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FACULTY OF AGRICULTURE, DEPARTMENT OF HORTICULTURE

ASSESSING THE PHYSIOLOGICAL POTENTIAL OF *TETRAPLEURA TETRAPTERA* (SCHUM. & THONN.) SEED LOTS USING THE ACCELERATED AGEING AND ELECTRICAL CONDUCTIVITY TESTS

By

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DEDICATION

This work is dedicated to my Parents, Evariste Sossou and Christine Ganse, who sent me to school, consistently encouraged and motivated me to aim high and reach the highest level of education; to my uncle and mentor Prof. Achille Assogba-djo of University of Abomey-Calavi (Benin) for his priceless help and pieces of advice and the rest of my family members for their prayers and encouragement.

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DECLARATION

I, hereby, declare that except for references to the work of other researchers which have been duly cited, this work is an original work and has not been submitted or presented in part or whole for any other degree in this University or elsewhere.

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ABSTRACT

High seed quality is indispensable for optimum establishment in the field. As a result, it is necessary to have seed vigour tests that allow rapid, objective and accurate evaluation of seed quality. The present study aimed to evaluate the efficiency of the electrical conductivity and accelerated ageing (traditional and salt saturated methods) tests for the determination of the physiological potential of *Tetrapleura tetraptera* seeds. Eight seed lots were tested for: water content, germination (emergence), seedling root, shoot and total length and seedling dry weight, leachate conductivity of 1, 2, 3, 4, 5, 6 and 24 hours soaking solutions, traditional and salt saturated accelerated aging in temperatures of 38 and 41°C for 48, 72 and 96 hours. The experiment was conducted in a 8x2x3 factorial (lots x temperature x exposure time), in a completely randomized design. The seedling dry weight were not effective to distinguish the physiological quality of *Tetrapleura tetraptera* seeds. The accelerated aging test influences, similarly, the percentage of germination and vigour, but showed low sensitivity in lots differentiation. The electrical conductivity readings were significantly affected by soaking time and only 24hours soaking evidenced promising role to a stratification of the physiological quality of *Tetrapleura tetraptera* seeds lots in accordance with seeds emergence and seedling length.

Key words: *Tetrapleura tetraptera*, germination, vigour.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

The contributions of forests to the welfare of humankind are particularly vast and farreaching. Forests play an important role in combating rural poverty, guaranteeing food security and providing decent livelihoods. The forest sector adds about $600 billion annually to global GDP and offers employment to over 50 million people (FAO, 2015). They offer favorable mid-term green growth opportunities and deliver vital long-term environmental services, such as clean air and water, conservation of biodiversity and alleviation of climate change (FAO, 2015). Despite their irrevocable importance, the rapidity of forest genetic resources erosion occasioned by deforestation is alarming. While in 1990 forests made up 31.6% of the word's land area, this has decreased to 30.6% in 2015 and this has taken place mostly in the tropics (FAO, 2015).

1.2 Problem statement

The global rate of deforestation is still high in many parts of the world particularly in the tropics, even though it has slowed in the last decade (FAO, 2014). The uppermost net annual loss of forests occurred in Africa and South America in the past five years (20102015), with 2.8 and 2 million hectares respectively (FAO, 2015). The tropical rainforest has been recognized as the most genetically diverse terrestrial ecosystem on earth (Gillespie *et al.,* 2004). This has exposed the rainforest to severe utilization for their timber and economically valuable non-timber resources which has therefore put several species of plants in danger of extinction (Whitmore, 1998). IUCN (2008) reported that, in subSaharan Africa, about 2000 tropical tree species are considered to fall into the categories of being

'near-threatened' to 'critically endangered'. Therefore, the urgent need to preserve the tropical rainforest cannot be overstressed.

Raising trees and preserving their seeds are means of supporting reforestation, combating desertification, safeguarding the environment and conserving biodiversity (Grainger, 1993). This requires the planting of large numbers of adapted species (Sacande and Pritchard, 2004). Planting of indigenous trees is considered to be an effective rehabilitation method for degraded tropical rain forests, because these trees provide benefits such as timber, food and medical products (Fisher, 1995; Lamb, 2011). *Tetrapleura tetraptera* (Schum and Thonn) is a highly valuable multipurpose indigenous species with nutritional, medicinal and economic value. However, the species has been overexploited, which in turn has resulted in the severe depletion of its natural population (Troup, 1986; Ibiang *et al.*, 2012). Moreover, like any other forest tree its regeneration cycle is long and the natural germination of the seeds is still a challenge to its establishment as a result of seed dormancy (Jimoh, 2005). This make the species not quiet adequate for use in a good and rapid rehabilitation programmes. There is therefore a need to establish sustained conservation measures and explore seed testing methods on the species to facilitate its use in forest rehabilitation programmes.

1.3 Justification

The use of highly valueable indigenous tree species in afforestation and conservation programmes is obstructed by problems related with seed handling and storage (Stubsgaard, 1992, Sacande *et al*., 2004). Engels and Ditlevsen (2004), have observed that studies on tropical forest tree seeds in general also remain more difficult compared to those on

agricultural species due to handling problems. Bonner (1992) reported that one of the gaps in our understanding of tropical seeds, is seed testing. Precise evaluations of seed vigour tests and their use for tree seeds is not yet possible (Bonner, 1998). This is as a result of the large genetic variation, primarily displayed in variable maturity and dormancy that occurs in most tree seed lots. According to Bonner (1998), none of the vigour testing handbooks include recommendations for seeds of trees or of any other woody species. Due to their current value, users of tree seeds (foresters, nursery managers, horticulturists, etc.) notice a need for vigour testing of tree seeds and have encouraged tree seed researchers to pursue that goal (Bonner, 1998). It is therefore important to generate knowledge on the appropriate seed handling and storage techniques as well as appropriate seed testing methods for them. Thus, developing an efficient rapid vigour testing methods for the species will be useful in estimating its physiological potential and therefore help to guide its ex-situ conservation.

1.4. Objectives

14.1 General objective

The general objective of this study was to assess the efficiency of different techniques of accelerated ageing and electrical conductivity tests for estimating the physiological potential of *Tetrapleura tetraptera* seeds.

1.4.1 Specific objectives

Assess the effect of various accelerated ageing conditions on proximate composition, seed membrane integrity, germination characteristics and seedling vigour *of Tetrapleura tetraptera* seeds.

Assess the efficiency of the various ageing conditions for *Tetrapleura tetraptera* seeds seed vigour test.

Assess the efficiency of the electrical conductivity test for *Tetrapleura tetraptera* seeds by determining the optimal soaking time for performance of the test.

Compare the results obtained after the various ageing procedures with electrical conductivity and other vigour parameters.

CHAPTER TWO

2 LITERATURE REVIEW

2.1 Species information

Tetrapleura tetraptera (Schum and Thonn), commonly known as 'prekesse' tree in Ghana, belongs to the family Fabaceae. It reaches a height of 20-35 m with the girth of 1.5-3 m (Orwa *et al.*, 2009). *Tetrapleura tetraptera* is a deciduous tree usually found in the lowland rainforest of tropical Africa and its distribution extends over large parts of tropical Africa, especially West and East Africa (Ojewale and Adewunmi, 2004). *Tetrapleura tetraptera* is highly valued and widely used in Africa traditional medicine for its nutritional and medicinal properties (Aniedi *et al.*, 2013). The fruits and seeds add good fragrance and flavour to food, thus increasing appetite (Essien *et al*., 1994; Aladesanmi, 2007), eliminate the pungent odour and inhibit fungal growth in cassava fufu (Okwu, 2004). The fruit is also used to make soup for nursing mothers to prevent post-partum contraction and manage convulsions, leprosy, inflammation and rheumatism (Enwere, 1998). The leaves possess strong molluscicidal activity (Adewunmi *et al*., 1991) and are important compound for the treatment of epilepsy (Akah and Nwabie, 1993). The aqueous fruit extract has been reported to possess hypoglycaemic properties (Ojewole and Adewunmi, 2004). The bark is active against cough and bronchitis and isused as a decoction in drinks. The bark is also used to treat rheumatism and fever. The root is used to treat gastrointestinal clinical

problems (Aladesanmi, 2007). *T. tetraptera* is likewise valued in timber as fairly hard heartwood (Orwa *et al.,* 2009). In spite of its social and economic value, the population of *T. tetraptera* is decreasing at an alarming rate as result of overexploitation, and lack of sustained conservation measures (Ibiang *et al.*, 2012). Only a small percentage of the seeds germinate in the field due to seed dormancy and this poses an obvious challenge in efforts aimed at its conservation (Jimoh, 2005).

2.2 Concept of seed quality and quality testing

Seed is a ripened fertilized ovule that provides an important means of reproduction, dispersal and serves as nutrition to seed-eating animals and fungi colonies (Wicklow, 1995). It can also be defined any plant part used to regenerate the next generation of the crop (Gardner *et al*., 1985). Seed, therefore represents the foundation of each plant production and guaranteeing its quality is the main concern of modern seed science. Seed quality can be defined as the inherent attributes of a seed that determine its germination potential and there after its growth characteristics (Mbora *et al.,* 2009). Seed quality often refers to its genetic traits, germination capacity, analytical purity, physical purity and storage potential (ISTA, 1987). Seed quality is affected by a variety of factors imposed on the seed during its formation, development, maturation, growth, harvesting, threshing or extraction, drying, cleaning, grading, packing, storage and marketing. The key factors which govern the seed quality include: genetic factors, ecological factors, agronomic packages and production technology, harvesting and post-harvest handling. Quality seed is indispensable for the success of seedling production in the nursery and their subsequence establishment in the field in forest plantations. Seed testing is an investigation of some physical and the physiological quality of a seed lot. Physical parameters that are considered during seed testing include seed weight, purity, viability, germination and moisture

(Schmit, 2000). Good seed testing is the cornerstone of any seed programme, no matter what kind of seeds: agricultural, forestry, agroforestry or ornamental. The main objectives of a seed testing programne comprise: the determination of the suitability of the seed for planting, the identification of any quality problems in a seed lot and their causes, the assessement of the need for drying and conditioning, the evaluation of seed quality regarding the quality standards or labelling specifications and providing a basis for price and consumer discrimination among seed lots in the market (Schmidt, 2000). ISTA (International Seed Testing Association) and AOSA (Association of Official Seed Analysts) have developed several guidelines which give recommendations for seed sampling, and a large number of quality tests; including purity, and moisture content, seed health,viability, germination.

2.3 Concept of seed vigour

Germination testing is the primary and worldwide accepted criterion for seed viability. However, the germination test alone may not provide sufficient information as to potential seed lot performance (ISTA, 1995). For more accurate information, seed quality assessment can be conducted by physical and physiological vigour tests. These vigour tests provide information on the potential behaviour of a seed lot under storage and field conditions (McDonald, 1999).

The development of an acceptable definition of seed vigour has been a fundamental issue in the establishment of vigour tests. Initially, seed vigour could not be considered as a single distinct physiological process such as germination or deterioration (Marcos-Filho *et al.*, 2015). McDonald (1993) reported that, seed vigour was suggested to be so intricate that it could not be adequately defined and this parameter could only be better understood under the framework of a concept. The first attempt to define seed vigour was made by Isely in

1957, suggesting that seed vigour is a “sum total of seed attributes, which favor stand establishment under favorable conditions”. The expression “wide range” was used for the first time by Woodstock in 1965, who defined seed vigour as a “condition of good health and natural robustness associated with rapid and complete germination under a wide range of environmental conditions”. Some years later, Pollock and Roos (1972), repoted that seed vigour might be the potential for seedling establishment in the field. This was reinforced by Bishnoi and Delouche (1980), who defined seed vigour as an aspect of seed quality which control field stand establishment. ISTA (2014) defimed seed vigour as, “sum of those properties that determine the activity and performance of seed lots of acceptable germination in a wide range of environments”. Therefore, any seed lot that has the potential to perform well (germination and establishment) under environmental conditions which are not only optimal for the species is considered as vigorous seed lot.

Losses of seed vigour in any seed lot are linked to a decrease in the ability of seeds to perform all the physiological functions that allow them the expression of their performance (Shaban, 2013). The causes of variations in vigour are several and diverse. The commonly known factors which influence vigour level include: genetic; environmental conditions; seed maturity at harvest; physical characteristics seed; mechanical integrity; deterioration and ageing and pathogens (ISTA, 1987). Recent studies have focused on the physiological causes of differences in seed vigour, particularly the role of seed ageing (Copeland and McDonald, 1995). Seed ageing resulting from its physiological deterioration generally starts at physiological maturity and continues during harvest, conditioning and storage as a result of the effect of harsh environmental conditions on the seed (Kapoor *et al.,* 2011). Seed deterioration becomes evident as its germination percentage reduces and produces weak seedlings, leading ultimately to its death (Tilebeni and Golpayegani, 2011).

Deterioration can occur in seed in a few days or years, depending on many environmental and biological factors. The speed of deterioration however changes critically from one speci to another and also among varieties of the same speci (Jatoi *et al*., 2001). Seed deterioration is inevitable and only the rate can be controled.

2.4 Seed Vigor Testing

Seed vigour is a more subtle measure of seed physiological quality. It is essential to know that vigour tests were not developed to forecast the exact amount of seedlings that will emerge in the field or the exact storage length without any reduction in its initial germination percentage. The aim of seed vigour testing is to offer an accurate identification of significant dissimilarities in physiological potential amongst seed lots of commercial value (Marcos-Filho *et al.,* 2015). Therefore, a reliable assessment of seed physiological potential includes the use of appropriate and standardized procedures and a proper understanding of test results. Meanwhile, no vigour test is universally accepted for all plant species. According to Miloševic *et al.* (2010), the biology, physiology and other specific features of plant species should be taken into account when choosing a vigour test.

