatile compounds, minerals, trace elements and many other phyto-pharmaceuticals (Aderogba *et al*., 2013). According to Kromhout *et al*. (2016), drinking three (3) cups of black and green teas per day reduces the risk of stroke and high blood pressure.

The essential oils in tea leaves are well-known for their antioxidant properties (Senanayake, 2013). In a study by Matsabisa *et al.* (2022) on South African indigenous teas, it was identified that indigenous tea plants exhibited outstanding radical scavenging activity in the various antioxidant essays performed. The antioxidant activity of the tea plants may be attributed to the existence of the superior polyphenols and flavanols found in indigenous tea plants.

Most consumers now make a preference for caffeine-free over caffeine beverages, because of its potential harm especially in babies (Tsao, 2010). Caffeine at concentrations of more than 400 mg per day may cause insomnia, nervousness and restlessness, stomach irritation, nausea and vomiting, increased heart rate and respiration as well as other associated side effects (Echeverri *et al*., 2010). Therefore, consumer consciousness toward caffeine–free health drinks is increasing (Nawrot *et al*., 2003). In the tea plants such as zinibar (*Lippia javanica*), haw haw (*Phyla dulcis*) and mosukujane (*Lippia scaberrimma*) assessed by Matsabisa *et al*. (2022), no caffeine was found. According to Joubert *et al*. (2008), South African tea consumers have a shifting preference to consuming indigenous or local tea. Therefore, the economic importance of the indigenous tea plants is growing continously.

2.2.3 Nutritional benefits of indigenous tea plants

Teas made from indigenous plants are rich in compounds and could play an important role in supplying nutrients and chemicals to compensate for low quality diets (Poswal *et al*., 2019). According to Klepacka *et al*. (2021), the importance of tea beverages in daily diet is mainly associated with their valuable mineral ingredients, most of all elements such as calcium, sodium, potassium, magnesium, and manganese. In a study by Matsabisa *et al*. (2022), it was found that indigenous tea plants contain energy levels ranging from 26-38 kg/100g. The energy levels on indigenous tea plants indicated that they are low calorie beverages when compared with modern beverages. Indigenous tea plants with low calories may be good for health-conscious tea drinkers and other health-conscious persons (Sikalidis *et al*., 2020). The low calorie beverages are potentially good for diabetics and health-conscious consumers (Pandey and Chauhan, 2019). Nakano *et al.* (1997), showed that it is probable that acid polysaccharides from rooibos tea were extremely safe and human immunodeficiency

virus (HIV) infection may be supressed by daily intake of alkaline extracts of rooibos tea.

2.3 Seed propagation and the development of indigenous tea plants

2.3.1 Seed germination of indigenous tea plants

Indigenous tea plants are propagated through seeds or vegetatively. However, the seed is the most preferred method of propagation for growers because propagation through cuttings is more expensive and labour intensive (Chiipanthenga *et al*., 2012). Seeds of indigenous tea plants are often harvested in the wild, however, natural populations of indigenous tea plants are very limited in indigenous habitats as a consequence of poor seed germination (Kalinganire *et al*., 2007). The hindrance in germination of indigenous tea seeds delays their propagation as a result of the effects of seed dormancy, leading to poor growth potential (Motsa *et al*., 2017a). A major problem with the use of indigenous tea plants inhibiting sustainable use, is the potential to germinate within indigenous environments, and failure to germinate under laboratory conditions or when cultivated (Nadjafi *et al*., 2006). The thriving establishment of newly germinated seedlings into young plants is dependent upon synchronizing the timing of germination with the growing season, where sufficient moisture and suitable temperatures are available (Ferreira *et al*., 2005.).

Honeybush seed does not have a good germination rate even under environmental conditions considered favourable for germination and therefore acknowledged to possess one or more forms of dormancy (Baskin and Baskin, 2004). The condition has been known to affect germination and may sometimes be an indication of divergent survival strategies (Baskin and Baskin, 2014). According to Koen *et al*. (2017) germination rate of honeybush seeds may be as low as 15%, or even less when not pre-treated. The age and species of the seed as well as the source have an effect on the germination of honeybush (Mbangcolo, 2008). In addition to seed dormancy, the germination of *C. sinensis* (L.) is affected by somatic embryogenesis, where somatic embryos of tea appear to exhibit desiccation sensitivity and fail to accumulate storage reserves which results in problems such as abnormal development, poor or abnormal germination as well as low frequency of normal conversions into seedling (Finneseth *et al*., 1996).

2.3.2. Seed dormancy in indigenous tea plants

Seed dormancy is an adaptive characteristic that enhance the distribution of germination over time in a population of seeds. It is part of the trait needed for the growth of plant seed and its survival (Stevens *et al*., 2020). Non-germination as a result of adverse conditions is regarded as “quiescence” and allows seed survival for further seedling development under harsh or extreme climatic conditions (Gomaa and Picó, 2011). Seed dormancy is regulated by genotypic and environmental factors during three stages in the persistent soil seed bank such as seed development, after-ripening and seed germination. During seed development some reserves are accumulated in seeds (Finch-Savage and Leubner-Metzger, 2006). During the process of after-ripening, seeds especially orthodox seeds have the ability to survive desiccation. The seed germination stands for mobilization of reserves under favorable conditions (primary dormancy) or non-dormant. The favorable conditions during after-ripening lead to the release of primary dormancy and seeds become non-dormant (Bareke, 2018).

Previous studies conducted by Bhattacharya *et al.* (2002) and Chandel *et al.* (1990), on *Camellia sinensis* (L.) tea seeds indicated that, several inferences can be made about the existence or absence of dormancy. For instance, Song *et al*. (2017) indicated that newly collected tea seeds at the natural dispersal time failed to germinate even after 60 days clearly revealing that the seeds were dormant. On the other hand, *Cyclopia* species indicate a form of physical and/or embryo-imposed (physiological) dormancy and will not germinate unless scarified. According to Sutcliffe and Whitehead, (1995), the physiological dormancy in *Cyclopia* species is attributed to organic substances present in the seeds.

Dormancy is necessary in the wild, where plants only rely on nature for survival. Seeds of indigenous plant species germinate poorly as a result of poor viability, but usually occur as a result of seed dormancy (Bareke, 2018). This can include embryo dormancy caused by immature embryos, physical dormancy as a result of seed-coat impermeability to water, inhabitation in the fruit or requirement for factors such as light (Rowart *et al*., 2007) or chilling. Some indigenous seeds may have a double or combinational dormancy, where both seed-coat and embryo dormancy are found in the seeds (Baskin *et al*., 2004).

Embryo dormancy: occurs as a result of morphology of underdeveloped embryos associated with a physiological component (morphophysiological dormancy) (Baskin and Baskin, 2014), or by the physiological responses of developed embryos to biochemical and physiological mechanisms that inhibits the event of germination (Bazin *et al*., 2011). The inability of embryo to differentiate may be a result of immaturity, however, with mature embryos, may be caused by metabolic impediment to elongation (Garbern and Lee, 2021). Seeds of certain species with small embryos complete the morphogenesis phase and acquire a completely differentiated embryo with regard to structure but do not undergo maturation (Gómez *et al*., 2006). Such seeds regularly hold proportionally great quantities of endosperm that sometimes completely gird a small embryo. In these seeds, embryos grow after scattering for germination, and dormancy is morphological (Hilhorst *et al.*, 2007). However, in other species, the embryo is mature at the moment of dispersion, but physiologic mechanisms cause dormancy not only as a result of the embryo but also by adjacent structures such as the endosperm (Hilhorst *et al*., 2007).

Physical dormancy: is imposed upon the seed from factors external to the embryo including the outer seed-coat or parts of the fruit covering (Hartmann *et al*., 2011). Physical dormancy precisely exists in hard seeds due to the hard seed-coat, which inherently hinders water and gaseous exchanges (Long *et al*., 2015). that are indispensable for kick-starting the germination process (Ferreira and Vieira, 2017). Physically dormant seeds have an outer seed or fruit cell layer that consists of macrosclereid cells. The existence of macrosclereid cells from the outer cell layer in the seed-coat or fruit wall in physically dormant seeds are accountable for inhibiting water uptake (Hilhorst *et al*., 2007). Sclereids are distinguished by the substantial secondary wall development and are normally non-living at maturity. During the later stages of seed development, cells of the outer integument coalesce and accumulate water-repellent materials within and on the surface of the macrosclereid cells (Singh, *et al*., 2012). This seals the seed and makes it impervious to water. These materials include lignin, suberin, and cutin (Ranathunge *et al*., 2011).

Combinational dormancy: occurs in seeds that have both physical and physiological dormancy. The combination of a hard seed-coat and a dormant embryo is interpreted

as a double safety mechanism to prevent early germination after out-of-season rains in arid regions and Mediterranean climates (Renzi *et al*., 2014). Combinational dormancy is present when the seed-coat induces physical dormancy, but the de-coated seed still cannot germinate until additional treatments such as stratification are applied (da Silveira Falavigna *et al.*, 2021).

Previous studies have proven that seeds of *C. sinensis* (L.) exhibit some form of dormancy (Song *et al.*, 2017). Since seeds were permeable to water and their weight increased to 97% within three days, dormancy was not a result of an impermeable seed-coat. Also, studies showed that after treatment of cracked *C. sinensis* seeds with gibberellic acid (GA_3), germination took place within a week, validating that embryo were totally developed and seeds had no morphological dormancy (Jaganathan *et al*., 2021). Cold stratification also enhanced germination remarkably. Collectively, all these outcomes indicated the presence of physiological dormancy in tea seeds. However, results from Bhattacharya *et al.* (2002) concluded that the tea seeds are non-dormant as germination took place within a week after sowing. *Cyclopia* species indicate a form of physical and/or embryo-imposed dormancy and will not germinate unless scarified. However, seeds of seeders *Cyclopia subternata* germinate promptly after scarification, while seeds of the resprouters such as *Cyclopia internedia* showed additional embryo dormancy (Sutcliffe and whitehead, 1995). Indigenous New Jersey tea (*Ceanothus americanus)* has also proven to have water-impervious seed-coats (Keogh and Bannister, 1994), which appear to impose a form of physical dormancy. Moreover, McMillan Browse (1994) observed that seeds of *Ceanothus* species have physiological dormancy and that they required up to 60 days or longer of chilling at 5°C to germinate.

2.3.3 Overcoming seed dormancy

The conditions required to free seeds from dormancy and enhance germination can be extremely variable among species, within a species or among seed sources of the same species (Luna and Wilkinson, 2019). Based on the plant species and type of dormancy, various pre-treatment methods to break dormancy and acquire fast germination must be applied (Mwase and Mvula, 2011). Artificial methods of breaking seed dormancy include pre-treatment with plant growth regulators (PGRs), stratification, and scarification and soaking in hot and cold water have been used to

break seed dormancy in indigenous plants (Luna *et al*., 2019). Seeds with both seed-coat and embryo imposed dormancy, that slows down a series of physiological and biochemical activities, cold stratification have been recognised as an essential method to break the dormancy (Finch-Savage and Leubner-Metzger, 2006).

Plant growth regulators: Plant growth regulators (PGRs) are extremely significant for the synchronization of seed dormancy and germination (El-Katony *et al*., 2021). The principal factors that influence seed dormancy include certain PGRs, and particularly among them, the abscisic acid is involved in the inhibition of germination, while gibberellins play part in the termination of seed dormancy (Dewir *et al*., 2011). Dormant seeds which require stratification, dry storage after ripening and light as a germination stimulator, are often treated with GA_3 to overcome dormancy (Gupta, 2003). Gibberellic acid is used to partly or completely replace the recommended time of cold moist stratification in a number of plant species. When seeds are released from dormancy, the receptors then start a signal transduction cascade, perhaps involving synthesis of or sensitisation to germination-promoting GAs that lead to the completion of germination (Bewley, 1997). Similarly, ethephon, an ethylene-releasing compound, is used to overcome seed dormancy in many species. In some species, ethylene alone is not enough to release seed dormancy, even when it promotes germination of non-dormant seeds of a given species (Thomas, 1992). Salicylic acid and its derivatives such as Acetylesalicylic are associated with increased germination percentages of tomato seeds (Szepesi *et al*., 2009).

Seed stratification: commonly know as cold or moist treatment, is used on seeds with internal dormancy from temperate areas, or high-elevation habitats in tropical regions. Some subtropical species may also benefit from a period of cool, moist stratification (Butler *et al*., 2007). In climates with four seasons, seeds sown in flats or containers in late summer or autumn and left outdoors during winter undergo natural stratification (Luna and Wilkinson, 2009). This pre-sowing method may be preferred if the species has double dormancy, requires a very long stratification or requires low temperatures or fluctuating temperatures for a long period of time. Artificial scarification involves placing seeds under refrigeration at 1 to 3°C for a period of time (Tang *et al*., 2019).

Seed scarification: Seeds with external or seed-coat imposed dormancy require scarification. This is any method of disrupting an impermeable seed-coat so that water and oxygen can enter the seeds (Baskin *et al*., 2000). In nature hard seed-coats are cracked or softened by fire, extreme temperatures, digestive acids in the stomachs of animals, or by the abrasion of blowing sand. After the seed-coat has been disrupted, oxygen and water pass into the seeds and germination can proceed (Yang *et al*., 2012). Seeds can be scarified many ways; however, the effectiveness of the pre-sowing method depends on the species and the thickness of the seed-coat. Whichever method is chosen, it is very important not to damage the endosperm, cotyledons, or embryo during the treatment. Scarification methods include mechanical, heat and chemical scarification (McDonald *et al*., 1996).

Mechanical scarification consists of filling, nicking, piercing, chipping the seed coat and de-coating, with a mounted needle, knife, hand-file or abrasive paper, this technique is especially best used for small quantities of seeds. Scarification of seeds by hand is most often used on large-seeded species (Oliveira *et al*., 2004). Scarification on the shoulder of the seed one quarter of the way round the circumference from the micropyle (ISTA, 1981) or the removal of one square millimetre of seed coat at the cotyledon end (Devkota *et al*., 2022) is sufficient. This is generally estimated to be the most consistent technique of pre-treatment and the subsequent germination percentage after this operation possibly approximates near the germination capacity (Moffett, 1952). This method consumes more time and high level of precision is required to effectively scarify the seed coat without disrupting the internal portions of the seed (Chachalis *et al*., 2008).

Many species, especially those from fire-adapted ecosystems, respond to germination signals from heat. Using either wet or dry heat to scarify the seeds can stimulate this response (Bradshaw *et al*., 2011). Using wet heat is an effective method for many small-seeded species because it provides rapid, uniform treatment that can be assessed within a few hours (Halmer, 2000). Wet heat treatments are effective for many tropical species including *Acacia*, *Cassia*, *Senna*, *Sesbania* and *Tamarindus* (Bhat and Karim, 2009).

Sulfuric acid is most commonly used on species with very thick seed-coats and with stony endocarps that surround the embryo (Schelin *et al*., 2003). It has been used on some species of *Acacia*, *Albizia*, *Cassia*, *Leucaena*, *Parkinsonia* and *Terminalia* (Dhan *et al*. 1992). Treatment lengths vary with the species and often among seed sources and it must be carefully monitored because seeds can be destroyed if the treatment is too long (Lippitt *et al*., 1994).

