

**Efficiency of Different Methods of Breaking Dormancy and
Seedling Development in *Parkia biglobosa* and *Prosopis africana***

BY

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CERTIFICATION

I certify that this research work was carried out by ADEYEMI, Christiana Oreoluwa under my supervision and approved as meeting the requirement of the Department of Plant Biology, University of Ilorin, Nigeria for the award of Doctor of Philosophy (Ph.D.) degree in Plant Biology.

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DEDICATION

This research work is dedicated to the glory of God, And to the memory of my late parents Deacon Emmanuel and Mrs. Eunice Adeyemi.

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ABSTRACT

Seed dormancy is an inactive phase of plant life that is greatly influenced by environmental and physiological conditions. High level of dormancy displayed in seeds of *Parkia biglobosa* (PB) and *Prosopis africana* (PA) delay seed germination, growth and development of the plants. This study was carried out to assess the efficiency of different methods of breaking dormancy to enhance seed germination and seedling establishment of PB and PA. The objectives of the study were to: (i) determine the proximate composition and secondary metabolites of the collected seeds; (ii) investigate the effect of different methods of breaking dormancy on the germination capacity; (iii) quantify the generated Reactive Oxygen Species (ROS) in the seeds; (iv) evaluate the effect of germination on the enzyme activities; (v) examine the seed coat morphology; and (vi) determine the elemental constituents of the seed coats.

Fruits of PB were collected from Jigawa (PBJG), Kwara (PBKW), Kaduna (PBKD) and Katsina (PBKT) while PA were from Niger (PANG), Kwara (PAKW), Kaduna (PAKD) and Kebbi (PAKB) states respectively. Seeds from the fruits were subjected to chemical, mechanical and hormonal treatments to break the dormancy. Chemical scarification was carried out using concentrated HCl, HNO₃ and H₂SO₄ for 5, 10, 15 and 20 minutes each. Mechanical scarification with sandpaper was used to remove part of the seed coat. Seeds were treated with auxin and gibberellin at 150, 200 and 250 mg/l for 12, 24 and 36 hours. Proximate composition, secondary metabolite, ROS levels, α -amylase, protease and lipase activities were evaluated using standard methods. Scanning electron microscope was used to examine the seed coat morphology. Elemental constituents of the seeds were determined using Electron Dispersive X-ray. Data were analysed using Analysis of Variance at $p < 0.05$.

The findings of the study were that:

- i. PBJG had the highest moisture content (9.29%) and crude fat (1.26%), PBKT produced the highest ash content (3.81%). Highest alkaloid (1.02%) and steroid contents (3.80%) were obtained in PBJG and PBKD, respectively;
- ii. PBJG, PBKW and PBKD had 100% germination capacity (GC) at 15 and 20 minutes H₂SO₄ scarification, PAKB and PANG had the highest GC at 20 minutes of scarification (93.3%). Only PBKW displayed 100% GC with mechanical scarification. The PBJG and PAKB treated with 250 mg/l of gibberellin at 30 hours produced the highest GC (76.7% and 70.0%), respectively;
- iii. hydrogen peroxide {32.72 μ mol g/dry weight (DW)}, superoxide anion (23.66 μ mol g DW⁻¹) and hydroxyl radicals (82.72) were contained in PBJG and PBKD, respectively;
- iv. activities of α -amylase (0.081 μ /mol/min), protease (0.34 μ /mol/min) and lipase (0.99 $\times 10^{-5}$ U/mol) were highest in the germinating seed of PBJG, PBKW and PBJG at 7 days of planting respectively;
- v. seeds morphology was undulate and thin with raised seed coat sculpture; and
- vi. calcium (29.6%) was the most abundant elemental constituents of the seed coat followed by potassium (26.2%) and antimony (23.4%).

The study concluded that dormancy breaking was most achieved using H₂SO₄ scarification. The study recommended that H₂SO₄ scarification be employed in the breaking of seed dormancy in PB and PA.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Seeds have been very important from the perspective of the biotechnology and plant industry. They may be used as food, for animal feed and for medicinal products, cosmetics and other industrial materials (Khakpoor *et al.*, 2015). Seed dormancy could play a key role in determining the appropriate time for seed germination and seedling establishment under harsh environmental conditions (Atiaet *al.*, 2009, Ahmed and Khan, 2010). Seed dormancy is very often regarded as the absence of germination. However, the absence of germination can have several causes: (1) the seed is nonviable; (2) the environment is non optimal for germination; or (3) the seed or dispersal unit is dormant. From this, a practical definition of dormancy can be formulated: “Dormancy is the absence of germination of a viable seed under conditions that are favorable to germination”. Seed dormancy interferes with the establishment of stands or the industrial use in crops and wild species germination (Rodríguez *et al.*, 2008).

Seed dormancy is simply a physiological problem that many crops or wild plants are facing (Tajbakhsh and Ghiyasi, 2008). Dormancy and germination of seeds depend on the genetic factors and environmental conditions that influence plant growth and development, as well as the post-harvest situation (Sarmadnia, 1996). Seed dormancy has been an intense focus of study in the last decades. Physiological and environmental regulation of dormancy has been studied in depth, and its molecular and genetic bases have been studied more recently. The study of dormancy has clear practical implications beyond the fact that dormancy is a highly fascinating biological process and consequently drives the attention of scientists.

Both farmers and seed companies encounter problems associated with dormancy or conversely with profound dormancy. Likewise, industrial processes such as malting, which depends on seed germination, can be largely hindered by the presence of dormancy or alternatively, due to precocious termination of dormancy and pre-germination in the region, face significant

reductions in the quality of the lots obtained. Long-term seed conservation (i.e., seed banks) is also subjected to limitations imposed by dormancy and the paucity of knowledge that exists on the mechanisms behind the phenomenon.

Germination of seeds is a crucial step that ensures continuity of the life cycle of plants. Seeds quit quiescence during the germination process, and gradually activate metabolism to synthesize the macromolecules required for successful germination. It has also been shown that the percentage of seed germination could be related to the efficiency of free radical scavenging in dry seeds, since this scavenging can only affect seed storage and vigor (Priestley, 1986; Bailly *et al.*, 1998). Germination requires high energy and oxidative phosphorylation, which can be quantified as early as a seed imbibes water and oxygen (Hourmant and Pradet, 1981; Cai *et al.*, 2011). Some authors have shown that ROS production during seed germination can be a beneficial biological reaction, linked to germination ability, seedling growth, and defense during germination from parasitic organisms (Schopfer *et al.*, 2001). That is why there is increasing interest in the functional role of ROS and the related enzyme scavenging systems in seed germination (Bailly *et al.*, 2001; Dučić *et al.*, 2002). Germination then involves integrating certain events that begin with dry seed absorption of water and end with embryonic axis elongation (Bewley, 1997). Seeds first imbibe water which allows metabolism to restart with an overall increase in respiratory activity (Bewley *et al.*, 2013). Enormous amounts of nutrients are prepared for sprout growth at germination. The sprout birth activates all the dormant enzymes within the seed to provide the best nutrition for the sprout (Khang *et al.*, 2016). During this process, massive breakdown of the reserve substances begins with the aid of amylolytic, proteolytic, and lipolytic enzymes and the products are transported to growing seedlings for their growth. At different times of seed germination, different proteins, enzymes and minerals play significant roles in different ways (Rahman *et al.*, 2007). Germinated seeds showed an increase in proteins, minerals, vitamins and enzymes from 25 to 4,000 percent (Azulay, 1997).

