The effect of scarification, temperature and light conditions on seed germination of *Artemisia afra* jacq. Ex willd.

by

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DISSERTATION

Submitted in fulfilment of the requirements for the degree of

Master of Agricultural Management

in

Plant production

in the

FACULTY OF SCIENCE AND AGRICULTURE

(School of Agriculture and Environmental Sciences)

at the

UNIVERSITY OF LIMPOPO

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2022

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DECLARATION

I declare that the dissertation hereby submitted to the University of Limpopo, for the degree Master of Agricultural Management (Plant production) has not been submitted previously by me or anybody for a degree at this or any other university; that it is my work in design and execution, and related materials contained herein had been duly acknowledged.

Letsoalo M.M	**Date**

DEDICATION

To my late mother and grandmother (rest in peace), the rest of my very beautiful and supportive family (Dikgomo-banareng) and the almighty God.

ACKNOWLEDGEMENTS

Philippians 4:13 “I can do all things through Christ who strengthens me”.

Above all, I would like to thank God almighty for keeping me and granting me strength and wisdom to complete this wonderful work. This work is a testimony of God’s grace. To my supervisory team, Dr A.R. Ndhlala and Prof P.W. Mashela, there are no enough words to express how grateful I am to learn from such excellent scientists. Special words of gratitude go to Dr Ndhlala, whom installed in me daily motivations to always do my best during presentations and all academic work. Thank you so much for pushing me to snap out of the challenges I was facing and focus on reaching the goal. ‘Thank you for installing in me the role of leadership’. Thank you for pushing me to greatness and believing in me when I did not believe in myself. To Prof. Mashela: thank you for training me into becoming a well-equipped scientist through your scientific writing lessons and presentations. Dr S. Mpai, thank you for your vast efforts, sleepless nights and for ensuring that my dissertation is of good standard. I will forever be indebted to you and are a great motivation as a woman in Science ‘nwa-Mpai’.

To Mr. N. Mutshekwa and Ms M. Malada, thank you for all the efforts ensuring that I get all the materials I need for research. Microbiology laboratory, thank you for a very healthy working environment. Mr. L. Ramoba from the Biochemistry laboratory, thank you so much for a healthy working environment and technical assistance. The Green Biotechnologies Research Centre of Excellence team, I am very grateful.

My friends and mates; Mothiba T.H., Mopai M.G., Mokgalabone T.T., Teffo K.P. and Ramakgasha M.J. who helped me with laboratory work, I appreciate your help. To my supportive family, more especially my aunt (Mologadi) and my brother (Mpho) and my little sister (Naledi). I am very grateful to the Department of Plant Production, Soil Sciences and Agricultural Engineering. The University of Limpopo. Department of Science and Innovation for the materials and most importantly the National Research Foundation for funding, thank you for making my dream come true.

PUBLICATIONS

Manuscript drafted

Tyson T. Mokgalabone, Mendy M. Letsoalo, Glas M. Mopai, Dineo G. Raphasha, Ndivhuho Mutshekwa, Phatu W. Mashela and Ashwell R. Ndhlala. 2021. Underutilised indigenous vegetables of the BaTswana culture: A review of their uses and availability in the Northwest Province of South Africa. Formatted according to the *South African Journal of Botany.*

Conference contribution

Letsoalo, M.M., Mashela, P.W. and Ndhlala, A.R. Priming using Moringa based biostimulant improved seed germination of *Artemisia afra* jacq. ex Willd, 4 -7 July 2022, Virtual 24th Indigenous Plant Use Forum (IPUF), University of Johannesburg, South Africa.

Letsoalo, M.M., Mashela, P.W. and Ndhlala, A.R. Priming using Moringa based biostimulant improved seed germination of *Artemisia afra* jacq. ex Willd, 21-23 September 2022, Bolivia lodge, 12th Faculty of Science and Agriculture Research Day (FSA), University of Limpopo, South Africa.

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GLOSSARY

®= Registered

µ= Micro (Mu)

%= Percentage

°C= Degrees Celsius

ARC= Agricultural Research Council

CRD= Completely Randomized Design

EM= Effective microorganisms

g= Gram

h= hour

H_2SO_4= Sulphuric acid

Min= Minute

Mg/l= Milligram per litre

M^2S^{-1}= Metre squared per second

Mol= Mole

MGT= Mean Germination Time

ml= Millilitre

P= Probability

SE= Standard Error

TTC= Triphenyl tetrazolium chloride

ABSTRACT

Artemisia afra Jacq. ex Willd is a common medicinal plant in Africa belonging to the Asteraceae plant family. It has been used for decades as a treatment for cold related illnesses and a variety of other ailments such as asthma, malaria and kidney disorders. As such this has led to its high demand and it is faced with threats of overharvesting. As the plant is naturally occurring and not widely cultivated, it's seed biology and germination properties are not documented. The optimum requirements (temperature and photoperiod) for seed germination as well as the effects of Moringa based biostimulant and different scarification methods were investigated on *A. afra* seeds in this study. The seeds were exposed to different scarification methods including physical scarification (rubbing using sandpaper), hot water soaking, cold water soaking, acid treatment (H_2SO_4) and fermentation (using effective microorganisms) while the untreated seeds served as the control under all conditions.

Moringa based biostimulant was also used as a seed priming agent at 0, 0.5, 1 and 3% levels. The seeds were then placed under a variety of photoperiods including constant light, alternating light (16/8 hours) and continuous darkness at different temperatures (15°C, 25°C and 35°C). Germination percentages and mean germination time (MGT) were calculated from the results and Statistix 10.0 software was used at the probability level of 5%, to compare the mean values. It was observed that under the different temperatures without any other treatments, the highest germination rate (70%) was achieved at a 25°C. No germination (0%) was obtained at both 15°C and 35°C in all the experiments including scarification methods and photoperiods.

Under the scarification treatments, soaking in hot and cold water scarification improved germination with final germination of 70%, compared to the other scarification methods (Physical at 20%, acid treatment at 20% and control at 10%). There was no germination observed (0%) for the fermented seeds in all the experiments. On the photoperiod treatments, the highest germination percentage of 70% was observed under alternating light condition. However, when mean germination time was calculated, continuous

darkness resulted in shortest MGT of 11 days compared to 13 days for both alternating light and for continuous light in this set of experiment. Seed priming with Moringa based biostimulant under alternating light conditions and at 25°C resulted in improved MGT. Under the investigated treatments, the use of 3% biostimulant exerted the highest improvement on germination with germination percentage of 62% and the shortest MGT of 7 days compared to the other treatments.

Generally the final germination (%) and time of *A. afra* seeds were affected by scarification, temperature, light and priming using Moringa based biostimulant. The information generated from this study is important because it will contribute in bridging the scientific gap on the information that has never been documented on optimum requirements for effective propagation of *A. afra* through seeds. As such this information will be useful for small scale farmers and medicinal plant growers who are willing to recover the lost populations of *A. afra* through seed propagation thus improving their supply and income.

Chapter 1: Research problem

1.1 Background

1.1.1 Description of the research problem

Artemisia afra Jacq. ex Willd., belonging to the Asteraceae family, is a common medicinal plant in Africa. It is used by people of different cultures and traditions for treatments of a variety of ailments such as coughs, fevers, colds, dyspepsia, loss of appetite, gastric de-rangements, colic, croup, whooping-cough, gout, asthma, malaria, diabetes, bladder and kidney disorders, influenza, and convulsions (Germishuizen and Meyer, 2003). The roots, stems and leaves of *A. afra* are used as enemas, poultices, infusions, lotions, inhaled (e.g. smoked or snuffed), or as an essential oil .In addition, *A. afra* is frequently used as a moth repellent, and in organic insecticidal sprays (Goldblatt and Manning, 2000).

There is a burgeoning need for the conservation of medicinal plants such as *A. afra* in South Africa because they are re-emerging as health aid. In fact, the rise is attributed by growing interest on the efficacy of this medicinal plant on preventing or control of non-communicable diseases. Apparently people have achieved awareness regarding the side effects of synthetic drugs and now prefer to use natural based formulations. Medicinal plants constitute one of the important overlooked areas of international development. They represent a form of biodiversity with the potential to do much good and not just in the healthcare. Indeed, the production and processing of medicinal plants such as *A. afra* offers the possibility of fundamentally upgrading the lives and well-being of people in rural regions (Hutchings, 1996).

It was estimated that 72% of Black South Africans, even in urban areas, subscribe to traditional health care systems involving the consumption of medicinal plants, and that more than 70,000 tons of plant material is consumed in South Africa each year, with at least 134,000 income-earning opportunities generated by the trade in medicinal plants and related products (Mander *et al*., 1996).

Besides the therapeutic impact on human health, medicinal plants are endangered due to improper harvest that leads to plant extinction in a long run. *Artemisia afra* is not easy to grow from seeds. The plant needs full sun and heavy pruning in winter to encourage development of new shoots in spring. Propagation by seeds is the major method by which plants reproduce in nature, and one of the most efficient and widely used propagation methods for cultivated crops (Leistner, 2000). Sowing seeds is the physical beginning of seedling propagation. Seed germination may be defined as the fundamental process by which different plant species grow from a single seed into a plant. This process influences both crop yield and quality. During the beginning stage of germination, the seeds take up water rapidly and this results in swelling and softening of the seed coat at an optimum temperature (Bewley *et al*., 2014).

1.1.2 Impact of the research problem

Artemisia afra is one of the oldest and best-known medicinal plants and is still used effectively today in South Africa by people of all cultures. The most widely utilized medicinal plant in southern Africa is undoubtedly *A. afra* (Liu *et al*., 2008; Diederichs, 2006; Van Wyk, 2008). In recent years, it has gained significant attention from the scientific community due to indigenous knowledge. Studies have been conducted either to verify or substantiate the traditional use of this herb. Further, its use is also being investigated in the modern diseases like diabetes, cardiovascular diseases, cancer, (Liu *et al*., 2002; Van Wyk, 2008) and related respiratory diseases (Diederichs, 2006).

Based on the above mentioned information, its high demand makes it difficult to find because the naturally occurring crops are endangered by over harvesting, hence depletion. As the effectiveness of medicinal plants is more widely acknowledged and accepted, over harvesting and extinction can result (Strangeland *et al.,* 2008), and small-scale farmers are also trying hard to meet burgeoning demand.

1.1.3 Possible causes of the research problem

Currently, in South Africa, *A. afra* is in popular demand for treatment of cold related illnesses and due to the pandemic caused by the Corona virus (Covid-19). Countries such as Madagascar proposed *A. afra* to be one possible treatment of the Covid-19 related disease. *Artemisia afra* is considered a “cure for all” remedy because of many ailments that it treats (Mukinda, 2007). According to the Red list of South African plants by SANBI. *A. afra* was found to be of least concern in Conservation status when the assessment was done in 2005. (Raimondo *et al*., 2009). Over the years, the great demand of this crop has led to its overharvesting and there is a risk of it getting depleted and getting extinct.

In an attempt to solve the over depletion rates of the crop, small scale farmers try to re-plant the crop, but a challenge arises regarding germination as a result of dormancy. Seed dormancy is a temporary interference in a viable seed, with germination processes inhibited for a specific period in a particular set of environmental conditions, after which germination might proceed provided interference factors were ameliorated by either natural or artificial means (Baskin and Baskin, 2004; Simpson, 1990). Generally, germination is a complex chemical process triggered by imbibition of water after potential dormancy factors had been released by appropriate triggers (Campbell, 1990). Dormant seeds fail to germinate even when all conditions for germination are favorable (Hartmann *et al.,* 2010).