2.2.1 Classification of vigour tests

A vigour test should provide a reproducible results which are more closely correlated with seed performance in the field under some conditions other than the germination test (ISTA, 1987). Several techniques have been developed and studied for seed vigour testing. These can be grouped in several ways. Hampton and Tekrony (1995) grouped vigour tests into two categories namely: the recommended tests in order to gain understanding into viability and nearer orientation concerning the percentage of field germination (conductivity test and accelerated ageing test) and the recommended tests that are still in improvement stage (cold test, Hiltner test, test of seedling growth, Tetrazolium test, etc..). According to

McDonald (1975) vigour tests can be classified into three groups: physical tests, physiological tests and biochemical tests. Physical tests involve determination of seed physical appearance such as size and mass. These tests are quick, affordable, and can be applied to large number of samples and are positively associated with seed vigour (Miloševic *et al.*, 2010). The physiological tests are conducted using germination and growth parameters (standard laboratory germination, cold test, accelerated ageing test, etc.). While, the biochemical tests are known as indirect methods for assessment of seed value and include tetrazolium test, conductivity measurements, enzyme activity and respiration.

ISTA (1987) divided vigour tests into two groups, namely direct and indirect tests. In the direct test the stress factors which are expected to reduce emergence in the field are imposed under controlled conditions in the laboratory (cold test, Hiltner test and accelerated ageing test). Indirect tests are those in which a seed characteristic measured in the laboratory is related to performance in the field (rate of germination measurement, rate of seedling growth, conductivity test, tetrazolium test, enzyme activity and respiration).

2.2.2 Some vigour tests for tree seed vigour evaluation

2.2.2.1 Cold Test

The cold test is known as the oldest vigour test technique. It is the primary test for the hybrid corn universal seed industry, initiated from the need to assess seedling emergence with a more subtle procedure than the standard germination test (Marcos-Filho *et al.*, 2015). The cold test has the aim of assessing the response of seed samples exposed to a combination of low temperature, high substrate water content and, preferably with presence of pathogens. These conditions contribute to a decrease in the speed and percentage germination or seedling emergence, according to the technique adopted to conduct the test.

Therefore, the vigour of a seed lot is proportional to the degree of seed survival when subjected to such an unfavorable environment. The cold test may be used for many crop species including, carrot (*Daucus carota L.*), field beans (*Phaseolus vulgaris*), lettuce (*Lactuca sativa* L), barley (*Hordeum vulgare L.*) sorghum (*Sorghum bicolor* L.), .), onion (*Allium cepa* L.), soybeans (*Glycine max* L.), rice (*Oryza sativa* L.), etc.. (Marcos-Filho *et al.,* 2015).

2.2.2.2 Electrical conductivity test (EC)

Electrical Conductivity (EC) test was first suggested by Fick and Hibbard, when they associated low germinability of timothy seeds to the excessive release of solutes during hydration (Marcos-Filho *et al.,* 2015). As confirmation, Priestley conducted in 1958 a study on cotton seeds and the results showed that the conductivity of the soaking solution was inversely related to germination. The test was then developed into a routine test for the estimation of the field emergence by Matthews and Bradnock in 1967, establishing a basic technique to evaluate the percentage of seedling emergence in peas (ISTA, 1987). The principle of the electrical conductivity test is that less deteriorated seeds show a higher speed of cell membrane repair during seed water uptake for germination and consequently release lower amounts of solutes to the external environment.

Measurement of various solutes from seeds has attracted remarkable consideration in tree seed research, mainly because it gives a quantitative approximation of seed quality (Bonner, 1974). Most consideration has been dedicated to the leachate conductivity as it is non-destructive, simple to carry out, rapid and involve low cost (Bonner, 1998). However, the large genetic variation that occurs in seed lots collected from wild populations has been reported as the biggest problem linked to the use of leachate conductivity to estimate tree seed vigour (Bonner, 1998). This was confirmed by Bonner and Agmata-Paliwal (1992)

who conducted a study on seed lots of red spruce (*Picea rubens*). The seed lots were aged for different periods prior to assessing leachate conductivity. An excellent association was revealed among conductivity and germination ($R^2 = 0.970$) when a single seed lot was aged and verified, while when 14 and 38 seed lots were tested the R^2 value dropped to 0.530 and 0.286, respectively.

2.3.2.4 Tetrazolium test

Tetrazolium is a rapid test to assess seed viability and vigour. This test is centered on colour variations of seed living tissues in contact with a solution of 2,3,5 triphenyl tetrazolium chloride, therefore reflecting the degree of activity of the dehydrogenase enzyme system thoroughly linked to seed respiration and viability (Marcos-Filho *et al.,* 2015). The triphenyl tetrazolium chloride is reduced by the terminal oxidase systems in living plant tissue from a colourless solution to a red, water insoluble formazan compound which precipitated within live cells while in the dead cells no reaction takes place (ISTA, 1987). Tetrazolium test is applied when seed must be sown directly after harvesting dormant seed or information on potential seed germinability is needed urgently (Hampton and Tekrony, 1995). The test is also used to assess different kinds of damage initiated by harvest or conditioning. Tetrazolium test requires particularly trained persons and does not identify the presence of pathogen or phytotoxic effect (Miloševic *et al.,* 2010).

2.3.2.5 Tests based on seedling growth

Tests based on seedling performance generally do not necessitate special apparatus apart from those used for germination and are simple to perform. Therefore, the tests based on seedling growth are excellent options to assess seed vigour in species such as forest, decorative, native and recalcitrant seeds, which have very little information about other

seed vigour tests (Marcos-Filho *et al.,* 2015). Speed of germination is one of the oldest seed vigour expressions. Rapid germination is an essential subdivision of the seed vigour concept as it generally relates to more rapid seedling emergence in the field (Marcos-Filho *et al.,* 2015). The capability of seeds to quickly germinate is a key indicator of seed physiological quality as a more subtle index of seed performance than germination percentage. This method was compared to other vigour tests and it was concluded that speed of germination, seedling emergence along with the first count of the standard germination test gave consistent estimates of seed vigour (Marcos-Filho *et al.,* 2015). Seedling growth and vigour classification are also important components of seed vigour. Several research on seed vigour testing have included at least one of these tests to demonstrate that the consistency and speed of seedling development are known components of seed vigour (Marcos-Filho *et al.,* 2015).

2.5 Accelerated ageing (AA) as vigour test

2.5.1 History

Accelerated ageing test is one of the most used tests to assess the vigour of various species of seeds and it provides data of high degree of uniformity (TeKrony, 1995). The development of this test was centered on the observations of Crocker and Groves, who suggested that, the process of seed deterioration in storage was triggered by protein coagulation and speeded up by increases in seed mass temperature. Marcos-Filho *et al.* (2015) reported that the test was suggested by Helmer in 1962 to be useful in seed vigour and storage potential assessment after they realized that clover seed reactions to temperatures of 35°C and 40°C and 100% relative humidity were associated with its vigour. Many similar studies were then conducted and led to a suggestion of a procedure to perform the ageing test (Marcos-Filho *et al.,* 2015). The methodology was improved by Delouche

and Baskin in 1973 some years later, but the recommended procedure was developed in 1978 by McDonald and Phannendranath. The accelerated ageing test has been effectively used to estimate seed vigour and storability in many agricultural crops and is one of the most accepted tests to assess seed vigour of various species (Marcos-Filho et al., 2015). The accelerated ageing technique has also attracted the attention of tree seed researchers as a means for evaluating the efficiency of ex-situ gene conservation methods (Chaisurisri, 1992).

2.5.2 Principle of accelerate ageing test

The accelerated ageing test is founded on the speeding up of the seeds deterioration rate, by subjecting them to high temperature and relative humidity, which are knows to be the most important environmental factors that can cause rapid seed deterioration (Marcos Filho, 1999). Less vigorous seeds deteriorate more rapidly then, showing a segregated reduction in viability. Thus seed vigour is measured by subsequent germination testing. Accelerated ageing test provides, valuable information on storage as well and its results are well related with field emergence (Lovato *et al.,* 2001; Marcos-Filho *et al.,* 2015). However, the need for accurate control of the test conditions (temperature and relative humidity) makes the ageing test more difficult than it might appear, but its basic concept has been attractive to tree seed workers (Hampton and TeKrony, 1995; Bonner, 1998).

2.5.3 Seed deterioration processes during seed ageing

Deterioration of seed is the loss of seed quality, viability and vigour as result of effect of harsh environmental conditions (Kapoor *et al.,* 2011). This process generally starts at physiological maturity and continues throughout harvest, conditioning and storage. Seed deterioration become obvious as a decrease in percentage germination, produces weak seedlings, loss of vigour, and eventually seed death (Tilebeni and Golpayegani, 2011).

Kapoor *et al.*, (2011) described the process of seed deterioration as cumulative, irreversible, degenerative and inexorable process. Seed deterioration is accompanied with numerous cellular, metabolic and chemical alterations as well as chromosome aberrations and damage to the DNA, impairment of RNA and protein synthesis, modifications in the enzymes and food reserves and loss of membrane integrity (Kibinza et al., 2006). The accumulation of these damaging changes results in a gradual loss of seed performance, starting with low germination rate, reduced field emergence, increased numbers of abnormal seedlings and ending with seed death. Accelerated ageing test increases the rate at which the various changes occur within the seed and providing in a relatively short period of time indices for estimating storage and seedling field emergence potentials.

2.5.4 Specific accelerated ageing conditions

2.5.4.1 Traditional accelerated ageing (TAA)

This accelerated ageing test gives significant information on storage and seedling field emergence potentials. During the traditional accelerated ageing (TAA), seeds of a particular moisture content are subjected to relatively high temperature (40 to 45°C, usually 41°C) and relative humidity (around 100%). Subsequently, seeds are tested for germination and higher vigour seed lots withstand the ageing conditions better than lower vigour seed lots and result in higher percentage of normal seedlings (Baalbaki *et al.*, 2009).

2.5.4.2 Salt saturated accelerated ageing (SSAA)

Vegetable seeds and other small-seeded crops occasionally give unsatisfactory result when subjected to accelerated ageing test. Small seeds absorb moisture more rapidly resulting in a large fluctuation in seed moisture content and lack of consistency among seed samples. This results in large dissimilarities in deterioration rate and a decrease of germination after the ageing process. Based on these facts, Jianhua and McDonald (1996) suggested the salt

saturated accelerated ageing (SSAA) test. The SSAA test uses similar apparatus and technique as the traditional accelerated ageing test but substitutes the distilled water with a salt saturated solution (usually NaCl). The SSAA test has been advocated to be more similar to "natural ageing" of seeds in difference to the traditional accelerated ageing method, as it stimulates a low increase in seed moisture content at high temperatures.

2.5.5 Accelerated ageing for testing tree seed vigour

Several studies were conducted to evaluate the efficacy of the accelerated ageing for tree seed vigour testing, with a few assessing the biochemical changes that occurred in the seeds during the ageing test. Pitel (1982) aged seeds of northern red oak (*Q. rubra* L.) and jack pine (*P banksiana* Lamb.) and monitored variations in amino acids, isoenzymes and proteins throughout the ageing period. Similar tests were carried out by Blanche *et al*. (1990) with acorns of water oak (*Q. nigra* L.) and variations in starch, reducing and nonreducing sugars and amino nitrogen were recorded. Marquez-Millano *et al*. (1991) monitored changes in saturated and unsaturated fatty acids when ageing slash pine. These studies gave results, which confirmed clearly, that rapid utilization of seed reserves during the accelerated ageing test accompanied a decline in germination and seed vigour (Bonner, 1998). These results confirm the concept that agricultural and tree seeds react in a similar way when exposed to accelerated ageing procedures.

The accelerated ageing (AA) test has received extensive testing among tree seeds (Bonner, 1998). Most accelerated ageing research with tree seeds have concentrated on temperate species, assessing the optimum times and temperatures for ageing. These species include slash pine (*Pinus elliottii* Engelm.); loblolly pine (*P. taeda*); white spruce (*Picea glauca*); pecan (*Carya illinoensis*); water oak (*Quercus nigra*); sissoo (*Dalbergia sissoo* Roxb.) (Bonner, 1998). Several other tree species have also been assessed, but revealed

unsatisfactory results regarding the ageing conditions. These include: sitka spruce (*Picea sitchensis*); sweetgum (*Liquidambar styraciflua L.*), white oak; sycamore (*Platanus occidentalis*), cherrybark oak, and green ash (*Fraxinus pennsylvunica*) (Bonner, 1998).

2.5.6 Some limitations of AA test evaluating tree seed vigour

One dissimilarity between some tree seeds and agricultural seeds is seed dormancy. Seed dormancy has been reported to have an influence on the interpretation of accelerated ageing results (Bonner, 1998). In many studies, tree seeds that normally exhibited dormancy showed an increase in the germination percentage when subjected to short AA durations (Bonner, 1984; Bonner, 1998; Chaisurisri *et al*., 1993; Blanche *et al*., 1990). Another problem that is created by tree seed dormancy in vigour testing procedures is the appropriate time for applying pre-germination treatment to break the seed dormancy (before or after the ageing treatment?) (Bonner, 1998). Research conducted using a number of species showed a clear difference in seed performance between chilling and no chilling after ageing (Bonner, 1998).

CHAPTER THREE

3 MATERIALS AND METHODS

This study was conducted in the laboratory and plant house of the National Tree Seed Centre located at the CSIR-Forestry Research Institute of Ghana in Kumasi and the laboratories of the Faculty of Agriculture and Natural Resources, Kwame Nkrumah University of Science and Technology, Kumasi, from October 2015 to April 2016.

3.1 Seed Source

Seeds of *Tetrapleura tetraptera* were obtained form the National Tree Seed Centre of the Forestry Research Institute of Ghana in October 2015. Eight seed lots were obtained from specific trees of similar age. The seeds were processed, kept for a month at ambient temperature (24-28°C) and subsequently used for the accelerated ageing and electrical conductivity experiments. Proximate composition and membrane deterioration were also assessed on the same seed samples.