α -amylase is found in germinating seeds of many species and it is an important enzyme which hydrolyses the reserve starch into sugars, providing thus necessary energy and osmotic adjustment to the growing embryo (Farooq *et al.*, 2005). Amylase and invertases are the important hydrolytic enzymes which are found in plants. The activity of amylase and invertase were studied on legume seeds (Koshiba and Minamikawe, 1983 and Morohashi, 1982). Protease activity during germination was studied in Mungbean (Chrispeels and Boutler, 1981) and legume seeds (Lichenfeld *et al.*, 1981).

The mobilization of storage lipids as a carbon source for the new seedling is catalysed by highly active lipases (Ben Miled *et al.*, 2000). Energy is required for post-germination processes and for the fueling of the seedling's early developmental stages before it becomes autotrophic (Mayer and Shain 1974).

Through nature, radicals have an unpaired electron that makes them extremely reactive and therefore capable of destroying all macromolecules including lipids, proteins, and nucleic acid. Reactive Oxygen Species refer to any oxygen derivative that is more reactive than an oxygen molecule (O_2) itself (Foyer and Noctor, 2009; Mittler, 2017; Mhamdi and Breusegem, 2018). Superoxide is a common reactive form of oxygen that is formed when molecular oxygen gains a single electron. Under different conditions particularly environmental stress, reactive oxygen species, such as superoxide anion radicals, hydrogen peroxide, and hydroxyl radicals, are generated (Zhu, 2000). Superoxide radicals can attack susceptible biological targets which include lipids, proteins and nucleic acids. Superoxide is produced as a by-product of O_2 metabolism and if not regulated causes many types of damage to cell, essential membrane lipids as well as proteins and nucleic acids (Noctor and Foyer, 1998; Hayyan *et al.*, 2016). Superoxide is known to denature enzymes, oxidize lipids and fragment DNA (Smirnoff, 1993). Oxidative phosphorylation and mobilization of food storage generate reactive oxygen species (ROS) that can cause structural and functional injury in cells. Therefore the enzymes that are responsible

for ROS scavenging are of particular importance for germination success. Oxidative phosphorylation paves the way for the yield of ROS, which can hamper germination if left unchecked (Thompson *et al.*, 1987). The over-production of ROS can result in oxidative stress and seed deterioration.

Phytohormones or plant growth regulators (PGRs) are endogenous substances (chemical) synthesized by plant for the promotion or inhibition of some metabolic processes in plants. In small amounts they modify the natural growth regulatory systems of the plant right from seed germination (i.e., it regulates physiological processes), a quick means of increasing crop production. They can be natural or synthetic however, they play a central role in morphology and physiology of plants. Plant growth-regulating substances are organic chemical compounds, produced naturally in plants or applied externally, and that, even in small amounts, can have positive and negative effects on growth, development and other physiological functions, alone or in combination with other growth regulators. They can be effective in the tissue where they are synthesized, or transported to other parts of the plant and sustain this effectiveness in other organs of the plant (Kerim *et al.*, 2016). Simpson (1990) reported that treating seeds with chemical promotes metabolic activity and induces germination. Plant hormones are therefore extremely important for the regulators of seed dormancy and germination (Koornneef *et al.*, 2002; Finkelstein, 2004). Therefore there is need to understand seed physiology and initial seedling development as an important phenomenon for successful germination rates, acids and hormonal dynamics involvement in overcoming dormancy and allowing the early germination.

1.2 Statement of Problem

In spite of the economic values of *Parkia biglobosa* and *Prosopis africana*, the populations of the plants are declining and no concerted effort has been made towards the conservation and replacement of these species either in the wild or in plantation (Ntui *et al.*, 2012). Reasons for this decline include: Urbanization, uncontrolled bush burning, charcoal and furniture making,

firewood for fuel, farming, excessive grazing by cattle and other animals like rodent, buffalo etc. In addition to eating young and tender seedlings, these animals may also physically destroy them during vegetation movement. A more important factor is the fact that only a small percentage of seeds germinate in the field, while much more are dormant; there is a need for vital and scientific investigation into how these plants can be raised or developed by various methods of breaking dormancy.

1.3 Justification

The trees serve as a wind breaker due to their anatomical structure and are also a good source of wood for furniture making, agroforestry, parkland and erosion control. In addition, the trees serve as shade in homesteads in dry areas and their nitrogen fixing ability improves soil fertility. It is therefore important to look for a way to get these tree plants back on tract. One of the ways as a plant physiologist is to provide an easy and appropriate method in breaking the seed dormancy which is the main problem faced by *Parkia biglobosa* and *Prosopis africana*. By using the methods proposed in this research, it would be a great contribution in raising these tree plants seedlings to nursery level for afforestation.

This is necessary because the methods of breaking the dormancy have not been satisfactorily effective and only few and scanty information are currently available in seedling establishment of these tree species in particular *Prosopis africana*. In order to make concise efforts to propagate these economic plants for crops / forests, it is necessary to develop and finalize dormancy breaking methods that would also provide good vigor for seedlings that could be used to establish planting and commercial cultivation of *Parkia biglobosa* and *Prosopis africana* crops as seedlings of these important plants are rarely seen growing in the wild and the existing trees are ageing and fast disappearing. It is therefore imperative to intervene to save these important trees from extinction commercial cultivation of *Parkia biglobosa* and *Prosopis africana* crops as seedlings of these important plants is rarely seen growing in the

wild, and the existing trees age and disappear quickly. So intervening to save these important trees from extinction is imperative.

1.5 Aim and Objectives of the Study

1.5.1 Aim

In view of the foregoing, the present study was to assess the efficiency of different physical and chemical dormancy breaking methods with the view of identifying cost effective, and reliable method of seed germination and seedling establishment of *Parkia biglobosa* and *Prosopis africana*

1.5.2 Objectives

The objectives of this study were to:

- i. determine the proximate composition and secondary metabolites of the collected seeds;
- ii. investigate the effect of different methods of breaking dormancy on the germination capacity;
- iii. quantify the generated Reactive Oxygen Species (ROS) in the seeds;
- iv. evaluate the effect of germination on the enzyme activities;
- v. examine the seed coat morphology; and
- vi. determine the elemental constituents of the seed coats.