1.1.4 Possible solutions

The populations of *A. afra* that were lost through overharvesting because of high demand can be regained by planting increased amounts of the crop. The re-planting of the crop will be done through sexual propagation. This method of propagation is done using seeds and it is the easiest and natural method of propagation with minimum disadvantages. Since it is well known that seeds of traditional or naturally occurring crops have very poor germination properties (Mander, 1998); several methods can be applied to improve their seed germination before planting. Enhancement of germination will be achieved by

breaking dormancy of the seeds through scarification and exposing the seed to different temperatures and light conditions because these are major requirements in germination. Seed priming is also another pre-planting method that will be used in this study to enhance germination. Seed priming is a process whereby germination process is regulated by means of managing the temperature and seed moisture content, this is done by taking the seed through the first biochemical processes within the initial stages of germination. The process involves advancing the seed to an equal stage of the germination process, to enable fast and uniform emergence when planted (Hussain *et al*., 2016).

1.1.5 General focus of the study

This study is focused on investigating the effect of scarification, temperature, and light condition on seed germination of *A. afra.* This will be achieved by applying different scarification methods on the seeds whilst exposing the seeds to different temperature regimes and varying light conditions. The study is also focused on investigating the effect of priming using a Moringa based biostimulant, Phytostim® on seed germination of *A. afra.*

1.2 Problem statement

Medicinal plants such as *A. afra* are becoming an important area of research and utilization as they were overlooked in the region (Hilliard, 1977; Diederichs, 2006). South Africa have different sources of health care, including traditional medicine and pharmaceuticals. The use of, *A. afra* is currently in demand for the treatment of cold-related illnesses and infections caused by the coronavirus. Countries such as Madagascar have proposed *A. afra* as a treatment for the novel coronavirus infection (Lu *et al*., 2020).

This led to over usage of the plant, depleting it, and putting it at risk of extinction. Depletion and extinction rates for slow growing; naturally scarce plant species are expected to be

greater than those for fast-growing, plentiful plant species. (UNESCO, 1998). Collection of seeds for cultivation *A. afra* could be one possible way to recover lost populations of this crop. Seeds including those of *A. afra* require moisture among all things and heat to sprout. In cases where conditions are not optimal, the seeds become dormant or rot. There is lack of information on how light and temperature affect the germination of these seeds and on methods of scarification that will help break the dormancy thereof. No information has been documented on how Moringa based biostimulant, Phytostim® affect germination of this crop. *Artemisia afra* has mostly been propagated asexually by cuttings and there is no enough information on propagation of this crop through seeds (DAFF, 2012).

1.3 Rationale of the study

Traditional healers have used *A. afra* to treat a variety of diseases, including respiratory symptoms, for decades. It has been used to treat coughs, fever, colds, chills, indigestion, lack of appetite, gastric derangements, colic, croup, gout, asthma, malaria, diabetes, bladder and kidney diseases, influenza, as well as convulsions. (Germishuizen and Meyer, 2003). It can be taken with water, inhaled as a smoke, or used with steam to relieve clogged nose and chest. This plant is used in a variety of ways to cure a variety of symptoms and disorders (Coetzee *et al*., 1999). Bronchitis is treated with *A. afra* syrup and mixtures can be used as a lotion to cleanse hemorrhoids and alleviate earache, In the Eastern Cape Province of South Africa, an infusion of this species' leaves or roots are also used to treat diabetes (Du- Preez *et al*., 2003). The leaves of *A. afra* are in high demand in South Africa where people want to treat Covid-19 and related symptoms.

Artemisia afra has become more difficult to find as demand and prices have risen in tandem with Covid-19 infection rates. Before the pandemic, the plant was easily accessible from street vendors and various traditional markets. People have recently begun abusing and overdosing on *A. afra* in attempt to avoid contracting Covid-19.The scientific community has paid close attention to *A. afra* in recent years (Van Wyk, 2008). There have been studies that confirm and support the use of this herb traditionally, but

no scientific reports were released on seed germination and propagation *of A. afra*. However, majority of seeds from our native plants have poor germination properties and it will be difficult to build up a sufficient stock for distribution to farmers when growing an indigenous crop such as *A. afra* (Mander, 1998).

As a result, there is a need to perform this study to explore, ways which improve germination and propagation of this crop, allowing farmers to cultivate *A. afra* to meet the high demand as well as close the scientific gap by researching on seed germination and propagation of *A. afra*.

1.4 Purpose of the study

1.4.1 Aims

- Determination of empirically based information on the effective scarification method, ideal temperature, and light conditions for *A. afra* seed germination.
- Determination of research based information on the effects of a commercially available Moringa based biostimulant, Phytostim® in seed germination of *A. afra* when applied using two priming methods.

1.4.2 Objectives

- To investigate the effect of scarification on the rate of germination of *A. afra*
- To investigate the optimal temperature for germination of *A. afra*
- To investigate the effect of photoperiod on the rate of germination of *A. Afra*
- To investigate the effects of a commercially available Moringa based biostimulant, Phytostim® in seed germination of *A. afra* when applied using two priming methods.

1.5 Reliability, validity, and objectivity

For this study, reliability of data is based on statistical analysis of data using Statistix 10.0 software at the probability level of 5%, validity is achieved through replicating the treatment as well as control and repeating the experiments in time, while objectivity is achieved by ensuring that the findings are discussed based on empirical evidence, to eliminate all forms of subjectivity (Leedy and Ormrod, 2005).

1.6 Bias

Bias was minimized by ensuring that the experimental error in each experiment was reduced using replications and by assigning treatments randomly within the selected research designs (Leedy and Ormrod, 2005).

1.7 Scientific significance of the study

This study intends on establishing whether priming, scarification, different temperature regimes and varying light conditions will influence seed germination of *A. afra*. This information will be shared with small-scale farmers and growers of traditional medicinal crops in general, to boost germination in their fields. This information will come in handy in growing *A. afra* and other medicinal crops with poor germinating properties or which have dormancy problems. The optimal light and temperature conditions for *A. afra* germination will be explored and used to improve optimal conditions for germination of this crop. Information on the priming method and effective dosage of Phytostim® when applied using two priming methods will be documented and shared among small scale farmers.

1.8 Structure of the dissertation

This dissertation begins with the description and detailed outlining of the research problem (Chapter 1), followed by the work done and not yet done on the research problem which is the literature review (Chapter 2). These will be followed by Chapter 3 which addresses each of the three objectives (The effect of scarification, temperature, and light conditions on seed germination of *A. afra* respectively. Chapter 4 will focus on how priming with Moringa based biostimulant, Phytostim® affect germination of *A. afra* seeds. In the final chapter (Chapter 5), findings in all chapters are abridged and integrated to provide the significance of the findings and recommendations with respect to future research, Finish up with a conclusion which tied together the entire study. This Dissertation followed the Harvard style using author-alphabets as approved by University of Limpopo Senate.

Chapter 2: Literature Review

2.1 Background

It is estimated that 80% of most developing countries of the world's population uses traditional medicines for primary health care services. The demand of medicinal plants has been increasing because of awareness of their efficacy and the increased rates in non-communicable diseases, as an effect, there is scarcity of medical plants. Due to high demands and intense harvesting practices, these plants are endangered in their natural habitat and have become scarce (Mander *et al*., 1996). The part of plants that are mostly under threat are bulbs, as an effect of unsustainable harvesting (Cunningham, 1988). There is a threat of extinction of many medical plants because there is an ever-increasing need for sustainable utilisation, instead of over harvesting, the plants that remain can mostly found in conservation areas, but even those are targeted for sustainable harvesting. It is extremely difficult to meet the already high demand of medical plants with only naturally available populations (Van Staden, 1999), so new strategies are needed to increase the populations of these plants.

Artemisia afra is a perennial, bushy, aromatic shrub, from a botanical family Asteraceae and it is originally from South-east Africa. It is one of the most popular medicinal plants in South Africa, which is easy to grow through cuttings, and it has been used by traditional medical practitioners for years to prevent and cure diseases. It is an important part of the herb garden, and with its silver-grey foliage it makes a beautiful display in the garden (Goldblatt and Manning, 2000). Its common names are African wormwood (English); wilde-als (Afrikaans); *umhlonyane* (*isiXhosa*); *mhlonyane* (*isiZulu*); *lengana* (Se*tswana*); *zengana* (Southern *Sotho*) (Pooley, 2003).

Artemisia afra grows in bushy, thick, and untidy clumps, it usually has tall stems up to 2 metres high, but sometimes very short, up to 0.6 meters. The stems are thick and woody at the base, becoming thinner and softer towards the top. Many smaller side branches shoot from the main stems (Germishuizen and Meyer, 2003). The stems are ribbed with

strong swollen lines that run all the way up. The soft leaves are finely divided, almost fern-like. The upper surface of the leaves is dark green whereas the undersides and the stems are covered with small white hairs, which give the shrub the characteristic overall grey colour (Germishuizen and Meyer, 2003).

Artemisia afra is a common species in South Africa with a wide distribution from the Cederberg Mountains in the Cape, northwards to tropical East Africa and stretching as far north as Ethiopia and it can be harvested throughout its growth (Leistner, 2000). Many of the other *Artemisia* species are aromatic perennials and are used for medicinal purposes.

Figure 2.1: *Artemisia afra* leaves and seeds (SANBI).

2.1. Introduction

Seed germination is a very essential stage in the life cycle of plants, since once seeds germinate, seedlings either start growing or they die, germination may also improve plant fitness, so that they are no easy damaged when attacked by animals or pathogens (Wenny, 2000). The time in which seeds germinate determines the conditions for subsequent seeding establishment and plant growth (Donohue *et al.,* 2010). As important as seed germination is in life cycles of plants, it is also often subjected to high mortality rates (Geraldine and Lisa, 1999).

Seed germination requirements are different across species and based on the environment in which the seeds are produced since seeds are sensitive to environmental conditions. Seed germination can occur only in response to specific combinations of environmental factors present in the field such as temperature, precipitation, or light (Donohue *et al.,* 2010). Germination is triggered by environmental conditions that are favourable to the overall plant growth, so that plant extinction can be prevented (Willis *et al*., 2014).

The process of germination begins with the saturation of water, followed by an increase in the respiratory activities and mobilisation of nutrient reserves, and finally resulting in initiation of growth and seedling emergence (Baskin and Baskin, 2014). Seeds are considered germinated when their coat breaks, seedlings emerge, the roots, hypocotyl and the cotyledons form (Busso, 2013). Germination begins with the activation of the seed embryo under certain environmental conditions, which results in the formation of the first true leaves (Hodge, 2014). However, germination can result with failure due to seed dormancy. Seed dormancy is the inability of an unscathed viable seed to complete germination under propitious conditions (Maldonado-Arciniegas *et al.*, 2018). In cases of seed dormancy, various treatment methods are used to improve the germination rate and percentage in seeds (Dayamba *et al*., 2010).

Furthermore, seed germination depends on inherent attributes and environmental factors including relative humidity, temperature and light. When these factors are at optimum, successful germination resulting in viable seed embryo emerge (Hodge, 2014; Bewley *et al.*, 2014). In addition, germination percentage is mainly used to indicate its completion, for example, 50% germination means 50% of the seed population has germinated. An embryo resumed to its normal physiological activities during seed germination, and plant emergence and growth were initiated. The ability of the seed to germinate at a particular time period is determined by different factors, such as external environmental conditions and of germination.

2.2. Work done on the problem statement

2.2.1. The effect of scarification on seed germination.

Different techniques can be applied to break seed dormancy and initiate seed germination, depending on the type of dormancy. There are two types of seed dormancy: exogenous and endogenous. Seeds with exogenous dormancy usually have pericarp and/or seed coat impermeable to oxygen and/or water. Endogenous dormancy occurs when germination is inhibited by chemicals in epidermis or adjacent interior membranes (Maldonado-Arciniegas *et al*., 2018).