3.2 Experimentation

All seed or samples were cleaned and further cleaned to obtain pure seeds for the experiments. 1000 seed weight, initial water content, germination and electrical conductivity tests and seedling vigour tests were conducted on samples taken from each lot. Accelerated ageing (AA) test was conducted on each seed lot using various combinations of relative humidity, temperature and ageing periods. Germination characteristics and seedling vigour were assessed on the seeds after ageing them. Fat, crude fiber, crude protein, ash and carbohydrate contents as well as electrolyte leakage rates were determined after the various ageing procedures.

3.2.1 1000 Seed weight (g)

The 1000 Seed weight was determined by randomly counting hundred (100) seeds in replicates of eight, weighed and recorded in grams. The mean weight was then multiplied by ten to obtain the thousand seed weight as per the procedure given under ISTA Rules (ISTA, 1996).

3.2.2 Moisture test

Seed moisture content was determined before and after the ageing test to assess moisture variations using the constant temperature oven method of 103±2 °C for 17h±1 (ISTA, 1993). This was carried out in duplicate samples of 5 grams each. The samples were ovendried in glass Petri dishes. At the end of the drying period, they were allowed to cool for about 45 minutes in a desiccator containing silica gel. After cooling, each sample was weighed again. All weights were recorded in grams to two decimal places. The moisture content was calculated on wet basis using the formula:

$$= \quad (\quad - \quad)/$$

where M1 = initial weight of seed sample; M2 = final weight of seed sample. The average of the two results is the percentage moisture content of the sample.

3.2.3 Seed germination (emergence) and seedling vigour tests

Germination test was done on each sample before and after the ageing test. *Tetrapleura tetraptera* seeds have been reported to have germination problems due to the seed coat dormancy (Onyekwelu, 1990; Maku *et al*., 2014; Omokhua *et al*., 2015). Seeds were then mechanically scarified by rubbing seeds between two rough surfaces of sand paper for three minutes as described by Onyekwelu (1990). Germination was assessed by sowing 50 seeds in two (2) replicates of 25 seeds each. Seeds were sown in plastic bowls filled with sterilized

river sand. Watering was done daily in the morning in a plant house. Germination data was recorded at two days interval for a four weeks period from the first day of germination. Seeds were counted as germinated when the plumule emerged from the seed coat and were free from visual fungal infection or deformation.

- **Germination capacity**

Germination capacity (GC, the percentage of total germinated seeds) was determined by the formula:

$$= \frac{\qquad\qquad\qquad\qquad}{} \times$$

- **Seedling vigour**

Seedlings were allowed to grow for two weeks from the final germination scoring day (six weeks from first germination count). Five normal seedlings were selected at random from each replication and seedling root length and shoot length were measured using ruler after which the seedlings fresh weights were taken. Mean seedling length (cm) was calculated. The five seedlings were placed in a paper envelope and then put in oven at 80°C for 24 h, after which they were weighed to determine mean seedling dry mass (g).

3.2.4 Accelerated ageing test

For this experiment, a pilot experiment was conducted on a single seed lot under four (4) temperature levels: 38°C, 41°C, 43°C and 45°C. The 38 and 41°C temperature levels gave consistent results and were therefore selected and applied on the eight seed samples. For the ageing experiment, two different tests were conducted namely: the traditional accelerated ageing (ageing at 100% relative humidity (RH)) and salt saturated accelerated aging (ageing at 76% relative humidity (RH)).

3.2.4.1 Traditional accelerated ageing (TAA)

For this accelerated ageing (AA) test, the most studied aspect is the interaction between temperatures/exposure periods. Seeds were aged at 100% RH using distilled water at 38 and 41°C for 48, 72 and 96 hours. Six (6) conditions of ageing differing in ageing temperature (38 and 41°C) and treatment length (48h, 72h and 96h) were then studied. The tests were conducted in clear plastic containers (boxes) having suspended wire mesh screen inside, in which 80g of seeds were spread to form a single layer. The screens were positioned in the plastic containers 6 cm above the water level (ISTA, 1987). The boxes were covered with lids and the samples were placed in an incubator at the various temperatures. After ageing, seeds of each treatment were subjected to moisture and germination tests as earlier described.

3.2.4.2 Salt saturated accelerated ageing (SSAA)

The salt saturated accelerated ageing (SSAA) test was carried out in a similar way as described for the traditional test, except that it was carried out using the procedure proposed by Jianhua and McDonald (1996) by replacing the water added to each individual compartment with the same amount of a NaCl saturated solution. This solution was obtained by dissolving 40 g of NaCl in 100 ml of water to establish an environment of 76% relative humidity.

3.2.5 Electrical conductivity test (EC)

This test was carried out to determine the electrolyte leakage rate and appropriate soaking period to estimate the physiological potential of the seed lots in comparison with ageing and germination result and to assess the effect of the various ageing procedures on the *Tetrapleura tetraptera* seeds. Twenty seeds in four replications were taken from each sample and weighed in grams to two decimal places prior to testing. Each replicate was

rinsed once with 20 ml of de-ionized water and placed in 150 ml beaker, with 50 ml of deionized water, and stirred. Two beakers with the same quantity of de-ionized water were set up as blank. All beakers were kept on laboratory bench at the room temperature (2428°C) at which the cell was calibrated and covered to avoid pollution and evaporation of water. The samples were stirred and the electrical specific conductivity were measured and recorded after 1, 2, 3, 4, 5, 6 and 24 hours. The conductivity dip cell was rinsed once in each of two beakers of rinse water between each sample measurement. The conductivity of the samples in the control beakers were measured and the mean value was subtracted from the readings for the seed samples (ISTA, 2007). Specific conductivity per grams of dry seed was calculated using the following formula:

#) = ______________________
!
! (µ
$ %

3.2.6 Proximate composition determination

Ash, fat, crude fibre, crude protein and carbohydrate content determination was done on a single lot before and after each ageing to access the effect of the various accelerated ageing conditions tested in this experiment on seed composition.

3.2.6.1 Ash content determination

A two gram (2 g) sample was weighed into earlier weighed container, placed in a furnace and burnt for 2 hours at 600°C. After ashing, the containers were allowed to cool below 200°C in the furnace to room temperature in a desiccator. The containers and their contents were weighed, and the ash content was calculated using the following formula (AOAC, 2002):

(A + B) – A = B

(A + C) - A = C

% Ash = C/B x 100

where A = container weight, B = sample weight, C = ash weight.

3.2.6.2 Crude fat content determination

The crude fat content was extracted with petroleum ether using Automatic Soxhlet apparatus. Two grams of each sample was put into a paper cover, plugged at the opening with glass wool. The sample was placed in the butt tube of the soxhlet extraction apparatus. The extraction flask was placed in an oven at 103°C for about 5 minutes, then cooled and weighed. The extraction continued with petroleum ether for 2-3 hours without disruption by gently warming after which the extraction flask was dismantled and allowed to cool. The ether was evaporated using water bath until there was no odour on the remaining ether. It was then cooled at ambient temperature and the flask and its extract were note down (AOAC, 2002). The crude fat content was calculated using the following formula:

$$\%' = \frac{\$ \quad \%}{\$ \quad \%} \times$$

3.2.6.3 Crude fibre content determination

Sample from the ether determination was moved into a digestion flask. 200 ml of boiling H_2SO_4 solution and anti-foaming agent was added. The flask was then associated with a condenser and heated for 30 min. The solution was then sieved through a linen cloth in a funnel and washed with boiling water. Then, the residue was transferred into the flask with 200 ml boiling sodium hydroxide (NaOH) solution and boiled for another 30 min. The

content were again sieved and washed with boiled distilled water and with 15 ml of 95% ethanol. The filtrate was moved into porcelain previously dried and weighed in an oven at 110°C to a constant weight and then cooled in a desiccator and weighed. The container and its contents were ignited in a muffle furnace at 550 °C for 30 min until the carbonaceous matter has been consumed. Samples were cooled in a desiccator and weighed. The percentage crude fibre was calculated using the following formula:

$$\% = \frac{(-)}{} \times$$

where A = weight of dry container and sample B = weight of burned container and ash, C = sample weight.

3.2.6.4 Crude protein content determination

The protein content was determined by the Kjeldahl method. This was done in three steps namely: digestion, distillation, and titration.

i. **Digestion of the sample**

Two grams (2g) of the sample (ground seeds) was weighed into a digestion flask and mixed with 25 mL concentrated H_2SO_4, selenium catalyst and few anti-bumping agents. The flask and its content were digested by heating till the solution became colorless.

ii. **Distillation of digest**

After the digestion was completed the digestion flask was allowed to cool and the solution moved into a 100 ml flask and the volume made up to the 100 ml mark with distilled water. A 25 ml of boric acid was poured into a 250 ml conical flask and a few drops of mixed indicator dded, turning the solution pink. The flask and its contents were placed under the

condenser with the tip of the condenser completely immersed in the boric acid solution. A 10 ml of the digested sample solution and about 50 ml of 40% NaOH solution were transferred into the decomposition flask and the funnel stopcock well closed. NH_3 liberated during the distillation was collected by the boric acid solution, changing it from pink to bluish-green.

iii. **Titration of distillate**

The nitrogen content was estimated by titrating the ammonium borate formed in the conical flask with 0.1M HCl solution till the solution changed from bluish-green to pink. Titre values of the replicate samples were recorded and percentage nitrogen calculated as shown below. A blank sample was run at the same time as the sample was being analyzed.

$$\%N = \frac{(T_s - T_b) \times M \times 14.007 \times 100}{W \times 1000}$$

% Crude Protein (CP) = Total Nitrogen (N_T) x 6.25 (Protein factor)

3.2.6.5 Total carbohydrate content determination

The total percentage carbohydrate content was assessed by adding the total values of crude protein, crude fibre, moisture and ash constituents of the sample and subtracting it from 100 (Onyeike *et al.*, 1995).

Thus: % carbohydrate=100–(% moisture + % crude fibre +% protein+%lipid+% ash).

3.3 Data Analysis

All data were subjected to analysis of variance (ANOVA) using Genstat (11th edition) Statistical Package. Tukey test was used to assess the difference in means within each

column at 5% probability. Correlation analysis was done to assess the relationship between ageing procedure, seed germination characteristics; seedling vigour parameters and electrolyte leachate conductivity.

CHAPTER FOUR

4.0 RESULTS

4.1 Initial characteristic of the eight seed lots

The characteristics of eight *Tetrapleura tetraptera* seed lots are reported below. These characteristics comprise: 1000 seed weight, seed moisture content, seed germination (emergence) percentage, seedling root and shoot length, seedling total length and seedling dry weight.

4.1.1 One thousand (1000) seed weight

The results of one thousand (1000) seed weight are showed in Table 4.1. The mean 1000 seed weight of the seed lots indicated that, there was significant difference ($p<0.01$) between the eight seed lots. Seed lot 7 was the heaviest followed by lot 2 and the lowest value was recorded in the lot 5.

4.1.2 Seed initial moisture content

The data relative to the initial moisture content is shown in Table 4.1. Seed moisture content ranged from 8.32% to 9.09% for the eight lots with an average of 8.66%. The statistical analysis showed significant differences ($p < 0.01$) among means with lot 8 having the heighest value and lot 5, the lowest. The moisture content variation among the samples was

0.77%, which was less than 2%, and required limits to obtain consistent results for accelerated ageing tests.

4.1.3 Seed germination before AA of seed lots

The germination (emergence) percentages the eight seed lots ranged from 49 to 65% with average of 56.50%. Significant differences ($p < 0.05$) were observed among means of the eight seed lots (Table 4.1). Results from the seedling emergence test indicated that lots 7 and 8 were of superior performance and lots 1 and 5 showed the poorest performance. Lots 2, 3, 4 and 6 were referred to as having intermediate quality.

4.1.4 Seedling root length, shoot length, total seedling length and seedling dry weight before AA test

Seedling shoot length and total seedling length showed significant differences ($p < 0.01$) among seed lots while seedling root length and seedling dry weight were not significant (Table 4.1).

Table 4.1: Characteristic of eight *Tetrapleura tetraptera* seed lots

Seed lot	1000SW	MC%	Vigour indicators				
			SE%	RL(cm)	SL(cm)	TL(cm)	DW(g)
1	135 bc[1]	8.48 cd	50 b	6.15	12.66 c	19.17 c	0.156
2	138.2 ab	8.55 cd	58 ab	7.5	13.72 ab	21.21 a	0.188
3	136.6 bc	8.47 cd	54 ab	6.85	12.53 c	19.38 c	0.188
4	135 bc	8.66 bc	59 ab	7.6	13.11 c	20.71 ab	0.257
5	132.2 c	8.32 d	49 b	7.15	13 bc	20.15 abc	0.193
6	134.7 bc	8.90 ab	53 ab	6.267	13.2 abc	19.47 bc	0.178
7	141.8 a	8.76 bc	65 a	7.1	13.7 ab	20.8 a	0.208
8	137.6 ab	9.09 a	64 a	7.4	14.07 a	21.47 a	0.193
Mean	136.39	8.66 0.1	56.50	7.05 1.8	13.25	20.30	0.20
CV%	0.3		2.5		0.7	0.1	2.1
Lsd (5%)	2.94**	0.18**	7.99*	0.80 ns	0.54**	0.76**	0.04 ns

ns = not significant; (*, **) = significant at 5% and 1%probability respectively; 1000SW = one thousand seed weight; CV (%): coefficient of variation; MC%= moisture content; SE = Seed emergence; RL (cm) = root length; SL (cm) = Shoot length; TL (cm) = seedling total length; DW (g) = seedling dry weight.

[1]Mean comparisons within each column by Tukey test, 5%.

4.1.5 Correlation amongst seed emergence test and four other vigour indicators

Correlation coefficients of seed emergence test and four seed vigour indicators of eight *Tetrapleura tetraptera* seed lots are shown in Table 4.2.