CHAPTER TWO

LITERATURE REVIEW

Throughout traditional tropical farming systems, it has been known that people depend on trees and shrubs to preserve and restore soil fertility (FAO, 2004). In this process, trees play a vital role and leguminous trees have added advantages in this regard over other trees. Judicious use of multipurpose trees in the farming system aids in the recycling of nutrients and water from deeper soil horizons, nitrogen fixation, soil thermo and water regulations, and prevention of leaching and run-off (FAO, 2004). Multipurpose trees also provide shelter for livestock and crops, provide animal feed and, in most cases, provide human food. In the farming system, these trees are capable of boosting both crop production through maintenance of soil fertility and livestock production through increased availability of high-quality feed. The efficiency of this, however, depends on the species used and the manner of their integration and management within the cropping system (Bayala, 2006). The value of the trees has long been recognized by farmers. In the production systems, they deliberately incorporate trees. Inquiry into current and past farming practices has clearly shown that rural people have a wealth of knowledge on which trees make agricultural crops grow more quickly, which provide fodder during dry seasons, and which help to keep soils for more productive farming on sloping land (Bayala, 2006). Trees are of great value to majority of the rural households' communities of the developing countries, because they depend these on forest resources to meet subsistence need for staple and supplementary foods for humans, timber for construction materials and fencing, fuel for cooking and heat, medicine, cash as source of income, local ecosystem services and farm inputs, such as animal feed and nutrients for crops, shade from the sun and shelter from wind, and, in many instances, stabilization of soils (Raintree, 1999). It is also recognized that farmers sometimes leave other tree species either as a source of wood for construction, fruit, medicinal purposes, yam stakes, and shade plants, among others. Although these species of multipurpose

trees are found on farmlands, their potential in agroforestry is not fully documented (Popoola and Tee, 2001).

The seeds of *Parkia biglobosa* and *Prosopis africana* are usually processed manually and hand pick; and in the process many unwanted materials such as pod particles and pulp in all the harvested seeds. This necessitate the need for cleaning of the seeds. To further process the harvested seeds, there is need to determine the engineering properties, the knowledge of this property is very relevant to agricultural engineers because of the importance and increase in the demand of the seeds of *Parkia biglobosa* and *Prosopis africana* as condiment. Together with complex modern technology for their production, handling, storage, processing, preservation and utilization (Adebayo and Nwankwo, 2013) a comprehensive information on the property of *Parkia biglobosa* and *Prosopis africana* seeds are important in the designing of equipment for the harvesting, transporting, storing, processing, cleaning, hulling and milling (Niveditha *et al.*, 2013).

The savanna tree species of economic importance in Nigeria including *Terminalia glaucescence*, *Acacia albida*, *Cassia lebbbeck*, *Afzelia africana*, *Prosopis africana*, *Dialum guineensis* and *Parkia biglobosa*. Their roles in the savanna cannot be underrated. Wild species of the savanna plants, especially tree legumes, have been a significant source of edible fruits, vegetables, medicinal plants, charcoal, gum, resin and timber for carnivals and construction, provision of shade for man and livestock (Ajiboye, 2006). The tree legumes are very drought resistant and are well adapted to the heat and poor soils of dry regions. These trees thrive in light sandy or rocky soil. They can grow and develop to about 20-40m tall. Most of the tree seeds can retain viability for a long period of time. Brasil (2009) tested that about 85 per cent of 260 leguminosae seeds had waterproof integument. Integument impermeability is related to the presence of waterproof layers of palisade cells (Baskin and Baskin, 1998). This kind of dormancy can be reversed by weakening the tegument by allowing water to pass and start

germination (Cavalheiro *et al.*, 2007) or it can also occur by dispersing animals through the action of acids during seed digestion (Goddard *et al.*, 2009).

The rough seed coat is one of the major obstacles in plant germination and reproduction for plant regeneration from seed. The main reason for being the hard seed relates to the physical and chemical properties of the seed coat (Tran and Cavanagh, 1984); thus, it appears that the force of water absorption and development is not adequate for seed coat splitting and germination (Ghaderi *et al.*, 2008). Studies have shown that rapid germination is caused by removing the thick crust wall (Baskin and Baskin, 1998). Seed dormancy has been broken using various methods. The Levitt (1974) study stated that the scarification of seed coats with concentrated sulfuric acid in seed dormancy induces accelerated germination due to lack of water penetration into the skin. Many Fabaceae Family species, like *Lupinus* spp. seeds exhibit dormancy which is primarily due to seed coat water impermeability. Scarification of Texas bluebonnet (*Lupinus texensis*) seeds with 30 to 60 min sulfuric acid improved the emergence of seedlings (Davis *et al.*, 1991). The mechanical dormancy can be broken with seed frill removal, seed crust abrasion, mechanical instruments (Black and Bewley, 2000; Kapland, 1996). Aliero (2004) studied the effects of different sulfuric acid treatments on *Parkia biglobosa* seed germination in 1, 2, 3, 4 and 5 seconds with 98 %, 90 %, 70 % and 50 % concentrations in 1, 3 and 5 minutes. He concluded that seeds by infusion in sulfuric acid displayed the highest level of germination percentage (50 percent) for 3 minutes relative to other treatments, while sulfuric acid treatment 90 percent for 3 and 5 minutes led to 28 and zero percent germination, respectively. Sxitus et al. (2003) worked on breaking the dormancy of the seeds of *Ulex europaeus* which have hard crust, concluding that sulfuric acid and sand paper treatments cause an increase in seed germination. In the other research, Rehman *et al.* (1999) on the dominance of hard-crust seed dormancy of *Acacia salicina*, it is reported that 98 percent of sulfuric acid originates germination of these seeds and that increasing connection

time with this acid solution causes increasing numbers of buds. It was reported that 98% of this seed treatment with sulfuric acid during 10 minutes has no effect on breaking seed dormancy but 30 minutes of seed infusion in said acid shows significant growth in germination. Rana and Nuatiyal (1989) also conducted similar experiment on the seeds of *Acacia farnesiana*; they observed that using sulfuric acid causes increasing in germination, but with increasing time of seed connection with acid originate injury to embryo structure that produce more abnormal seedling. Research of Uzen and Aydin (2004) signify positive effect of scratching on breaking dormancy and germination stimulus on some seeds of *Medicago* genus. The results of various treatments with different chemicals (thiourea, KNO₃, HCl, HNO₃, and H₂SO₄) confirmed that *Rhynchosia capitata* seeds display dormancy due to their hard seed coat (Ali *et al.*, 2011). Different chemicals have been widely used for breaking dormancy of many hard seed coat species, such as *Astragalus hamosus*, *Medicago orbicularis* (Patane and Gresta, 2006), and *Albizia* spp. (Tigabu and Oden, 2001). In the present study, seed scarification with H₂SO₄ and sandpaper was the best treatment for removing hard seed dormancy which caused the highest percentage of germination. Aliero (2004) also reported that scarification of seed to break seed dormancy in seeds of *Parkia biglobosa* was effective. Tavili *et al.* (2010) expressed that chilling at 4°C for 10 days has had a positive effect on increasing and enhancing germination of two species of *Descurainia sophia* and *Plantago ovata*. Zare *et al.* (2011) reported that scarification with sandy paper and 98 percent sulfuric acid treatments are the most effective treatments for breaking dormancy and improving germination in two species of *Prosopis koelziana* and *Prosopis juliflora*, and that treatment with 98 percent sulfuric acid at 15 min gave the best results. Jabarzare *et al.* (2010) assessed the pre-chilling effect at 5°C on seed germination and seedling establishment at *Artemisia sieberi*. and the result showed a positive pre-chilling effect. Ansari *et al.* (2013) tested heat shock treatment in *Secale montanum* under ageing conditions for improving seed germination characteristics. The findings were different