Endogenous dormancy can be reduced by gibberellic acid and stratification, since they leach out inhibitors, whilst exogenous dormancy is broken down by physical and chemical treatments, which removes and penetrates the seed coat (Matilla, 2008). Seed scarification by soaking in sulphuric acid is mostly used to reduce barriers delaying germination (Hartmann *et al*., 2010).Soaking of seeds assists in improving, unifying, and increasing seed germination (Finch-Savage, 2013).

In order to trigger the germination process in seeds, dry or mature seeds absorb moisture through the seed coat (Kimball *et al*., 2008). Imbibition of water in the seed is required

since seeds lose a massive amount of water during different stages, such as when they mature or after they have matured, and during storage or processing, the process causes the seed to expand resulting in the rupture of the seed coat. When the seeds have water, the metabolism of the embryo which enables it to ensure growth will be influenced (Kimball *et al*., 2008).

Different stratification treatments have been tested, and successfully employed under greenhouse conditions, these treatments are, smoking and soaking in water (Dayamba *et al*., 2010). Smoking as a stratification method has not only been used on species from fire-prone ecosystems but also on species from fire-free ecosystems (Dayamba *et al*., 2010). Farshad *et al.* (2012) performed different scarification on *Allium hirtifolium* and reported that sulphuric acid scarification and gibberellic acid treatments in all experiments had no effect on seed germination when applied separately, but in the second experiment the highest germination percentage of 30% and germination rate of 4.2 days were observed when sulphuric acid scarification and gibberellic acid treatments were combined for 20 min. However, this combination could not completely overcome the seed dormancy.

Table 2.2.1.1: The effects of Gibberellic acid and sulphuric acid scarification on *Allium hirtifolium* seed germination.

Gibberellic-acid concentration (mg·L-1)	Soaking-duration in sulfuric acid (min)	Germination (%)
Control		0
100	5	5
	10	6.60
	20	6.60
200	5	5
	10	8.30
	20	11.30
500	5	16.60
	10	18.30
	20	21.60
100	5	21.60
	10	23
	20	30

Source: Farshad *et al.* (2012)

Further reports proved that when sandpaper, sulphuric acid scarification and cold stratification are applied separately, for 30 days, seed germination did not improve, but the highest germination percentage of 53% was observed when sulphuric acid was combined with cold stratification. However, the results of the combination of sandpaper stratification and cold stratification did not differ significantly from that obtained by sulphuric acid was combined with cold stratification. The results proved that the seeds had double dormancy and required both scarification and cold stratification (Farshad *et al*., 2012).

Table 2.2.1.2: The effects of cold stratification, scarification with sulphuric acid and sandpaper on *Allium hirtifolium* seed germination.

Cold scarification (days)	Scarification	Germination (%)
0	Sulphuric acid 10 min	0
0	Sandpaper	0
30	No scarification	3
30	Sulphuric acid 10 min	53
30	Sandpaper	50

Source: Farshad *et al.* (2012)

Farshad *et al.* (2012) reported that the highest germination percentage of 86.6% was obtained from scarification by sulfuric acid for 5 minutes combined with cold stratification for 60 days. This combination produced results like those that was obtained after 10- or 20-min of sulfuric acid treatment plus with 60 days of cold stratification: both combinations showed more than 80% germination percentage. By increasing the cold stratification duration from 15 to 60 days, germination percentage and germination rate were increased. Both scarification and cold stratification are needed for at least 60 days to attain an acceptable number of germinated seeds. Duration of soaking in sulphuric acid did not affect germination. Germination value of seeds was significantly affected by scarification. Without scarification, none of the seeds germinated.

Table 2.2.1.3: The effects of cold stratification and scarification with sulphuric acid on *Allium hirtifolium* seed germination.

Cold stratification (days)	Soaking duration in sulphuric acid (min)	Germination (%)
Control		0
15	5	30
	10	28.80
	20	28.80
30	5	40
	10	53.30
	20	48.30
45	5	68.30
	10	73.30
	20	70
60	5	86.60
	10	81.60
	20	80

Source: Farshad, *et al* (2012)

Maldonado-Arciniegas *et al*. (2018) used *Vachellia macracantha* seeds to test germination after different scarification methods were employed. As a result, it was reported that when chemical scarification was used seeds did not germinate at any soaking time. Seed coats may act as physical barriers limiting water penetration, gas exchange and embryo expansion (Huang *et al.*, 2017).

Maldonado-Arciniegas *et al.* (2018) also reported that physical treatments such as cold scarification significantly improved seed germination percentage, through all physical treatments compared to the control. This was due to acid not being able to enter the seed coat, which did not allow entry of water to the seed embryo, because of the concentration of the acid. Quality and fitness and physical scarification methods appeared to be more

effective than chemical scarification. Mechanical treatments, such as rubbing with sandpaper, break down the integrity of the seed coat, which facilitates water absorption and embryo expansion (Huang *et al*., 2017).

Loayza-Cabezas *et al*. (2018) used *Myroxylon balsamum* seeds to test germination of seeds after scarification and reported that scarification of *M. balsamum* increased germination. The highest germination rate when complete scarification had been done was reached in 37 days, 52 days when a longitudinal cut was made, and 72 days when the seed were not scarified.

Table 2.2.1.4: The germination capacity of *Myroxylon balsamum* seed in response to scarification.

Scarification	Germination (%)
Not scarified	13
Scarified	87
P	< 0.0001

Source: Loayza-Cabezas *et al.* (2018)

Physical dormancy is one of the classes of seed dormancy, based on five classes with different characteristics (Baskin and Baskin, 2004). The physical dormancy of seeds is caused when the seed coat is not permeable (Baskin, *et al,* 2000). Loayza-Cabezas *et al.* (2018) showed in the results that *M. balsamum* seeds have a physical dormancy behaviour and the scarification method used removed the physical dormancy caused by the pericarp and seed coat impermeable to water, increasing the germination percentage and the germination rate.

Werker (1980) reported that water resistant substances such as wax that covers the seed coat and restrict water penetration during seed ripening influences physical dormancy. Loayza-Cabezas *et al. (*2018) identified that the *M. balsam* was found to be hard on the

seed coat, and it is water impermeable. This substance keeps water out of the reach of the seed, contributing to the physical dormancy in *M. balsamum* seeds.

Nava *et al.* (2010) used *Lupinus campestris* seeds to test germination using different scarification methods and reported that seeds immersed 90 min in sulphuric acid showed the highest germination percentage of 50%. Scarification treatments of immersion in acid for 30 and 60 minutes resulted in a lower germination than the one immersed for 90 minutes in acid. Seeds in the control treatment which were soaked in distilled water had less than 5% germination percentage. The results of this conducted experiments showed that seeds of *L. campestris* has a very low germination percentage when scarification treatment is not used to break their dormancy. The immersion of *L. campestris* seeds in water did not improve their germination percentage so this shows that seed dormancy in *L. campestris* is generally better broken by acid treatment than by hormonal treatment.

Mackay *et al.* (2001) observed that seeds *of L. arboreus* that were not scarified had a very low germination percentage of less than 5% and the immersion of seeds in H_2SO_4 improved germination percentage to 80%. In this study, seeds of *L. campestris* scarified with H_2SO_4 had the germination percentage of almost 50%. Luera *et al.* (2021) used *Ebenopsis ebano* to determine the effect of scarification on germination, the seeds were soaked in different acids and the results showed that when soaking seeds in sulfuric acid treatment then soaking time had a significant effect on the germination likelihood in both the unstirred and stirred. The average germination time in the stirred treatment was 9 days with 20 minutes of soaking and the overall of less than 3% when independent of sulfuric acid soaking time in both the stirred and unstirred treatments. When seeds were treated with gibberellic acid alone, only 6.4% of *Ebenopsis* seeds germinated. In the control, no seeds germinated, and the likelihood of germination ranged from 3% when treated with 5 mg/L of gibberellic acid to 15% with 100 mg/L gibberellic acid, but these differences were not significant, and all treatment levels were not different from the control. At 100 mg/L gibberellic acid to 27 days at 10 and 500 mg/L gibberellic acid, average time to germination ranged from 18 days, but these differences were also not significant.

Table 2.2.1.5: The summary of results for chemical seed treatments for *Ebenopsis ebano*. Germination likelihood (%), average time to germination (days).

Treatment	Dose	Germination (%)	Days of germination
Sulfuric acid stirred	Control	4	22
	3 min	4	1
	6 min	24	10.2
	9 min	68	14.8
	12 min	60	11.2
	15 min	92	11.2
Sulfuric acid not stirred	Control	0	-
	10 min	0	-
	20 min	60	9.3
	30 min	84	7.9
	40 min	92	7.5
	50 min	100	7.6
	60 min	92	7.9
Gibberellic acid cracked			
Gibberellic acid not cracked	Control	0	-
	5 mg/l	5	9
	10 mg/l	10	26.5
	50 mg/l	3	21
	100 mg/l	15	18
	500 mg/l	10	27

Source: Luera *et al.* (2021)

2.2.2. The effect of temperature on seed germination.

Artemisia afra is planted in temperate and subtropical regions. It grows actively in the summer months, and it can withstand extremely low temperatures during winter months. It grows well where rainfall is more than 650 mm per year. Its natural habitat is in the higher-rainfall areas of the coastal regions and central escarpment area. *Artemisia afra* have higher germination under low temperature regimes than other species, suggesting they can emerge in early spring. However, this prediction needs to be viewed with caution because the low germination of some species at low temperatures might be due to physiological dormancy that would have been broken by cold stratification during winter, resulting in the ability of seeds to germinate at low spring temperatures (Baskin and Baskin, 2014).

Seasonally fluctuating temperatures are an important factor determining germination time of non-dormant seeds, and species in different sites show different germination behaviours in response to temperature fluctuation (Liu *et al*., 2013).Some studies have reported that seed germination and establishment success increase with increasing seed mass in a variety of environmental conditions (Moles *et al*,2005). In addition, large seeds with large reserves of stored food produce larger seedlings that can perform better under unfavourable conditions than seedlings from small seeds (Kitajima and Fenner, 2000). However, Baskin and Baskin, (2014) showed that there was no significant relationship between seed germination and seed size, but there was much variation regarding whether large or small seeds germinate best.

The germination percentage usually increases linearly with temperature up to an optimal temperature, after which the germination percentage decreases sharply (Tolyat *et al.,* 2014). For most perennial or winter annual plants, their favourable germination temperature ranges from 10–20°C, and these ecological habits are quite essential; for them to adapt to warm climates (Washitani and Masuda, 1990) Sometimes, high, or low temperatures also result in secondary dormancy, known as thermos-dormancy (Huo *et al.*, 2013).

It has been reported that high temperatures reduce seed germination in many species, and this thermal inhibition is usually attributed to high levels of endogenous abscisic acid,

which is realized by the transcriptional activation of endogenous abscisic acid signalling genes (Chiu *et al.,* 2012). High temperatures up-regulate endogenous abscisic acid biosynthesis genes and down-regulate catabolism genes (Toh *et al.*, 2008).

Guo *et al.* (2020) used *Pinus bungeana* seeds to test germination using different temperatures and found that they were all significantly different among the incubation temperatures, the highest germination of greater than 90% was achieved when the seeds were cultured for 30 days at 20°C, and a slightly lower temperature of 15°C was also an effective temperature for a high germination percentage of about 85%. Higher temperature regimes of 25°C and 30°C hindered radicle protrusion and significantly lowered the germination percentage less or equal to 5%. No seed germinated at a temperature of 10°C.