The correlation coefficient (r) showed highly positive significant association of seed emergence with seedling shoot length (r = 0.70), total seedling length (r = 0.72) and significant association with seedling root length (r = 0.48), and seedling dry weight (r = 0.44). Seedling total length showed highly significant correlation with seedling shoot length (r = 0.83) and root length (r = 0.81) but no significant relationship with seedling dry weight. In addition, seedling dry weight was significantly correlated with seedling root length (r = 0.50) but not significantly correlated with seedling shoot length (0.39).

Table 4.2: Correlation coefficients (r) amongst seed emergence test, seedling shoot length, seedling root length, total seedling length and seedling dry weight

Test	SE	SL	RL	TL	DW
SE	1				
SL	0.70**	1			
RL	0.48*	0.35ns	1		
TL	0.72**	0.83**	0.81**	1	
DW	0.44*	0.15ns	0.50*	0.39ns	1

SE= Seed emergence, SL= Shoot length, RL= Root length, TL= Total length, DW= Dry weight. ns= non-significant *, ** = significant difference at $p < 0.05$ and $p < 0.01$, respectively.

4.2 Electrical conductivity for *Tetrapleura tetraptera* seed vigour test

4.2.1 Classification of the eight *Tetrapleura tetraptera* seed lots using electrical conductivity test

Table 4.3 shows that changes occurred in the electrical conductivity values of leachates from the seed lots when they were soaked for different periods (hours). The results show significant differences in conductivity readings for all the soaking periods ($p < 0.05$) except those of 5 and 6 hours, which showed no significant differences among means. For the soaking periods of 1, 2 and 24 hours, lot 7 showed the lowest conductivity reading (3.70 $\mu Scm^{-1}g^{-1}$; 3.70 $\mu Scm^{-1}g^{-1}$ and 11.1$\mu Scm^{-1}g^{-1}$ respectively) followed by lot 8 (5.08 $\mu Scm^{-1}g^{-1}$; 5.08 $\mu Scm^{-1}g^{-1}$ and 12.5 $\mu Scm^{-1}g^{-1}$). Based on the principle that vigorous seeds release lower amounts of solutes when soaked for a specific time, the seed lots 7 and 8 can therefore be characterized as lots of superior quality. The soaking period of 24 hours permitted the characterization of lots 1 (35.7 $\mu Scm^{-1}g^{-1}$, highest value) as inferior to the other ones but 1 and 2 hours of soaking did not allow such separation. In addition, 24 hours soaking permitted the classification of lots 2, 3, 4, 5 and 6 as intermediate quality. Soaking periods of 3, 4, 5 and 6 hours did not allow the separation of the eight *Tetrapleura tetraptera* seed lots into different vigour levels.

Table 4. 3: Conductivity ranges of the eight *Tetrapleura tetraptera* seed lots after soaking for different periods

Seed lot	1h Soak (µScm−1g−1)	2h Soak (µScm−1g−1)	3h Soak (µScm−1g−1)	4h Soak (µScm−1g−1)	5h Soak (µScm−1g−1)	6h Soak (µScm−1g−1)	24h Soak (µScm−1g−1)
1	7.52 a[1]	7.52 a	7.52 a	9.34 a	9.34	9.34	35.7 a
2	7.67 a	7.67 a	7.66 a	8.88 a	8.88	10.18	15.3 ab
3	7.71 a	7.71 a	7.70 a	7.70 a	7.70	11.56	27.0 ab
4	7.21 a	7.21 a	7.20 a	7.20 a	8.96	8.96	14.4 ab
5	7.88 a	7.88 a	9.93 a	9.93 a	13.88	13.87	30.0 ab
6	7.25 a	7.25 a	9.06 a	9.06 a	12.69	12.69	27.2 ab
7	3.70 b	3.70 b	5.54 a	5.54 a	7.40	7.40	11.1 b
8	5.08 ab	5.08 ab	7.15 a	7.15 a	7.15	7.15	12.5 ab

[1] Mean comparisons within each column by Tukey test, 5%.

Mean	6.75	6.75	7.72	8.10	9.50	10.15	21.7
CV%	4.2	4.2	12.7	15.0	6.3	7.8	8.5
Lsd (5%)	1.99*	1.99*	3.68*	3.78*	4.60 ns	4.63 ns	13.74*

ns = not significant, (**,*) = significant at 1% and 5% probability respectively.

4.2.2. Correlation amongst seed emergence test, seedling vigour tests and electrical conductivity test

The correlation result showed negative association of conductivity test with all the other vigour indicators tested in this study; seed emergence, seedling root length, seedling shoot length, seedling total length and seedling dry weight (Table 4.4). Soaking periods of 1, 2, 3, 4, 6 and 24 hours showed highly significant correlation (-0.69; -0.69; -0.55; -0.60; 0.57; -0.76 respectively) with seed emergence while 5 hours soaking was significantly correlated (-0.49). Result from 1 and 2 hours soaking were significantly correlated with seedling shoot length and seedling total length but showed no significant correlation with seedling root length and seedling dry weight. Soaking periods of 3, 4, 5 and 6 hours were not significantly correlated with seedling root length, seedling shoot length, seedling total length and seedling dry weight. Concerning the soaking period of 24 hours, the correlation analysis showed the highest coefficient (r = -0.76). In addition, 24 hours soaking was highly correlated with all the other vigour indicators: seedling root length (r = -0.58), seedling shoot length (r = -0.66), seedling total length (r = -0.75) and seedling dry weight (r = -0.53).

Table 4.4: Correlation coefficients (r) of electrical conductivity recorded after seven periods of soaking, and five other vigour indicators, of eight seed lots.

Test	SE	SL	RL	TL	DW	1h soak	2h soak	3h soak	4h soak	5h soak	6h soak	24h soak
SE	1											
SL	0.70**	1										
RL	0.48*	0.35ns	1									
TL	0.72**	0.83**	0.81**	1								
DW	0.44ns	0.15ns	0.50*	0.39ns	1							
1h soak	-0.69**	-0.51*	-0.27ns	-0.48*	-0.15ns	1						
2h soak	-0.69**	-0.51*	-0.27ns	-0.48*	-0.15ns	1.00**	1					
3h soak	-0.55**	-0.24ns	-0.16ns	-0.24ns	-0.08ns	0.53*	0.53*	1				
4h soak	-0.60**	-0.24ns	-0.24ns	-0.29ns	-0.22ns	0.56**	0.56**	0.87**	1			
5h soak	-0.49*	-0.22ns	-0.26ns	-0.29ns	-0.06ns	0.44ns	0.44ns	0.76**	0.74**	1		
6h soak	-0.57**	-0.42ns	-0.26ns	-0.42ns	-0.12ns	0.60**	0.60**	0.78**	0.73**	0.87**	1	
24h soak	-0.76**	-0.66**	-0.58**	-0.75**	-0.53*	0.62**	0.62**	0.63**	0.70**	0.61**	0.68**	1

SE= Seed emergence, SL= Shoot length, RL= Root length, TL= Total length, DW= Dry weight. ns= non-significant *, ** = significant difference at $p < 0.05$ and $p < 0.01$, respectively.

4.3 Accelerated ageing for *Tetrapleura tetraptera* seed vigour test 4.3.1 Effect of accelerated ageing on *Tetrapleura tetraptera* seed moisture content

Seeds, after the exposure to the traditional and the salt saturated accelerated ageing methods, showed different moisture absorption patterns as indicated by the figures 4.1, 4.2, 4.3 and 4.4. The seed moisture content, after the traditional ageing periods (figure 4.1 and 4.2), ranged from 9.12 to 18.37%, depending on the period and temperature combination. Variations in moisture content between seed lots for seeds subjected to traditional accelerated ageing method were from 0.6 to 8.16% depending on the period and temperature combination. For seeds exposed to the salt saturated solution, the moisture content presented smaller and more uniform values, which ranged from 9.06 to 12.79% for the two temperature regimes after the ageing periods (figure 4.3 and 4.4), with a moisture variation form 0.27 to 2.04%. It appears from the result that seed moisture content increased after exposure to the various ageing conditions but the traditional accelerated ageing conditions result in large variation in the seed moisture content as compared to the salt saturated conditions. However the ageing temperature of 38°C showed greater impact on the seed moisture content of the eight lots for both traditional and salt saturated methods.

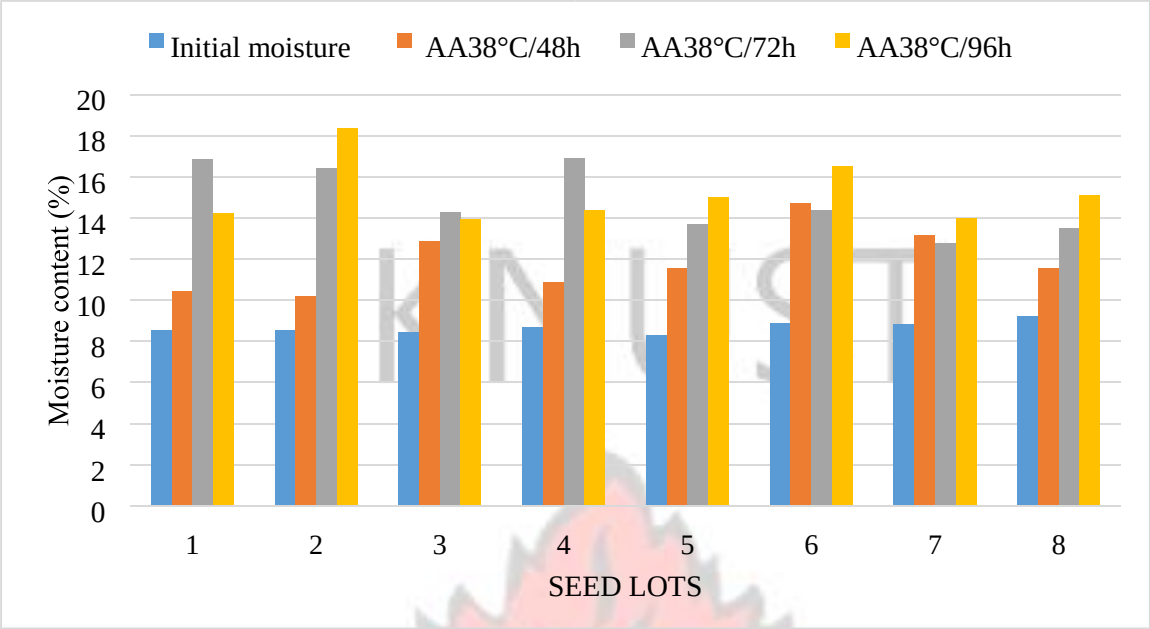

Figure 4.1: Seed moisture contents variation after being exposed to the traditional accelerated ageing (TAA) method during three ageing periods (48, 72 and 96 hours) and temperature of 38°C.

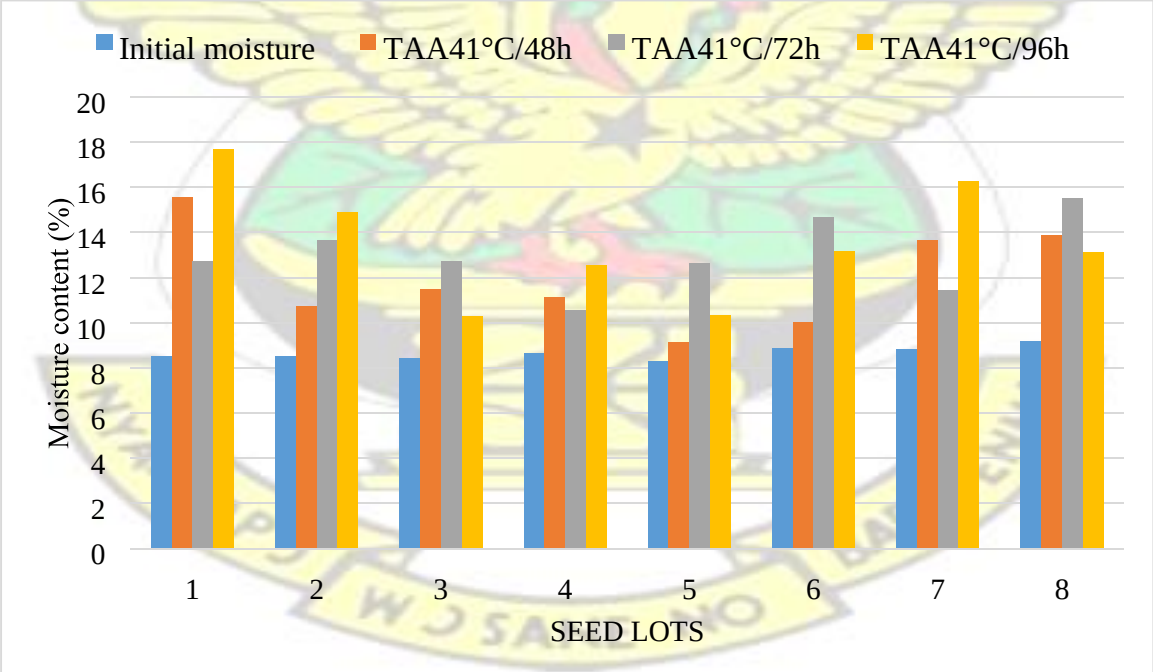

Figure 4.2: Seed moisture contents variation after being exposed to the traditional accelerated ageing (TAA) method during three ageing periods (48, 72 and 96 hours) and temperature of 41°C.