based on the temperature used. Hormone pretreatment is a commonly used priming strategy to improve seed germination in stressful conditions (Jisha *et al.* 2013, Masood *et al.* 2012, Hu *et al.* 2013). For example, pretreated rye seeds (*Secale montanum*) with gibberellic acid (GA3) have increased germination under water-deficit conditions (Ansari *et al.* 2013). Khan *et al.* (2009) showed in pepper (*Capsicum annum* L.), that pretreatment with acetylsalicylic acid and salicylic acid resulted in increased germination uniformity and high salinity seedlings establishment. Ethylene was also used to minimize the effect of high temperatures on the germination of lettuce seeds (*Lactuca sativa* L.) (Nascimento, 2004). According to Khakpoor *et al.*, (2015), *Salvia verticillata* treated for germination with gibberellic acid showed a decrease in percentage of germination of seeds with an increase in gibberellic acid treatment time. Furthermore, seed skin scraping with sandpaper, 10, 20 and 30 minute citric acid treatment, and 24 hour gibberellic acid treatment, improved the percentage of germination compared to the control treatment. Okunlola *et al.* (2010) reported that pretreatment of *Parkia* seeds with soaking in 100% sulphuric acid for one minute, mechanical scarification with sandpaper, soaking in boiling water for two minutes, soaking in cold water for 24 hours improved seed germination. The response of *Parkia* seed to various observed treatments showed 100 percent for wet heat, 90 percent for scarification (sand paper) and 70 percent for HCl of 50 percent concentration, while the untreated seed control showed 60 percent germination (Abubakar and Maimuna, 2013).

2.1 *Parkia biglobosa*

2.1.2 Description of *Parkia biglobosa*

The genus *Parkia* was described in 1826 by Robert Brown. He named it after Mungo Park, a Scot who in 1795-1797 and 1805 made 2 spectacular exploration voyages into West Africa's interior. *Parkia biglobosa* is a perennial deciduous tree that belongs to the Mimosodae family and the Leguminosae family (now Fabaceae) (Akande *et al.*, 2010) and is found in the

rainforest and arid areas of some African countries (Ademola *et al*, 2013). The tree is not usually grown, but can be seen in population of two or more in the West African savannah area, common in the entire West African savannah region and rising up to the edge of the Sahel zone in the West African savannah region (Campbell-Platt, 1980). *Parkia biglobosa* tree ranges from Senegal to Sudan and its range is in savannah land as it is characteristic of transition areas locally on farmland from Sahelian to Sudanese eco-zones. It is common in Nigeria particularly in Northern and South West Nigeria (Odunfa, 1982). Locust bean tree is found throughout the North Central Nigerian savannah lands covering the states of Benue, Kaduna, Kwara, Kogi, Nassarawa and Plateau (Tee *et al.*, 2009). The tree is common around villages when land is cleared or sometimes planted, where it is left standing. Upon planting, the tree will begin bearing fruit from five to seven years (Musa, 1991). The tree is about 7 to 20 meters high and has bears pods which occur in large bunches and vary in length from 120 to 300 mm. Fruits from African locust bean tree during the months of December through March of the year. The fruits are ready for harvest in April and each have a tough pericarp with many leguminous pods. The pods contain yellow powdery pulp which incorporates seeds (Simonyan, 2012). The seeds have hard, black tests which make them less vulnerable to androdent infestation of insects (Oladele *et al.*, 1985, Oyewole *et al.*, 1986, Oni, 1990). At maturity the pod length averages 232 mm ($173 \leq L \leq 290$ mm) with a mean diameter of about 15 mm ($10 \leq d \leq 20$ mm) (Oni, 1990).

Parkia biglobosa is a perennial deciduous tree with a height of 7 to 20 m although under exceptional conditions it can exceed 30 m. A mature African locust bean tree aged from 20 to 30 years will bear around one ton of harvested fruit and above (Simonyan, 2012). Crown broad, spreads high on a stiff bole with branches low down; amber gum exudes from wounds; dark brown, dense, fissured, dark grey bark. Leaves alternate, dark green, bipinnate to 30 cm long, pinnae up to 17 pairs of 13-60 pairs of leaflets of distinctive shape and venation, 8-30 mm x

1.5-8 mm. Leaflets were kept on a rachis wide. Peduncles 10-35 cm in length; capitula 4.5-7 cm long and 3.5-6 cm in diameter; biglobose but much larger distal portion. African locust bean tree also bears alternate, dark green bi-pinnate leaves and hermaphrodite flowers (Adewumi, 1997; Simonyan, 2012). Hermaphrodite orange or red flowers in colour: calyx 10-13 (16 max.) mm; corolla 10-14 (17 max.) mm long, lobes very short 1-3 mm long, connate in the center and free or connate at the base; filaments exerted about 4 mm beyond calyx mouth. Nectar-secreting flowers: 6-7 mm long calyx. Staminal flowers: calyx about 5.5-7 mm long, filaments exerting 2-3 mm beyond the mouth of the calyx. Pods, when mature, pink brown to dark brown, about 45 cm long and 2 cm wide; can contain up to 30 seeds embedded in a yellow pericarp; hard-tested seeds, large (mean weight 0.26 g / seed) with large cotyledons forming around 70% of their weight.

2.1.3 Economic value of *Parkia biglobosa*

Parkia biglobosa species has important socio-economic and cultural values for local people. It is also a food species whose importance is well known both at the regional and international levels (Ouédraogo, 1995). Among thirty-one woody medicinal plants used in traditional medicine in Benin, it is ranked fifth important, and fourth from a list of eighteen priority woody food plants to preserve. The species helps to enrich physico-chemical soil characteristics in conjunction with crops, which in turn helps to increase crop yields. *Parkia biglobosa* pods are popular chimpanzee, baboon and other primate foods. It occurs on a wide range of natural and semi-natural habitats such as open savannah forests, but it is most prominent and widespread in anthropic communities, especially bush fallow and wooded fields where semi-permanent agriculture occurs. On rocky slopes, stony ridges or sandstone hills too, the tree will grow. It is a helophyte which is immune to fire. *Parkia biglobosa* occurs in a number of agro-ecological zones, ranging from high and well-distributed tropical forests to arid areas where mean annual

rainfall may be less than 400 mm. It has the potential to endure drought conditions due to its deep taproot system and the ability to suppress transpiration.

Elevation: 0-300 m; annual mean rainfall: 400-700 mm; annual mean temperature: 24-28°C.