Nogemane (2017) used *Greyia radlkoferi* seeds to determine germination at different temperatures. It was reported that temperature significantly affected the final germination percentage of *G. radlkoferi* seeds with most temperature treatments showing statistically significant differences. With the increase in temperature from 15°C to an optimum point of 25°C seed germination percentage increased and decreased thereafter at 30°C. The highest germination percentage was 81% at 25°C followed by seeds exposed to 20°C at 78%. However, the difference in germination percentage between the two temperature treatments was not significant ($P > 0.05$). No significant seed germination occurred at 15°C and 30°C while seed germination did not occur at 10°C. At 30°C germination percentage of 47% was recorded and the lowest germination of 32% was recorded at 15°C.

Table 2.2.2.1: The temperature effects on the days to beginning of germination and days to ending of germination of *Greyia radlkoferi*

Temperature (°C)	Onset of germination (days)	End of germination (days)
10	-	-
15	12	13
20	6	11
25	6	11
30	9	13

Source: Nogemane (2017)

Table 2.2.2.1 shows that the germination time of Greyia radlkoferi was significantly affected by temperature. The seeds of *G. radlkoferi* started to germinate 6 days after sowing and germinated up to 13 days after sowing. The first seeds to germinate were exposed to 20°C and 25°C of temperatures on day 6 after sowing, followed by 30°C on day 9 and 15°C on day 12. Seeds exposed to 10°C of temperature did not germinate over the whole period of the experiment.

Sparg *et al*. (2005) used *Merwilla natalensis* seeds to determine germination rated across different temperatures and reported that the germination of *M. natalensis* seeds is not temperature dependent, because seeds germinated over a wide temperature range (10–40°C). A temperature range of 10–40°C and alternating temperatures did not significantly influence the percentage germination. Temperature did, however, affect germination time. At high (40°C) and low (10°C) temperatures, the germination time was delayed. Although germination in this study was delayed at both 10 and 40°C, the percentage germination was not affected. The optimum temperature for germination of *M*. *natalensis* seeds was 26°C.

Table 2.2.2.2: The effects of temperature on the seed germination of *Merwilla natalensis*

Temperature(°C)	Germination (%)	Germination time(days)
10	97.5	10
20	100	4
25	87.5	2
30	95	2
35	95	3
40	92	6

Source: Sparg *et al.* (2005)

Yang *et al.* (2014) used seven *Stipa* species to determine the effect of temperature on their germination and reported that in all seven *Stipa* species temperature had considerate effects on percentage and rate of germination. In general, germination percentage increased at average temperatures and then decreased as temperature increased to above average rates, however, *S. purpurea* seeds germinated to a high percentage if greater than 90% at the temperature of 5°C to 35°C. Germination of *Stipa* species from the cold temperature habitats appeared to be more tolerant to high temperature than that of species from the warm temperature habitats. For example, germination of *S. grandis*, *S. purpurea*, and *S. penicillata* seeds from habitats with low temperature and high rainfall was 93%, 97%, and 51% at the temperature of 35°C, respectively, whereas for seeds of *S. glareosa*, *S. breviflora*, *S. gobiea*, and *S. bungeana*, which are from habitats with high temperatures and low rainfall the germination was 27%, 45%, 13%, and 46% respectively at the temperature of 35°C.

Mohammad and Faezeh (2017) used *Eryngium caeruleum* seeds to test germination in different temperatures and reported that germination of seeds of the *E. caeruleum* occurred within the constant temperature range of 5 ºC to 30 ºC. There was an increase in the seed germination of all the species when temperature Increased from 5 to 15 ºC.

The highest germination percentage of seeds was at 10 ºC and 15 ºC. there was a decrease in seed germination as temperature increased from 15 ºC up to 30 ºC, and at a high temperature of 35 ºC germination decreased to zero.

Loayza-Cabezas *et al.* (2018) used *Myroxylon balsamum* seeds to test germination using different temperature regimes and reported that there was no significant difference between the factors of temperature regimes in the germination percentage of *M. balsamum*, but the cumulative germination percentage on the 23/27°C temperature regime was reached at 19 days before that on the 19/23°C regime.

Table 2.2.2.3: The effects of temperature regimes in the seed germination of *Myroxylon balsamum*.

Temperature	Germination (%)
19/23°C	82
23/27°C	87
P	0.528

Source: Loayza-Cabezas *et al.* (2018)

Different literature shows that temperature affects the germination of different plants differently, depending on the characteristics of the plant and other factors such as dormancy and seed size.

2.2.3. The effect of photoperiod on seed germination.

Light is a critical factor for germinating seeds, root cuttings, seedling emergence and growth and when propagating using tissue culture (van Wyk, 2011). In fact, it acts directly on germination. Light can be controlled by growing plants in structures such as, green or glass house, shade nets, incubator, and growth chambers. Light can limit or improve the growth of plants depending on light intensity and photoperiod (McMahon *et al*., 2002). Seeds responds differently to light depending on their species.

Some seeds germinate more readily under light and some in complete darkness, whilst others are barely affected by light. Light is not an essential factor for the germination of all species, but it helps to reduce the detrimental effects of germination when temperatures are higher than optimum (Aud and Ferraz, 2012).

Light can regulate environmental signals in seed germination of desert plants, since dessert seeds can change dormancy from primary to secondary and this would cause them to require light to trigger germination (Baskin and Baskin, 1995). Phytochrome found in seeds mediates the genetic characteristic of seeds to require light to germinate (Jones and Hall, 1979). This phytochrome stimulates the formation of growth promoting hormones to start germination (Gul and Weber, 1999). In some species light is temperature dependant, whilst in others it can affect germination independently (Khan and Ungar, 1997).

Guo *et al.* (2020) used *Pinus bungeana* seeds to test germination in dark and light, it was reported that no significant differences were obtained between light and dark germination conditions, and the interaction of the light condition and temperature regime was also not significant, this showing that that the germination response pattern of the tested pine seeds to temperature is the same under different light conditions.

Nava *et al.* (2010) used scarified *L. campestris* seeds to test germination in light and darkness and reported that germination percentage was very low in scarified seeds that were maintained under completely dark conditions than the ones germination in light conditions. The study also showed that light has a positive effect on germination, Kaye (1997) did not find differences in germination percentage of seeds of *Luminus latifolius* under light or darkness conditions.

Sparg *et al.* (2005) reported that from the results of continuous light and dark germination experiments of *M. natalensis* seeds, it was observed that light may not be an important requirement for germination since seeds germinated equally well under both light and dark conditions.

Table 2.2.3.1: The effects of light conditions on the seed germination of *Merwilla natalensis*.

Light treatment	Germination (%)	Germination time (days)
Continuous dark	95	3
Continuous light	95	3

Source: Sparg *et al.* (2005)

Jabarzare *et al.* (2011) used *Artemisia sieberi* seeds to determine how light affects germination and the results showed that continuous darkness reduced germination percentage in a significant level compared with the 12-hour photoperiod. In plant number 2 the highest germination percentage was obtained in the 12-hour photoperiod, and it was observed under dark treatment. In the light treatment, plant number 1 showed a high percentage of germination. In plant number 4, the lowest, percentage and rate of germination were observed with dark treatment. In this research it was concluded that germination rate is not significantly affected by light.

Table 2.2.3.2: The effect of light and darkness on different plants for all factors on *Artemisia sieberi* seeds.

Treatment	Plant	Germination (%)
Light	1	95
Darkness	1	58
Light	2	95
Darkness	2	83
Light	3	85
Darkness	3	72
Light	4	94
Darkness	4	33

Source: Jabarzare *et al.* (2011)

Loayza-Cabezas *et al.* (2018) used *Myroxylon balsamum* seeds to test germination of seeds in high and low light and reported that light intensity does not affect seed germination. Species that can tolerate growing in the shade can germinate, grow, and survive in low light (Osunkoya *et al*., 1994). Loayza-Cabezas *et al.* (2018) showed that *M. balsamum* germinated well in both low and high lights, 65% germination in the absence of light and 67% germination with 367 µmol $m^{-2}s^{-1}$ light intensity, under a similar temperature regimen (20/30 °C).

Table 2.2.3.3: Germination capacity of *Myroxylon balsamum* seed in response to light intensity.

Light intensity (L)	Germination (%)
124 µmol m^{-2} s^{-1}	84
32 µmol m^{-2} s^{-1}	85
P	0.7755

Source: Loayza-Cabezas *et al*. (2018)

2.2.4 The effect of Seed priming on germination

Seed priming is a low-cost effective hydration technique to stimulate seed germination. During priming, seeds go through a physiological process, i.e., controlled hydration and drying which results in enhanced and improved pre-germinative metabolic process for rapid germination (Musa *et al*., 1999). Seed priming can synchronize seed germination, and increase emergence (Rafi *et al.,* 2015). Seed priming techniques have multiple benefits such as reduce the use of fertilizers, enhance crop yield by synchronized seed germination, and induce systemic resistance in plants which is both cost-effective and eco-friendly.

Poor seed germination and seedling emergence are general problems in most of the farms, which ultimately results in the poor establishment of seedlings and low crop yield.

Priming can play a potential role to minimize this problem. Priming can positively affect growth, both at the initial and later developmental stages. Yield can be affected by the growth promotion of plants (Elouaer and Hannachi, 2012).

To invigorate the seeds, accelerate the germination process, and alleviate the environmental stress, different seed priming methods have been developed including Hydro-priming, Halo-priming, Osmo-priming, Hormone-priming, Solid matrix priming and Bio-priming. Though the choice of a priming method is important but most importantly is its efficiency which is affected by many factors and depends on the plant species. Factors like priming duration, priming agent and oxygen supply to seed have a notable effect on seeds of various crops (Bajehbaj, 2010). Following the above factors, some physical and chemical parameters such as osmotic potential, temperature, presence or absence of light, aeration, and seed condition can also affect priming and determine the germination rate and time, seedling vigor, and further plant development (Abraha and Yohannes, 2013).

2.3. Work not done on the problem statement

A lot of literature has been written on germination, and the effect of different conditions on the germination of other plants, but there is no research done on the effect of the conditions on the germination of *A. afra* seeds. *Artemisia afra* is one of the most popular and commonly used herbal medicinal plants in southern Africa. However, there is very limited research conducted on this species in relation to seed germination to improve its sustainable production. Such research is a necessity to improve propagation strategies for medicinal plants.

Chapter 3: The effect of different temperature regimes, light conditions and scarification methods on seed germination of *Artemisia afra* Jacq. ex Willd

3.1 Introduction

Indigenous cultures have relied on plants to supply their medicinal needs for centuries (Taylor *et al.,* 2001). In South Africa alone, it is estimated that about 27 million people depend on traditional medicine for their health care needs (Fennell *et al*., 2004; James *et al.*, 2018). According to McGeocha *et al*. (2008), "Over exploitation is a growing problem for many medicinal species in Africa". For example, in Tanzania alone, there are nine plants of medicinal value that are reported to be of conservation concern (Strangeland *et al.,* 2008). In South Africa, there is one species (*Encephalartos woodi*) that is known to be extinct in the wild due to over harvesting for medicine at the time it was discovered by scientists in the 19th century (Grouch *et al.,* 2003).

Although there is still a drive towards sustainable harvesting, increasing demand coupled with the loss of habitats is quickly leading to the only real solution being the cultivation of important medicinal plants (Fennell *et al*., 2004). Although it is agreed that there is a need for the cultivation of medicinal plants, there is a lack of relevant information available as to the specific requirements of these plants (Makunga *et al.,* 2008; Fennell *et al*., 2004; McGeocha *et al*., 2008). Little information exists on the effects of cultivation practices on the growth and biological activity of African medicinal plants (Fennell *et al.,* 2004).