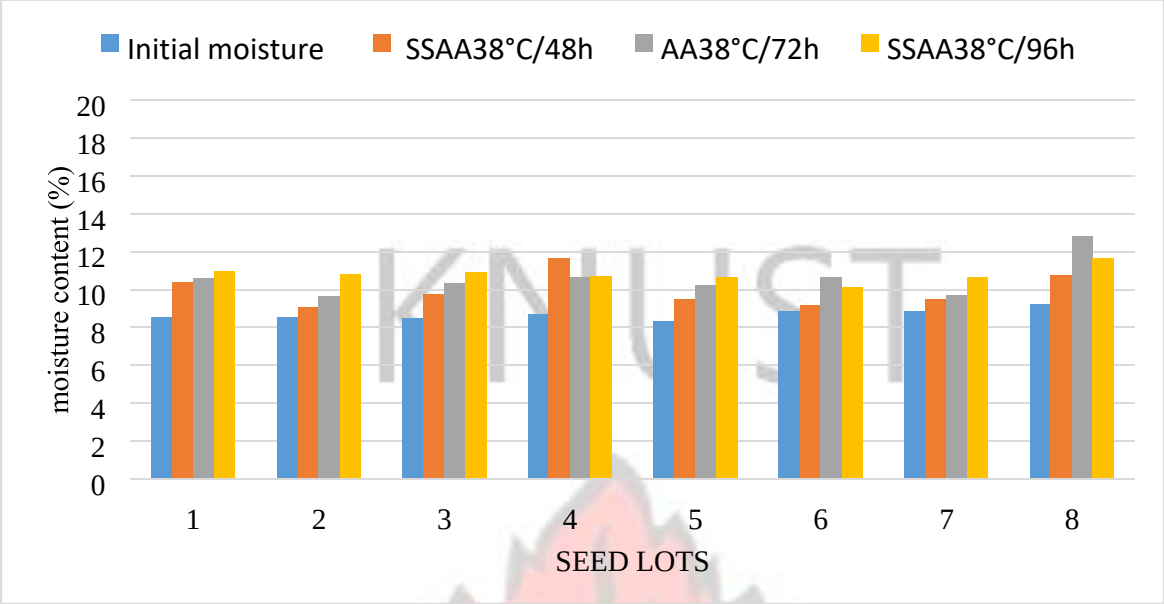

Figure 4.3: Seed moisture contents variation after being exposed to the salt saturated accelerated ageing (SSAA) method during three ageing periods (48, 72 and 96 hours) and temperature 38°C.

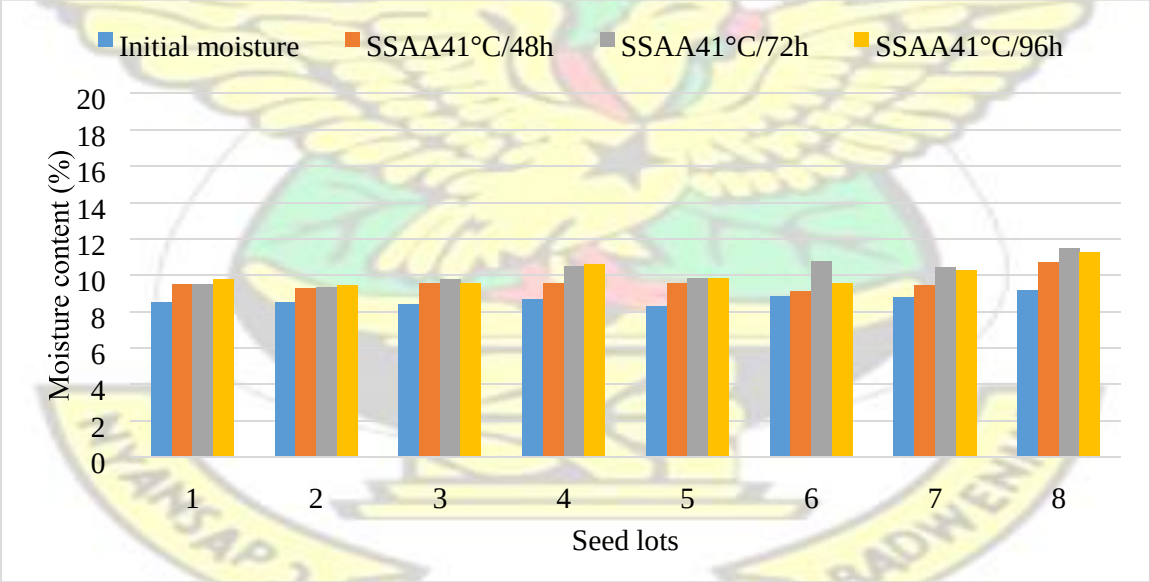

Figure 4.4: Seed moisture contents variation after being exposed to the salt saturated accelerated ageing (SSAA) method during three ageing periods (48, 72 and 96 hours) and temperature of 41 °C.

4.3.2 Effect of accelerated ageing conditions on *Tetrapleura tetraptera* seed membrane deterioration

The electrical conductivity of *Tetrapleura tetraptera* seeds increased with increasing ageing and soaking time (Figures 4.5, 4.6, 4.7, 4.8). The results showed an increase in the conductivity reading of leachate solution of seeds that were subjected to accelerated ageing treatement as compared with the control. It appears that for both traditional and salt saturated methods, longer period of exposure resulted in higher seed leachage. In a similar way, the ageing temperature of 41°C showed greater seed leachage than 38°C. However, the salt saturated accelerated ageing method showed less impact on the conductivity readings of the leachate solutions as compared with the traditional method.

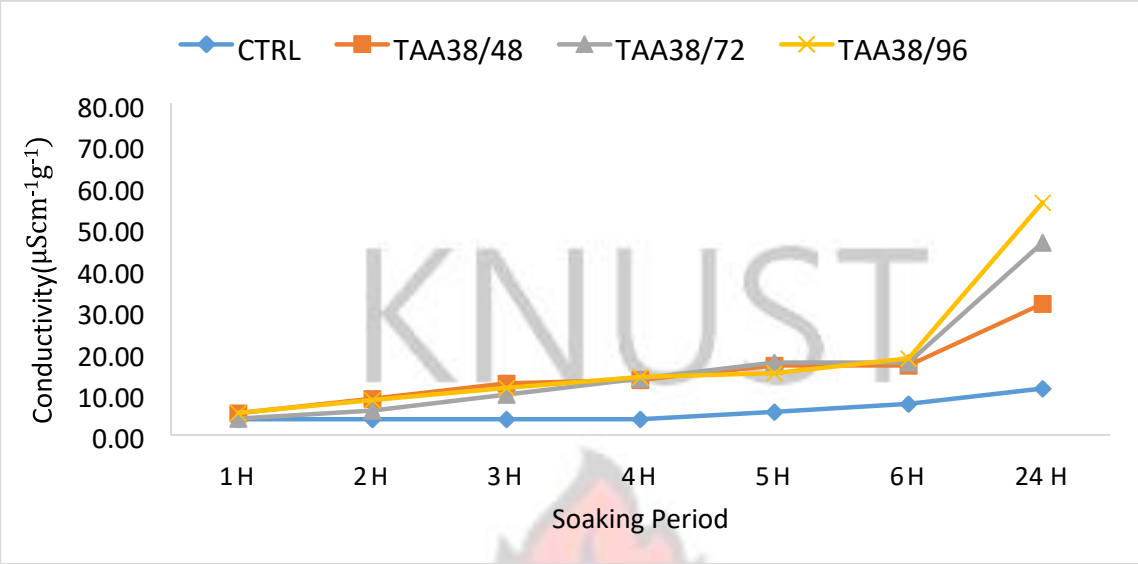

Figure 4.5: Leachate conductivity of *Tetrapleura tetraptera* seed, as affected by traditional accelerated ageing (TAA) method during three ageing periods (48, 72 and 96 hours) and temperatures of 38°C.

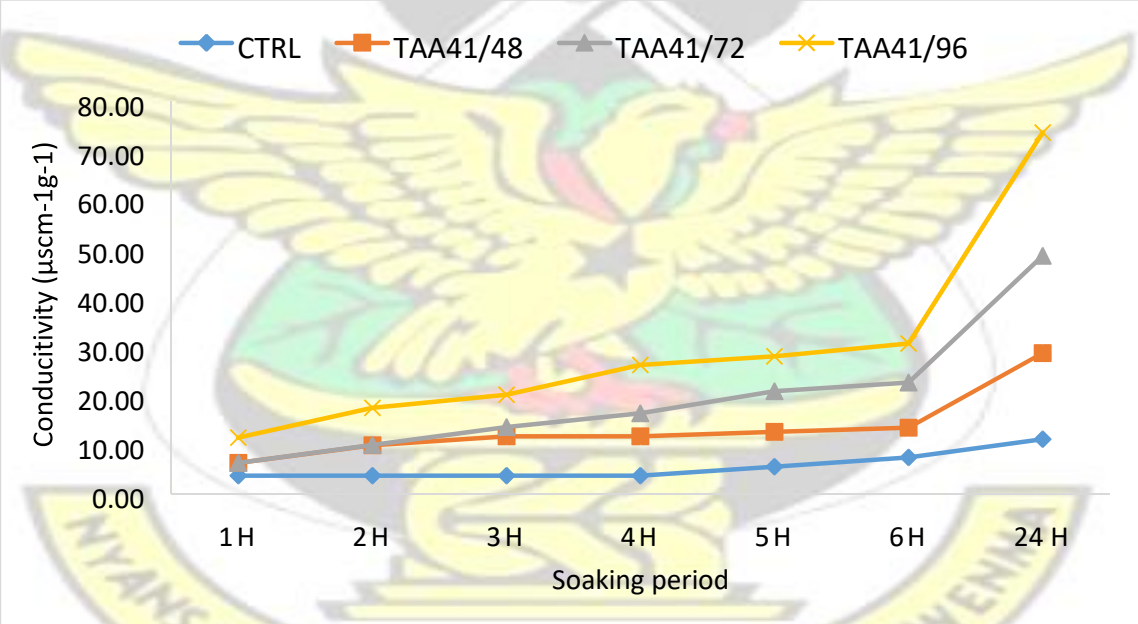

Figure 4.6: Leachate conductivity of *Tetrapleura tetraptera* seed, as affected by traditional accelerated ageing (TAA) method during three ageing periods (48, 72 and 96 hours) and temperatures of 41°C.

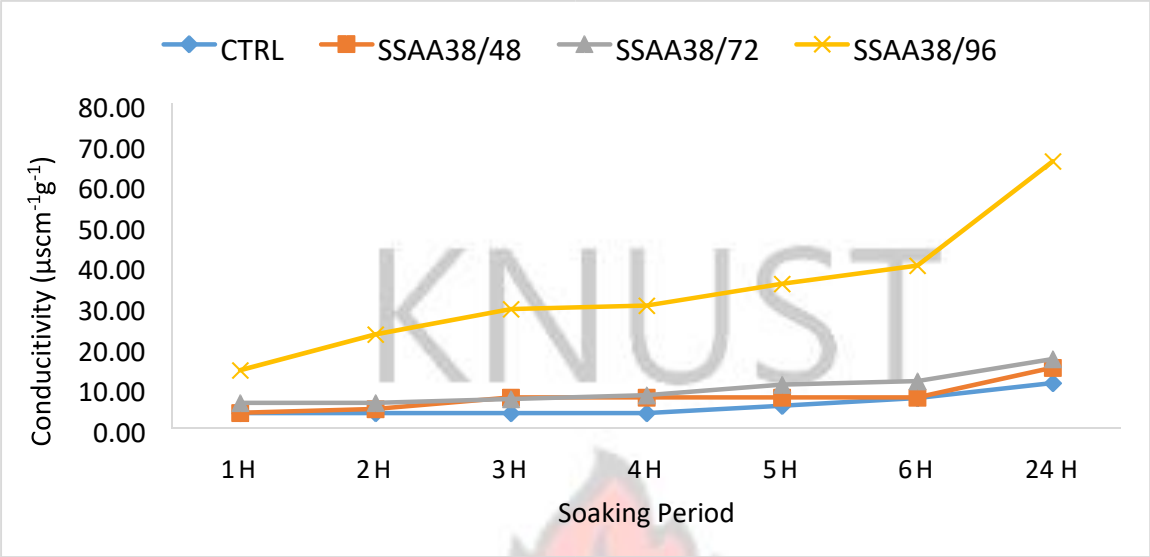

Figure 4.7: Leachate conductivity of *Tetrapleura tetraptera* seed, as affected by salt saturated accelerated ageing (SSAA) method during three ageing periods (48, 72 and 96 hours) and temperatures of 38°C.

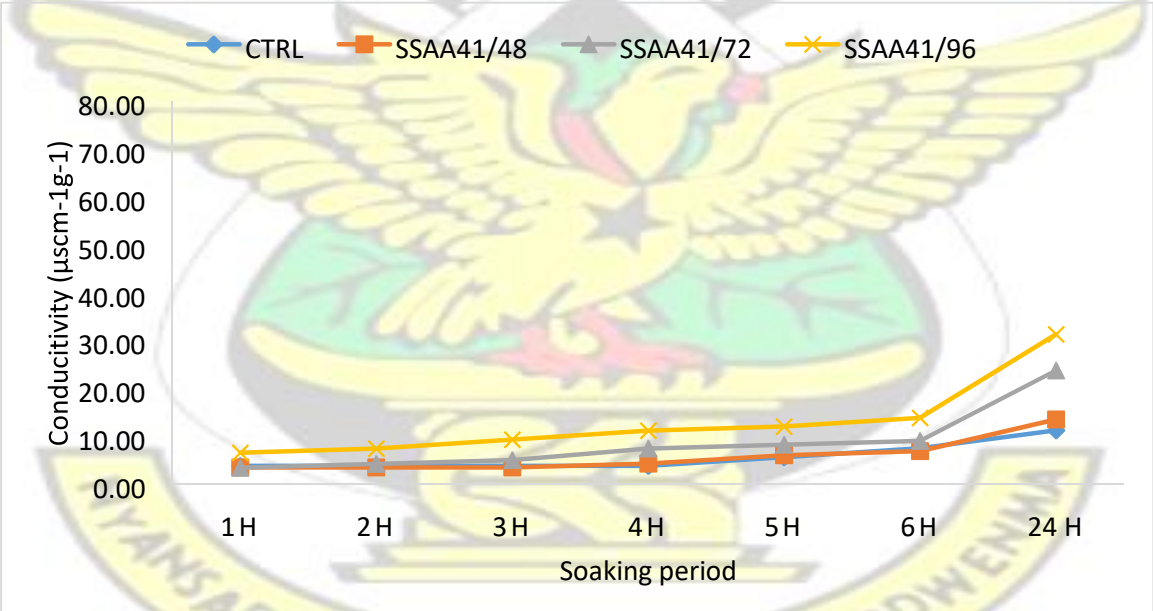

Figure 4.8: Leachate conductivity of *Tetrapleura tetraptera* seed lot, as affected by salt saturated accelerated ageing (SSAA) method during three ageing periods (48, 72 and 96 hours) and temperatures of 41°C.