In seeds of *C. subternata,* dormancy can be overcome by scarification which results into high percentages in germination. However, scarification of *C*. *internedia* only partially removed the dormancy (Mbangcolo *et al*., 2013). Wild fires can modify seed germination response through both physical and chemical signals involved in the germination process (Rivas *et al*., 2006). Previous studies on *C. subternata and C. internedia* indicated that germination was partially dependent on ethylene. The stimulating effect of smoke and ethylene was inhibited after exposure to 2, 5-norbonadene, indicating that ethylene in the smoke was responsible for the germination stimulation (Whitehead and Sutcliffe, 1995). Therefore, the presence of compounds such as ethylene and short-chain fatty acids evident in smoke when vegetation is burnt could stimulate seed germination in *Cyclopia* species and many other species (Whitehead and Sutcliffe, 1995). On seeds of *Camellia*, scarification by cracking the seed-coat increased the mass of the seeds more quickly than the intact seeds. However, treatment of the cracked seeds with GA_3 increased seed germination percentage and seeds started germinating within a week. Also, cold stratification improved germination significantly in seeds of *Camellia* (Jaganathan *et al*., 2021).

Hot water soaking: Hot water treatment has been reported to enhance germination of hard seed-coated seeds by elevating water and oxygen permeability of the seed-coat (Aydin and Uzun, 2001). The treatment of seeds with hot water to improve germination is a better, safe and cost-efficient alternative to scarification methods such as sulfuric acid and sand-paper. It is possible that hot water treatments could vary with different exposure times to different temperature treatments (McDonnell *et al*., 2012). Hot water methods could be used efficiently only when the treatment is immediately followed by soaking, forced sprouting or seedling. Soaking seeds in water within the range 60 to

90°C is often as effective as soaking at 100°C but, there is less chance of damage at the lower temperatures (Ashraf and Foolad, 2005).

Cold water soaking (24 hours): Seeds with dormancy can be soaked as an initial step in stratification (Tuckett *et al*., 2010), or to plant the seeds shortly after the soak (Himanen and Nygren, 2014). Since the water content of seeds increases during the soaking process, germination can begin faster in the seed bed. Soaking can also be used to categorize poor-quality seed by skimming the floating, presumably low-vigour seeds after soaking. This can be done after seed extraction and before storing seeds (Shafaei *et al*., 2016), or at a nursery prior planting (Himanen and Nygren, 2014). Soaking seeds in still or aerated water results in fast germination and seedling emergence (Himanen and Nygren, 2014). Seeds with hard coats that prevents the embryo from being damaged will germinate more quickly if they are soaked than seeds that are directly planted in the soil without soaking (Lippitt *et al*., 1994). The duration of the soak in water determines the softness of the seed-coat after the soaking process. As that occurs, the water reaches the embryo (Giami, 2001).

2.4 Work not done on the research problem

South Africa is recognised by producing wild herbal tea such as honeybush tea and rooibos tea (Marnewick *et al*., 2000). Honeybush, rooibos and bush tea have been used for many years in the South African villages as herbal tea or medicinal tea (van Wijk 19860). There are available reports that show the propagation and domestication of these tea species and ways to improve their seed quality and seedling vigour for commercial purposes. However, not much is known about the propagation of *Jatropha zeyheri* by seed, the presence of seed dormancy, the resulting seedling establishment and development of this herbal indigenous tea plant. Therefore, the research was undertaken to investigate pre-sowing methods that will improve seed germination and the resulting seedling establishment, growth and development of *J. zeyheri*. Different ethnic groups of people in South Africa have been collecting the *J. zeyheri* plant from the wild and using it for many years as medicine, for nutritional benefits and as a tea beverage. However, suitable cultivation of the plant for domestication has not yet been identified and established. Therefore, the researcher aims to investigate ways in which seed germination and seedling development of *J. zeyheri* can be enhanced for the domestication of the plant.

CHAPTER 3
EFFECT OF DORMANCY BREAKING MECHANISMS ON GERMINATION OF *JATROPHA ZEYHERI* TEA PLANT

3.1 Introduction

Jatropha zeyheri is commonly propagated from seeds, and often seeds have low viability, which results in poor germination rates and poor seedling establishment (Devappa *et al*., 2011). In *Jatropha* species, high viability of seed had been documented to be determined by the observed genotypes, time after harvest, storage conditions, environmental features of plant growing, pre-sowing and after sowing treatments (Marcello *et al*., 2015). Some authors reported that loss of seed viability and germination as a result of medium and long-term storage (Duong *et al*., 2013), whereas others suppose that the existence of seed-coat, may be accountable for a physical dormancy, and moreover creates the necessity to eradicate this hampering by pre-sowing treatments (Windauer *et al*., 2012).

Notably, the seed of *J. zeyheri* is enclosed in a hard testa, which many horticulturist approve the hard testa to be responsible for germination failure or poor germination in many horticultural crops. The existence of the hard testa in *J. zeyheri* restricts water and air penetration into the seeds mainly because the testa exerts physical dormancy mechanisms to the growing embryo (Holmes *et al*. 1987). In order to improve germination in dormant seeds, seeds should be exposed to pre-germination treatments before sowing, with the aim to break the seed-coat, favour the embryo hydration and consequently increase the germination as related to untreated seeds (Fredrick *et al*., 2017).

Physical dormancy breaking techniques such as soaking the seeds in cold and hot water for a few hours or overnight, helps in triggering the plant growth regulators (PGRs) in the seeds that signal the cells to start germinating (Płażek *et al*., 2018). Seed scarification techniques, namely seed coat clipping, piercing and de-coating are also used to free seeds from dormancy as a result of the hard testa. Seed clipping is a technique that involves the use of a pair of nail clippers to cut a small nick of the seed coat to expose a small lighter inner part of the seed, allowing enough water imbibition required for germination process to take place (Das *et al*., 2017). Seed

piercing is a technique that uses a sharp needle to puncture through the seed-coat just enough to allow it to imbibe water more easily (Homola *et al*., 2021). De-coating is a technique that involves the complete removal of the seed-coat to improve germination and extends the range of temperature at which the seeds can germinate easily (Liyanage *et al*., 2020).

In other *Jatropha* species, Islam *et al*. (2009) demonstrated that *Jatropha curcus* seeds, kept under stone sand and moistened with water for 72 h before sowing, showed a significantly higher germination percentage than the untreated and directly sown seeds. In a study by Windauer *et al*. (2012) on some *Jatropha* species, testing the effect of different temperatures (from 15 to 35°C) on germination percentage. It was indicated that incubation of seeds at 25°C before sowing resulted in increased final germination percentage, even if at 30°C, seeds germinated quicker than any other temperature. Furthermore, positive outcomes were achieved for seeds of several tropical tree species, previously treated with hot water (Wang and Hanson, 2008). Interestingly, in another study conducted on *J. curcus*, findings indicated that among all the pre-sowing treatments performed on the seeds, only seeds treated by cracking the seed coat significantly influenced seed germination (Nyamayevu and Mashingaidze, 2018).

Generally, freshly harvested *J. zeyheri* seeds express dormancy challenges and a complete after-ripening stage accompanied by seed pre-treatment mechanisms are essential before seed sowing (Devappa *et al*., 2011). In order to improve seed germination in *J. zeyheri* tea plant, dormancy breaking techniques have to be employed. The objective of this study, therefore, was to determine whether germination of *J. zeyheri* seeds under controlled conditions could be enhanced through physical dormancy breaking mechanisms.

3.2 Materials and methods

3.2.1 Study area and plant materials

Mature seeds of *J. zeyheri* were collected at Khureng village, Lepelle-Nkumpi Municipality (24° 33’53” S, 29° 23’4” E), Limpopo Province of South Africa (SA) during the month of September (summer) in the year 2021. Seeds were transported to Limpopo Agro-Food Technology Station (LATS), at the University of Limpopo,

(23°53’10” S,29°44’15” E), and shelled under laboratory (LATS) conditions prior to germination studies.

3.2.2 Plant material preparations and procedures

Shelled seeds were first imbibed under tapwater for an hour prior to applying the treatments, which involved various physical dormancy breaking techniques, except for the reference seeds which were left intact. All treated seeds were placed inside petri dishes that contained moistened filter paper. Watering with distilled water was done every other day to keep the filter papers moist at all times. The petri dishes with seeds were placed under a growth chamber set at 25°C and light conditions at 8/16 hours of day and night.

Dormancy breaking procedures used on *Jatropha zeyheri* seeds

a. Clipping

The seed coat of the imbibed seeds wereslightly cut at the bottom-end of the seed with a surgical blade, carefully to avoid damage to the inside of the embryo.

b. Piercing

Seed coats were pierced at the bottom-end of the seeds with a sharp needle. The procedure was done carefully to avoid any damages to the embryos.

c. Cold water soak

Seeds were soaked for an hour in a glass beaker with distilled water prior to placing them inside petri dishes.

d. Hot water soak

Jatropha zeyheri imbibed seeds were soaked for an hour in a glass beaker with boiled (40-60°C) distilled water for 5 to 10 seconds, then quickly transferred to cold water for 1second in order to avoid embryo damage.

e. De-coating

The seed coat of the imbibed seeds was carefully and completely removed with a surgical blade to avoid any injury to the embryo.

3.2.3 Study treatments and design

The study consisted of six (6) aforementioned dormancy breaking techniques as treatments, namely, clipping, piercing, cold water soak, hot water soak and de-coating, while the untreated, but imbibed intact seeds served as the reference. Each treatment

consisted of 20 seeds. The experiment was laid out in a completely randomised design (CRD) with 10 replications (n = 60).

3.2.4 Data collection

Radicle extrusion from seeds served as a germination yardstick. Germinated seeds were counted daily and expressed as germination percentage (GP) (AOSA, 1990).

GP = $\frac{Germinated\ seeds}{Total\ seeds} \times 100$.

Three seedling performance tests, namely, mean germination time (MGT), germination index (GI) and germination rate (GR), were calculated using the Ellis and Roberts (1981) method.

MGT = ΣDn/Σn, with n being the number of seeds that germinated on day D, where D is the number of days from initiation to the completion of the germination process.

GI = $\frac{No.of\ germinated\ seedlings}{Days\ of\ first\ count}$+.....+$\frac{No.of\ germinated\ seedlings}{Days\ of\ final\ count}$

GR = $\frac{Number\ of\ all\ germinated\ seeds}{Total\ experiment\ duratiom}$.

3.2.5 Data analysis

Germination performance data were subjected to analysis of variance (ANOVA) using SAS software (SAS Institute, Inc. 2018). Fisher's Least Significant Difference test was used to separate the means at the probability level of 5%. Unless stated otherwise, all treatment means significant at 5% level of probability were discussed.

3.3 Results

After 21 days of establishing the treatments, erratic germination was observed among the pre-treated seeds (Figure 3.1). Germination occurred in the intact imbibed seeds (reference), as well as seeds treated with clipping and de-coating methods. Seeds treated with piercing, cold and hot water soaking pre-treatment failed to germinate (no data collected).

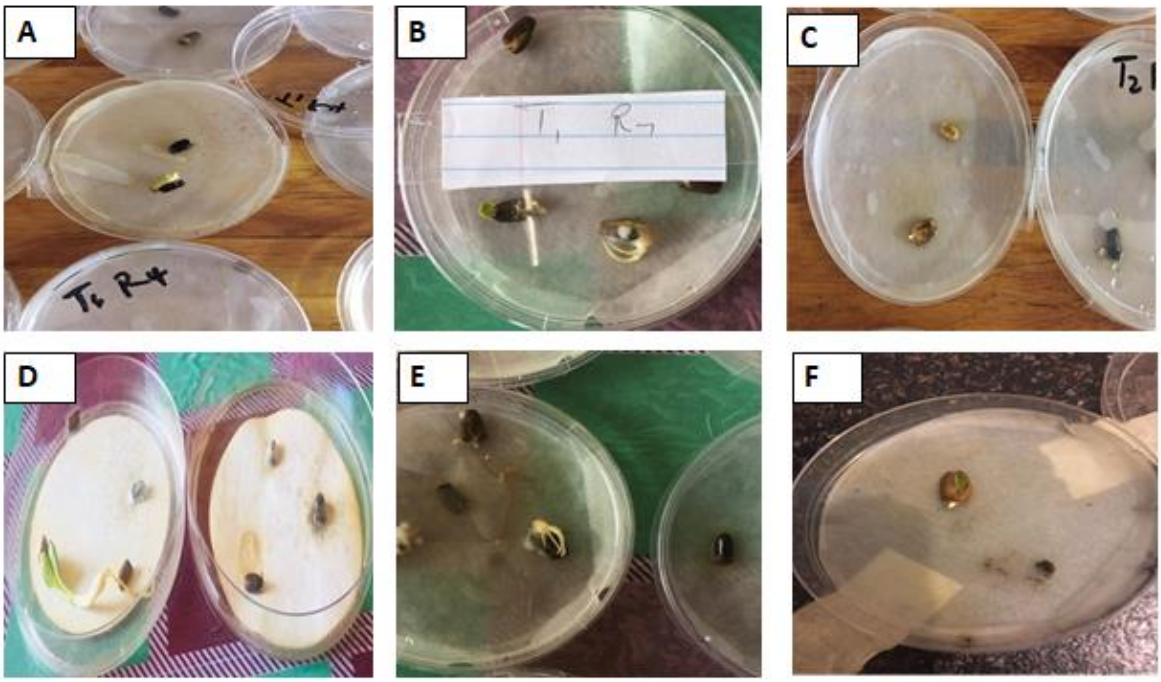

Figure 3.1. Laboratory sown *Jatropha zeyheri* seeds showing germination of the intact (A, B), clipped (C, D) and de-coated (E, F) seeds.

Dormancy breaking techniques had significant (P≤0.05) effect on MGT, GP, GI and GR of *J. zeyheri* seeds (Appendix 3.1- 3.4), contributing 57, 55, 51 and 55% to total treatment variation (TTV) in the respectively (Table 3.1). Relative to the intact imbibed seeds (reference), MGT for the clipped seeds was 3 days, whereas for the de-coated seeds, it occured at 7 days (Table 3.2). For the clipped seeds, 11% GP and 0.29 GI at a GR of 0.05 was recorded. However, for the de-coated seeds, 16% GP and 0.3 GI at a GR of 0.08 was achievedwhen compared with the reference. Seeds treated that werepierced, cold and hot water soaked completely failed to germinate.

Table 3.1. Sum of squares for mean germination time (MGT), germination percentage (GP), germination index (GI) and germination rate (GR) of pre-treated *Jatropha zeyheri* seeds sown under laboratory conditions.

Source	DF	MGT (days)		GP		GI		GR	
		SS	TTV%	SS	TTV%	SS	TTV%	SS	TTV%
Replication	9	34.793	6	193.33	4	0.14796	7	0.00438	4
Treatment	5	342.812	57*	2473.33	55*	1.12493	51*	0.05608	55*
Error	45	221.597	37	1826.67	41	0.91270	42	0.04142	41
Total	59	599.201	100	4493.33	100	2.18558	100	0.10189	100

*Significant at P≤0.05, nsNot significant at P≥0.05.

TTV = Total Treatment Variation, SS = Sum of Squares, DF = Degree of Freedom.

Table 3.2. Response of pre-treated *Jatropha zeyheri* seeds to mean germination time (MGT), germination percentage (GP), germination index (GI) and germination rate (GR) sown under laboratory conditions.