Naturally occurring species such as *A. afra* are particularly affected by seed dormancy, and require special practices to break dormancy and induce germination. For germination to occur, moisture must be able to enter seeds (Alvarado and Bradford, 2002). The process of scarifying seeds can vary from scratching and cracking seed coats, soaking seeds briefly in hot water or extreme acid. Scarification is pretreatment of seeds which aims to break seed dormancy and accelerate the occurrence of uniform seed germination.

It can also be described as a way to provide a permeable condition of seeds through puncturing, burning, breaking, filing, and scratching with knives, needles, sandpaper, and other tools (Bradford, 1990).

Temperature is the most important driving force influencing crop development rate (Bewley, 1997). The effects of temperature on plant development are the basis for models used in predicting germination timing. Temperature and moisture are among the very important environmental factors in the germination of non-dormant seeds. These two factors, singly or in combination, can influence on germination percentage and rate. Plants have base or minimum, optimum, and ceiling temperatures for seed germination. The minimum or base temperature is the lowest temperature which seeds can be germinated (Ramin, 1997). The optimum temperature is the temperature which seeds reach its highest germination rate, and the maximum or ceiling temperature is the temperature above which seeds cannot germinate. Use of cardinal temperatures makes it possible to estimate geographical limitations and select the suitable time before planting seeds (Kebreab and Murdoch, 2000).

Cardinal temperatures of germination usually depend on the range of adaptability of the related species and insure the coincidence of germination with desirable conditions for the following stages of development. Seed germination of any plant takes place in a specific range of temperatures called the cardinal temperatures. In this range, there are minimum, optimum, and maximum temperatures that have applications in presenting models for predicting germination (Bewley and Black, 1994). Germination rate increases with rises in temperature up to the optimum one and declines at temperatures exceeding it.

Plant growth rate increases with rises in temperature from the base to the optimum temperatures and declines at temperatures between the optimum and the ceiling ones. Seeds germinate in a wide range of temperatures, but their maximum germination considerably changes at the upper and lower thresholds of this range.

The temperature range which germination is at its maximum varies depending on species and seed quality (Bewley and Black, 1994). Seeds of each plant type need an optimum temperature for germination. Sometimes, some seed types germinate at low temperatures such as zero or close to zero degrees, but these temperatures prevent germination of other seed types. For most plants, the minimum and maximum germination temperatures are 15-30 and 30-40°C, respectively. There are numerous reports suggesting germination percentage and rate increase up to a point with rises in temperature. Temperature can influence germination percentage and rate by affecting seed deterioration by reducing seed dormancy and through all other germination processes (Ramin, 1997).

Light, a key crucial environmental signal, controls diverse biological processes in plants such as seed dormancy and germination, photo morphogenesis, phototropism, shade avoidance, and flowering. Light is a much vital requirement for some plants as darkness is to others; it all depends on the nature of the plant in question (Guo *et al*., 2020). Light serves as an external trigger for seed germination process, and some plants would not sprout till light shines on them. Some biologists consider seeds that require light to germinate to be photo dormant. Others feel it just one of many environmental factors (like temperature and water) that are necessary for germination. In general, light requiring seeds tend to be very small (Gul and Weber, 1999).

During the process of germination in plants there is a stage known as photo absorption stage, this stage is a distinguishing phase for most plants as they have different responses to light for their germination. Some plants are photoblastic (they need light to grow), while others are non-photoblastic (they do not need light to grow). When seeds have absorbed water and oxygen, they tend to respond better to light, and if they are positively photoblastic, then just the right amount light will provoke their growth (Gul and Weber, 1999).

As a way of overcoming depletion rates of medicinal plants, cultivation is the proposed solution and this study aimed on gathering information on the methods and conditions that enhance seed germination of *A. afra*.

To achieve this, different scarification techniques, varying temperature regimes and alternated light conditions were investigated on how they affect seed germination of *A. afra.*

3.2 Objective of the study

The objective of this chapter was to determine the effect of temperature, different light conditions and different scarification methods on seed germination of *A*. *afra* seeds.

3.3 Material and methods.

3.3.1 Description of study location

The study was carried out at the University of Limpopo's Green Biotechnologies Research Centre of Excellence in South Africa (23°53′10″S, 29°44′15″E). The experiment was conducted in temperature-controlled growing chambers in the laboratory.

3.3.2. Seed collection, viability and moisture tests

The seeds of *A. afra* were obtained from the Agricultural Research Council, Roodeplaat in Pretoria, South Africa. The seeds were stored at 4 °C prior usage in seed germination tests. The seed viability of the obtained seeds was tested using a 2, 3, 5-triphenyl tetrazolium chloride (TTC) solution (ISTA, 1999). Seeds were soaked in a 1% solution of TTC for 24 h at 25 ± 2 °C in the dark. Seeds were then cut longitudinally under a microscope and the red-stained embryos were considered as representing viable seeds.

Moisture content of the seeds was determined by drying them at 110 °C in a pre-set incubator until there was no further loss in seed weight. Moisture content, expressed as percentage, was calculated on the basis of fresh weight using the equation below:

Moisture content of seeds (%) = $\frac{fresh\ weight - dry\ weight}{fresh\ weight} \times 100$ (Bewley and Black, 1982).

3.3.3 Research design

The experiment was set up in a Completely Randomized Design (CRD), with ten seeds in each petri dish, replicated four times.

3.3.4. Experimental procedures

3.3.4.1 Effect of temperature and photoperiod

Figure 3.1: Growth chamber containing *Artemisia afra seeds* experiment.

Ten *A. afra* seeds were placed in each Petri dish (90 mm) with the bottom plate lined with two layers of filter paper (Whattman No.1) and placed incubators set at constant temperatures of 15, 25, and 35°C to assess the impacts of varied temperature regimes. Apart from the temperature settings, the following light (photoperiod) conditions were set on the incubators: constant light given off by cool-white fluorescent lamps (Photosynthetic Photon Flux of 40.5 mol. m^2s^{-1}), alternating light (16 h dark and 8 h light photoperiod), and constant dark period where the Petri dishes were wrapped with paper foil which do not permit entry of light. The filter papers were dampened with distilled water when there is a need and the experiment was conducted for a period of 40 days. The experiment was replicated four times. Data was collected on a daily bases by recording of shoot emergency. For the constant dark experiments, green safe light (0.3 $molm^2s^1$) was used for inspecting the seeds. The data collected was used to determine the Mean Germination Time (MGT) and germination percentages.

The equation developed by Ellis and Roberts (1981) was used to compute MGT as stated below.

Mean Germination Time (MGT) = $\frac{\sum(n \times d)}{N}$

Where **n** denotes the number of seeds that were able to germinate on daily basis, **d** being the number of days since the commencement of the test, and **N** denoting the total number of seeds germinated at the termination point of the experiment.

3.3.4.2 Effect of scarification

Different scarification procedures were carried out to investigate their effect on *A. afra* seed germination.

3.3.4.2.1 Non-treatment

The non-scarified seeds were used as the control groups.

3.3.4.2.2 Chemical scarification (Acid Treatment)

A batch of *A. afra* seeds was exposed to 93% technical grade sulphuric acid as described by Hartman *et al.* (2001).The procedure was done by placing the seeds in contact with the acid in a glass beaker for a minute then rinsing them with distilled water. The seeds were then placed in Petri dishes as described in section 3.3.4.2.1 above, in 25°C incubators set at the three photoperiod conditions (complete darkness, continuous light and 16h/8h alternating light). Each petri dish contained 10 seeds and was replicated 4 times. After 40 days of daily monitoring and data collection as in the section above, MGT and germination percentages were calculated. A temperature of 25°C was used for this experiment because there was no germination observed at 15 and 35°C as per results of section 3.3.4.2.1 above.

3.3.4.2.3 Scarification by fermentation

The investigation of fermentation on the effect of *A. afra* seeds germination was done using the effective microorganisms and molasses mixture. The fermentation process was done by adding 18 ml of Effective Microorganisms solution in 1 liter plastic bottle, 18 ml of molasses was also added, as well as 6.25 g of fine white sugar. The sugar was dissolved in 500 ml chlorine free water and 500 ml water was added to the overall solution and mixed well (Siquera *et al.*, 1991). After the solution has settled, *A. afra* seeds were added to the mixture and left to ferment for 72h. After the 72h fermentation process, the seeds were then placed in petri dishes as described in section 3.3.4.2.1 above, in 25°C incubators set at the three photoperiod conditions (complete darkness, continuous light and 16h/8hr alternating light). Each petri dish contained 10 seeds and was replicated 4 times. After 40 days of daily monitoring and data collection as in the section above, MGT and germination percentages were calculated. A temperature of 25°C was used for this experiment because there was no germination observed at 15 and 35°C as per results of section 3.3.4.2.1 above.

3.3.4.3.4 Physical scarification by rubbing

A batch of *A. afra* seeds was rubbed softly for a minute using sandpaper to break dormancy by softening the seed coat (Dayamba *et al*., 2010). After rubbing, the seeds were then placed in petri dishes as described in section 3.3.4.2.1 above, in 25°C incubators set at the three photoperiod conditions (complete darkness, continuous light and 16h/8hr alternating light). Each petri dish contained 10 seeds and was replicated 4 times. After 40 days of daily monitoring and data collection as in the section above, MGT and germination percentages were calculated. A temperature of 25°C was used for this experiment because there was no germination observed at 15 and 35°C as per results of section 3.3.4.2.1 above.

3.3.4.4.5 Cold water soaking

A batch of *A. afra* seeds were soaked in 150 ml cold distilled water in a glass beaker for 24 hours. After soaking, the seeds were removed from the beaker and dried using a paper towel and then placed in petri dishes as described in section 3.3.4.2.1 above, in 25°C incubators set at the three photoperiod conditions (complete darkness, continuous light and 16h/8h alternating light). Each petri dish contained 10 seeds and was replicated 4 times. After 40 days of daily monitoring and data collection as in the section above, MGT and germination percentages were calculated. A temperature of 25°C was used for this experiment because there was no germination observed at 15 and 35°C as per results of section 3.3.4.2.1 above.

3.3.4.4.6 Hot water soaking.

Distilled water was boiled and left to cool off for few minutes (to avoid destroying the embryo of seeds by boiling water) and a batch of *A. afra* seeds were then added to soak in water (150 ml) for 24 hours. After 24 hours had elapsed, the seeds were removed from the beaker and dried on a paper towel and then placed in petri dishes as described in section 3.3.4.2.1 above, in 25°C incubators set at the three photoperiod conditions

(complete darkness, continuous light and 16h/8hr alternating light). Each petri dish contained 10 seeds and was replicated 4 times. After 40 days of daily monitoring and data collection as in the section above, MGT and germination percentages were calculated. A temperature of 25°C was used for this experiment because there was no germination observed at 15 and 35°C as per results of section 3.3.4.2.1 above.

3.4. Statistical analysis

The data was analyzed using Statistix 10.0 software and the means were separated using the least significant difference at 5% level of significance.

3.5 Results and Discussion

A quick and easy method of checking the viability of seeds using the tetrazolium test was used in the study. The seed viability of *A. afra* obtained from the ARC was 96%. The moisture content of the seeds was found to be 4%.