Type of soil: prefers well drained, deep, grown soils, but also found on shallow, skeletal soils and thick laterites

Parkia biglobosa, a multipurpose tree, has a hard-tested grain legume seed and these seeds have a mean weight of about 0.3 g / seed and cotyledons make up about 70 per cent of the weight. Such seeds are borne in pods that can contain up to 30 seeds embedded in a pericarp yellow. The pods change color from pink brown to dark brown as maturity sets in.

The size of the *Parkia biglobosa* seeds varies within the pod, with those in the centre. The seeds vary in shape, some are oval, some are more or less flat and some are almost spherical. The seed has a hard dark or brown coat with a hard golden cotyledon-yellow (Adewumi, 1997). The seeds are wrapped in a coat that is durable, elastic and fairly dense and has very low permeability. The low permeability limits free and easy water movement through the coats. The adhesive interface force which binds the coat to the seed is relatively high. It causes challenging and inefficient dry hulling of locust bean seed. When dehulled in dry condition, most of the beans are shattered (Adewumi and Igbeka, 1996). According to Esenwah and Ikenebomeh (2008), the high cost of animal protein has centered attention on several possible leguminous sources of vegetable protein for humans. Among the plant species, grain legumes are considered a major source of dietary protein in which African locust bean seeds are rich in protein and are typically fermented into a savory food condiment named dawadawa that is used as a flavor intensifier for soups and stews and nutritional values and also adds protein to protein-poor diets (Dike and Odunfa, 2003; Chukwu *et al.*, 2010). The seeds fermented to make dawadawa are a high in protein, black, strong-smelling, tasty food. Odunfa (1981) recorded the frequent consumption of fermented locust bean seed in Ghana, Nigeria, Sierra Leone and Togo.

It is called “Iru” in Yoruba, “dawadawa” in Hausa and “ogiri-igala” in Igbo parts of Nigeria. It is referred to as “kinda” in Sierra Leone and “Kpalugu” in Ghana. It is a source of a natural nutritious condiment that often features in both rural and urban dwellers' traditional diets in at least seventeen Western African countries including Nigeria (Hopkins and White, 1984). The estimated average intake of condiment per head per day is 10 g, 4 g and 2 g respectively for Nigeria, Togo and Ghana (Campbell- Platt, 1980). The dried fermented seeds are kept without refrigeration for more than a year in traditional earthenware pots, and small amounts are crumbled into traditional soups and stews, which are usually eaten with sorghum or millet-based dumplings and porridge. Because of the savory taste and the seed's high protein and fat values, it is sometimes described as a meat or cheese substitute but is typically not eaten in large quantities. Dawadawa is rich in protein, vitamin B2 and lipids. The beans' fat is nutritionally useful (about 60 per cent is unsaturated). Seeds are used as a supplement for coffee.

Seeds are sometimes embedded in a mealy pulp called dozim which is high in value for oil. This contains up to 29% crude protein and up to 60% saccharose, is rich in vitamin C and high in fat. The pulp is eaten raw or processed into a refreshing beverage and is used as a sweetener. It is then pressed into a cake for storage. In severe droughts the fruit provides food in emergency. Young pods are rarely roasted and eaten on embers. Leaves are edible but rarely eaten. The leaves are combined with cereal flour and used in sauces and eaten or fermented into balls. Whole pods, including cattle, are eaten by domestic stock (Douglas, 1996, Obiazoba, 1998). The young seedlings are edible, and livestock are extensively browsed. *Parkia biglobosa* trees have an important attribute that most of their leaves stay green throughout the dry season and branches are lopped and used as fodder. Calcium, sodium, potassium and phosphorus are rich in seeds (Sabiiti and Cobbina, 1992). The leaves are nitrogen rich and used as animal feed and manure (Adewumi, 1997). *Parkia biglobosa* attracts bees, and is among beekeepers a

popular tree. Sometimes branches are lopped in for firewood. Pods and roots for the musical instruments are used as sponges and strings. Wood is whitish, moderately heavy, 580-640 kg / cubic m when air is seasoned, relatively hard and solid; smells bad when freshly felled, but seasoning does not take long and only rarely causes distortion of the shape; easy to use by hand or power tools; well worked by nails, glues, varnishes and paints; often useful as a light structural timber, for example, for vehicle bodies, agricultural implements, boxes, crates and barrels, furniture, mortars and pestles, bowls, planks and carvings (Booth and Wickens, 1988). Mucilage from part of the fruit is made into a fluid and used to harden terrestrial floors and to give black glaze in pottery; gum exudate is proteinaceous and contains galactose, arabinose, glucuronic and 4-0 methyglucuronic acid as the constituent sugars. The carbon gum is extracted from the bean and applied as thickeners and emulsifiers to foods and cosmetics (Yudkin, 1985). Husks of pods combined with indigo improve the luster of colouring materials. Seeds and bark contain tannin, so tanning is achieved using bark. Fruit pulp can ferment into an alcoholic drink. Bark and pods contain piscicides, and may be responsible for the alkaloid parkin that occurs in pods and bark. Tobacco is applied to burnt husks to improve the pungency. Pulp is ostensibly a purifier of water, but might just sweeten and mask the taste of foul water.

Nutrient recycler: *Parkia biglobosa* tree plays a vital role in both stabilizing and providing protection for the soils in farming areas and extracting nutrients from deeper soil layers (Campell-Platt, 1980). *Parkia biglobosa* is also used in agroforestry due to its ability to fix atmospheric nitrogen in the soil (Cobley and Steele, 1976), and the seeds are recorded to maintain long-term viability (Leopold and Kreidemann, 1975). Traditionally, the pod shell is used to extract a material that helps to harden beaten natively produced house floors, and is an important tannin source for the leather industry. Together with tannins, the presence of fibers in the husk makes this a good raw material for particle board production (Owoyale *et al.*, 1986). The bean coat is used to enhance the luster of the fabric with indigo dye, while the tree bark

produces red tannin for dyeing leather. The *Parkia biglobosa* fruit pulp is sweet which indicates the presence of natural sugars and therefore a potential source of energy. The appealing yellow color indicates the presence of essential precursors of retinol (Vitamin A), phyto-nutrients, probably carotenoids. It also constitutes a good source of ascorbic acid (Vitamin C) (Akoma *et al*, 2001). The bean content of around 67.30 percent carbohydrate makes it a potential good energy source (Uwaegbute, 1996). It is a source of dietary fiber that is essential for good bowel movement and helps prevent obesity, colon diabetes and cancer and other gastrointestinal tract disorders in humans. The amount of vitamin C (ascorbic acid) in the seed is (191.20ug/100 g), which is higher than the most commonly eaten food and sufficient compared to the recommended daily intake of 30mg/65 kg of adult human body weight (Olson and hogdes, 1987). Vitamin C is very effective in synthesizing collagen, promoting the absorption of iron and engaging in glucocorticoid biosynthesis. *Parkia biglobosa* is of economic importance in Nigeria especially as it provides a cheaper source of protein for many inhabitants (Asonye *et al* 2014). According to Gernmah *et al.* (2007), *Parkia biglobosa* fruit pulp's nutritional and anti-nutritional composition showed an 8.41 percent moisture content, 6.56 percent protein, 1.80 percent fat, 11.75 percent crude fiber, ash. 4.18 per cent and 67.30% glucose. The sugar content was 9.00 ° Brix; total carotenoids, 49.175ug/100 g, and 191.20mg/100 g ascorbic acid (Vitamin C). Anti-nutritional factors / toxin analyzes revealed a value of 60.00mg / 100 g of phytic acid; 17.80mg/100 g crude saponins; 81.00mg/100 g tannins; 204.60mg/100 g total phenols and 17.30mg/100 g hydrocyanic acid (HCN).