Treatments	MGT (days)	GP	GI	GR
	[Y]Variable	Variable	Variable	Variable
Intact dry seeds	0.800[c]	1[b]	0.013[b]	4.76E-03[b]
Clipping	2.933[b]	11[a]	0.290[a]	0.052[a]
Piercing	0[c]	0[c]	0[b]	0[b]
Cold water soak	0[c]	0[c]	0[b]	0[b]
Hot water soak	0[c]	0[c]	0[b]	0[b]
De-coating	6.525[a]	16[a]	0.297[a]	0.076[a]

[Y]Column means with the same letter were not significantly different (P≤0.05) according to Fisher's Least Significant Difference test.

3.4 Discussion

In this study, findings indicated that among the six pre-sowing treatments applied relative to reference seeds, clipped and de-coated seeds successfully influenced germination, with the applied treatments showing different effects on the tested germination variables of *J. zeyheri* seeds, particularly MGT, GP, GI and GR. Other tested treatments (piercing, cold and hot water soaking) failed to stimulate germination in *J. zeyheri* seeds. It was evident that the de-coating pre-treatment technique was found be more effective with respect to higher GP, GI and GR, although the treatment took almost 7 days for the seeds to finally germinate (MGT), which is acceptable for most horticultural crops. According to Muralidhara *et al*. (2016), de-coating pre-sowing treatment enhances early germination (MGT), which could a result of complete removal of the hard seed-coat that inhibits water penetration exerting mechanical restrain to the growing embryo. Therefore, it can be suggested that complete removal of the hard seed-coat exposed the *J. zeyheri* seed, enabling imbibition as a result of sufficient water absorbed by the seed for the germination process to take place. These

results were in agreement with those of Padma and Reddy (1997), where easy absorption of water by seeds increased the alpha amylase activity and also helped with early emergence of the plumule, the findings from the present study indicates that clipped seeds resulted in early (3 days) MGT, but very low GR, GI and GP.

According to Lozano-Isla *et al*. (2017), *Jatropha* seeds normally take about 10 to 14 days to germinate. Contrary to this study, relative to the reference, the process of germination was evident from the 3rd day when seeds were treated with the clipping method. However, the completely de-coated seeds took longer (7 days) to germinate, although in horticulture, it is common for most seeds to germinate readily after 7-14 days and fruit trees between 10-20 days, which was found acceptable to the researched crop. Literature states that MGT is a measure of the time taken by the sown seed to germinate (Chauhan and Johnson, 2011). It can be argued that for the clipped seeds to germinate at 3 days, it was a result of the small surface area that was damaged in order to allow moisture/water to penetrate the inside seed. Due to the small surface area that was removed by clipping, the inside seeds received less shock from being fully exposed to the outside environmental conditions which could be detrimental to the inside cotyledons and growing embryonic plant. On the other hand, extended MGT observed in de-coated seeds could be a result of complete exposure of the cotyledon to the external environmental conditions, which necessitated the bare seed to adapt to the new growing condition, causing delays in MGT. According to Nourmohammadi *et al.* (2019), de-coating seed treatment had a more positive effect on germination, as compared to other physical treatment methods. However, since the embryo is suddenly exposed to ambient factors, it might be subjected to moisture stress and also the delicate embryo may be affected, while removing the entire seed-coat (Long *et al*., 2015). In a study conducted by de Jesus *et al.* (2014), mechanical scarification such as cracking the seed-coat showed significantly better results when compared with seeds without seed-coat. This could be the result of the function of the seed-coat as a protectant to the inside embryo.

Generally, seed germination is the vital and final event in the life of a seed. It represents both the achievement and the completion of the basic function of a seed (Rajjou *et al*., 2012). Seed germination is the process of reactivation of the metabolic activity of the seed embryo; resulting in the emergence of radicle (root) and plumule

(shoot), thus leading to the production of a seedling. It is a very complex process as it involves many biochemical, physiological and morphological changes within a seed for germination to be initiated (El-Maarouf-Bouteau, 2022). Generally, seed germination includes water absorption by the process of imbibition and osmosis by the dry seed, which softens the seed coat and other coverings and causes hydration of the protoplasm. After water uptake, the synthesis and activation of enzymes take place (Hegde and Maness, 1996). Activation of enzymes is partly from reactivation of stored enzymes and partly by the synthesis of the enzymes during germination initiation process. Finally, cell elongation occurs where hydration, synthesis and activation of enzymes help in the elongation of cells, which results in the emergence of radicle (Rosental *et al*., 2014).

In terms of GP, the highest value (16%) was observed in seeds treated with de-coating technique, whereas lowest value (11%) was achieved in the clipped seeds, when compare with the reference seeds. The high GP in de-coated seeds could bea result of improved moisture and air absorption as a result of the removal of mechanical resistance that was imposed by the hard seed-coat which restricts embryo growth. Low GP observedd in seeds treated with clipping method, could be attributed to the small surface area that was created to permit water and air penetration into the seed. Although the clipping treatment improved the permeability, the seed-coat remained resistant to allow the imbibed seed and developing embryonic plant to break free from the hard seed-coat, thus exerting mechanical barrier. It was reported that even after seeds are exposed to suitable conditions for germination, due to the resistance of the seed-coat, the developing embryo could still fail to rupture the hard seed-coat (Bao and Zhang, 2010). Similar observations were reported in seeds of *Parinari curatellifolia* (Mobola-plum) (Mwang'ingo, 2004) where incomplete removal of the seed-coat enhanced permeability, but was inadequate in overcoming mechanical resistance imposed by the seed-coat. The author indicated the need for the complete removal of the seed-coat, which ultimately enhanced germination.

Generally, GP is an estimate of the viability of seeds within a population that indicates the proportion of the number of seeds which have produced normal seedlings within specified periods under favourable conditions (Liu *et al*., 2020). Usually, GP is calculated from the number of normal seedlings from the total number of seeds

evaluated and within a population of seeds, it can be determined by performing germination tests, which allow growers to determine what will germinate in the field (Batty *et al*., 2001). For commercially purchased seeds, information about the GP of a seedlot is often printed on the packet or on a tag attached to the seed bag. Often, if growers have seeds purchased from the previous year or have harvested open-pollinated seeds, depending on how long the seed is stored, their GP decreases.

With regard to GI, the highest value (0.297) was also observed in de-coated seeds and the lowest (0.290) GI was found in seeds treated with clipping method, however both methods were not significantly different to each other. Germination index, commonly known as emergence index (EI) under field conditions, is an estimate of the time (in days) it takes a certain GP of a seed to occur. It is a parameter that combines the percentage and time of germination, thus the faster a seed lot has germinated, the higher the GI (Kader, 2005). Results of this study was in agreement with a study by Adams and Farrish (1992), who proved that de-coating treatment provided the fastest and most complete germination of water oak (*Quercus nigra*) seeds. It was further explained that seeds that had their seed-coats removed completely started the germination process at 9 days after planting and 89% had germinated by day 15, which indicates the best results when compared to 'drilled-hole' treatment and seeds with cracked seed-coats. For domestication of indigenous tea plants rapid seed germination methods are desirable. Therefore, the time required for germination could be reduced by the applications of de-coating seed treatment before sowing, optimising storage conditions and suitable germination temperature (Getachew and Derero, 2011). In contrast, the low GI found in seeds subjected to clipping method might be due to an insufficient water absorption that resulted in a small seed-coat rupture, consequently affecting the germination speed (Cho *et al*., 2020).

In terms of GR, the highest rate was also obtained in de-coated seeds whereasthe lowest rate was observed in clipped seeds. Generally, GR is explained as the number or percentage of seeds that germinated in a seed-lot (Soleymani, 2019). In the de-coated seeds of *J. zeyheri*, the rate of germination was the highest when compared seeds that were clipped. The presence of seed-coat functions as a mechanical barrier to the development of the primary root during germination because of the reduction in oxygen and carbon dioxide diffusion and imbibition (Hillel and Kozlowski, 2012). There

is a correlation between seed-coat thickness and germination rate. The thicker the seed-coat the greater the relative increase in plant emergence after seed-coat removal. Therefore, smooth seed-coats usually corresponds with faster GR (Zhou *et al*., 2010). In a study conducted by Nourmohammadi *et al.* (2019) on the effects of dormancy-breaking methods on germination and seedling growth of the water impermeable seeds of *Gleditsia caspica* (Fabaceae), seed-coat removal enhanced seed GR from 0.2 to 12.5 seeds per day.

Generally, indigenous plants are known to have germination difficulties and their establishment is challenging (Kloppenburg, 2010). Attempts to germinate several indigenous plant species in an indigenous mixture was found not to be uniform (Mainz and Wieden, 2019). Indigenous plants are often characterized with poor seed-set, yield seeds bearing very short life-span or fail to produce viable seeds (Bykova *et al*., 2012). Predominantly, indigenous plant species are sown by direct seeding (Pedrini *et al*., 2020). However, direct seeding of plants has numerous challenges to growers, namely, difficulties sourcing quantities of viable seeds, lack of information on optimum sowing times and variability in germination duration (Pedrini *et al*., 2020). Low germination in a range of indigenous plant species is often a result of seed dormancy, which is the failure of a viable seed to germinate despite being exposed to favourable environmental conditions (Stevens *et al*., 2020). Dormancy may be the result of physiological mechanisms such as seed still being contained in fleshy fruit and hard seed coat and mechanisms such as embryo dormancy and chemical inhibitors in the seed (Baskin and Baskin, 2004). In seeds of *J. curcas* species, the major difficulty in their cultivation is the poor germination which comes from their hard seed-coats and exerts a physical exogenous dormancy (Holmes *et al*., 1987).

3.5 Conclusion

It can be concluded that differences in the germination parameters were observed in this study, with some treatments resulting in zero germination. Treated seeds with de-coating methods were found to be the high germinators, characterised by high GI, GR and GP, whereas seeds treated with clipping were observed as the low germinators, bearing low GI, GR and GP, when compared with the reference (intact seeds). Although a GP of less than 50% was achieved in this study, more work need to be done to improve this percentage. The study has demonstrated that pre-sowing

treatments could enhance germination in *J. zeyheri* indigenous. However, further studies need to be conducted with more viable and vigorous seeds to enhance germination results. Should one decide to use the de-coating treatment, the best way would be to take into consideration the time that the de-coated seeds are exposed to the external environmental conditions, which could be detrimental to the exposed cotyledons, and as a result increase MGT.

CHAPTER 4
EVALUATION OF GROWTH AND DEVELOPMENT OF *JATROPHA ZEYHERI* SEEDLINGS GROWN ON DIFFERENT GROWING MEDIA UNDER GREENHOUSE CONDITIONS

4.1 Introduction

The effective establishment of commercial plantations need complete understanding of the soil requirements of the species. Tea plants are grown in a variety of soils from one country to another, with the most important feature being soil pH (Wang *et al*., 2020). The best soil conditions recommended for the growth of tea plants include a light, friable loam with porous sub soil, well-drained deep and well-aerated soils, for tea plants are highly intolerant to stagnant water (Layomi Jayasinghe *et al*., 2019). For economic tea production, other characteristics must be considered which includes slope of the field, graveness and rockiness of the soil. Soil depth of less than 50 cm, graveness of more than 50% and rockiness of 20% affect the growth of tea plants adversely (Pramanik, 2016). Tea plants growing in shallow and compacted soils are likely to suffer from drought and waterlogging during rainy months (Layomi Jayasinghe *et al*., 2019).

Generally, tea that grows into shrubs require fertile acidic mountain soils, with a pH range of 4.5 to 5.5, at least 2 m deep, well-structured with a high mineral contents and well-developed humus-containing horizon (Valdes-Rodriguez *et al.,* 2011). Dystrophic soils that commonly occur in valleys and areas with a rainfall exceeding 1000 mm per annum are highly suitable for tea plants (Daly and Mitchell, 2000). The harvested leaf yield of tea plant can generally reach 4 to 5 tons/ha per year under favourable climatic and soil conditions with proper management (Donald, 2004). Dutta *et al*. (2010) documented that in the absence of soil constrains and under proper management, tea plant yields at higher elevations are higher than that obtained from tea stands at lower elevations.

Previous study reported that *Jatropha* species grows properly in aerated sandy and loamy soils of at least 45 cm depth (Gour, 2006). On the other hand other species of *Jatropha* namely, *Jatropha curcas*, *Jatropa cinerea* and *Jatropa integerrima* can grow very well even on gravel, sandy and saline lands (Valdes-Rodriguez *et al*., 2011).

Heavy clay soils are less favourable and should be avoided, mainly where drainage is reduced, as some species of *Jatropha* such as *J. curcas* are intolerant to waterlogged conditions (Uwuigbe, 2017).

Jatropha species are recognised for being capable of surviving in immensely poor dry soils, in conditions regarded as marginal for agriculture and can even root into rock crevices. The root system performs well under arid and semi-arid conditions (Kumar *et al*., 2016). However, survival capacity does not mean that high productivity can be attained from *Jatropha* under marginal agricultural environments. Generally, *Jatropha* is not sensitive to soil type and can grow on moderately acidic soils, predominantly, lateritic soil, dry red soil and deep fertile soils with suitable aeration (Mahajan *et al*., 2009). Lateritic soils are typically formed under tropical climates experiencing alternative wet and dry seasons (Singh *et al*., 2013). Red and lateritic soils are generally acidic and have low cation exchange capacity, low to moderate base saturation (Bishworjit and Athokpam, 2013). These soils are dominated by kaolinite clay and rich in sesquioxides. Surface crusting, poor inherent fertility, phosphorus fixation, aluminium toxicity, soil erosion among others are the major constrains in the soil (Bishworjit and Athokpam, 2013).

Findings on *J. curcas* plants sown in south-west Mexico, proved that in the absence of fertilizers, the annual average plant height increase in sandy, loam and clay soils was 60, 100, and 120 cm, respectively, indicating reduced growth in sandy soils when compared with loam and clay soils (Valdes-Rodriguez *et al*., 2011). *Jatropha zeyheri* tea plant is commonly propagated from seeds, and most often seeds usually exhibit low viability, leading to erratic germination and poor seedling establishment (Devappa *et al*., 2011). Finding proper soil(s) with suitable properties for germination, growth and development of *J. zeyheri* seedlings is of important as it will enhance the domestication possibilities of this tea plant for local communities and the tea industries at large in South Africa. The objective of this study, therefore, was to evaluate the growth and development of *J. zeyheri* seedlings sown on different growth media under controlled conditions.

4.2 Material and methods

4.2.1 Study area and plant material

The experiment was conducted under greenhouse conditions at the Green Biotechnologies Research Centre of Excellence (GBRCE), University of Limpopo, (23°53'10" S, 29°44'15" E), Limpopo Province of South Africa. The area had ambient temperatures averaging 28°C during the day and 21°C at night, with maximum temperatures controlled using thermostatically activated fans, wet wall and a net covering the greenhouse roof. The successful dormancy breaking technique (de-coating) used in the previous chapter (Chapter 3), was used to treat *J. zeyheri* seeds for use in this chapter.

4.2.2 Plant material preparations and procedures

The de-coated *J. zeyheri* seeds were planted in four various growth media types, namely sand, Hygromix, compost and loam soil, which were mixed together. Sand was sourced locally, while loam soil [containing 65% sand, 5% silt, 30% clay, 1,6 organic, electrical conductivity (EC) 0,148dS.m^{-2} and pH (H_2O) 6.5], was obtained from GBRCE site and then steam pasteurized (300°C for 1 hour). Hygromix and compost media was purchased (Hygrotech®, West Pretoria, South Africa). The growth media mixtures were prepared by combining each medium in a concrete media mixture (3:1 v/v) and mixing for 3 minutes. The media mixtures were then transferred into 10 cm plastic pots and each pot contained a single *J. zeyheri* de-coated seed planted to a depth of 2 cm. All pots with seeds were immediately irrigated to full capacity with tapwater after planting, thereafter each pot was then irrigated with 250 ml tap water every other day when the moisture content dropped below 50%, which was measured with a Tensionmeter. After seedling emergence, when seeds were 5 cm high with 2 developed leaves, each pot was fertilised with 0.63 g Multifeed P (Plaaskem®, Witfield) to provide 0.12 g N, 0.05 g P, 0.10 g K, 0.59 mg Mg, 0.23 mg Zn, 0.65 mg B, 0.05 mg Mol, 0.05 mg Fe, 0.20 mg Mn and 0.50 mg Cu per ml water.