4.3.3 Effect of accelerated ageing conditions on *Tetrapleura tetraptera* seed proximate composition

4.3.3.1 Ash content (%)

The mean ash content of seeds that were subjected to salt saturated accelerated ageing showed no significant ($p<0.01$) difference for the ageing periods but there was a significant ($p<0.01$) difference among means for the two temperatures and the interaction temperatures and periods (Table 4.6). Concerning seeds that were subjected to traditional method, there were no significant differences among the treatments (Table 4.5). However, both traditional and salt saturated ageing methods yielded, an increase in the ash content as compared to the control treatment (Table 4.5, 4.6).

4.3.3.2 Crude protein (%)

Analyzing the protein content after seeds were submitted to traditional and salt saturated accelerated ageing methods using different temperatures for different exposure times, no significant ($p<0.01$) effect was observed between the two factors for the traditional method (Table 4.5) but for the salt saturated method the means showed significant ($p<0.01$) differences (Table 4.6). The mean protein content fell from 21.4% in the control to 17.5% and 17.2% (38 and 41°C respectively) for the salt saturated method and to 17.1% and 16.7% (38 and 41°C respectively) for traditional method. It appears then, that the traditional accelerated ageing had greater effect on the seed protein content than the salt saturated one, even though the difference between the means were not significant ($p<0.01$) and the temperature of 41°C affected the protein content in a greater way than 38°C.

4.3.3.3 Carbohydrate (%)

The differences in carbohydrate content was not significant ($p<0.01$) for the traditional method (Table 4.5). The salt saturated method showed significant ($p<0.01$) differences for the temperatures and period but the interaction of the two factors was not significant (Table 4.6). However both traditional and salt saturated methods resulted in an increase in the carbohydrate content as compared to the control treatment.

4.3.3.4 Fat (%)

The mean fat content of seeds that were subjected to the traditional method showed no significant differences ($p<0.01$) (Table 4.5). Though there was a significant ($p<0.01$) difference due to the temperatures and periods alone, the interaction of the two factors produced no significant ($p<0.01$) effect in the salt saturated method (Table 4.6). Yet both traditional and salt saturated methods resulted generally in an increase in the carbohydrate content as compared with the control treatment.

4.3.3.5 Crude fibre (%)

The crude fibre content of seeds that were subjected to the traditional method showed no significant differences ($p<0.01$) (Table 4.5). For seeds that were subjected to the salt saturated methods, there was a significant ($p<0.01$) difference for the temperatures and period and their interactions (Table 4.6). There was an increase in the crude fibre content with the combination of 41°C and 72h having the highest value (13.4%) and 38°C and 72h having the lowest (10.7%).

Table 4.5: Proximate composition of *Tetrapleura tetraptera* seeds after being exposed to the traditional ageing method during three ageing periods (48, 72 a nd 96 hours) and two temperatures (38 and 41°C).

	Ash%				Crude protein%				Carbohydrate%				Fat%				Crude fibre%			
	48h	72h	96h	Mean	48h	72h	96h	Mean	48h	72h	96h	Mean	48h	72h	96h	Mean	48h	72h	96h	Mean
38° C	3.8	3.7	3.5	3.7	17.3	18.4	15.6	17.1	53.7	50.0	52.3	52.0	8.3	8.5	8.6	8.5	9.9	11.2	11.0	10.7
41° C	3.6	3.7	3.5	3.6	16.9	18.1	15.2	16.7	53.8	50.7	52.5	52.3	8.8	8.4	8.5	8.5	9.8	11.1	11.0	10.6
CTRL	3.5	3.5	3.5	3.5	21.4	21.4	21.4	21.4	51.4	51.4	51.4	51.4	7.0	7.0	7.0	7.0	10.1	10.1	10.1	10.1
CV%	6.2				5.4				6.8				7.2				11.0			
	Lsd (5%)																			
temp	0.1674 ns				2.650 ns				4.98 ns				0.861 ns				3.323 ns			
Period	0.2050 ns				3.245 ns				6.10 ns				1.054 ns				4.070 ns			
Temp x period	0.2899 ns				4.589 ns				8.62 ns				1.490 ns				5.756 ns			

ns = not significant, (*, **) = significant at 5% and 1% probability, respectively.

Due to their high coefficient of variation (CV), data of ash, crude protein and crude fibre (Table 4.3), were transformed by square root method.

Table 4.6: Proximate composition of *Tetrapleura tetraptera* seeds after being exposed to the salt saturated accelerated ageing method during three ageing periods (48, 72 and 96 hours) and two temperatures (38 and 41°C).

	Ash%				Crude protein%				Carbohydrate%				Fat%				Crude fibre%			
	48h	72h	96h	Mean	48h	72h	96h	Mean	48h	72h	96h	Mean	48h	72h	96h	Mean	48h	72h	96h	Mean
38° C	3.2	3.0	3.6	3.2	18.7	19.6	14.2	17.5	53.4	53.5	55.0	54.0	7.5	6.3	7.3	7.0	11.5	10.7	12.8	11.6
41° C	3.8	3.8	3.4	3.7	19.3	16.2	16.1	17.2	48.7	50.7	50.7	50.1	9.3	8.3	8.5	8.7	11.1	13.4	12.3	12.3
CTRL	3.5	3.5	3.5	3.5	21.4	21.4	21.4	21.4	51.4	51.4	51.4	51.4	7.0	7.0	7.0	7.0	10.1	10.1	10.1	10.1
CV%	4.4				2.1				0.9				3.7				1.1			
	Lsd (5%)																			
Temp	0.213**				0.511 ns				0.697**				0.408**				0.193**			
Period	0.261 ns				0.626**				0.854**				0.499**				0.236**			
Temp x period	0.369**				0.885**				1.208 ns				0.706 ns				0.334**			

ns = not significant, (*, **) = significant at 5% and 1%probability, respectively.

4.3.4 Effect of accelerated ageing conditions on *Tetrapleura tetraptera* seed germination characteristics

4.3.4.1 Effect of traditional accelerated ageing conditions on *Tetrapleura tetraptera* seed germination percentage

The data relative to the effect of the traditional accelerated ageing conditions on *Tetrapleura tetraptera* seed germination percentage are shown in Table 4.7. The results showed that germination percentages were significantly different ($p<0.01$) among the temperatures and periods tested in this study and among seed lots. The interactions of seed lots, temperature and exposure period also showed significant ($p<0.01$) differences among means. Germination percentage was generally low after 48h exposure but increased at 72h exposure before falling again at 96h. However, the 41°C temperature caused greater reduction of germination than 38°C.

4.3.4.2 Effect of salt saturated accelerated ageing conditions on *Tetrapleura tetraptera* seed germination

The data relative to the effect of the salt saturated salt saturated accelerated ageing conditions on *Tetrapleura tetraptera* seed germination percentage are shown in Table 4.8. There was a significant difference ($p<0.01$) germination percentage among the exposure temperatures and periods and among seed lots. The interactions of seed lots, temperature and period also showed significant ($p<0.01$) differences among means. Germination percentage was generally low after 48h exposure but increased gradually at 72h and 96h exposure.

Table 4.7: Germination percentage of eight *Tetrapleura tetraptera* seed lots, after being exposed to the traditional ageing method during three ageing periods (48, 72 and 96 hours) and two temperatures (38 and 41°C).

Seed lot	Initial GP(%)	Accelerated ageing conditions					
		38°C			41°C		
		48h	72h	96h	48h	72h	96h
1	50	48	32	18	10	18	12
2	58	24	42	41	21	21	17
3	54	31	42	32	53	36	22
4	59	32	42	36	46	56	34
5	49	32	50	45	38	55	33
6	53	38	44	37	42	34	24
7	65	32	50	41	32	28	16
8	64	59	40	34	32	36	29
		CV%=6.1					

Lsd (5%)	
Seed lot	3.487**
Temp	1.743**
period	2.135**
Seed lot x Temp	4.931**
Seed lot x period	6.039**
Temp x period	3.019**
Seed lot x Temp x period	8.540**

ns = not significant, (*, **) = significant at 5% and 1%probability respectively.

GP= germination percentage

Table 4.8: Germination percentage of eight *Tetrapleura tetraptera* seed lots after being exposed to the salt saturated accelerated ageing method during three ageing periods (48, 72 and 96 hours) and two temperatures (38 and 41°C).

Seed lot	Initial GP (%)	Accelerated ageing conditions					
		38°C			41°C		
		48h	72h	96h	48h	72h	96h
1	50	22.5	30	26	32	39	42
2	58	38	14	10	24	48	40
3	54	40	28	17	26	32	46
4	59	32	32	24	30	30	52
5	49	34	38	38	54	50	60
6	53	34	28	30	26	48	58
7	65	23	28	42	26	49	38
8	64	26	36	30	32	26	48
CV%= 5.8							

Lsd (5%)	
Seed lot	3.241**
Temp	1.621**
period	1.985**
Seed lot x Temp	4.584**
Seed lot x period	5.614**
Temp x period	2.807**
Seed lot x Temp x period	7.940**

ns = not significant, (*,**) = significant at 5% and 1% probability respectively.

.GP= Germination percentage

4.3.5 Effect of accelerated ageing conditions on *Tetrapleura tetraptera* seedling vigour

4.3.5.1 Effect of traditional accelerated ageing conditions on *Tetrapleura tetraptera* seedling root length

A significant ($p<0.01$) interactive effect among seed lot, temperature and exposure period (Table 4.9) was observed when the seeds were subjected to the traditional accelerated ageing method at different temperatures and exposure times. The two temperatures had similar effects on seedling root length but the exposure time of 96h had greater effect than the other ones (48 and 72°C). It appeared from the result that seedling root length decreased with increase of the ageing period.

4.3.5.2 Effect of salt saturated accelerated ageing conditions on *Tetrapleura tetraptera* seedling root length

The seedling root length was not significantly ($p<0.01$) affected by the combination of temperature and exposure period after seeds were subjected to the salt saturated accelerated ageing condition. However interaction of seed lots x temperature x period significantly ($p<0.01$) affected seedling root length. Also, the two temperatures had similar effects on seedling root length but the exposure time of 96h had greater effect than the other ones (Table 4.10).

Table 4.9: Seedling root length of eight *Tetrapleura tetraptera* seed lots after being exposed to the traditional accelerated ageing method during three ageing periods (48, 72 and 96 hours) and two temperatures (38 and 41°C).

Seed lot	Initial Root length (cm)	38°C			41°C		
		48h	72h	96h	48h	72h	96h
		Root length (cm) after TAA					
1	6.15	8.18	6.97	6.67	7.34	7.59	5.57
2	7.50	7.25	8.74	7.76	6.31	7.25	5.30
3	6.85	7.20	7.45	7.15	8.00	7.45	6.46
4	7.60	7.90	7.85	7.10	7.25	7.14	5.95
5	7.15	5.77	6.70	5.96	6.85	7.25	6.57
6	6.27	6.25	6.45	5.80	6.65	7.20	6.10
7	7.10	6.53	7.10	4.90	6.81	5.75	5.95
8	7.40	7.90	5.65	4.25	7.20	7.10	6.60
				CV%= 7.2			

Lsd (5%)	
Seed lot	0.4005 * *
Temp	0.2002 ns period
0.2452 **	
Seed lot x Temp	0.5663 **
Seed lot x period	0.6936 **
Temp x period	0.3468 ns
Seed lot x Temp x period	0.9809 **

ns = not significant, (*, **) = significant at 5% and 1% probability respectively.

Table 4.10: Seedling root length of eight *Tetrapleura tetraptera* seed lots after being exposed to the salt saturated accelerated ageing method during three ageing periods (48, 72 and 96 hours) and two temperatures (38 and 41°C).

Seed lot	Initial Root Length (cm)	38°C			41°C		
		48h	72h	96h	48h	72h	96h
		Root length (cm) after SSAA					
1	6.15	7.25	6.93	6.75	6.90	6.74	6.40
2	7.50	7.30	7.38	7.25	7.73	7.25	6.40
3	6.85	6.50	7.92	6.70	7.40	7.25	6.55
4	7.60	8.10	7.05	7.00	6.70	7.25	6.90
5	7.15	7.10	7.40	7.15	7.03	7.48	6.25
6	6.27	7.20	7.10	7.23	6.65	7.80	6.45
7	7.10	7.50	7.80	7.15	6.90	7.33	7.20
8	7.40	7.65	7.85	6.65	7.58	7.35	7.60
		CV%= 5.2					

Lsd (5%)	
Seed lot	0.3026 **
Temp	0.1513 ns
period	0.1853 **
Seed lot x Temp	0.4279 ns
Seed lot x period	0.5240 ns
Temp x period	0.2620 ns
Seed lot x Temp x period	0.7411 **

ns = not significant, (*, **) = significant at 5% and 1% probability respectively.

4.3.5.3 Effect of traditional accelerated ageing conditions on seedling shoot length of *Tetrapleura tetraptera* seed lots

Table 4.11 shows the effect of the traditional accelerated ageing conditions on seedling shoot length. The results showed significant ($p<0.01$) effect of the temperatures and exposure periods on seedling shoot length but the differences among seed lots were not significant. Moreover, the interactions of temperature x period, and seed lots x temperature x period were not significant ($p<0.01$).