3.5.1 Temperature requirements and effects of photoperiod on *A. afra* seed germination

3.5.1.1 Effect of temperature

The experiment on temperature variation demonstrated a significant effect on different temperature regimes ($p< 0.05$) on germination rate of *A. afra* seeds. There were variations on germination rate of *A. afra* seeds as shown in Figure 3.2 at different temperatures. No germination was observed at temperatures of 15°C and 35°C. However, the maximum percentage germination (70%) was at 25°C. This implies that temperature is among the very important environmental factors in the germination of non-dormant seeds (Washitani and Masuda, 1990).Temperature has an influence on germination percentage and rate by affecting seed deterioration and reducing seed dormancy and through other germination processes which are imbibition, respiration, mobilization of reserves during seed germination, and role of growth regulators and development of the embryo axis into a seedling (Ramin, 1997). A temperature of 15°C is relatively lower and 35°C can also be regarded as a higher temperature depending on the type of seeds in question. The failure

of *A. afra* seeds to germinate under both 15°C and 35°C may be owed to the idea that very low and very high temperatures have attributes to inhibit germination.

Germination is affected by temperature in three primary ways including moisture, hormone production and enzyme activity. Seeds need sufficient moisture for imbibition and high temperature may increase water loss through evaporation and decrease moisture, which will have a negative effect on germination (IPCC, 2007). In the case of hormones, there are two hormones that are responsible for regulating germination, which are gibberellins and abscisic acids. Abscisic acids inhibit germination because they promote dormancy while gibberellins enhance germination (Finch-Savage and Leubner-Metzger, 2006). Through environmental factors, most importantly temperature; genes that control the production of gibberellins are up-regulated, dormancy is released, and germination occurs (Finch-Savage and Leubner-Metzger 2006). Seed germination percentage usually increases linearly with temperature up to an optimal point, after which the germination percentage decreases sharply (Tolyat *et al.,* 2014). For most perennial or winter annual plants, their favourable germination temperature ranges from 10–20°C, and these ecological habits are quite essential for them to adapt to warm climates (Washitani and Masuda, 1990). Sometimes, high, or low temperatures also result in secondary dormancy, known as thermos-dormancy (Huo *et al.*, 2013).

There was a significant difference in germination rate ($p < 0.05$) as a result of variations in photoperiod. Continuous light conditions had a positive effect on germination and resulted in the maximum germination rate followed by alternating light conditions. Continuous darkness did not favour germination for *A. afra* seeds and resulted in lowest germination rate at 25 °C. In the case of continuous darkness, this study observed similar results to the study conducted by Jabarzare *et al.* (2011) whereby *Artemisia sieberi* seeds were investigated on their response to different light conditions on germination and the results showed that continuous darkness reduced germination percentage in a significant level compared with the 12-hour alternating photoperiod.

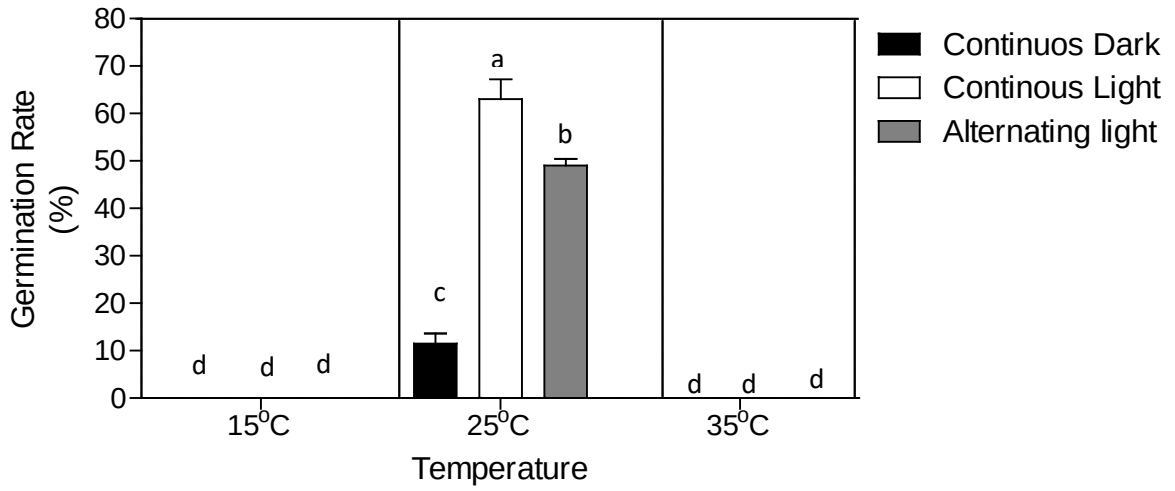

Figure 3.2: Final germination in percentage of *Artemisia afra* seeds at different temperatures and photoperiods. Bars (± SE) with different letters are significantly different ($p < 0.05$).

3.5.2 Germination Percentage of different scarification methods at varying photoperiods

There were significant differences ($p< 0.05$) in final germination percentage as affected by both scarification and photoperiod. Referring to Figure 3.3, maximum germination (final) was exhibited by cold water and hot water scarification methods at a germination percentage of 70% for both treatments under continuous light and alternating light conditions respectively. Minimum germination rate of 0% (no germination) was observed from fermentation in all light conditions. This may be due to pronounced chemical compositional changes as a result of proteolytic processes within the seeds, leading to degradation and alteration of storage proteins (Biehl *et al*., 1982).

Scarification in general resulted in higher germination percentage as compared to non-treated seeds (control), whereby cold water and hot water soaking performed generally better in enhancing germination than other scarification methods such as physical scarification (rubbing). Seed scarification by soaking reduces barriers delaying

germination (Hartmann *et al*., 2010), and soaking of seeds assists in improving, unifying, and increasing seed germination (Finch-Savage, 2013).

The reason why physical scarification (rubbing with sandpaper) resulted in lower germination rates may be attributed by the idea that sandpaper may have destroyed the seed coat and damaged the inner parts of the seeds. The aim of using sand paper was to just soften the seed coat to allow entry of moisture. The effectiveness of mechanical scarification may vary depending on genus and species of the seed (Kimura and Islam, 2012).

The use of Sulfuric acid (H_2SO_4) in this study also resulted in relatively lower final germination percentage. Scarification using a chemical solution is expected to soften the seeds which have a hard-shell structure so the water and other substances that are useful for the germination process can enter to the seeds optimally (Tjitrosoepomo, 1999). The use of sulfuric acid as a scarification method did not effectively enhance germination in this study because *A. afra* seeds do not have very hard seed coat.

In order to trigger the germination process in seeds, dry or mature seeds absorb moisture through the seed coat (Kimball *et al*., 2008). Imbibition of water in the seed is required since seeds lose a massive amount of water during different stages, such as when they mature or after they have matured, and during storage or processing, the process causes the seed to expand resulting in the rupture of the seed coat. When the seeds have water, the metabolism of the embryo which enables it to ensure growth will be influenced (Kimball *et al*., 2008).

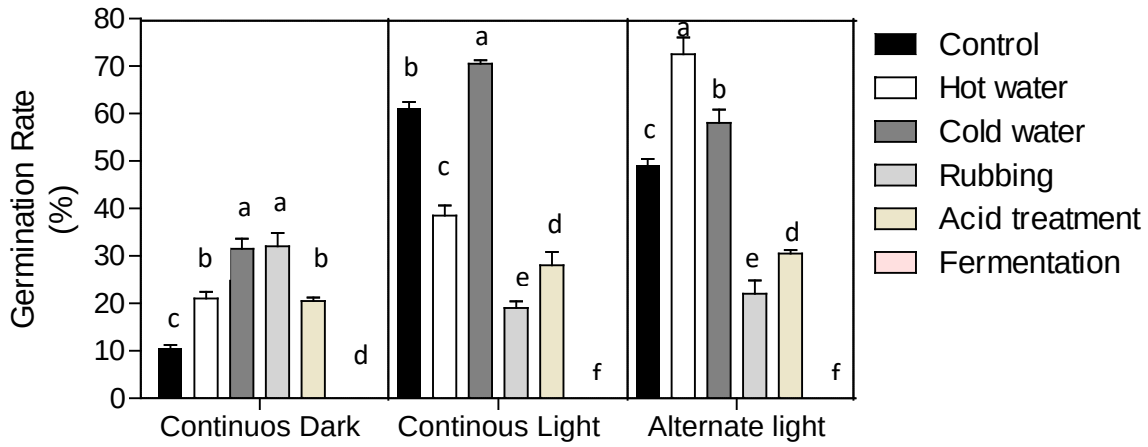

Figure 3.3: Final germination (%) of *Artemisia afra* seeds at 25°C as influenced by different scarification methods and different photoperiods. Bars (± SE) with different letters are significantly different ($p < 0.05$).

3.5.3 Mean Germination Time of different scarification methods at varying photoperiods.

The findings of this study show that scarification and photoperiod had an effect on the average time it takes for seeds to germinate (MGT). Cold water scarification under complete darkness had positive effect on MGT of *A. afra* seeds whereby the seed achieved the lowest MGT compared to all other conditions (Figure 3.4). Under complete darkness, seeds in cold water scarification achieved the lowest MGT (11 days), whilst acid treated seeds took the longest period to start germinating (17 days). In continuous light condition (Figure 3.5), cold water scarified seeds were faster to germinate (13 days) compared to the other methods. There was no significant difference in MGT for control and hot water scarified seeds (15 days). The longest MGT (22 days) was observed from seeds that had undergone physical scarification (rubbing).

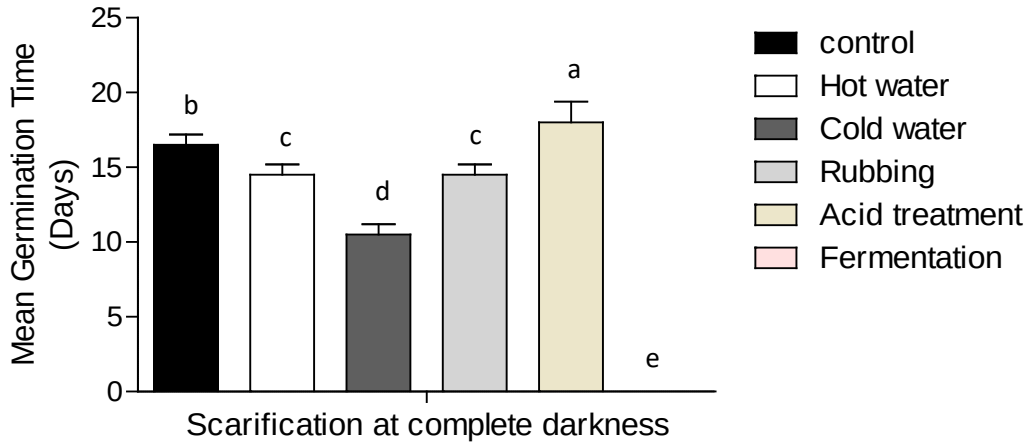

Figure 3.4: Mean germination time of *Artemisia afra* seeds as influenced by different scarification methods under completely dark conditions Bars (± SE) with different letters are significantly different ($p < 0.05$).

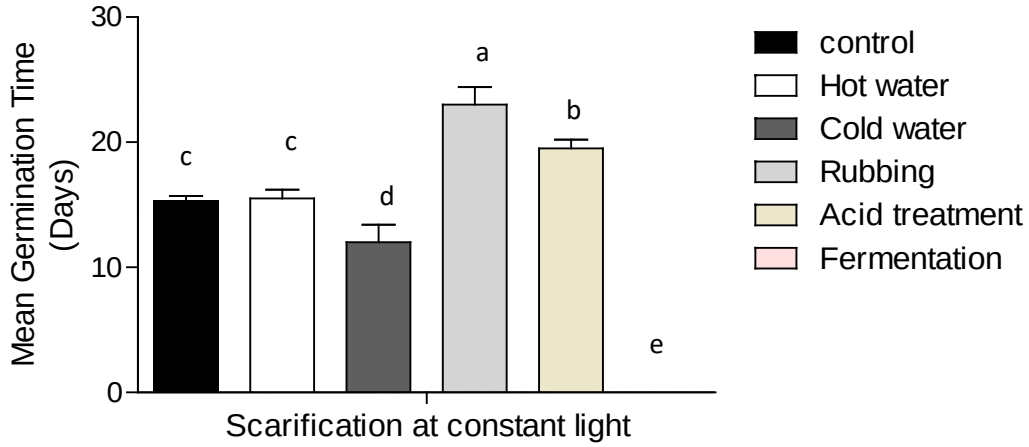

Figure 3.5: Mean germination time of *Artemisia afra* seeds as influenced by different scarification methods under constant light conditions. Bars (± SE) with different letters are significantly different ($p < 0.05$).