4.2.3 Study treatments and design

Six treatments comprising different growing media mixtures (3:1 v/v) namely, sand + loam, loam + compost, loam + Hygromix, sand + compost, sand + Hygromix were used. Sand alone served as the reference medium. The experiment was laid out in a randomised complete block design (RCBD), replicated 10 times (n = 60).

4.2.4 Data collection

Emerged seedlings with at least 2 cotyledons and measuring 2 mm in length above the growing media mixtures were considered as an indicator for successful germination. The number of germinated seeds per plant was recorded daily until no further germination was observed. Seed germination variables, namely, mean germination time (MGT), germination percentage (GP), germination index (GI), and germination rate (GR) were calculated using the formulas explained in chapter 3. Ninety (90) days after the treatments were applied, seedling performance variable namely, stem diameter (SD), chlorophyll content (CC), seedling length (SL) and number of shoots (NS) were measured, stem diameter (SD) (mm) was measured using a digital Vernier caliper (Mitutoyo 530-119, Japan). Before taking the SD on each seedling, the Vernier caliper was set to zero in order to obtain accurate results. The outside jaws were placed on the seedling stem and a reading was recorded for all the germinated seedlings. Chlorophyll content (CC) ($\mu mol.m^2$) was measured using a chlorophyll meter (SPAD-502, MINOLTA South Africa). The chlorophyll meter was placed on two leaves per seedlings, after taking the reading from the leaves, an average value was obtained from the readings of the two leaves which determined the CC in the seedlings. For seedling length (SL), a ruler was used to measure the length of the seedlings. The ruler was placed at the bottom of the stem just above the roots to the tallest leaf and a measurement was taken. Number of shoots (NS) was also counted and recorded. Seedlings were then severed at the soil surface, root systems removed from pots and washed with tapwater to remove soil particles and blotted dry. Both shoots and roots were oven-dried at 70°C for 72 h. Dry shoot and root mass were weighed using a weighing scale (HCB1002 Max:1000g x 0,01g, AE South Africa) and the values were recorded.

4.2.5 Data analysis

Seedling performance and plant variables data were subjected to analysis of variance (ANOVA) using SAS software (SAS Institute, Inc. 2018). When the treatments were significant at the probability level of 5%, the associated mean sum of squares were partitioned to determine the percentage contribution of sources of variation to the total treatment variation (TTV) among the means. Fisher's Least Significant Difference test

was used to separate the means at the probability level of 5%. Unless stated otherwise, only treatment means significant at 5% level of probability was discussed.

4.3 Results

4.3.1 Seedling performance variables in *Jatropa zeyheri* seedlings

Erratic seedling emergence and development was observed among the different growth media mixtures used (Figure 4.1). At 90 days after seed sowing, seedling emergence was observed on sand + compost, sand + Hygromix as well as sand + loam growing media mixtures. On the other hand, seeds planted on growth media mixture containing loam + Hygromix and loam + compost, including seeds planted in sandy soil (reference) failed to emerge (no data was collected).

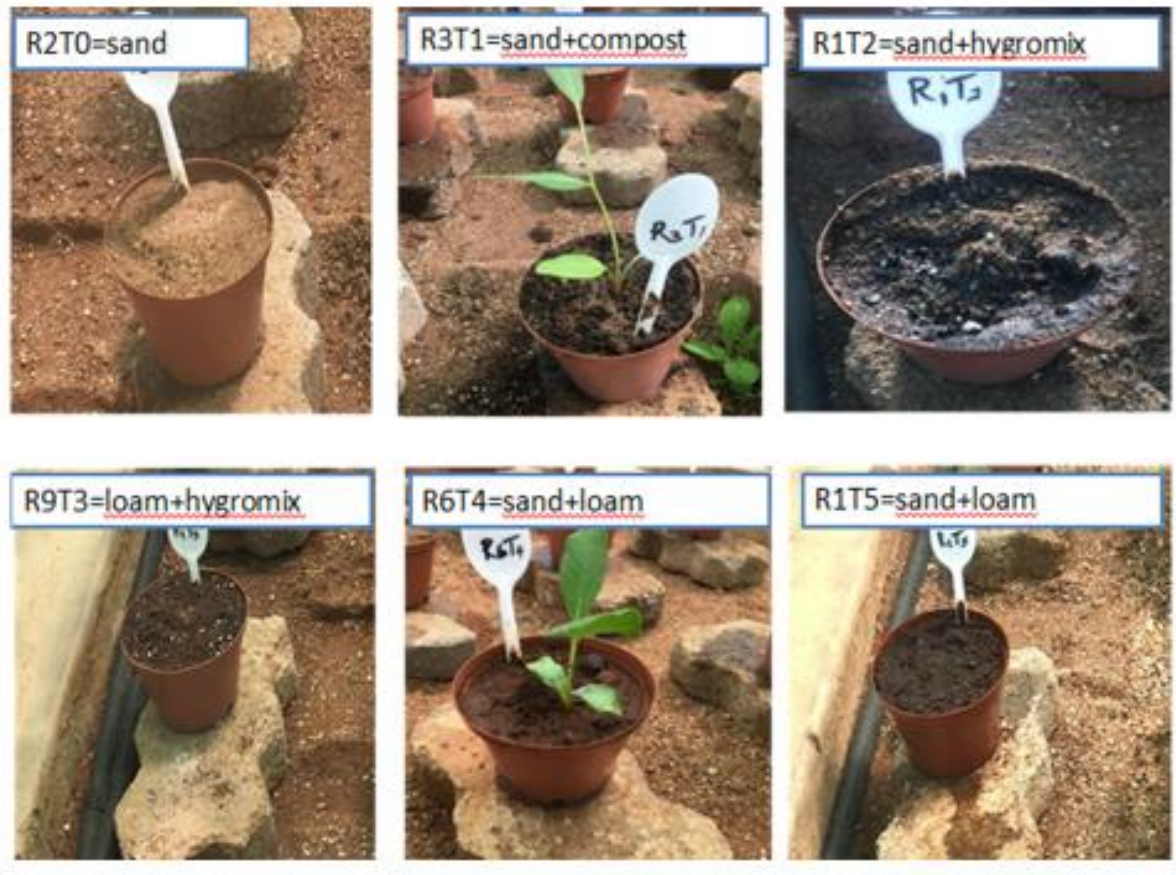

Figure 4.1. Seedling growth and development of *Jatropha zeyheri* tea plant on six different growing media mixtures under greenhouse conditions at 90 days.

Treatments had significant (P≤0.05) effect on MGT, GP and GR of *J. zeyheri* seedlings, whereas no significant effects were observed on GI (Appendix 4.1- 4.4).

Treatments contributed 25, 21, 9 and 22% to TTV in MGT, GP, GI and GR, respectively (Table 4.1). Relative to the reference medium (sand), MGT for the de-coated *J. zeyheri* seeds sown on sand + compost occurred at 2 days, whereas emergence in sand + loam occurred at 7 days. The longest MGT (26 days) was observed in sand + Hygromix growing media (Table 4.2). Germination percentage and GR for seeds sown on sand + compost was 1% and 1.1, respectively. Moreover, germination percentagefor seeds sown on sand + loam were 2% and 3.33E-03 whereas in seeds sown on sand + Hygromix, was 4% and 4.44E-03, respectively, when compared withthe reference.

Table 4.1. Sum of squares for mean germination time (MGT), germination percentage (GP), germination index (GI), germination rate (GR) of pre-treated *Jatropha zeyheri* seeds sown on different media mixtures under greenhouse conditions.

Source	DF	MGT (days)		GP (%)		GI		GR	
		SS	TTV%	SS	TTV%	SS	TTV%	SS	TTV%
Replication	9	3486.8	16	68.333	11	2457.3	15	1.152E-04	14
Treatment	5	5435.1	25*	128.333	21*	1365.0	9^{ns}	1.893E-04	22*
Error	45	12695.7	59	421.667	68	12288.3	76	5.514E-04	64
Total	59	21617.6	100	618.333	100	16110.6	100	8.560E-04	100

*Significant at P≤0.05, ^{ns}Not significant at P≥0.05.

TTV = Total Treatment Variation, SS = Sum of Squares, DF = Degree of Freedom

Table 4.2. Responses of *Jatropha zeyheri* seedling performance on mean germination time (MGT), germination percentage (GP) and germination rate (GR), grown on different growing media under greenhouse conditions.

	MGT (days)	GP	GR
Growing medium	[Y]Variable	Variable	Variable
Sand	0[b]	0[b]	0[c]
Sand + compost	1.7[b]	1[b]	1.1
Sand + Hygromix	26.4[a]	4[a]	4.44E-03[a]
Loam+ Hygromix	0[b]	0[b]	0[c]
Sand + loam	7[b]	2[ab]	3.33E-03[ab]
Loam + compost	0[b]	0[b]	0[c]

Column means with the same letter were not significantly (P≥ 0.05) different according to Fisher's Least Significant Difference test.

4.3.2 Plant performance variables in *Jatropa zeyheri* seedlings

Treatments had no significant (P≥0.05) effects on SD (cm), CC, SL (cm), NS, DSM (g) and DRM (g) of *J. zeyheri* plantlets (Appendix 4.5- 4.10). Treatments contributed 16, 13, 9, 12, 11 and 11% to TTV (Table 4.3).

Table 4.3 Sum of squares for stem diameter (SD), chlorophyll content (CC), seedling length (SL), number of shoots (NS), dry shoot mass (DSM) and dry root mass (DRM) of *Jatropha zeyheri* seedlings sown on different media mixtures under greenhouse conditions.

Source	DF	SD (cm)		CC		SL (cm)		NS		DSM (g)		DRM (g)	
		SS	TTV %	SS	TTV %	SS	TTV %	SS	TTV %	SS	TTV %	SS	TTV %
Replication	9	1.434	8	273.84	10	69.744	9	5.017	9	0.004	10	0.026	12
Treatment	5	2.992	16^{ns}	337.75	13^{ns}	68.196	9^{ns}	6.483	12^{ns}	0.005	11^{ns}	0.022	11^{ns}
Error	45	14.262	76	2003.4	77	625.921	82	42.683	79	0.034	79	0.161	77
Total	59	18.688	100	2615.0	100	763,861	100	54.183	100	0.043	100	0.209	100

*Significant at P≤0.05, ^{ns}Not significant at P≥0.05.

TTV = Total Treatment Variation, MSS = Sum of Squares, DF = Degree of Freedom

4.4 Discussion

In this study, findings showed erratic seedling growth and development among the six different growing media mixtures relative to sand (reference). Seedling emergence and development only occurred on sand + compost, sand + Hygromix as well as sand + loam growing media mixtures indicating that germination was successful in the respective media mixtures. These treatments also indicated varying effects on the tested germination performances of *J. zeyheri* seeds, namely, MGT, GP, GI and GR. In contrast, seedlings in growth media mixture containing sand (reference), loam + Hygromix as well as loam + compost failed to emerge, indicating that the process of germination was not successful. In terms of seedling growth and development, there were no significant differences among the treatment means. The non-significant difference is probably as a result of similarities in the physical characteristics of the media used, mainly sand.

The results of sand + compost indicated a faster germination time (MGT) where germination occurred in almost 2 days compared to seeds sown in sand + loam and sand + Hygromix. Compost addition to sand has been proven to potentially decrease deep percolation (Cogger, 2005). Deep percolation is responsible for rapid water losses under gravitational force. Compost helps in increasing the water holding capacity of sandy soils, which quickly lose the irrigation water by deep percolation, this is because the addition of compost results in the decrease in large pore spaces (Mondal *et al*., 2021). The enhanced water-holding allowed seeds to absorb enough moisture needed for germination, which led to faster germination of seeds sown in sand and compost mixture. Mean germination time of 7 days and 26 days was noticed in sand + loam and sand + Hygromix, respectively. Hygromix has been proven to exhibit good physical and chemical properties that enhances seedling emergence (Oagile *et al*., 2016). Sand on the other hand has different properties such as weak structure or no structure and poor water retention properties (Bonicelli *et al*., 2015) which is likely to dry off rapidly when irrigated. These properties of sand may have resulted in seeds not receiving enough water suitable for germination and only after sometime seeds had enough moisture to kick-start the germination process. The results from the sand + compost and sand + loam indicated that the treatments had suitable physical properties. With the time it took for seedlings to emerge, it is evident that treatments were responsive enough for seeds to easily germinate. There was no

significant difference between sand + compost and sand + loam indicating that the treatments had similarities in the physical characteristics.

In terms of GP, the highest value of 4% was observed in seeds planted on sand + Hygromix, whereas seeds planted on sand + loam and sand + compost had low values of 2 and 1% GP, respectively. Mathowa *et al*. (2016) indicated that Hygromix revealed superior absolute number of seedling emergences as compared to coco-peat and germination mix. It was stated in the literature that the observed superiority exhibited by the Hygromix could probably be attributed to its good physical properties and water holding capacity that supported seed germination percentages. The relative balance between air and water in a growth media's pore space is critical for seed germination and plant growth (Nkongolo and Cron, 1999) and this is potentially a trait of the sand + Hygromix growth media mixture. The low GP on sand + loam and sand + compost could have been a result of poor seed viability. Hoveka (2016) indicated that seeds of *J. zeyheri* usually have low viability. Additionally, *J. zeyheri* plant is documented to grow from mature seed, but freshly harvested seeds express dormancy challenges. Another explanation for the low GP value is that, because the seeds had their seed-coats removed before planting, contamination of the growth media might have tempered with the quality of the imbibed and developing seed, which resulted in seed rotting as the removal of the seed-coat made them susceptible to contamination. Again, when the growth media is too wet it can cause seeds to rot and discontinue the germination process (Farru *et al*., 2022).

The GR, which is explained as the speed of seed germination, for sand + compost growth media it was the highest (1.1) among the tested growth media. Sand + Hygromix and sand + loam had GR values of 4.44E-03 and 3.33E-03, respectively. This indicates a significant difference between sand + compost and the other two treatments. The high value of GR on sand + compost may be due to the good drainage and good aeration of the treatment as compared to sand + Hygromix and sand + loam. In contrary, according to literature (Valdés-Rodríguez and Pérez-Vázquez, 2019), *Jatropha* species thrive well on drained sandy loam soils. The presence and proportion of fine particles to large particles of the two treatments may have resulted in mixtures being too wet. The results of waterlogged growing media are poor aeration which

potentially leads to seed deterioration resulting in poor germination and failure of seedling emergence (Drew and Lynch, 1980).