4.3.5.4 Effect of salt saturated accelerated ageing conditions on seedling shoot length *Tetrapleura tetraptera* seed lots

For salt saturated accelerated ageing, both seed lot and temperature tested in this study showed no significant effect on seedling shoot length, whereas the exposure period significantly ($p<0.01$) decreased the seedling shoot length (Table 4.12). However, the interactions of seed lots x temperature x period were significant ($p<0.01$).

Table 4.11: Seedling shoot length (cm) of eight *Tetrapleura tetraptera* seed lots after being exposed to the traditional accelerated ageing method during three ageing periods (48, 72 and 96 hours) and two temperatures (38 and 41°C).

Seed lot	Initial Shoot Length	38°C			41°C		
		48h	72h	96h	48h	72h	96h
		Shoot length (cm) after TAA					
1	12.66	11.90	12.16	10.68	12.13	11.79	10.37
2	13.72	12.60	12.20	12.22	11.78	12.59	11.20
3	12.53	11.70	11.37	11.05	11.43	11.60	10.17
4	13.11	10.73	11.87	10.98	11.47	12.15	10.94
5	13	11.43	11.93	10.53	11.67	11.90	10.40
6	13.2	13.32	13.20	9.25	10.88	11.47	10.75
7	13.7	12.72	11.60	10.33	12.05	10.93	10.00
8	14.07	12.88	11.70	11.20	11.75	9.88	10.05
CV%= 5.4							

Lsd (5%)	
Seed lot	0.5038 ns
Temp	0.2519 ** period
0.3085 **	
Seed lot x Temp	0.7125 ns
Seed lot x period	0.8726 **
Temp x period	0.4363 ns
Seed lot x Temp x period	1.2340 ns

ns = not significant, (*, **) = significant at 5% and 1%probability respectively.

Table 4.12: Seedling shoot length (cm) of eight *Tetrapleura tetraptera* seed lots after being exposed to the salt saturated accelerated ageing method during three ageing periods (48, 72 and 96 hours) and two temperatures (38 and 41°C).

Seed lot	Initial Shoot Length (cm)	38°C			41°C		
		48h	72h	96h	48h	72h	96h
		Shoot Length (cm) after SSAA					
1	12.66	12.88	11.57	11.48	12.45	13.01	12.60
2	13.72	12.38	12.38	11.50	13.24	12.90	12.44
3	12.53	13.45	12.50	12.35	11.90	11.45	11.65
4	13.11	13.35	11.45	12.85	12.60	12.55	10.31
5	13	13.62	14.35	11.70	12.39	12.73	11.80
6	13.2	12.15	13.35	11.57	13.58	13.40	12.35
7	13.7	12.40	13.20	12.15	13.25	12.70	11.50
8	14.07	12.98	13.67	11.55	13.36	12.94	11.12
CV%=4.8							

Lsd (5%)	
Seed lot	0.4911 ns
Temp	0.2455 ns period
0.3007 **	
Seed lot x Temp	0.6945 **
Seed lot x period	0.8506 ns
Temp x period	0.4253 ns
Seed lot x Temp x period	1.2029 **

ns = not significant, (*, **) = significant at 5% and 1% probability respectively.

4.3.5.5 Effect of traditional accelerated ageing conditions on *Tetrapleura tetraptera* seedling total length

Table 4.13 shows the effect of the traditional accelerated ageing conditions on seedling total length. Seed lots, temperature and period of exposure significantly ($p<0.01$) affected the seedling total length but the interaction between the three factors was not significant ($p<0.01$). It appears from the result that temperature of 41°C had greater effect on seedling total length than 38°C and the period of 96h significantly ($p<0.01$) decreased the seedling total length.

4.3.5.6 Effect of salt saturated accelerated ageing conditions on *Tetrapleura tetraptera* seedling total length

For seeds that were subjected to the salt saturated accelerated ageing conditions, only the time of exposure significantly ($p<0.01$) affected the seedling total length. Also the interaction of the three factors (seed lots x temperature x period) was not significant ($p<0.01$). It appeared from the result that seedling total length decreased as the exposure time increased (Table 4.14).

Table 4.13: Seedling total length (cm) of eight *Tetrapleura tetraptera* seed lots after being exposed to the traditional accelerated ageing method during three ageing periods (48, 72 and 96 hours) and two temperatures (38 and 41°C).

Seed lot	Initial Seedling total length	38°C			41°C		
		48h	72h	96h	48h	72h	96h
		Seedling total length (cm) after TAA					
1	19.17	20.08	19.13	17.35	19.46	19.38	15.94
2	21.21	19.85	20.94	19.98	18.09	19.84	16.50
3	19.38	18.90	18.82	18.20	19.43	19.05	16.63
4	20.71	18.63	19.72	18.08	18.72	19.29	16.89
5	20.15	17.20	18.63	16.49	18.52	19.15	16.98
6	19.47	19.57	19.65	15.05	17.52	18.67	16.85
7	20.8	19.25	18.70	15.23	18.86	16.68	15.95
8	21.47	20.78	17.35	15.45	18.95	16.98	16.65
CV%= 4.7							

Lsd (5%)

Seed lot 0.6964 * *

Temp 0.3482 ** period

0.4265 **

Seed lot x Temp 0.9849 ns

Seed lot x period 1.2063 * *

Temp x period 0.6031 ns

Seed lot x Temp x period 1.7059 ns

ns = not significant, (*, **) = significant at 1% and 5% probability respectively.

Table 4.14: Seedling total length of eight *Tetrapleura tetraptera* seed lots after being exposed to the salt saturated accelerated ageing method during three ageing periods (48, 72 and 96 hours) and two temperatures (38 and 41°C).

Seed lot	Initial Seedling total length	38°C			41°C		
		48h	72h	96h	48h	72h	96h
		Seedling total length (cm) after SSAA					
1	19.17	20.13	18.50	18.23	19.35	19.75	19.00
2	21.21	19.68	19.75	18.75	20.96	20.15	18.84
3	19.38	19.95	20.42	19.05	19.30	18.70	18.20
4	20.71	21.45	18.50	19.85	19.30	19.80	17.21
5	20.15	20.72	21.75	18.85	19.41	20.20	18.05
6	19.47	19.35	20.45	18.80	20.23	21.20	18.80
7	20.8	19.90	21.00	19.30	20.15	20.03	18.70
8	21.47	20.63	21.52	18.20	20.94	20.29	18.72
CV%= 3.5							

Lsd (5%)	
Seed lot	0.5698 ns
Temp	0.2849
ns period	0.3489 **
Seed lot x Temp	0.8058 **
Seed lot x period	0.9868 ns
Temp x period	0.4934 ns
Seed lot x Temp x period	1.3956 ns

ns = not significant, (*, **) = significant at 1% and 5% probability respectively.

4.3.5.7 Effect of traditional accelerated ageing conditions on *Tetrapleura tetraptera* seedling dry weight

Table 4.15 shows the effect of the traditional accelerated ageing conditions on seedling dry weight (g). The traditional accelerated ageing showed no significant ($p<0.01$) effect on the seedling dry weight for the three factors and there interactions.

Table 4.15: Seedling dry weigth (g) of eight *Tetrapleura tetraptera* seed lots after being exposed to the traditional accelerated ageing method during three ageing periods (48, 72 and 96 hours) and two temperatures (38 and 41°C).

Seed lot	Initial Seedling dry weight	38°C			41°C		
		48h	72h	96h	48h	72h	96h
		Seedling dry weight (g) After TAA					
1	0.156	0.186	0.202	0.186	0.328	0.187	0.142
2	0.188	0.211	0.200	0.199	0.285	0.210	0.173
3	0.188	0.194	0.184	0.136	0.195	0.178	0.216
4	0.257	0.194	0.227	0.172	0.152	0.225	0.214
5	0.193	0.137	0.193	0.173	0.137	0.191	0.198
6	0.178	0.153	0.225	0.143	0.114	0.250	0.122
7	0.208	0.145	0.199	0.180	0.548	0.077	0.038
8	0.193	0.118	0.198	0.194	0.118	0.106	0.172
		CV%=17.5					

Lsd (5%)
Seed lot 0.07557 ns
Temp 0.03779 ns period 0.04628 ns
Seed lot x Temp 0.10687 ns
Seed lot x period 0.13089 ns
Temp x period 0.06545 ns Seed lot x Temp x period 0.18511 ns

ns = not significant, (*, **) = significant at 5% and 1% probability respectively.

Due to their high coefficient of variation (CV), data of seedling dry weight (Table 4.15), were transformed by square root method and means were reported on the back transformed data.

4.3.5.8 Effect of salt saturated accelerated ageing conditions on *Tetrapleura tetraptera* seedling dry weight

For seed subjected to the salt saturated accelerated ageing, there was a significant ($p<0.01$) differences among seed lots and the effect of temperature and exposure period on seedling dry weight was also significant ($p<0.01$). However, the interaction of the three factors was not significant ($p<0.01$) (Table 4.16).

Table 4.16: Seedling dry weigth of eight *Tetrapleura tetraptera* seed lots, after being exposed to the salt saturated accelerated ageing method during three ageing periods (48, 72 and 96 hours) and two temperatures (38 and 41°C).

Seed lot	Initial Seedling dry weight	38°C			41°C		
		48h	72h	96h	48h	72h	96h
		Seedling dry weight (g) after SSAA					
1	0.156	0.226	0.191	0.122	0.374	0.375	0.250
2	0.188	0.330	0.190	0.170	0.390	0.373	0.280
3	0.188	0.320	0.280	0.344	0.346	0.396	0.324
4	0.257	0.386	0.219	0.401	0.426	0.229	0.221
5	0.193	0.276	0.318	0.222	0.363	0.368	0.328
6	0.178	0.244	0.245	0.151	0.349	0.380	0.280
7	0.208	0.200	0.279	0.212	0.364	0.438	0.336
8	0.193	0.263	0.283	0.240	0.387	0.431	0.321
		CV%= 7.9					

Lsd (5%)	
Seed lot	0.03653 *
Temp	0.01826 *
period	0.02237 *
Seed lot x Temp	0.05166 *
Seed lot x period	0.06327 *
Temp x period	0.03164 ns
Seed lot x Temp x period	0.08948 ns

ns = not significant, (*, **) = significant at 1% and 5% probability respectively.

Due to their high coefficient of variation (CV), data of seedling dry weight (Table 4.16), were transformed by square root method and means were reported on the back transformed data.

4.3.6 Classification of the eight *Tetrapleura tetraptera* seed lots into different vigour levels using the accelerated ageing tests

Tables 4.17 and 4.18 show the separation of seed lots into different categories based on the traditional and salt saturated methods, respectively. It appears from the result that none of the ageing conditions tested in this study showed a classification similar to those of seed emergence, electrical conductivity tests or any of the other vigour tests. However, traditional accelerated ageing, in general, pointed out lot 1 as having a lower physiological potential.

The correlation analysis of the results obtained from the traditional accelerated ageing test and the others vigour tests conducted in this study showed no significant relationship between the ageing conditions, seed emergence and conductivity tests (Table 4.18). A significant positive correlation was found between 38°C/96h (traditional method) and seedling total length (r = 0.50), 41°C/72h (traditional method) and seedling dry weight (r = 0.59) and 41°C/96h (traditional method) and seedling dry weight (r = 0.55). However, no significant relationship was found between the ageing conditions and seedling root length and shoot length. The salt saturated accelerated ageing conditions tested showed in general negative correlation with seed emergence but only 41°C/96h was significant (r = -0.52).

Table 4. 17: Classification of seed lots using the traditional accelerated ageing method

Seed lot	38°C			41°C		
	48h	72h	96h	48h	72h	96h
	Germination (%)					
1	48 ab	32 b	18 d	10 b	18c	12 d
2	24 c	42 ab	41 ab	21 ab	21 bc	17 cd
3	31 c	42 ab	32 c	53 a	36 b	22 bc
4	32 c	42 ab	36 bc	46 a	56 a	34 a
5	32 c	50 a	45 a	38 ab	55 a	33 a
6	38 cb	44 ab	37 bc	42 ab	34 bc	24 bc
7	32 c	50 a	41 ab	32 ab	28 bc	16 cd
8	59 a	40 ab	34 bc	32 ab	36 b	29 ab

Mean comparisons within each column by Tukey test, 5%.

Table 4. 18: Classification of seed lots using the salt saturated method

seed lot	38°C			41°C		
	48h	**72h**	**96h**	**48h**	**72h**	**96h**
	Germination (%)					
1	22.5 b	30b	26 cd	32 b	39 abc	42 b
2	38 ab	14d	10 e	24 b	48 ab	40 b
3	40 a	28bc	17 de	26 b	32 bc	46 ab
4	32 ab	32ab	24 cd	30 b	30 c	52 ab
5	34 ab	38a	38 ab	54 a	50 a	60 a
6	34 ab	28bc	30 bc	26 b	48 ab	58 a

7	23 b	28bc	42 a	26 b	49 ab	38 b
8	26 ab	36a	30 bc	32 b	26 c	48 ab

Mean comparisons within each column by Tukey test, 5%.

Table 4.19: Correlation coefficients (r) of electrical conductivity, six conditions of traditional accelerated ageing test and five other vigour indicators, of eight seed lots.