The embryo or cotyledon is rich of nitrogen, phosphorus, potassium, magnesium, iron, and zinc. Compared with the cotyledon, which is a rich source of lipids, carbohydrates, proteins, fibers and ascorbic acid, the testa is high in calcium, manganese and sodium (Alabi 1991). The yellow powdery pulp is rich in carbohydrates (Oladele *et al.*, 1985), and rich in oil for soap production (Owoyale *et al.*, 1986).

The husk is abundant in tannins, making it a valuable leather industry source of tannins. The presence of fibers in the husk, along with tannins, makes the husk a good raw material for particle board processing (Owoyale *et al.*, 1986). One of *Parkia biglobosa*'s important features is the inflorescence. This inflorescence is widely recognized in Southwest Nigeria, most notably Ekiti State, Ondo State, and Kogi State, and is generally consumed. The inflorescences are grounded and used as condiments as well as food supplements according to the indigenous peoples of the aforementioned states (Ojo, *et al* 2015). In Nigeria, the fried, fermented seeds are eaten raw. This produces a tasty black scent, rich in lipid (29%), protein (35% 0, and carbohydrate (16%) (Amoo and Ayisire, 2005). It is a good source of fat and calcium for rural residents, too. The tree is highly recognized as a medicinal species used as mouth wash; for toothache or ear complaints, vapor inhalant. It is used to cure leprosy, bronchitis, pneumonia, skin infections, sores, ulcers, malaria and many others (Sofowora, 1993).

According to Alabi *et al.* (2005), the proximate study of *Parkia* seed showed that cotyledon has a high lipid, basic protein, carbohydrate, total soluble sugar and starch value compared to 16.86, 34.02, 20.70, 25.66, and 10.25 respectively.

Parkia biglobosa, a common savanna tree was widely prescribed for its various medicinal virtues in traditional medicine. *Parkia biglobosa* has been reported to be effective in treating arterial hypertension, piles, amoebiasis, bronchitis, cough, burn, zoster and abscess (Millogo-Kone *et al.*, 2008; Olaleye *et al.*, 2013). Millogo-Kone *et al.*, 2008, recently reported the in vitro antioxidant property and the leaf's hypotensive effect; Olaleye *et al.*, 2013. The bark is used as a mouthwash, a vapor inhalant for toothache, or an ear complaint. It is macerated in leprosy baths and used for bronchitis, pneumonia, skin infections, sores, ulcers, and fever, malaria, diarrhoea, and sterility washings. Roots are used to treat sore eyes in a lotion. Branches are used to clean teeth; bark stains are red in the mouth and contain saponins which cleans teeth (Orwa *et al.*, 2009). Bark is used as a mouthwash, toothache vapor inhalant,

or for ear complaints. It is macerated in leprosy baths and used as a poison remedy for bronchitis, pneumonia, skin infections, sores, ulcers, bilharzia, fever washes, malaria, diarrhoea, severe colic and vomiting, sterility, venereal diseases, guinea worm, oedema, and rickets. Leaves are used for eye sore, burns, haemorrhoids and toothache lotions. Seed is taken for pain, and pulp is used as a diuretic and a mild purgative for fevers. Roots are used for sore-eyes in a lotion (Orwa *et al.*, 2009).

2.1.4 Processes involved in production of *Parkia biglobosa* condiment

According to Olaoye (2010a), the processes involved in the manufacture of Iru condiment are shown in the flow chart (Fig. 1) and it is one of the main locust bean products directly from the raw material.