Generally, in the natural environment soil is the only growth media for indigenous plant species. The importance of soils in the environment has been recognised since the earliest times, particularly in relation to agriculture (Lambers *et al*., 2011). Tropical ecosystems are characterised with low fertility as much of the nutrient stock is stored in the biomass rather than in the soil (Gourlet-Fleury *et al*., 2011). Clearance of tropical vegetation often by burning helps to enhance soil nutrient status. However, due to weed invasion, rapid depletion of soil nutrients occurs (Omotayo and Chukwuka, 2009), resulting in poor establishment of indigenous plants and also plants fail to reach their full growth potential in the natural environment (Lake and Leishman, 2004).

The selection of proper growth media is one of the most essential factors to consider in nursery plant production. A growth media can be defined as a substance through which roots grow and extract water and nutrients (Barrett *et al*., 2016). In indigenous plant nurseries, a growth media can consist of indigenous soil, but is more commonly artificial soil composed of materials such as peat moss or compost (Krishnapillai *et al*., 2020). Potting media are soils or materials similar to soil which aids in the physical support of plants grown in them (Ekpo and Sita, 2010). The use of appropriate growth media or substrate is important for production of quality horticultural crops as this has a direct effect on the development and subsequent maintenance of the extensive functional rooting system (Bhardwaj, 2014).

A number of growth media have been evaluated on various plants by previous researchers (Kumar *et al*., 2016). Some growth media are of natural origin while others are man-made and are produced artificially in factories (Bhat *et al*., 2013). Different media combinations such as sewage sludge, sawdust, spent mushroom compost, coconut-coir dust, rice husk and varying soil types are currently used globally as growing media (Mehmood *et al*., 2013). It is important to take into careful consideration the phyisco-chemical characteristics and composition of growth media as they are important determinants of plant growth and production (Riffat *et al*., 2011). Pore spaces, cation exchange capacity, water holding capacity, bulk density, pH, soluble salts and distribution of particle size are essential physical and chemical traits to be

taken into consideration before the selection of a growth media (Riffat *et al*., 2011). A good growth media would supply adequate anchorage to the plant, serve as a reservoir for nutrients and water, allow the diffusion of oxygen to the roots and enable gaseous exchange between roots and atmosphere outside the root substrate (Dhanasekaran *et al*., 2020). Favourable environments with adequate water and nutrient availability must be provided by growth media to allow the plant to develop an excellent root system that favours the luxurious growth (Neelam and Ishtiaq, 2001).

Generally, tea plant cultivation is most suitable in slightly acidic soils in the absence of calcium. However, the availability of iron in the sub-soil is desirable (Adhikary *et al*., 2019). Indigenous tea plants such as honeybush (*Cyclopia*) prefer well-drained sandy to sandy loam-type soils, with a low pH that ranges below 5 with low phosphorus (Biswas and Motalib, 2012). However, low pH ranges lead to iron deficiency as a result of manganese competition with iron uptake. At low pH iron readily forms insoluble precipitates thus rendering both nutrient unavailability for plant uptake (El-Jaoual and Cox, 1998). The soils must be free from plant-parasitic nematodes (DAFF, 2016). *Aspalathus linearis* (rooibos) plant requires deep sandy soil for easy enlargement of roots. The soil must have good drainage with pH of 4.5 to 5.5 (Van Schalkwyk, 2018). *Jatropha* species such as *J. curcus*, *J. ingerrima* and J*. cinerea* thrive well in a wide variety of pH levels as long as the soil is characterised by good drainage, preferably sandy soils (Valdes-Rodriguez *et al*., 2011). *Jatropha curcus* has the ability to grow in alkaline soils and it can also tolerate saline irrigation water. In contrast *J. ingerrima* cannot survive in saline soils as it is intolerant to salt (Kumar *et al*., 2010).

4.5 Conclusion

Growth media had no significant effect on seedling variables, however, growers are recommended to select growing media that will result in optimum seed germination and subsequent seedling growth and development for plants to reach their full yield potential. Seeds sown on sand + compost indicated better results with regard to MGT and GR. However, MGT results for seeds sown on sand + loam were low compared to seeds sown on sand + compost, but the results were found acceptable to the researched crop. Sand + Hygromix had poor results of MGT. Treatments, sand + loam and sand + Hygromix indicated poor GR. However, sand + Hygromix treatment showed a higher GP value when compared with sand + compost and sand +loam.

With GP of all treatments with emerged seedlings being below 50%, more work can be done to improve this percentage. In this study *J. zeyheri* seeds have proven to do well on sand + Hygromix compared to the rest of the treatments. This indicates that if growers wish to domesticate the tea plant, sand + Hygromix can potentially yield great results. However, further studies should be conducted with viable and vigorous seeds to improve the results. Should one decide to use sand + Hygromix as their growth media they must take into consideration the properties of sand. To improve MGT and GR, sand must be less in the media mixture to allow the Hygromix to retain water that will be sufficient for seed to imbibe and start the germination process earlier.

CHAPTER 5
SUMMARY, SIGNIFICANCE OF FINDINGS, RECOMMENDATIONS AND CONCLUSIONS

5.1 Summary of findings

The study investigated the effect of dormancy breaking mechanisms in field collected *Jatropha zeyheri* tea plant and evaluated the growth and development of *J. zeyheri* seedlings on different growth media under greenhouse conditions. Erratic germination were observed among seeds treated with clipping and de-coating pre-treatment methods when compared to the intact imbibed seeds (reference). However, failed germination was evident on seeds treated with piercing, cold and hot water soaking. Relative to the reference, mean germination time (MGT) for the clipped seeds was shorter, whereas MGT for de-coated seeds took longer. Germination percentage (GP) and germination index (GI) for de-coated seeds were high compared with the clipped seeds. Germination rate (GR) was higher in de-coated seeds and low clipped seeds. The de-coated seed treatment method was viewed as a better treatment to promote germination in *J. zeyheri* seeds.

Poor seedling emergence and development was observed among the different growing media mixtures used in this study. Erratic seedling emergence occurred on sand + compost, sand +Hygromix and sand + loam. In contrary, seeds planted on loam + Hygromix and loam + compost and sandy soil failed to emerge. The de-coated *J. zeyheri* seeds grown on sand + compost and sand + loam had a shorter MGT compared to the. The longest MGT was observed in sand + Hygromix growth media. Sand + compost and sand + loam had poor germination percentages and GR, but sand + Hygromix had good germination percentages and GR. However, growth media mixtures had no significant difference on plant performance.

5.2 Significance of the findings

The study indicated significant differences in seed pre-treatment methods as well as different growth media mixtures, but not the seedling development variables. Seed treatment and the best growth media are essential for plant domestication, in such a

way that it will allow horticulturists and household individuals consuming the plants as a beverage to grow this plant with ease. The de-coated seed treatment released seeds from physical dormancy and germination was enhanced, though low in the clipped seed treatment method. Findings on the suitable dormancy breaking technique for dormant *J. zeyheri* seeds is important in the tea industry as availability of starting plant material will not be a challenge to horticulturists and growers. The sand + Hygromix media mixture for the plant will result in the best seedling growth and development, and allow the plant to grow to its full potential thus increasing yields. This will also allow the plant to be easily available in nurseries, increasing its commercialization, as a source of economic development through job creation and reducing porverty in rural households.

5.3 Recommendations

Findings from the study indicated that pre-treatment of *J. zeyheri* seeds resulted in better results compared to untreated seeds. The results indicated that before planting it is best that seeds undergo treatment since seeds which showed better results in the pre-treatment experiment were those treated with de-coating method. It is advisable for growers to use the de-coating pre-treatment method for good seedlings establishment under greenhouse conditions before transplanting Care should be taken that the growth medium to be used is heat sterilized because when the seed-coat is removed, seeds become susceptible to soil borne organisms which feed on the exposed embryo as well as contamination which reduce the quality of seeds thus hampering germination. Also, the different growth media mixtures indicated that the emergence of seedlings was high on certain growth media mixtures when compared with the reference medium. If ever growers want to grow this tea plant, it is advisable to use a suitable growth media that will allow seed germination and increase emergence of seedlings such as those indicated by sand + Hygromix.

5.4 Conclusions

The results of the study indicated that it is important to apply pre-treatment methods to seeds of *J. zeyheri* as it was evident that the seeds possesses physical dormancy as a result of the hard seeds coat. Treated seeds resulted in high values of germination variables compared to untreated seeds. However, the results of the study were not satisfactory as germination of the treated seeds was below 50%. The results may have been due to poor seed quality. Therefore further studies with more viable seeds are

necessary to improve germination of the treated seeds. The results of the study also indicated that de-coated seeds under greenhouse conditions germinated with low levels of germination in the different growth media mixtures. The results indicated that seeds had better GP when planted on sand + Hygromix compared to the other treatment but took longer to germinate. Further studies should be conducted with the sand + Hygromix growth media mixture in order to improve MGT as well as GP.

REFERENCES

Adams, J. C. and Farrish, K. W. 1992. Seedcoat removal increases speed and completeness of germination of water oak. *Tree Planters' Notes-US Department of Agriculture, Forest Service (USA)*, 43: 52-53.

Aderogba, M. A., Ndhlala, A. R., Rengasamy, K. R. and Van Staden, J. 2013. Antimicrobial and selected in vitro enzyme inhibitory effects of leaf extracts, flavonols and indole alkaloids isolated from Croton menyharthii. *Molecules*, 18: 12633-12644.

Adhikary, S., Khan, M. Z., Arobe, S., Dey, S. and Billah, S. M. 2019. Soil chemical analysis of kazi and kazi organic tea garden and compared to ordinary tea gardens of Bangladesh. *Open Journal of Soil Science*, 9: 91-102.

Araya, H. T. 2007. Seed germination and vegetative propagation of bush tea *(Athrixia phylicoides).* Doctoral dissertation Submitted to the University of Pretoria.Tshwane, South Africa.

Ashraf, M. and Foolad, M. R. 2005. Pre-sowing seed treatment—A shotgun approach to improve germination, plant growth, and crop yield under saline and non-saline conditions. *Advances in agronomy*, 88: 223-271.

Association of Official Seed Analysts (AOSA). 1990. Rules for testing seeds (revised). *Journal of Seed Technology,* 12: 1-122.

Aydin, I. and Uzun, F. 2001. The effects of some applications on germination rate of Gelemen Clover seeds gathered from natural vegetation in Samsun. *Pakistan Journal of Biological Sciences*, 4: 181-183.

Bao, J. P. and Zhang, S. L. 2010. Effects of seed coat, chemicals and hormones on breaking dormancy in pear rootstock seeds (*Pyrus betulaefolia Bge*. and *Pyrus calleryana Dcne*.). *Seed Science and Technology*, 38: 348-357.

Bareke, T. J. 2018. Biology of seed development and germination physiology. *Advanced Plants Agricultural Research*, 8: 336.

Barrett, G. E., Alexander, P. D., Robinson, J. S. and Bragg, N. C. 2016. Achieving environmentally sustainable growing media for soilless plant cultivation systems–A review. *Scientia Horticulturae*, 212: 220-234.

Basey, A. C., Faint, J. B. and Kramer, A. T. 2015. Producing native plant materials for restoration: 10 rules to collect and maintain genetic diversity. *Native Plants Journal*, 16: 37-53.

Baskin, C. C. and Baskin, J. M. 1998. Seeds: Ecology, biogeography and evolution of dormancy and germination. Academic Press, New York. pp. 387-399

Baskin, J. M. and Baskin, C. C. 2004. A classification system for seed dormancy. *Seed Science Research,* 14: 1-16.

Baskin, J. M. and Baskin, C. C. 2014. What kind of seed dormancy might palms have?. *Seed Science Research*, 24:17-22.

Baskin, J. M., Baskin, C. C. and Li, X. 2000. Taxonomy, anatomy and evolution of physical dormancy in seeds. *Plant Species Biology*, 15: 139-152.

Baskin, J. M., Davis, B. H., Baskin, C. C., Gleason, S. M. and Cordell, S. 2004. Physical dormancy in seeds of *Dodonaea viscosa* (*Sapindales, Sapindaceae*) from Hawaii. *Seed Science Research*, 14: 81-90.

Batty, A. L., Dixon, K. W., Brundrett, M. and Sivasithamparam, K. 2001. Constraints to symbiotic germination of terrestrial orchid seed in a mediterranean bushland. *New Phytologist*, 152: 511-520.

Bazin, J., Langlade, N., Vincourt, P., Arribat, S., Balzergue, S., El-Maarouf-Bouteau, H. and Bailly, C. 2011. Targeted mRNA oxidation regulates sunflower seed dormancy alleviation during dry after-ripening. *The Plant Cell*, 23: .2196-2208.

Bewley, J. D. 1997. Seed germination and dormancy. *Plant Cell*, 9: 1055-1066.

Bhardwaj, R. L. 2014. Effect of growing media on seed germination and seedling growth of papaya cv. 'Red lady'. *African Journal of Plant Science*, 8: 178-184.

Bhat, N. R., Suleiman M. S., Thomas, B., Lekha V. S., George, P. and Ali, I. S. 2013. Growing substrate for organic lettuce production in Kuwait. *World Journal of Agricultural Science*, 9: 143-147.

Bhat, R. and Karim, A. A. 2009. Exploring the nutritional potential of wild and underutilized legumes. *Comprehensive Reviews in Food Science and Food Safety*, 8: 305-331.

Bhattacharya, A., Nagar, P. and Ahuja, P. S. 2002. Seed development in *Camellia sinensis* (L.) O. Kuntz*e. Seed Science Research,* 12: 39-46.

Bishworjit, N. and Athokpam, H. S. 2013. Characterization, and evaluation for crop suitability in lateritic soils. *African Journal of Agricultural Research*, 8: 4628-4636.

Biswas, A. and Motalib, M. A. 2012. Comparative study on tea soils of South India and Bangladesh. *Tea Journal Bangladesh*, 41: 27-36.

Bonicelli, A., Giustozzi, F. and Crispino, M. 2015. Experimental study on the effects of fine sand addition on differentially compacted pervious concrete. *Construction and Building Materials*, 91: 102-110.

Bradshaw, A. D. and Chadwick, M. J. 1980. The restoration of land: the ecology and reclamation of degraded land. University of California Press. Los Angeles. USA.

Bradshaw, S. D., Dixon, K. W., Hopper, S. D., Lambers, H. and Turner, S. R. 2011. Little evidence for fire-adapted plant traits in Mediterranean climate regions. *Trends in Plant Science*, 16: 69-76.

Bricker, M., Pearson, D. and Maron, J. 2010. Small-mammal seed predation limits the recruitment and abundance of two perennial grassland forbs. *Ecology*, 91: 85-92.

Buijs, G. 2020. A perspective on secondary seed dormancy in *Arabidopsis thaliana*. *Plants*, 9: 749.

Butler, D. W., Green, R. J., Lamb, D., McDonald, W. J. F. and Forster, P. I. 2007. Biogeography of seed-dispersal syndromes, life-forms and seed sizes among woody rain-forest plants in Australia's subtropics. *Journal of biogeography*, 34: 1736-1750.

Bykova, O., Chuine, I., Morin, X. and Higgins, S. I. 2012. Temperature dependence of the reproduction niche and its relevance for plant species distributions. *Journal of Biogeography*, 39: 2191-2200.

Chachalis, D., Korres, N. and Khah, E. M. 2008. Factors affecting seed germination and emergence of Venice mallow (*Hibiscus trionum*). *Weed Science*, 56: 509-515.

Chandel, K., Lakhanpaul, S., Singh, I. and Chaudhury, R. 1990. Germination and desiccation tolerance of tea (*Camellia sinensis* (L) O.*Kuntze)* seeds and feasibility of cryopresevation*. Sri lankan Journal of Tea Science,* 59: 89-94.