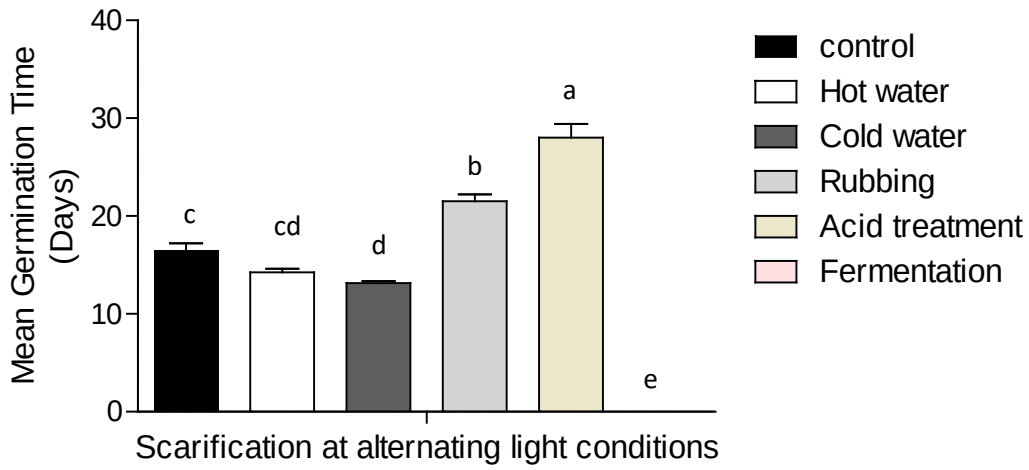

Figure 3.6: Mean germination time of *Artemisia afra* seeds as influenced by different scarification methods under alternating light conditions. Bars (± SE) with different letters are significantly different ($p < 0.05$).

There were variations in the amount of time (in days) that *A. afra* seeds took to germinate under different photoperiods. In alternating light conditions (Figure 3.6), cold water achieved the lowest MGT (13 days) while acid treated seeds achieved the longest MGT (27 days). A study by Moyo *et al*. (2022) showed that constant darkness and alternating light/ dark photoperiod conditions, and high temperatures favor seed germination in *Amaranthus dubius.*

Light is a critical factor for germinating seeds, root cuttings and seedling emergence (Van Wyk, 2011). Seeds responds differently to light depending on their species. Some seeds germinate more readily under light and some in complete darkness, whilst others are barely affected by light.

3.6 Conclusion

It was observed that the most favorable temperature for germination of *A. afra* seeds was 25°C. The best scarification method in this study was found to be hot and cold water scarification because there was an improved germination rate at these methods. Seeds scarified using cold water scarification had significantly lower MGT. Alternating light conditions were the most favorable photoperiod because the highest germination rate was achieved in this condition. Completely dark condition did not favor germination of *A. afra* because the germination rate in this case was the lowest. This study showed that different scarification methods, temperature and varying photoperiods affects germination of *A. afra* seeds. As such, the information obtained from results of this study can be shared among small scale farmers who are willing to propagate *A. afra* through seeds to recover the lost populations.

Chapter 4: The effect of two seed priming methods using a Moringa based biostimulant on seed germination of *Artemisia afra* Jacq. ex Willd seeds

4.1 Introduction

Seed priming treatment is done before sowing seeds, which involves hydration of seeds plentiful enough to enable metabolic events before germination to take place, although preventing radicle emergence to occur (Hussain *et al*., 2016). Priming is an approach that involves treating seeds with different organic or inorganic chemicals and or with high or low temperatures (Elouaer and Hannachi, 2012). Priming triggers the physiological processes in a seed that lead to its germination, by imbibing the seed to a certain level. These processes are halted at precisely the right stage and the seed is carefully dried back. In this way, all the seeds in the lot are left in the same state: ready to germinate quickly and uniformly after sowing (Rehman *et al*., 2015). Seed priming is a delicate and complex process and there are many different seed priming techniques. Each seed type has its own traits and characteristics and the various conditions that crops are grown in all present their own specific problems (Rehman *et al*., 2015). Each crop challenge needs a much targeted approach.

Priming entails imbibition of seeds in different solutions for a specified duration under controlled conditions, drying back them to their original moisture content, so that radicle does not emerge before sowing. This stimulates various metabolic processes that improve germination and emergence of several seed species, particularly seeds of vegetables, small-seeded grasses, and ornamental species and also reverses the detrimental effects of seed deterioration (Selvarani and Umarani, 2011). Seed priming is an easy, highly effective, low cost and low risk technique. Primed seeds are more have numerous advantages such as uniformity, early and faster appearance.

Moringa based biostimulant (Phytostim®) is derived from *Moringa oleifera* Lam. extracts, which contain a wide number of bioactive compounds. These compounds are able to improve various physiological processes that stimulate plant growth and development and increase nutrient use efficiency, reducing chemical fertilizers without adverse effects on yields and their qualities (Bulgari *et al*., 2015).

Moringa based biostimulant (Phytostim®) contain powerful natural antioxidants, which can be used by farmers to improve growth and yield attributes of various crops, and to overcome environmental stresses (Howladar, 2014). This study was aimed at investigating the effects of a commercially available Moringa based biostimulant, Phytostim® in upregulating seed germination of *A. afra* when applied using two priming methods.

4.2 Objective of the study

The objective of this study was to investigate the effects of a commercially available Moringa based biostimulant, Phytostim® in upregulating seed germination of *A. afra* when applied using two priming methods.

4.3 Material and methods.

4.3. 1 Description of study location

The study was conducted at the University of Limpopo's Green Biotechnologies Research Centre of Excellence in South Africa (23°53′10″S, 29°44′15″E). The experiment was conducted in temperature-controlled growing chambers in the laboratory.

4.3.2. Seed collection

The seeds of *A. afra* were obtained from the Agricultural Research Council, Roodeplaat in Pretoria, South Africa. The seeds were stored at 4 °C prior to usages in seed germination tests. Prior to experimentation, seed viability and moisture composition were determined as described in Section 3.3.2.

4.3.3 Research design

The experiment was set up using a Completely Randomized Design. Each Petri dish contained ten seeds. Each treatment was replicated four times.

4.3.5 Experimental procedures

4.3.5.1 Effect of two different priming methods using a Moringa based biostimulant on the germination of *A. afra*

Figure 4.1. Moringa based biostimulant, Phytostim® (Source: Moringa products South Africa).

A commercially available Moringa based biostimulant, Phytostim® and locally produced by Moringa Nutrition (Pty) LTD was used in the study.
Different concentrations, 0, 0.5, 1.0 and 3 %, of the biostimulant were prepared from the commercial stock by dilution with distilled water.The prepared concentrations were used in the study as outlined below, with the 0% biostimulant treatment being the control solution.

4.3.5.1.1 Seed priming by soaking (Treatment 1)

Treatment 1, seed priming by soaking consisted of a batch of *A. afra* seeds that was soaked for 24 hours in the prepared Moringa based biostimulant concentrations (0, 0.5, 1.0 and 3 %). After 24h, the seeds were removed from the beaker and dried on a paper towel and batches of ten (10) seeds were placed in petri dishes (90 mm) with the bottom plate lined with two layers of filter paper (Whattman No.1) and placed in an incubator set at a constant temperature of 25°C and alternating light (16 h dark and 8 h light photoperiod). The filter papers were dampened with distilled water when there was a need and the experiment was conducted for a period of 40 days. The experiment was replicated four times. Data was collected on a daily basis by recording of shoot emergency. The data collected was used to determine the MGT and germination percentages as described in section 3.3.4.1.

4.3.5.1.2 Seed priming by continuous application (Treatment 2)

Treatment 2, seed priming by continous application consisted of batches of ten (10) *A. afra* seeds that were placed in petri dishes (90 mm) with the bottom plate lined with two layers of filter paper (Whattman No.1) and placed in an incubator set at a constant

temperature of 25°C and alternating light (16 h dark and 8 h light photoperiod). The petri dishes were labeled and the filter papers were dampened with 0, 0.5, 1.0 and 3.0 % of the prepared Moringa based biostimulant concentrations instead of distilled water (except for the 0% treatment) when there was a need. The experiment was conducted for a period of 40 days. The experiment was replicated four times. Data was collected on a daily bases by recording of shoot emergency. The data collected was used to determine the MGT and germination percentages as described in section 3.3.4.1.

4.4. Statistical analysis

The data was analyzed using Statistix 10.0 software and the means were separated using the least significant difference at 5% level of significance.

4.5 Results and discussion

4.5.1 Seed priming by soaking (Treatment 1)

Application of different concentrations of Phytostim® biostimulant significantly ($p<0.05$) affected germination rate and MGT in *A. afra* seeds. Seeds soaked with Moringa based biostimulant at the concentration of 3% dose improved seed germination up to 22% in just 7 days. This result was outstanding when compared to the other doses, 0.5% and 1% doses which resulted in two-fold lower rates of germination and had MGT of 10 and 11 days respectively.

Figure 4.2 shows that the control resulted in a lower germination rate than all other doses and this proves that the application of biostimulant through soaking played a very huge role in enhancing Final germination percentage. Biostimulants are diverse substances which improve growth and development of plants through nutrient uptake, nutrient efficiency, and tolerance to abiotic stress (European Biostimulants Industry Council, 2012). These biostimulants enhance plant development from seed germination up to maturity and improve plant adaptability to diverse environments. Priming is known to activate cellular and molecular changes through several pathways involved in different

metabolic processes in order to alleviate the deleterious effects of stress (Jisha and Puthur, 2016).

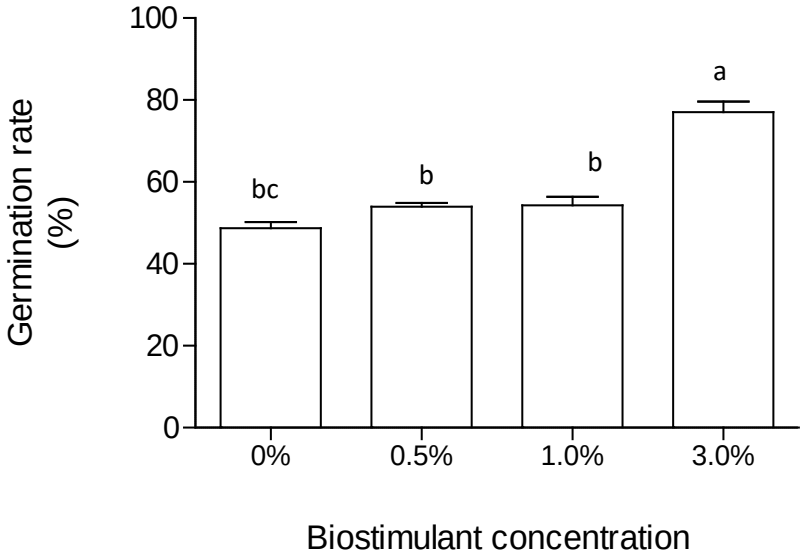

Figure 4.2: The effect of priming of *Artemisia afra* seeds through soaking in different concentrations of a Moringa-based biostimulant on the seed germination rate (%). Bars (± SE) with different letters are significantly different ($p < 0.05$).