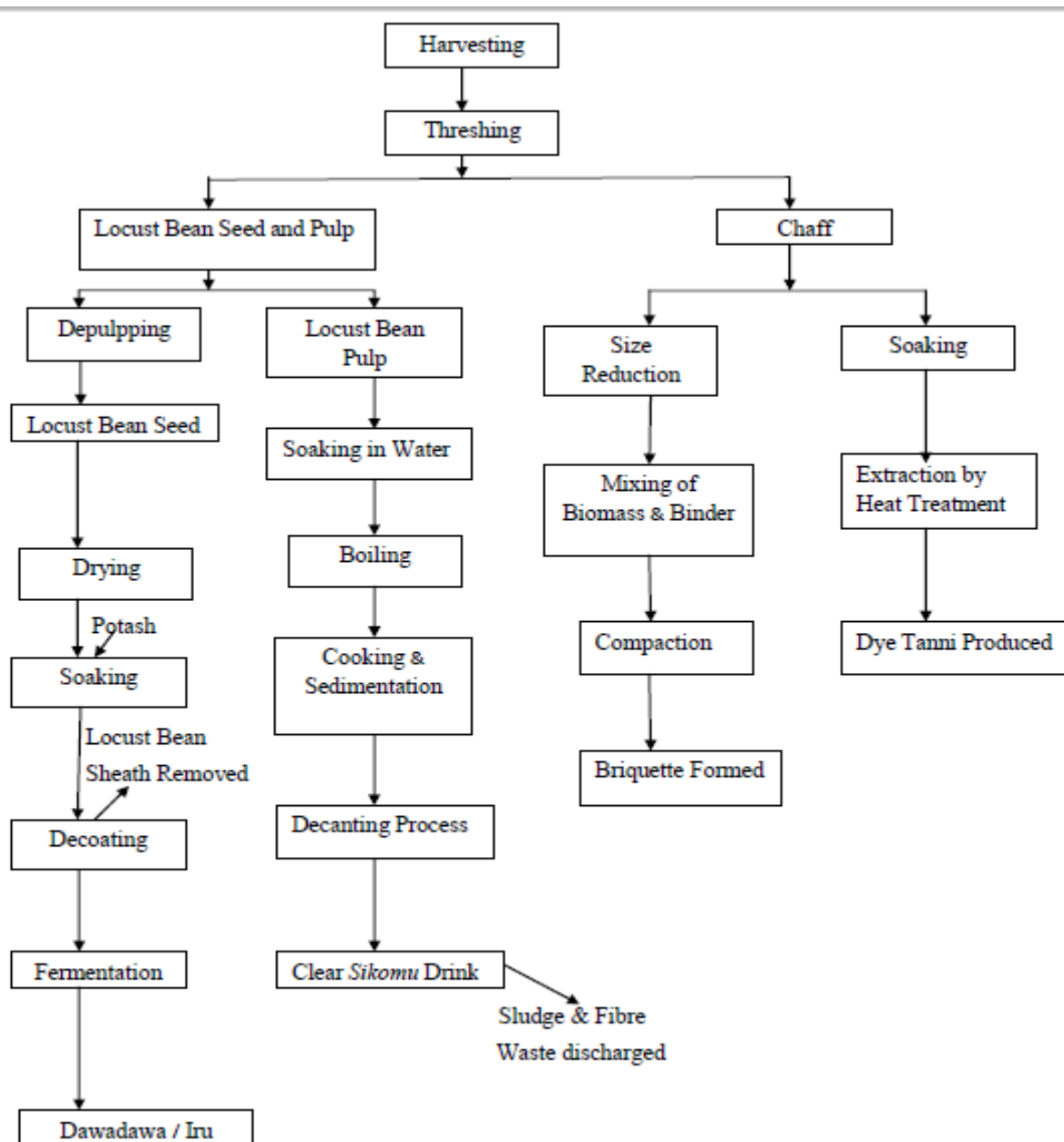


Fig. 1: Processing Procedure for Production of Major African Locust Bean Products (Olaoye, 2010a)

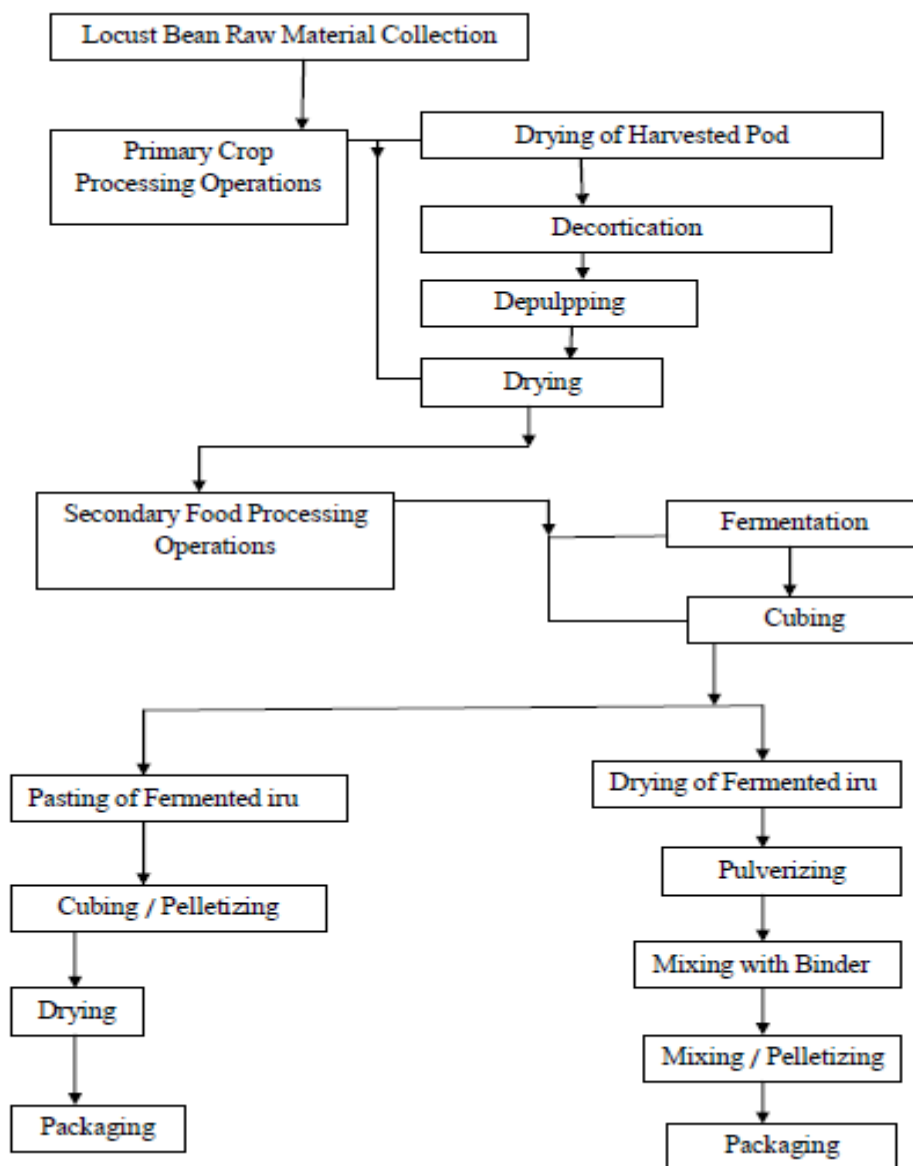


Fig. 2: Flow Chart of Unit Operations Involved in the Iru Cube Production (Olaoye, 2010a)

2.2. *Prosopis africana*

2.2.1 Description of *Prosopis africana*

Prosopis is a genus in the pea family of flowering plants, Fabaceae. It includes about 45 species of spiny trees and shrubs found in the Americas, Europe, Western Asia, and South Asia subtropical and tropical areas. *Prosopis* species are typically fast-growing, drought-resistant, nitrogen-fixing trees or shrubs that in arid and semi-arid areas are adapted to poor and saline soils and sometimes establish very deep root systems (Pasiecznik *et al.*, 2001). Their wood is generally hard, dense and durable. Their fruits are seeds, and can contain high amounts of sugar. The generic name means "burdock" in late Latin and originated in the Greek language (Umberto, 2000). According to Spore-CTA 1993, the genus *Prosopis* consists of 44 species of trees and shrubs that are mainly found in arid and semiarid regions of the world.

Prosopis africana is a flowering plant species in the genus *Prosopis* found in Africa especially in Nigeria and Senegal (Agboola, 2004). It is a perennial leguminous tree of the Mimosoidae subfamily (Keay *et al.* 1964), and is found to flourish mostly in western Africa's savanna regions (Agboola, 2004). African mesquite, iron tree, gele (Malinke) (traditional djembe wood) or dark tree (Wikipedia, 2015), Arabic (abusurung, abusuruj); English (iron wood); Wolof (jaxan-jaxan, ir)(Orwa *et al.*, 2009). In Nigeria, *Prosopis africana* is variously called *Okpei* (Igbo), *Ayan* (Yoruba), *Okpeghe* (Idoma and Tiv), and *Kiriyaor Kiriaya* (Hausa), (Ezike *et al.*, 2010). *Prosopis africana* is not cultivated, it is often referred to as a wild, endangered but edible plant (Ola-Adams and Onyeachusim, 1993); as a lost crop (Okafor, 1993) or as a small crop (Agboola, 2004). They can be found in between lat in Nigeria. 70 N and 100 N (Agboola, 1995) in two or more populations of trees, such as *Parkia biglobosa* and *Daniella oliveri* (Fasidi *et al.*, 2000). Fruit setting, ripening and dropping occurs between November and March in *Prosopis africana* in Nigeria. During the months of January and March, the trees produced many legume pods.

Prosopis africana reaches 4-20 m in height; has an open crown and slightly rounded buttresses; bark is very dark, scaly, slash orange to red-brown with white streaks.