Chauhan, B. S. and Johnson, D. E. 2011. Ecological studies on Echinochloa crus-galli and the implications for weed management in direct-seeded rice. *Crop Protection*, 30: 1385-1391.

Chiipanthenga, M., Maliro, M., Demo, P. and Njoloma, J. 2012. Potential of aeroponics system in the production of quality potato (*Solanum tuberosum* L.) seed in developing countries. *African Journal of Biotechnology*, 11: 3993-3999.

Cho, J. S., Jang, B. K., Lee, S. M., Lee, I. J. and Lee, C. H. 2020. Factors affecting the dormancy and germination of bleeding heart [*Lamprocapnos spectabilis* (L.) Fukuhara] seeds. *Plant Biology*, 22: 514-521.

Cogger, C. G. 2005. Potential compost benefits for restoration of soils disturbed by urban development. *Compost Science and Utilization*, 13: 243-251.

da Silveira Falavigna, V., Severing, E., Lai, X., Estevan, J., Farrera, I., Hugouvieux, V., Revers, L. F., Zubieta, C., Coupland, G., Costes, E. and Andrés, F. 2021. Unraveling the role of MADS transcription factor complexes in apple tree dormancy. *New Phytologist*, 232: 2071-2088.

Daly, D. C. and Mitchell, J. D. 2000. 14. Lowland Vegetation of Tropical South America: An Overview. *Imperfect Balance*, 14: 391-454.

Darvesh, A. S., Carroll, R. T., Bishayee, A., Geldenhuys, W. J. and Van der Schyf, C. J. 2010. Oxidative stress and Alzheimer's disease: dietary polyphenols as potential therapeutic agents. *Expert review of neurotherapeutics*, 10: 729-745.

Das, M., Rana, V. S. and Abirami, K. 2017. Seed germination and seedling growth of *Artemisia annua* L. *Medicinal Plants-International Journal of Phytomedicines and Related Industries*, 9: 29-35.

de Jesus, V. A. M., Braccini, A. L., Scapim, C. A., Santos, F. L., Mariucci, G. E. G. and Suzukawa, A. K. 2014. Effect of seed coat on the seed germination and seedling development of *Calophyllum brasiliense* Cambess. (*Clusiaceae*). *Acta Scientiarum. Biological Sciences*, 36: 451-456.

Department of Agriculture, Forestry and Fisheries (DAFF). 2016. Honeybust tea production guideline. Plant production. Pretoria, Republic of South Africa.

Devappa, R. K., Makkar, H. P. and Becker, K. 2011. *Jatropha* diterpenes: A review. *Journal of the American Oil Chemists' Society*, 88: 301-322.

Devkota, L., He, L., Midgley, J. and Haritos, V. S. 2022. Effect of seed coat microstructure and lipid composition on the hydration behavior and kinetics of two red bean (*Phaseolus vulgaris* L.) varieties. *Journal of Food Science*, 87: 528-542.

Dewir, Y. H., El-Mahrouk, M. E. S. and Naidoo, Y. 2011. Effects of Some Mechanical and Chemical Treatments on Seed Germination of *'Sabal palmetto*' and *'Thrinax morrisii*' Palms. *Australian Journal of Crop Science*, 5: 248-253.

Dhan, P., Sidhu, O. P. and Behl, H. M. 1992. Fodder potentials of some tree and shrub species grown on substandard (alkaline) soil site. *Indian Journal of Agricultural Biochemistry*, 5: 57-62.

Dhanasekaran, D., Ramya, K., Kumar, S. R. and Sathappan, C. T. 2020. Optimization of media and nutrition for foliage plants grown under modular vertical green walls, *Journal of Ornamental Horticulture*, 23: 51-60.

Donald, P. F. 2004. Biodiversity impacts of some agricultural commodity production systems. *Conservation Biology*, 18: 17-37.

Drew, M. C. and Lynch, J. 1980. Soil anaerobiosis, microorganisms, and root function. *Annual Review of Phytopathology*, 18: 37-66.

Duong, T. H., Shen, J. L., Luangviriyasaeng, V., Ha, H. T. and Pinyopusarerk, K. 2013. Storage behaviour of *Jatropha curcas* seeds. *Journal of Tropical Forest Science,* 25: 193-199.

Dutta, R., Stein, A., Smaling, E. M. A., Bhagat, R. M. and Hazarika, M. 2010. Effects of plant age and environmental and management factors on tea yield in Northeast India. *Agronomy Journal*, 102: 1290-1301.

Echeverri D., Montes F. R., Cabrera, M., Galán, A. and Prieto, A. 2010 Caffeine's vascular mechanisms of action. *Internaional Journal of Vascular Medicine.* 2010: 834060.

Ekpo, M. O. and Sita S. K. 2010. Influence of growing media on stem diameter and ecological characteristics of *Pinus patula* seedlings in Switzerland. *World Journal of Agricultural Science*, 6: 652-659.

El-Jaoual, T. and Cox, D. A.1998. Manganese toxicity in plants. *Journal of Plant Nutrition*, 21: 353-386.

El-Katony, T. M., Deyab, M. A., El-Adl, M. F. and Ward, F. M. E. N. 2021. Extracts of the brown alga *Dictyota dichotoma* (Hudson) JV Lamouroux alleviate salt stress in rice (*Oryza sativa* L.) during germination. *Journal of Plant Growth Regulation*, 40: 986-999.

Ellis, R. A. and Roberts, E. H. 1981. The quantification of ageing and survival in orthox seeds. *Seed Science Technology,* 9: 373-409.

El-Maarouf-Bouteau, H. 2022. The Seed and the Metabolism Regulation. *Biology*, 11: 168.

Erhabor, J. O., Omokhua, A. G., Ondua, M., Abdalla, M. A. and McGaw, L. J. 2020. Pharmacological evaluation of hydro-ethanol and hot water leaf extracts of *Bauhinia galpinii* (*Fabaceae*): A South African ethnomedicinal plant. *South African Journal of Botany*, 128: 28-34.

Erukainure, O. L., Ijomone, O. M., Chukwuma, C. I., Xiao, X., Salau, V. F. and Islam, M. S. 2020. Dacryodes edulis (G. Don) HJ Lam modulates glucose metabolism, cholinergic activities and Nrf2 expression, while suppressing oxidative stress and dyslipidemia in diabetic rats. *Journal of ethnopharmacology*, 255: 112744.

Farru, G., Dang, C. H., Schultze, M., Kern, J., Cappai, G. and Libra, J. A. 2022. Benefits and limitations of using hydrochars from organic residues as replacement for peat on growing media. *Horticulturae*, 8: 325.

Ferreira, J. F., Laughlin, J. C., Delabays, N. and de Magalhães, P. M. 2005. Cultivation and genetics of *Artemisia annua* L. for increased production of the antimalarial artemisinin. *Plant Genetic Resources*, 3: 206-229.

Ferreira, M. C. and Vieira, D. L. M. 2017. Topsoil for restoration: Resprouting of root fragments and germination of pioneers trigger tropical dry forest regeneration. *Ecological Engineering*, 103: 1-12.

Finch-Savage, W. E. and Leubner-Metzger, G. 2006. Seed dormancy and the control of germination. *New phytologist*, 171: 501-523.

Finneseth, C. L. H., Layne, D. R. and Geneve, R. L. 1996. Morphological development of pawpaw [*Asimina triloba* (L.) Dunal] during seed germination and seedling emergence. *HortScience*, 31: 633c-633.

Fredrick, C., Muthuri, C., Ngamau, K. and Sinclair, F. 2017. Provenance and pretreatment effect on seed germination of six provenances of *Faidherbia albida* (Delile) A. Chev. *Agroforestry Systems*, 91: 1007-1017.

Freese, K. E., Kokai, L., Edwards, R. P., Philips, B. J., Sheikh, M. A., Kelley, J., Comerci, J., Marra, K. G., Rubin, J. P. and Linkov, F. 2015. Adipose-derived stems cells and their role in human cancer development, growth, progression, and metastasis: A systematic review. *Cancer Research*, 75: 1161-1168.

Fung, K. F., Zhang, Z. Q., Wong, J. W. C. and Wong, M. H. 2003. Aluminium and fluoride concentrations of three tea varieties growing at Lantau Island, Hong Kong. *Environmental Geochem Health*, 25: 219-232.

Garbern, J. C. and Lee, R. T. 2021. Mitochondria and metabolic transitions in cardiomyocytes: lessons from development for stem cell-derived cardiomyocytes. *Stem Cell Research and Therapy*, 12: 1-25.

Getachew, S. and Derero, A. 2011. Seed handling and optimizing germination performance of tree. *Trends in Tree Seed Systems in Ethiopia*, 21: 30.

Giami, S. Y. 2001. Quality attributes of three new improved lines of Nigerian lima beans (*Phaseolus lunatus* L. Walp.). *Plant Foods for Human Nutrition*, 56: 325-333.

Gomaa, N. H. and Picó, F. X. 2011. Seed germination, seedling traits, and seed bank of the tree *Moringa peregrina* (*Moringaceae*) in a hyper-arid environment. *American Journal of Botany*, 98: 1024-1030.

Gómez, L. D., Baud, S., Gilday, A., Li, Y. and Graham, I. A. 2006. Delayed embryo development in the ARABIDOPSIS TREHALOSE-6-PHOSPHATE SYNTHASE 1 mutant is associated with altered cell wall structure, decreased cell division and starch accumulation. *The Plant Journal*, 46: 69-84.

Gour, V. K. 2006. Production practices including post-harvest management of *Jatropha curcas*. In *Proceedings of the Biodiesel Conference Towards Energy Independence—Focus on Jatropha*, 5: 223-251.

Gourlet-Fleury, S., Rossi, V., Rejou-Mechain, M., Freycon, V., Fayolle, A., Saint-André, L., Cornu, G., Gerard, J., Sarrailh, J. M., Flores, O. and Baya, F. 2011. Environmental filtering of dense-wooded species controls above-ground biomass stored in African moist forests. *Journal of Ecology*, 99: 981-990.

Goussous, S. J., Samarah, N. H., Alqudah, A. M. and Othman, M. O. 2010. Enhancing seed germination of four crop species using an ultrasonic technique. *Experimental Agriculture*, 46: 231-242.

Graeber, K., Linkies, A., Steinbrecher, T., Mummenhoff, K., Tarkowská, D., Turečková, V., Ignatz, M., Sperber, K., Voegele, A., De Jong, H. and Urbanová, T. 2014. DELAY OF GERMINATION 1 mediates a conserved coat-dormancy mechanism for the temperature-and gibberellin-dependent control of seed germination. *Proceedings of the National Academy of Sciences*, 111: 3571-3580.

Gupta, V. 2003. Seed germination and dormancy breaking techniques for indigenous medicinal and aromatic plants. *Journal of Medicinal and Aromatic Plants Science*, 25: 402-40.

Halmer, P. 2000. Commercial seed treatment technology. Seed Technology and Its Biological Basis. Sheffield Academic Press. Sheffield, England. pp. 257-286.

Hamilton, A. C. 2004. Medicinal plants, conservation and livelihoods. *Biodiversity and Conservation*, 1: 1477-1517.

Hartmann, H. T., Kester, D. E. and Davies, T. F. 1990. Principal of Propagation by Seed: Plant Propagation, Principals and Practices. 4th ed. Prentice Hall International Inc., New York. pp. 104-136.

Hartmann, H. T., Kester, D. E., Davies, Jr. F. T. and Geneve, R. L. 2011. Hartmann and Kester's Plant propagation: Principles and Practices. 8th ed. Prentice-Hall, Inc., Englewood Cliffs, New Jersey. p. 915

Hayat, K., Iqbal, H., Malik, U., Bilal, U. and Mushtaq, S. 2015. Tea and its consumption: Benefits and risks. *Critical Reviews in Food Science Nutrition*, 55: 939–954.

Hegde, S. and Maness, N. O. 1996. Changes in Hemicellulose-associated Pectin during Softening of Peach fruit. *Horticiltural Science*, 31: 640-640.

Hilhorst, H. W., Bradford, K. and Nonogaki, H. 2007. Definitions and hypotheses of seed dormancy. *Annual Plant Reviews: Seed Development, Dormancy and Germination*, 27: 50-71.

Hillel, D. and Kozlowski, T. T. 2012. Soil moisture and seed germination. *Water Deficits and Plant Growth*, 3: 65-89.

Himanen, K. and Nygren, M. 2014. Effects of seed pre-soaking on the emergence and early growth of containerized Norway spruce seedlings. *New Forests*, 45: 71-82.

Hishe, M., Asfaw, Z. and Giday, M. 2016. Review on value chain analysis of medicinal plants and the associated challenges. *Journal of Medicinal Plants Studies*, 4: 45-55.

Holmes, R. J., Mcdonald, J. N. A. W. and Juritz, J. 1987. Effects of clearing treatment on seed bank of the Alinene invasive shrubs *Acacia saligna* and *Acacia Cyclops* in the Southern and South Western Cape, South Africa. *Journal of Applied Ecology*, 24: 1045-1051.

Homola, T., Prukner, V., Artemenko, A., Hanuš, J., Kylián, O. and Šimek, M. 2021. Direct treatment of pepper (*Capsicum annuum* L.) and melon (*Cucumis melo*) seeds by amplitude-modulated dielectric barrier discharge in air. *Journal of Applied Physics*, 129: 193-303.

Hoveka, L. 2016. *Jatropha zeyheri* Sond. South African Biodiversity Institute. Pretoria. South Africa http://pza.sanbi.org/jatropha-zeyheri.

International Seed Testing Association ISTA. 1981. Amendments to International Rules for Seed Testing1976. International Seed Testing Association. Zurich. Switzerland.

Islam, A. K. M. A., Anuar, N. and Yaakob, Z. 2009. Effect of genotypes and pre-sowing treatments on seed germination behavior of *Jatropha*. *Asian Journal of Plant Sciences*, 8: 433.

Jaganathan, G. K., Bayarkhuu, N., He, L., Liu, B., Li, J. and Han, Y. 2021. Germination ecology of *Trachycarpus fortunei* (*Arecaceae*), a species with morphophysiological and shoot dormancy. *Botany*, 99: 601-608.

Jonville, M. C., Kodja, H., Strasberg, D., Pichette, A., Ollivier, E., Frederich, M., Angenot, L. and Legault, J. 2011. Antiplasmodial, antiinflammatory and cytotoxic activities of various plant extracts from the Mascarene Archipelago. *Journal of. Ethnopharmacology*, 136: 525-531.

Jose, S. 2011. Managing native and non-native plants in agroforestry systems. *Agroforestry Systems*, 83: 101-105.

Joubert, E., Gelderblom, W. C. A., Louw, A. and de Beer, D. 2008. South African herbal teas: Aspalathus linearis, Cyclopia spp. and Athrixia phylicoides—A review. *Journal of ethnopharmacology*, 119: 376-412.

Joubert, E., Joubert, M. E., Bester, C., De Beer, D. and De Lange, J. H. 2011. Honey bush (*Cyclopia* spp.): From local cottage industry to global markets-The catalytic and supporting role of research. *South African Journal of Botany*, 77: 887-907.

Juliani, H. R., Koelliker, Y., Bucuk, M., Welch, C., Tepper B., Jefthas, E. and Simon, J. E. 2009. Quality and consumer studies in the USA of African herbal teas for the natural product industry development in SubSahara Africa. *ACS Symposium Series,* 1021, 23: 403-420.