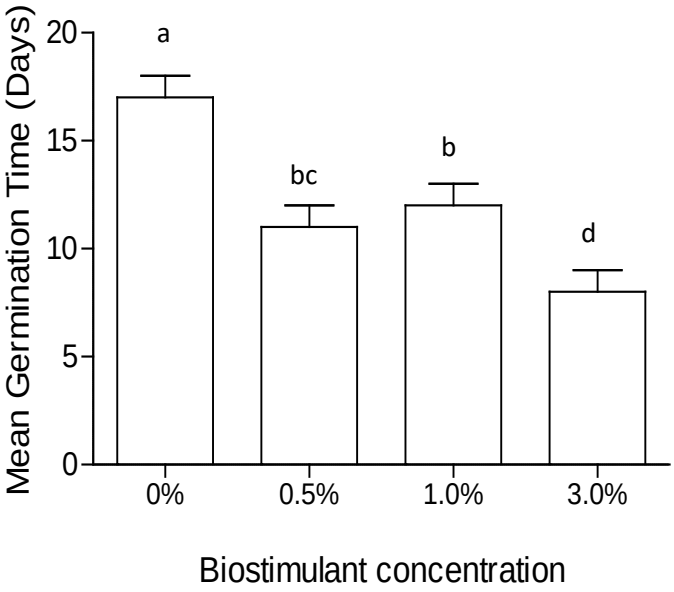

Figure 4.3: The effect of priming of *Artemisia afra* seeds through soaking in different concentrations of a Moringa-based biostimulant on the MGT. Bars (± SE) with different letters are significantly different ($p < 0.05$).

4.5.1 Seed priming by continuous exposure (Treatment 2)

The application of different concentrations of a Phytostim® biostimulant through continuous application had significant effect on Final germination percentage and MGT of *A. afra* as depicted in Figure 4.4. A biostimulant dose of 0.5% and the control (untreated) showed similar rate of germination which was higher than the 1 and 3% doses. However, high biostimulant dose (3%) delayed germination (MGT) up to 17 days in comparison to control which had an MGT of 15 days. Continuous application of the biostimulant at a dose of 1% achieved the lowest MGT than all other doses (Figure 4.5).

In this study continuous application of Phytostim® biostimulant to the seeds of *A. afra* at a 0.5% dose, played remarkable role for accomplishment of germination phases (Coolbear and McGill, 1990). Seed priming with suitable priming agents and concentrations induce some physiological and biochemical changes in the seed, which result in improved crop performances in terms of enhanced germination potential, seedling vigor, and final yield (Farooq *et al*.,2008 ; Afzal *et al.*, 2012). Continuous application of high doses of the biostimulant, 1% and 3% was detrimental and significantly reduced germination rate and led to increased MGT. High concentrations of biostimulant have been associated with inhibition of germination and plant growth and development. In agreement with our findings, Ertani *et al*. (2009) reported better growth of plants when low doses of biostimulant were applied in the range of 0.01–0.1 g/L. In another study, Quartieri *et al*. (2002) tested the effect of an animal-derived biostimulant as a foliar application in potted kiwifruit plants and found an improvement in hypogeal plant dry weight. In nursery-grown passionfruit, Morales-Pajan and Stall (2004) observed that foliar applications with an animal-derived biostimulant increased the seedling growth.

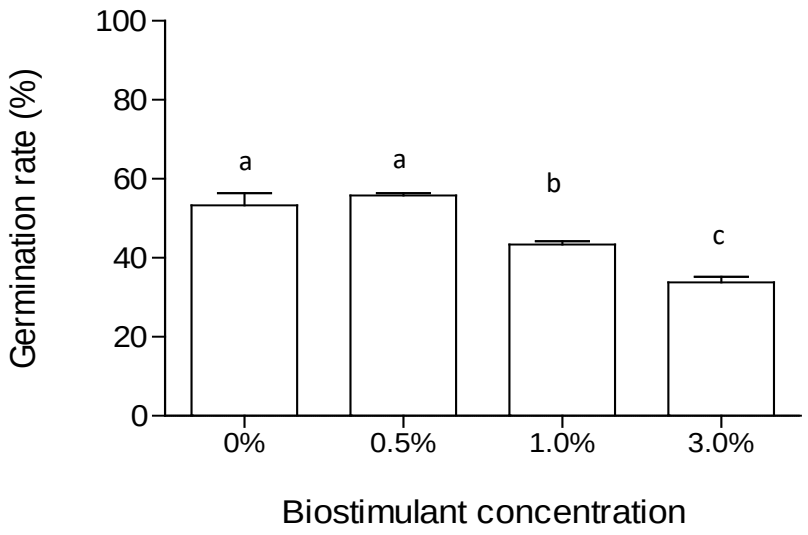

Figure 4.4: The effect of priming of *Artemisia afra* seeds through continuous application of different concentrations of a Moringa-based biostimulant on the seed Final germination percentage (%).Bars (± SE) with different letters are significantly different ($p < 0.05$).

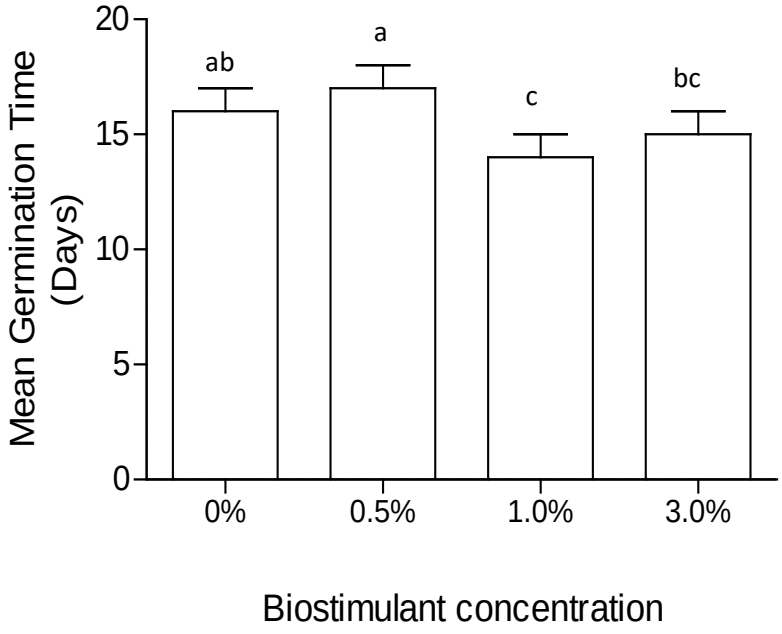

Figure 4.5: The effect of priming of *Artemisia afra* seeds through continuous application of different concentrations of a Moringa-based biostimulant on the MGT. Bars (± SE) with different letters are significantly different ($p < 0.05$).

Priming helps in improving germination rate because it regulates imbibition and active metabolic processes of germination before radicle emergence (Farooq *et al*., 2005). Seed priming helps the plants to cope with the adverse effects of unfavorable environmental conditions (Ashraf and Foolad, 2005; Chen *et a*l., 2010). According to Liu *et al*., (2002) priming improves the activities of anti-oxidative metabolites, such as superoxide dismutase and peroxidase, during seed germination. Priming helps the plants to accelerate cell division, transport stored proteins and hasten the speed of seed germination (De Castro *et al*., 2000). In this study priming through continuous application had a positive effect and significantly increased Final germination percentage.

4.6 Conclusions

Priming A*. afra* seeds with a commercially based Moringa based biostimulant proved to have an effect on its seed germination. Priming through soaking in 3% biostimulant resulted in an improved Final germination percentage and fewer days of shoot emergency (MGT) whereas 0.5% and 1% were not significantly different ($P<0.05$) from the control. Contrary to this, priming with continuous application produced different results. In this case, priming with application of 0.5% was not significantly different from the control ($P<0.05$). Priming with continuous application of 3% biostimulant did not improve germination rate as well as the MGT. Therefore it implies that high concentrations of the biostimulant are inhibiting to germination. These results can be useful in providing small scale farmers on priming agent that can improve germination of *A. afra* which is currently in high demand.

Chapter 5: Summary of findings, significance, recommendations and conclusions.

5.1 Summary of findings

Due to the increased demand for *A. afra* as a medicinal plant, the issue of overharvesting is now a major concern. The way to meet the burgeoning need of this is through cultivation to recover the lost populations and meet the demand. As medicinal crops are naturally occurring, the germination requirements of such seeds are not well documented. As such the optimum conditions for germination of *A. afra* were studied.

Findings of this study reported that scarification, temperature, photoperiod, and priming using Moringa based biostimulant have an effect on Final germination percentage of *A. afra* seeds and Mean germination Time. The optimum temperature for germination of *A. afra* is 25, the best scarification method was through soaking in hot and cold water. These two methods improved germination rate of *A. afra* than all the other scarification methods. Seeds germinated better when exposed to alternating light conditions than when placed in complete darkness or complete darkness. This implies that *A. afra* seeds need to be exposed to both darkness and light alternating at the 16/8-hour period.

Soaking the seeds in Moringa based biostimulant achieved the best results at the 3% dose, and this also resulted in lowest mean germination time than the other doses. In continuous application of the Moringa based biostimulant: The high dose (3%) had a detrimental effect on the Final germination percentage as it resulted in lowest germination rate. The best dose for this method was the 0.5 % dose as its application had an outstanding Final germination percentage. The 1% dose in this application method resulted in lowest MGT. via soaking and continuous application also significantly improved germination.

5.2 Significance

The results of this study showed that scarification approach that promotes greater germination is cold and hot water soaking.

This information will be carefully documented and employed in the future to improve *A. afra* seed propagation. This information will also be shared with small-scale farmers and growers of traditional medicinal crops in general, to boost germination in their fields. The optimal photoperiod was found to be alternating light condition. A 25°C temperature was found to be the most favorable for *A. afra* seed. The most effective dosage of the Moringa based biostimulant was found to be 3% when applied through soaking prior germination, and when applied continuously the lower dose of 0.5% was found to be more effective in enhancing germination. These findings will be employed in sexual propagation of *A. afra* as the optimal conditions for germination of this crop.

5.3 Recommendations

It is recommended to use the 25°C temperature when propagating *A. afra* through seeds when growing in a controlled environment. The seeds must also be placed under alternating light conditions because this combination according to this study resulted in high germination rate. When breaking dormancy, soaking the seeds in either hot or cold water 24 hours prior sowing is recommended. When using Moringa based biostimulant as a method of priming, soaking in 3% of the biostimulant or continuously applying 0, 5% of the biostimulant is the most recommended approach. These doses with their respective application methods have proven to enhance germination rate *of A. afra*. In future studies, the effect of scarification, light conditions, temperature and priming using Moringa based biostimulant on seedling vigor, uniformity and growth attributes on *A. afra*; must be studied. Since this study focused only on the effect of the above mentioned on germination, further studies should be conducted on further growth attributes of this crop.

5.4 Conclusions

In conclusion germination of *A. afra* seeds was affected by scarification, temperature, light and priming using Moringa based biostimulant. The information generated from this study is important because it will contribute in bridging the scientific gap on the information that has never been documented on optimum requirements for effective propagation of *A. afra* through seeds.

As such this information will be useful for small scale farmers and medicinal plant growers who are willing to recover the lost populations of *A. afra* through seed propagation thus improving their supply and income.

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