TEST	SE	SL	RL	TL	DW	EC 24h soak	TAA 38°C 48h	TAA 38°C 72h	TAA 38°C 96h	TAA 41°C 48h	TAA 41°C 72h	TAA 41°C 96h
SE	1											
SL	0.70**	1										
RL	0.48*	0.35ns	1									
TL	0.72**	0.83**	0.81**	1								
DW	0.44ns	0.15ns	0.50*	0.39s	1							
EC 24h soak	-0.76**	-0.66**	-0.58**	-0.75**	-0.53*	1						
TAA38/48	0.09ns	0.20ns	-0.12ns	0.05ns	-0.33ns	0.06ns	1					
TAA38/72	0.24ns	0.20ns	0.22ns	0.26ns	0.41ns	-0.23ns	-0.50*	1				
TAA38/96	0.27ns	0.43ns	0.38ns	0.50*	0.42ns	-0.44ns	-0.52*	0.84**	1			
TAA41/48	0.04ns	-0.14ns	0.13ns	-0.02ns	0.31ns	-0.08ns	-0.24ns	0.37ns	0.35ns	1		
TAA41/72	-0.08ns	-0.12ns	0.28ns	0.09ns	0.59**	-0.11ns	-0.14ns	0.38ns	0.45*	0.62**	1	
TAA41/96	0.02ns	0.07ns	0.42ns	0.29ns	0.55**	-0.11ns	0.04ns	0.38ns	0.44ns	0.61**	0.88**	1

SE= Seed emergence; SL= Shoot length; RL= Root length; TL= Total length; DW= Dry weight; TAA= Traditional Accelerated Ageing; ns= non-significant; *, ** = significant difference at $p < 0.05$ and $p < 0.01$, respectively.

Table 4.20: Correlation coefficients (r) of electrical conductivity, six conditions of salt saturated accelerated aging test, and five other vigour indicators, of eight seed lots.

TEST	SE	SL	RL	TL	DW	EC 24h soak	SSAA 38°C 48h	SSAA 38°C 72h	SSAA 38°C 96h	SSAA 41°C 48h	SSAA 41°C 72h	SSAA 41°C 96h
SE	1											
SL	0.70**	1										
RL	0.48*	0.35ns	1									
TL	0.72**	0.83**	0.81**	1								
DW	0.44ns	0.15ns	0.50*	0.39ns	1							
EC 24h soak	-0.76**	-0.66**	-0.58**	-0.75	-0.53	1						
SSAA38/48	-0.29ns	-0.22ns	-0.03ns	-0.16ns	0.04ns	0.05ns	1					
SSAA38/72	-0.10ns	-0.12ns	-0.07ns	-0.12ns	0.07ns	0.20ns	-0.25ns	1				
SSAA38/96	0.11ns	0.17ns	-0.17ns	0.01ns	0.09ns	0.02ns	-0.54*	0.58**	1			

SSAA41/48	-0.37ns	-0.17ns	0.08ns	-0.06ns	-0.04ns	0.30ns	0.01ns	0.63**	0.45*	1		
SSAA41/72	-0.28ns	0.06ns	-0.21ns	-0.09ns	-0.32ns	0.20ns	0.00nn	-0.31ns	0.26ns	0.15ns	1	
SSAA41/96	-0.52*	-0.22ns	-0.10ns	-0.20ns	0.08ns	0.28ns	0.28ns	0.46*	0.24ns	0.48*	0.06ns	1

SE= Seed emergence; SL= Shoot length; RL= Root length; TL= Total length; DW= Dry weight; SSAA= Salt Saturated Accelerated Ageing; ns= non-significant; *, ** = significant difference at $p < 0.05$ and $p < 0.01$, respectively.

CHAPTER FIVE

5 DISCUSSION

5.1 Initial characterization of *Tetrapleura tetraptera* seed lots

The germination test (emergence) results varied between 49 and 65%. It is evident that germination percentages recorded for all the eight lots of the species tested even before the samples were subjected to the accelerated ageing conditions were relatively low, that is below 70%, even after some level of mechanical scarification was carried out on the seed lots. This may probably be due to the problem of dormancy in the seed as reported by Jimoh (2005). This permitted the classification of the lots into different vigour levels, in which lots 7 and 8 were of superior performance and lot 1 showed the poorest performance (Table 4.1). Torres *et al*. (1998) and Marcos Filho (2005) reported that one single test will be less effective to satisfactorily assess the physiological quality of different lot of seeds. Several tests must therefore be used for such purpose in order to increase the accuracy of the resultant information. Seedling root, shoot and total length were then assessed and the results, in a similar way as the emergence test, permitted a satisfactory differentiation among seed lots. However, data from seedling dry weight were not efficient in the classification of seed lots since the differences among means were not significant. Moreover, there was a high significant positive correlation between seed emergence and seedling growth results. It therefore appeared from the results obtained that, the germination tests (emergence) together with the early growth of seedling could be used in the classification of *Tetrapleura tetraptera* seed lots in different vigour levels.

5.2 Electrical conductivity for *Tetrapleura tetraptera* seed vigour test

It was found in this study that different soaking times were associated with significant differences in conductivity of *Tetrapleura tetraptera* seeds. This could be as result of the

thickness of the seed coat affecting the soaking procedure. This is becasue the species had very hard seed coats, which slowed down soaking rate. These results were divergent from those reported by Ramos *et al.* (2012), who found that different soaking times were not associated with any significant differences in conductivity in *Kielmeyera coriacea* seeds. Form the soaking times tested in this study, 24h showed a classification similar to those of seed emergence and seedling vigour tests, in which lot 7 (11.1 $\mu Scm^{-1}g^{-1}$) were of superior performance and lot 1 (35.7 $\mu Scm^{-1}g^{-1}$) showed the poorest performance. In addition, results from the conductivity test of 24h soaking were highly significantly correlated with the other vigour tests used in this study. Therefore, it could be inferred that for *Tetrapleura tetraptera* seed a soaking time of 24 hours could be applied to obtain satisfactory results. The long period of 24 hours soaking needed to show high significant differences in conductivity of leachate from seed lots may be as a result of the hard seed coat of the seed which made it difficult for water to penetrate into the seed to cause leakages of solutes from the soaked seed lots.

5.3 Accelerated ageing for *Tetrapleura tetraptera* seed vigour test

5.3.1 Seed moisture content

In this study, the initial seed moisture content was almost identical. The maximum variation among the eight *Tetrapleura tetraptera* seed lots was 0.77% (8.32 to 9.09%). This was within the limits of two percent points required for the consistency of the ageing results (Marcos-Filho, 1999). Concerning the moisture content after the ageing treatments, results were in general similar for the eight lots under study but varied with the ageing methods, temperature and exposure time. The traditional accelerated ageing showed large variations in the seed moisture content (from 0.6 to 8.16% depending on the period and temperature combination), as a result of the high relative humidity (100% RH) to which seeds were

subjected. The variation in moisture content of seeds subjected to the traditional accelerated ageing exceeded the tolerable limit for conducting the test (3 to 4%), according to Marcos Filho (1999). However, the moisture content of seeds subjected to the salt saturated conditions showed smaller and more uniform values (0.27 to 2.04% difference), after the ageing periods, as compared to those observed for seeds aged using the traditional method. The observations of Jianhua & McDonald (1996) were then confirmed as the use of saturated salt method contributed to slow down of moisture absorption by seeds in the accelerated ageing test. In addition, the use of salt-saturated solutions also contributes to reduce or inhibit the growth of fungi, thus reducing the sources of variation for the results (Jianhua & McDonald, 1996).

5.3.2 Membrane deterioration of seeds exposed to the accelerated ageing tests

Membrane deterioration occurs at an early stage of the seed deterioration. The loss of membrane integrity is believed to be one of the primary reasons for loss of viability (Malik and Jyoti, 2013). Seed membrane deterioration results in an increase in cell permeability, allowing large quantities of cellular components to diffuse out when the seed is soaked in water (Brasavarajappa *et al*., 1991). An increase in conductivity of the leachate solution of the accelerated-aged seed as compared to seeds that were not subjected to the ageing treatments was observed. This indicated that accelerated ageing triggered *Tetrapleura tetraptera* seed membrane deterioration and thereby released its seed coat dormancy. Under adverse conditions the increase in membrane permeability leads to increased leaching of seed constituents and hence loss in viability (Malik and Jyoti, 2013). According to Chaisurisri *et al*. (1993), the increase in membrane permeability in the accelerated-aged seed is possibly due to changes that occured in the molecular structure of the membrane. The increase in the metabolism during ageing reduced food reserves and, subsequently,

seed vigour declined (Blanche *et al*., 1990). High electrolytes leakage rate is associated with a decline in seed germination, field emergence and seedling vigour (Malik and Jyoti, 2013).

5.3.3 Effect of accelerated ageing on proximate composition of *Tetrapleura tetraptera* seeds

The analysis of the changes in proximate composition of *Tetrapleura tetraptera* seeds exposed to traditional accelerated ageing showed no significant ($p<0.01$) differences among the temperatures and periods of exposure. However when compared to the initial proximate composition of seeds of the species, there was a large variation in the crude protein, carbohydrate and fat content. The crude protein decreased from 21.4% in the initial seed to a minimum of 15.2% depending on the temperature and ageing period. Concerning the carbohydrate and fat content, there was an increase form 51.4 and 7.0% respectively in the initial to a maximum value of 53.8 and 8.8% in seeds exposed to the traditional ageing treatment. The ash and crude fibre content also showed an increase in the seed subjected to ageing treatment as compared to the initial values.

With regard to seed exposed to the salt saturated accelerated ageing, the results showed generally significant differences ($p<0.01$) in the means as affected by temperature and ageing period as well as their interactions. The comparison of the result obtained after the salt saturated ageing treatments were applied with the result of the initial seed composition showed that seed composition varied in similar way, as when seeds were subjected to the traditional method. The crude protein decreased from 21.4% in the initial seed to a minimum of 14.2% depending on the temperature and ageing period. The carbohydrate and fat content increased form 51.4 and 7.0% respectively in the initial to a maximum value of 55.0% and 9.3% in seeds exposed to the salt saturated ageing treatments. The ash and

crude fibre content also showed an increase in the seed subjected to ageing treatment as compared to the initial values.

The results obtain from this experiment are in accordance with those obtained by Verma *et al*. (2003), who stated that the carbohydrate content increased while the protein content decreased in deteriorating seeds.

5.3.4 Effect of accelerated ageing test on seed germination and seedling growth

The average germination percentage after both traditional and salt saturated accelerated ageing at 38 and 41°C for 48 to 96 h of each seed lot was lower than the average initial percentage. However, there were significant ($p<0.01$) differences among means due to the temperatures and periods of exposure and their interactions. For the traditional method, the ageing period of 72h had the highest average germination percentage while for salt saturated method the highest average percentage were found at 96h. These observations are probably due to the fact that seed lots exposed to the traditional ageing process for more than 72 hours, that is 96 hours, suffered from fungal infection due to dramatic increase in seed moisture as noted during the experiment. This eventally led to reduced germination compared to samples exposed to the salt saturated ageing method for 96 hours which limited the rate of water uptake by seeds and therefore prevented fungal infection as observed during the experiment reported earlier by Marcos-Filho *et al*. 2015. It may also be attributed to the seed membrane disruption, which is more rapid in seeds subjected to the traditional method. Obviously, the changes in seed composition after accelerated ageing tests also might have affected the germination percentage. Bonner (1998) reported that in many tests, short accelerated ageing durations have increased the germination of tree seeds that normally exhibit dormancy.

The seedling length and dry weight showed similar trend after seeds were subjected to the various accelerated ageing treatments but results were generally not significantly affected by the three factors (Seed lot, Temperature and period) and their interactions.

5.3.5 Accelerate ageing for *Tetrapleura tetraptera* seed vigour testing

In general, germination of seeds after being exposed to accelerated ageing was used in the seed lots classification into vigour levels in this study. From the result of the traditional accelerated ageing test, the inferiority of seed lot 1 is evident as detected from the seed emergence, seedling vigour and conductivity test. However, the traditional accelerated conditions tested in this trial showed low sensitivity for differentiation of the other seed lot into vigour levels. In the same way, the salt saturated method showed no sensitivity for differentiation of the other seed lots into vigour levels. As confirmation, there were no significant correlation between the accelerated ageing result and any of the other vigour test assessed in this experiment. It appears then from the result that the accelerated ageing tests used in this study were not sensitive for differentiation of *Tetrapleura tetraptera* seed lots into different vigour levels.

CHAPTER SIX

6 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study revealed that seed emergence as well as seedling early growth parameters are good vigour indicators for *Tetrapleura tetraptera* seeds and the results showed high significant, positive correlations. However they are not recommended since they are time consuming methods.

Electrical conductivity test has the advantages of providing rapid and reliable results and the technique is not destructive. The present study showed that different soaking times had significant effects on the results of conductivity testing *Tetrapleura tetraptera* seeds, suggesting that 24 hours soaking is the most efficient. Electrical conductivity test therefore, proved to be a feasible option for vigour testing of *Tetrapleura tetraptera* seeds, as the results obtained were consistent and highly correlated with seed emergence and seedling length.

With regard to the accelerated ageing test, the results showed significant effect of the ageing conditions tested on seed proximate composition, germination and seedling vigour of *Tetrapleura tetraptera* seeds. However, despite the traditional method pointing out the lot with the lowest physiological potential, none of the conditions tested for the two accelerated ageing methods was sensitive enough to classify the seed lots into different vigour levels.

It is possible seed dormancy in *Tetrapleura tetraptera* as reported by Jimoh (2005) might have influenced the results of the tests in this study. According to Bonner (1998), seed dormancy has an influence on the interpretation of accelerated ageing results. In the current study seed lots of *Tetrapleura tetraptera* used for the ageing tests were rubbed with sandpaper as a form of mechanical scarification to break the hard and impermeable seed coat of the seeds. Even though this enhanced germination compared with unscarified seeds, germination of seed lot even before they were exposed to the accelerated ageing test conditions fell below 70%.

6.2 Recommendations

The information obtained in this study should help in the decision making process at different stages of *Tetrapleura tetraptera* seed production and utilization in aforestation

programmes, including operations for the selection of lots for sowing. However, further investigations are required to assess other conditions (temperature and time of exposure combination) of ageing taking into account seed dormancy and enzyme activity to yield better understanding.

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