Kader, M. A. 2005. A comparison of seed germination calculation formulae and the associated interpretation of resulting data. *Journal and Proceeding of the Royal Society of New South Wales*, 138: 65-75.

Kalinganire, A., Weber, J. C., Uwamariya, A. and Kone, B. 2007. Improving rural livelihoods through domestication of indigenous fruit trees in the parklands of the Sahel. *Fruit Trees*, 10: 186-203.

Keogh, J. A. and Bannister, P. 1994. Seed structure and germination in *Discaria toumatou* (*Rhamnaceae*). *Weed Research,* 34: 481-490.

Kildisheva, O. A., Erickson, T. E., Madsen, M. D., Dixon, K. W. and Merritt, D. J. 2019. Seed germination and dormancy traits of forbs and shrubs important for restoration of North American dryland ecosystems. *Plant Biology*, 21: 458-469.

Klepacka, J., Tońska, E., Rafałowski, R., Czarnowska-Kujawska, M. and Opara, B. 2021. Tea as a source of biologically active compounds in the human diet. *Molecules*, 26: 1487.

Kloppenburg, J. 2010. Impending dispossession enabling repossession: biological open source and the recovery of seed sovriety. *Journal of Agrarian Change*, 10: 367-388.

Koen, J., Slabbert, M. M., Bester, C. and Bierman, F. 2017. Germination characteristics of dimorphic honeybush (*Cyclopia spp*.) seed. *South African Journal of Botany*, 110: 68-74.

Krishnapillai, M. V., Young-Uhk, S., Friday, J. B. and Haase, D. L. 2020. Locally produced cocopeat growing media for container plant production. *Tree Plant Notes*, 63: 29-38.

Kromhout, D., Spaaij, C. J. K., de Goede, J. and Weggemans, R. M. 2016. The 2015 Dutch food-based dietary guidelines. *European Journal of Clinical Nutrition*, 70: 869-878.

Kumar, A., Sharma, S. and Mishra, S. 2010. Influence of arbuscular mycorrhizal (AM) fungi and salinity on seedling growth, solute accumulation, and mycorrhizal dependency of *Jatropha curcas* L. *Journal of Plant Growth Regulation*, 29: 297-306.

Kumar, P., Srivastava, V. C. and Jha, M. K. 2016. *Jatropha curcas* phytotomy and applications: Development as a potential biofuel plant through biotechnological advancements. *Renewable and Sustainable Energy Reviews*, 59: 818-838.

Lake, J. C. and Leishman, M. R. 2004. Invasion success of exotic plants in natural ecosystems: The role of disturbance, plant attributes and freedom from herbivores. *Biological Conservation*, 117: 215-226.

Lambers, H., Brundrett, M. C., Raven, J. A. and Hopper, S. D. 2011. Plant mineral nutrition in ancient landscapes: High plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. *Plant and Soil*, 348: 7-27.

Layomi Jayasinghe, S., Kumar, L. and Sandamali, J. 2019. Assessment of potential land suitability for tea (*Camellia sinensis* (L.) O. Kuntze) in Sri Lanka using a GIS-based multi-criteria approach. *Agriculture*, 9: 148.

Leakey, R. R. 2019. From ethnobotany to mainstream agriculture: socially modified Cinderella species capturing 'trade-ons' for 'land maxing'. *Planta*, 250: 949-970.

Leedy, P. D. and J. E. Ormrod. 2005. Practical Research: Planning and Design. Pearson Education: New Jersey.

Leubner-Metzger, G., Petruzzelli, L., Waldvogel, R., Vögeli-Lange, R. and Meins F. 1998 Ethylene-responsive element binding protein (EREBP) expression and the transcriptional regulation of class I β-1,3-glucanase during tobacco seed germination. *Plant Molecular Biology*, 38: 785–795.

Li, B. and Foley, M. E. 1997. Genetic and molecular control of seed dormancy. *Trends in Plant Science*, 2: 384-389.

Linkies, A., Graeber, K., Knight, C. and Leubner-Metzger, G. 2010. The evolution of seeds. *New Phytologist*, 186: 817-831.

Lippitt, L., Fidelibus, M. W. and Bainbridge, D. A. 1994. Native seed collection, processing, and storage for revegetation projects in the western United States. *Restoration Ecology*, 2: 120-131.

Little, T. M and Hills, F. J. 1981. Statistical Methods in Agricultural Research. University of California: Davis, USA.

Liu, U., Cossu, T. A., Davies, R. M., Forest, F., Dickie, J. B. and Breman, E. 2020. Conserving orthodox seeds of globally threatened plants ex situ in the Millennium Seed Bank,

Royal Botanic Gardens, Kew, UK: the status of seed collections. *Biodiversity and Conservation*, 29: 2901-2949.

Liu, Z. Q., Ma, L .P., Zhou, B., Yang, L. and Liu, Z. L. 2000. Antioxidative effects of green tea polyphenols on free radical initiated and photosensitized peroxidation of human low density lipoprotein. *Chemistry and Physics of Lipids*, 106: 53-63.

Liyanage, G. S., Offord, C. A. and Sommerville, K. D. 2020. Techniques for breaking seed dormancy of rainforest species from genus *Acronychia*. *Seed Science and Technology*, 48: 159-165.

Long, R. L., Gorecki, M. J., Renton, M., Scott, J. K., Colville, L., Goggin, D. E., Commander, L. E., Westcott, D. A., Cherry, H. and Finch-Savage, W. E. 2015. The ecophysiology of seed persistence: a mechanistic view of the journey to germination or demise. *Biological Reviews*, 90: 31-59.

Lozano-Isla, F., Miranda, P. V. and Pompelli, M. F. 2017. Germination behaviour of *Jatropha curcus* L. after different imbibition times. *Peruvian Journal of Agronomy*, 1: 32-38.

Luna, B., Chamorro, D. and Pérez, B. 2019. Effect of heat on seed germination and viability in species of *Cistaceae*. *Plant Ecology and Diversity*, 12: 151-158.

Luna, T. and Wilkinson, K. M. 2009. Collecting, processing, and storing seeds. *Nursery Manual for Native Plants: Guide for Tribal Nurseries*, 1: 113-131.

Madikizela, B. and McGaw, L. J. 2017. Pittosporum viridiflorum Sims (*Pittosporaceae*): A review on a useful medicinal plant native to South Africa and tropical Africa. *Journal of Ethnopharmacology*, 205: 217-230.

Mahajan, R. A., Pongen, I. M., Dutt, S., Chand, R. and Rana, R. C. 2009. Oil content variation of purging nut (*Jatropha curcas Linn*.) seeds in Uttarakhand. *Annals of Forestry*, 17: 97-100.

Mainz, A. K. and Wieden, M. 2019. Ten years of native seed certification in Germany–a summary, *Plant Biology*, 21: 383-388.

Makunga, N. P., Philander, L. E. and Smith, M. 2008. Current perspectives on an emerging formal natural products sector in South Africa. *Journal of Ethnopharmacology*, 119: 365-375.

Mander, M., Ntuli, L., Diederichs, N. and Mavundla, K. 2007. Economics of the traditional medicine trade in South Africa care delivery. *South African Health Review*, 2007: 189-196.

Marcello, L., Nicla, C., Luca, G. and Maurizio, M. 2015. Effects of pre-sowing treatments on *Jatropha curcas* seed germination and seedling growth. *African Journal of Agricultural Research*, 10: 2553-2561.

Marnewick, L. J., Gelderblom, W. C. A. and Joubert, E. 2000 An investigation on the antimutagenic properties of South African herbal tea. *Mutation Research*, 471: 157-166

Maroyi, A. 2013. Traditional use of medicinal plants in south-central Zimbabwe: Review and perspectives. *Journal of Ethnobiology and Ethnomedicine*, 9: 1-18.

Mathibela, K. M. 2013. An investigation into aspects of medicinal plant use by traditional healers from Blouberg Mountain, Limpopo Province, South Africa. Doctoral dissertation Submitted to the University of Limpopo. Sovenga, South Africa.

Mathowa, T., Tshegofatso, N., Mojeremane, W., Matsuane, C., Legwaila, G. M and Oagile, O. 2016. Effect of commercial growing media on emergence, growth and development of tomato seedlings. *International Journal of Agronomy and Agricultural Research*, 9: 83-91.

Matsabisa, M. G., Bala, A., Tripathy, S., Digashu, M. M., Rautenbach, F., Dassarma, B., Erhabor, J. O., Braga, F. C., Mukherjee, P. K., Tang, M. and Kang, Y. 2022. Study on South African Indigenous Teas—Antioxidant Potential, Nutritional Content, and Hypoxia-Induced Cyclooxygenase Inhibition on U87 MG Cell Line. *Molecules*, 27: 3505.

Mbangcolo, M. M. 2008. Aspects of honeybush tea (*Cyclopia species*) propagation. Doctoral dissertation Submitted to the University of Stellenbosch. Stellenbosch, South Africa.

Mbangcolo, M. M., Reinten, E. Y. and Agenbag, G. A. 2013. Effect of different seed treatments on germination of *Cyclopia* spp. Seeds. *Agriprobe*, 30: 22-24.

McDonald Jr, M. B., Copeland, L. O., Knapp, A. D. and Grabe, D. F. 1996. Seed development, germination and quality. *Cool-Season Forage Grasses*, 34: 15-70.

McDonnell, A., Grant, M. and Coons, J. M. 2012. Effects of hot water on breaking seed dormancy of the endangered Kankakee mallow (*Iliamna remota* Greene (*Malvaceae*). *Erigenia*, 25: 8-13.

McMillan Browse, P. 1994. The propagation of *Ceanothus* from seed. *New Plantsman,* 1: 218-219.

Mehmood, T., Ahmad, W., Ahmad, K. S., Shafi, J., Shehzad, M. A. and Sarwar, M. A. 2013. Comparative effect of different potting media on vegetative and reproductive growth

of floral shower (*Antirrhinum majus* L.). *Universal Journal of Plant Science*, 1: 104-111.

Moffett, A. A. 1952. Differential germination in the black wattle (*Acacia mollissima* Willd.) caused by seed treatment. *Republic of Wattle Research Institute of South Africa*, 52: 39-50.

Mondal, M., Biswas, B., Garai, S., Sarkar, S., Banerjee, H., Brahmachari, K., Bandyopadhyay, P. K., Maitra, S., Brestic, M., Skalicky, M. and Ondrisik, P. 2021. Zeolites enhance soil health, crop productivity and environmental safety. *Agronomy*, 11: 448.

Mongalo, N. I. 2014. Antibacterial activity of Waltheria indica Linn (*Sterculiaceae*), collected from Blouberg area, Limpopo Province, South Africa. *African Journal of Biotechnology*, 13: 3198-3203.

Mongalo, N. I., Soyingbe, O. S. and Makhafola, T. J. 2019. Antimicrobial, cytotoxicity, anticancer and antioxidant activities of *Jatropha zeyheri* sond. roots (*Euphorbiaceae*). *Asian Pacific Journal of Tropical Biomedicine*, 9: 307-314.

Motsa, M. M., Bester, C., Slabbert, M. M., Ngwenya, M. Z. and Booyse, M. 2017. Natural fecundity and germination characteristics of selected *Cyclopia* (Honeybush) species: Preliminary findings. *Journal of Agricultural Science*, 9: 154-167.

Motsa, M. M., Slabbert, M. M., Bester, C., Mokwena, L. and Taylor, M. 2017. Volatile organic compounds from germinating seeds of *Cyclopia* species as affected by temperature. *Seed Science and Technology*, 45: 43-55.

Muralidhara, B. M., Reddy, Y. T. N., Srilatha, V. and Akshitha, H. J. 2016. Effects of seed coat removal treatment on seed germination and seedling attributes in Mango varieties. *International Journal of Fruit Science*, 16: 1-9.

Mutshekwa, N. 2017. Effect of time-based hot air drying method on chemical composition of *Jatropha zeyheri* tea. Mini-dissertation Submitted to the University of Limpopo. Sovenga, South Africa.

Mwang'Ingo, P. L., Teklehaimanot, Z., Maliondo, S. M. and Msanga, H. P. 2004. Storage and pre-sowing treatment of recalcitrant seeds of Africa sandalwood (*Osyris lanceolata*). *Seed Science and Technology*, 32: 547-560.

Mwase, W. F. and Mvula, T. 2011. Effect of seed size and pre-treatment methods of Bauhinia thonningii Schum. on germination and seedling growth. *African Journal of Biotechnology*, 10: 5143-5148.

Nadjafi, F., Bannayan, M., Tabrizi, L. and Rastgoo, M. 2006. Seed germination and dormancy breaking techniques for *Ferula gummosa* and *Teucrium polium*. *Journal of Arid Environments*, 64: 542-547.

Nakano, M., Itoh, Y., Mizuno, T. and Nakashima, H. 1997. Polysaccharide from Aspalathus linearis with strong anti-HIV activity. *Bioscience, Biotechnology, and Biochemistry*, 61: 267-271.

Nawrot P., Jordan S., Eastwood J., Rotstein J., Hugenholtz A. and Feeley M. 2003 Effects of caffeine on human health. *Food Additives and Contamination,* 20: 1-30.

Neelam A. and Ishtiaq M. 2001. Response of Eucalyptus camaldulensis seedlings to different soil media. *Sarhad Journal of Agriculture*, 17: 75-79.

Nkongolo N. V. and Caron J. 1999. Bark particle sizes and the modification of the physical properties of peat substrates. *Canadian Journal of Soil Science*, 79: 111- 116.

Nourmohammadi, K., Kartoolinejad, D., Naghdi, R. and Baskin, C. C. 2019. Effects of dormancy-breaking methods on germination of the water-impermeable seeds of *Gleditsia caspica* (*Fabaceae*) and seedling growth. *Folia Oecologica*, 46: 115-126.

Nyamayevu, T. and Mashingaidze, A. B. 2018. Influence of duration of storage at room temperature, pre-sowing seed treatment and fruit colour harvest index on germination and seedling growth of *Jatropha curcas* L. *Agroforestry Systems*, 92: 1221-1235.

Oagile, O., Ramalekane, O., Mojeremane, W., Matsuane, C., Legwaila, G. M. and Mathowa, T. 2016. Growth and development response of Kale (*Brassica oleracea var. Acephala* L.) seedlings to different commercial growing media. *International Journal of Plant Soil Science*, 12: 1-7.

Oliveira, M. A., Grillo, A. S. and Tabarelli, M. 2004. Forest edge in the Brazilian Atlantic forest: drastic changes in tree species assemblages. *Oryx*, 38: 389-394.

Omotayo, O. E. and Chukwuka, K. S. 2009. Soil fertility restoration techniques in sub-Saharan Africa using organic resources. *African Journal of Agricultural Research*, 4: 144-150.

Oyebode, O. A., Erukainure, O. L., Ibeji, C. U., Koorbanally, N. A. and Islam, M. S. 2019. Phytochemical constituents, antioxidant and antidiabetic activities of different extracts of the leaves, stem and root barks of Alstonia boonei: An in vitro and in silico study. *Botany Letters*, 166: 444-456.

Padma, M. and Reddy, Y. N. 1997. Effects of cracking and seedcoat removal on seed germination of mango (*Mangifera indica* L.). *Journal of Research Angrau*, 25: 18-21.

Pandey, A. K and Chauhan, O. P. 2019. Monk fruit (*Siraitia grosvenorii*)-health aspects and food applications. *Pantnagar Journal of Research*, 17: 191-198.

Pedrini, S., Gibson-Roy, P., Trivedi, C., Gálvez-Ramírez, C., Hardwick, K., Shaw, N., Frischie, S., Laverack, G. and Dixon, K. 2020. Collection and production of native seeds for ecological restoration. *Restoration Ecology*, 28: S228-S238.

Philippi, T. 1993. Bet-hedging germination of desert annuals: Beyond the first year. *The American Naturalist*, 142: 474-487.

Płażek, A., Dubert, F., Kopeć, P., Dziurka, M., Kalandyk, A., Pastuszak, J. and Wolko, B. 2018. Seed hydropriming and smoke water significantly improve low-temperature germination of *Lupinus angustifolius* L. *International Journal of Molecular Sciences*, 19: 992.

Poswal, F. S., Russell, G., Mackonochie, M., MacLennan, E., Adukwu, E. C. and Rolfe, V. 2019. Herbal teas and their health benefits: a scoping review. *Plant Foods for Human Nutrition*, 74: 266-276.

Pramanik, M. K. 2016. Site suitability analysis for agricultural land use of Darjeeling district using AHP and GIS techniques. *Modeling Earth Systems and Environment*, 2: 1-22.

Rajjou, L., Duval, M., Gallardo, K., Catusse, J., Bally, J., Job, C. and Job, D. 2012. Seed germination and vigor. *Annual Review of Plant Biology*, 63: 507-533.

Ranathunge, K., Schreiber, L. and Franke, R. 2011. Suberin research in the genomics era—new interest for an old polymer. *Plant Science*, 180: 399-413.

Renzi, J. P., Chantre, G. R. and Cantamutto, M. A. 2014. Development of a thermal-time model for combinational dormancy release of hairy vetch (*Vicia villosa* ssp. *villosa*). *Crop and Pasture Science*, 65: 470-478.

Riffat, A., Noreen F., Misbah R., Khalid M. Q., Ishfaq A., Khalid S. K. and Atif K. 2011. Influence of different growth media on the fruit quality and reproductive growth parameters of strawberry (*Fragaria ananassa*). *Journal of Medicinal Plants Research*, 26: 6224-6232.

Rivas, M., Reyes, O. and Casal, M. 2006. Influence of heat and smoke treatments on the germination of six leguminous shrubby species. *International Journal of Wildland Fire*, 15: 73-80.

Rosental, L., Nonogaki, H. and Fait, A. 2014. Activation and regulation of primary metabolism during seed germination. *Seed Science Research*, 24: 1-15.

Samie, A., Tambani, T., Harshfield, E., Ramalivhana, J. N. and Bessong, P. O. 2010. Antifungal activities of selected Venda medicinal plants against *Candida albicans,*

Candida krusei and *Cryptococcus neoformans* isolated from South African AIDS patients. *African Journal of Biotechnology,* 9: 2965-2976.

Schelin, M., Tigabu, M., Eriksson, I., Sawadogo, L. and Oden, P. C. 2003. Effects of scarification, gibberellic acid and dry heat treatments on the germination of Balanites aegyptiaca seeds from the Sudanian savanna in Burkina Faso. *Seed Science and Technology*, 31: 605-617.

Senanayake, S. N. 2013. Green tea extract: Chemistry, antioxidant properties and food applications–A review. *Journal of functional foods*, 5: 1529-1541.

Shafaei, S. M., Masoumi, A. A. and Roshan, H. 2016. Analysis of water absorption of bean and chickpea during soaking using Peleg model. *Journal of the Saudi Society of Agricultural Sciences*, 15: 135-144.

Sikalidis, A. K., Kelleher, A. H., Maykish, A. and Kristo, A. S. 2020. Non-alcoholic beverages, old and novel, and their potential effects on human health, with a focus on hydration and cardiometabolic health. *Medicina*, 56: 490.

Singh, A. V., Rahman, A., Kumar, N. S., Aditi, A. S., Galluzzi, M., Bovio, S., Barozzi, S., Montani, E. and Parazzoli, D. 2012. Bio-inspired approaches to design smart fabrics. *Materials and Design*, 36: 829-839.

Singh, T. B., Patra, S. K. and Devi, K. N. 2013. Study on Sesquioxide Content and Lime Requirement of Lateritic Soils. *International Journal of Bio-resource and Stress Management*, 4: 518-522.

Soleymani, A. 2019. Safflower (*Carthamus tinctorius* L.) seed vigor tests for the prediction of field emergence. *Industrial Crops and Products*, 131: 378-386.

Song, D., Jaganathan, G. K., Han, Y. and Liu, B. 2017. Seed dormancy in *Camellia sinensis* L.(*Theaceae*): effects of cold-stratification and exogenous gibberellic acid application on germination. *Botany*, 95: 147-152.

Statistical Analysis System (SAS). 2018. User Installation Guide for SAS® 9.1.3 Foundation for Microsoft® Windows®. SAS Institute Inc., Cary, North Carolina, USA.

Stevens, A. V., Nicotra, A. B., Godfree, R. C. and Guja, L. K. 2020. Polyploidy affects the seed, dormancy and seedling characteristics of a perennial grass, conferring an advantage in stressful climates. *Plant Biology*, 22: .500-513.

Sutcliffe, M. A. and Whitehead, C. S. 1995. Role of ethylene and short-chain saturated fatty acids in the smoke-stimulated germination of *Cyclopia* seed. *Journal of Plant Physiology*, 145: 271-276.

Szepesi, A., Csiszár, J., Gémes, K., Horváth, E., Horváth, F., Simon, M. L. and Tari I. 2009. Salicylic acid improves acclimation to salt stress by stimulating abscisic aldehyde oxidase activity and abscisic acid accumulation, and increases Na^+ content in leaves without toxicity symptoms in *Solanum lycopersicum* L. *Journal of Plant Physiology*, 166: 14-925.

Tahaei, A., Soleymani, A. and Shams, M. 2016. Seed germination of medicinal plant, fennel (*Foeniculum vulgare* Mill), as affected by different priming techniques. *Applied Biochemistry and Biotechnology*, 180: 26-40.

Tang, Y., Zhang, K., Zhang, Y. and Tao, J. 2019. Dormancy-breaking and germination requirements for seeds of *Sorbus alnifolia* (Siebold & Zucc.) K. Koch (*Rosaceae*), a mesic forest tree with high ornamental potential. *Forests*, 10: 319.

Taylor, A. G. P. S., Allen, P. S., Bennett, K. J., Burris, J. S. and Misra, M. K. 1998. Seed enhancements. *Seed Science Research*, 8: 245-256.

Terborgh, J., Feeley, K., Silman, M., Nunez, P. and Balukjian, B. 2006. Vegetation dynamics of predator-free land-bridge islands. *Journal of Ecology*, 92: 253-263.

Thomas, T. H. 1992. Some reflections on the relationship between endogenous hormones and light-mediated seed dormancy. *Plant Growth Regulation*, 11: 239-248.

Tsao R. 2010.Chemistry and Biochemistry of Dietary Polyphenols. *Nutrients.* 2: 1231-1246.

Tuckett, R. E., Merritt, D. J., Hay, F. R., Hopper, S. D. and Dixon, K. W. 2010. Dormancy, germination and seed bank storage: a study in support of ex situ conservation of macrophytes of southwest Australian temporary pools. *Freshwater Biology*, 55: 1118-1129.

Uwuigbe, E. U. 2017. Effects of different soil textures on early propagation of physic nut (*Jatropha curcas* L.). *Journal of Agriculture and Food Environment*, 4: 75-86.

Valdés-Rodríguez, O. A and Pérez-Vázquez, A. 2019. Seedling characteristics of three oily species before and after root pruning and transplant. *Plants*, 8: 258.

Valdes-Rodriguez, O. A., Sanchez-Sanchez, O., Perez-Vazquez, A. and Ruiz-Bello, R. 2011. Soil texture effects on the development of *Jatropha* seedlings–Mexican variety 'piñón manso'. *Biomass and Bioenergy*, 35: 3529-3536.

Van Schalkwyk, R. 2018. Soil water balance and root development in Rooibos (*Aspalathus linearis*) plantations under Clanwilliam field conditions. Doctoral dissertation Submitted to the University of Stellenbosch, Stellenbosch, South Africa.

Van Wijk, Y. 1986. The Practical Book of Herbs: Growing and Using Herbs in South Africa. Chameleon Press C.C., Cape Town, South Africa.

Van Wyk, B. and Gericke, N. 2007. People's plants: A guide to useful plants of Southern Africa, First Edition, Third Impression, Briza Publications, Pretoria, South Africa.

Van Wyk, B. E. 2011. The potential of South African plants in the development of new medicinal products. *South African Journal of Botany*, 77: 812-829.

Van Wyk, B. E. 2015. A review of commercially important African medicinal plants. *Journal of Ethnopharmacology*, 176: 118-134.

Van Wyk, B. E. and Gorelik, B. 2017. The history and ethnobotany of Cape herbal teas. *South African Journal of Botany*, 110: 18-38.

Wang, Y. R. and Hanson, J. 2008. An improved method for breaking dormancy in seeds of Sesbania sesban. *Experimental Agriculture*, 44: 185-195.

Wang, Y., Yao, Z., Pan, Z., Wang, R., Yan, G., Liu, C., Su, Y., Zheng, X. and Butterbach-Bahl, K. 2020. Tea-planted soils as global hotspots for N_2O emissions from croplands. *Environmental Research Letters*, 15: 104018.

Whitehead, C. S. and Sutcliffe, M. A. 1995. Effect of low temperatures and different growth regulators on seed germination in *Cyclopia* spp. *Journal of Plant Physiology*, 147: 107-112.

Windauer, L. B., Martinez, J., Rapoport, D., Wassner, D. and Benech-Arnold, R. 2012. Germination responses to temperature and water potential in Jatropha curcas seeds: a hydrotime model explains the difference between dormancy expression and dormancy induction at different incubation temperatures. *Annals of Botany*, 109: 265-273.

Yang, X., Baskin, J. M., Baskin, C. C. and Huang, Z. 2012. More than just a coating: ecological importance, taxonomic occurrence and phylogenetic relationships of seed coat mucilage. *Perspectives in Plant Ecology, Evolution and Systematics*, 14: 434-442.

Yineger, H., Yewhalaw, D. and Teketay, D. 2008. Ethnomedicinal plant knowledge and practice of the Oromo ethnic group in southwestern Ethiopia. *Journal of Ethnobiology and Ethnomedicine*, 4: 1-10.

Zhou, S., Sekizaki, H., Yang, Z., Sawa, S. and Pan, J. 2010. Phenolics in the seed coat of wild soybean (*Glycine soja*) and their significance for seed hardness and seed germination. *Journal of Agricultural and Food Chemistry*, 58: 10972-10978.

APPENDICES

Appendix 3.1 Analysis of variance for mean germination time (MGT) for seeds of *Jatropha zeyheri* under laboratory conditions after 30 days.

Source	DF	SS	MS	F	P
Treatment	5	342.81	68.562	14.44	0.0000
Error	54	256.39	4.748		
Total	59	599.20			

Appendix 3.2 Analysis of variance for germination percentage (GP) for seeds of *Jatropha zeyheri* under laboratory conditions after 30 days.

Source	DF	SS	MS	F	P
Treatment	5	2473.33	494.66	13.22	0.0000
Error	54	2020.00	37.40		
Total	59	4493.33			

Appendix 3.3 Analysis of variance for germination rate (GR) for seeds of *Jatropha zeyheri* under laboratory conditions after 30 days.

Source	DF	SS	MS	F	P
Treatment	5	0.056	0.011	13.22	0.0000
Error	54	0.045	0.000		
Total	59	0.101			

Appendix 3.4 Analysis of variance for germination index (GI) for seeds of *Jatropha zeyheri* sown under laboratory conditions after 30 days.

Source	DF	SS	MS	F	P
Treatment	5	1.124	0.224	11.45	0.0000
Error	54	1.060	0.019		
Total	59	2.185			

Appendix 4.1 Analysis of variance for mean germination time (MGT) for seeds of *Jatropha zeyheri* sown under greenhouse conditions after 90 days.

Source	DF	SS	MS	F	P
Replication	9	3436.8	387.42	3.85	0.0054
Treatment	5	5435.1	1087.03		
Error	45	12695.7	282.13		
Total	59	21617.6			

Appendix 4.2 Analysis of variance for germination percentage (GP) for seeds of *Jatropha zeyheri* sown under greenhouse conditions after 90 days.

Source	DF	SS	MS	F	P
Replication	9	68.33	7.59	2.74	0.0304
Treatment	5	128.33	25.66		
Error	45	421.66	9.37		
Total	59	618.33			

Appendix 4.3 Analysis of variance for germination index (GI) for seeds of *Jatropha zeyheri* sown under greenhouse conditions after 90 days.

Source	DF	SS	MS	F	P
Replication	9	2457.3	273.02	1.00	0.4289
Treatment	5	1365.0	273.00		
Error	45	12288.3	273.07		
Total	59	16110.6			

Appendix 4.4 Analysis of variance for germination rate (GR) for seeds of *Jatropha zeyheri* sown under greenhouse conditions after 90 days.

Source	DF	SS	MS	F	P
Replication	9	1.152E-04	1.280E-05	0.03	0.0176
Treatment	5	1.893E-04	3.786E-05		
Error	45	5.514E-04	1.225E-05		
Total	59	8.506E-04			

Appendix 4.5 Analysis of variance for stem diameter (SD) for seedlings of *Jatropha zeyheri* sown under greenhouse conditions after 90 days.

Source	DF	SS	MS	F	P
Replication	9	1.4341	0.15935	1.89	0.1153
Treatment	5	2.9922	0.59843		
Error	45	14.2618	0.31693		
Total	59	18.6881			

Appendix 4.6 Analysis of variance for chlorophyll content (CC) for seedlings of *Jatropha zeyheri* sown under greenhouse conditions after 90 days.

Source	DF	SS	MS	F	P
Replication	9	273.84	30.4263	1.52	0.2035
Treatment	5	337.75	67.5508		
Error	45	2003.43	44.5207		
Total	59	18.6881			

Appendix 4.7 Analysis of variance for seedling length (SL) for seedlings of *Jatropha zeyheri* sown under greenhouse conditions after 90 days.

Source	DF	SS	MS	F	P
Replication	9	69.744	7.7493	0.96	0.4533
Treatment	5	68.196	13.6392		
Error	45	625.921	14.2255		
Total	58				

Appendix 4.8 Analysis of variance for number of shoots (NS) for seedlings of *Jatropha zeyheri* sown under greenhouse conditions after 90 days.

Source	DF	SS	MS	F	P
Replication	9	5.0167	0.55741	1.37	0.2546
Treatment	5	6.4833	1.29667		
Error	45	42.6833	0.94852		
Total	59	54.1833			

Appendix 4.9 Analysis of variance for dry shoot mass (DSM) for seedlings of *Jatropha zeyheri* sown under greenhouse conditions after 90 days.

Source	DF	SS	MS	F	P
Replication	9	0.00447	4.970E-04	1.30	0.2792
Treatment	5	0.00487	9.747E-04		
Error	45	0.03363	7.473E-04		
Total	59	0.04297			

Appendix 4.10 Analysis of variance for dry root mass (DRM) for seedlings of *Jatropha zeyheri* sown under greenhouse conditions after 90 days.

Source	DF	SS	MS	F	P
Replication	9	0.02593	2.881E-03	1.23	0.3123
Treatment	5	0.02195	4.391E-03		
Error	45	0.16105	3.579E-03		
Total	59	0.20893			