Foliage drooping; alternating, bipinnate leaves; 10-15 cm long rachis with 3-6 pairs of opposite pinnae (5-8 cm long); 9-16 pairs of leaflets, oblong lanceolate, 12-30 mm, pubescent; a typical gland between pairs of pinnae and leaflets. Greenish-white to yellow flowers, fragrant, in thick 6-10 cm long axillary spikes; pubescent calyx with glabrous petals; 10 free-standing stamens; anthers with a small apical gland. Pods dark brown, cylindrical, thick and rough, smooth, up to 15x 3 cm, compartmented with woody walls; about 10 loose, rattling seeds per pod with a thin, inter-marginal line around it.

Flowering in it's natural habitat happens just before the rainy season. Seeds ripen from February to March.

Prosopis africana is the only species of tropical African *Prosopis* that occurs in the zone between the Sahel and savannah forests from Senegal to Ethiopia. It has disappeared from vast parts of the southern Sahel and adjacent savannahs of Sudan due to extensive overexploitation.

Altitude: Up to 1000 m, mean annual temperature: 0-40°C, Mean annual rainfall: Up to 500 mm

Soil type: Frequently on fallow land, on sandy clayey soils over laterite. It fairly tolerates most soil types.

It is a native of Benin, Cameroon, Central African Republic, Chad, Cote d'Ivoire, Egypt, Ethiopia, Gambia, Ghana, Guinea, Guinea-Bissau, Liberia, Nigeria, Senegal, Sierra Leone, Sudan, Tanzania, Togo, Uganda

2.2.2 Economic value of *Prosopis africana*

The fermented seeds are used as a food condiment in many places, particularly for Nigerian Idoma, Ighala, Ebira, and Tiv (mostly Guinean savanna) who use them to prepare a spice called ukpehe (Agboola, 2005). This is a fermented soup condiment that is rich in protein and fatty

acids (Sanni *et al.*, 1993a,b). The pods have been a common food source for human populations, for example in North America's Sonoran Desert, where Indian tribes made flour and dough from ripe pods with the dried or toasted pulp (Simpson, 1977). Flour made from *Prosopis* pulp is known as 'patay' in Northern Argentina, and is still eaten today (Ochoa, 1996). *Prosopis pallida* pods are ground into flour in Peru for making bread, cakes or a rich porridge, pods of are boiled into syrup called 'algarrobina'. Another food product from *Prosopis* is 'yupisín', a beverage which is obtained by water extraction of the sugars from the pod. A fermented beverage, 'aloja' can be produced and is used as a substitute for beer or wine (Ochoa, 1996). Dried pods have historically been pounded in a pestle and mortar to produce a coarse flour, or ground using a variety of stone mills (Felger, 1977). Other processes of *Prosopis* pods are described by Meyer *et al.*, (1982), Saunders *et al.* (1986); Del Valle *et al.* (1987) and Grados and Cruz (1996). The lack of starch in the bread formulations is a weakness of the amounts of *Prosopis* flour. Mixing 5-25 percent of *Prosopis* flour with wheat flour produces products with good taste. Sweet bread containing 5% *Prosopis pallida* flour is appropriate in texture and taste, although up to 25% has been used in biscuit making, which also reduces the amount of extra sugar required (Cruz, 1999). Flour is the main product included in this article, but there is also an impressive range of other food products from *Prosopis* pods. The development of a protein isolate (Baião *et al.* 1987) and a protein-enriched flour (Ruiz, 1997) from seeds of *Prosopis juliflora* have been recorded in Brazil, and its application in bread making. *Prosopis pallida* pulp flour can also be used as an ingredient in many other food preparations, such as cakes, ice creams and other desserts. *Prosopis pallida* pulp flour has been converted into an instantly soluble powder, and could be used as a cocoa powder

Young leaves and shoots are a much sought after forage towards the end of the dry season. Thus, branches are often broken or lopped off. Cattle eat goose bumps.

The wood has a high calorific value of approximately 1720 joules / kg and produces excellent firewood and coal.

The wood is heavy to heavy to medium, with fine grain. Sapwood is small, light yellow to light brown distinct from dark red-brown heartwood. After drying the latter takes on a dark wine-red colour. Pleasant smell when freshly cut. The wood is difficult to saw and plane and blunts the cutting tools. It cannot be nailed without predrilling; however, it is durable and easy to carve, turn and glue. It has many applications over its entire distribution area, depending on the dimensions it is available in. In Senegal it is used for works of art and crafts, whereas in Ghana it is used for pestles, mortars, mallets, cudgels, furniture, joinery, sleepers in the construction of railway lines, boat building and handles of axes.

Prosopis africana produces gum. The bark and roots contain 14-16 per cent tannin and a coloring substance which gives leather a reddish tint. Pounded dry fruits fit as a poison for fish. Medicine: In medicine, almost all parts of the tree are used, particularly the leaves for the treatment of headache and toothache as well as various other head ailments. Leaves and bark are combined to treat rheumatism. The bark are obtained for the remedies for skin diseases, caries, fevers and eyewash. The roots are diuretic and are used to treat gonorrhea, tooth and stomach ache, dysentery and bronchitis. In Mali, the leaves, bark, twigs and roots are used to treat and alleviate the cramps of bronchitis, dermatitis, rotting of the teeth, dysentery, malaria and diarrhea. In Ghana, boiled roots act as a poultice for sore throat, root decoction for toothache, and bark for wounds or cuts as a dressing or lotion. Other Products: *Prosopis africana* pod ashes are a source of potash for soap production in Ghana. With the planting of *Prosopis africana*, soil conservation is strengthened. It is suitable in dry areas for shade at homesteads. As it can fix atmospheric nitrogen, it has the potential to improve soil fertility and also provides a useful mulch for soil.

In the Sahel, *Prosopis africana* has great potential for agroforestry parkland systems and for advanced agroforestry technologies, where it grows well in valleys and rocky soils.

Prosopis africana has been known to be essential to mankind in various ways and is used for medicinal purposes in almost all parts of this tree. Specifically the leaves are used to treat headache, toothache, as well as other head ailments. The leaves and bark are combined to treat rheumatism while the pounded dry fruit is used by the fishermen as fish poison. The young leaves and shoots are used as forage; cattle like to eat the pods as well. Specifically the leaves are used to treat headache, toothache, as well as other head ailments. The leaves and bark are combined to treat rheumatism while the pounded dry fruit is used by the fishermen as fish poison. The young leaves and shoots are used as forage; cattle like to eat the pods as well. Hence it can also be used as a fuel. Bark and root can be used as tannin or coloring material, and pod ashes are a source of potassium to make soap used to treat skin disease, fevers and eyewashes. They contain 14–16 per cent tannin and a coloring substance which gives leather a reddish tint. Phosphorus, potassium and calcium are the major mineral elements of the seeds which suggest that consumption of the seed or its products could contribute partially to the overall daily intake of these elements. The pod pulp of *Prosopis africana* contains an energy value of 1168 J, 96 percent protein, 3 percent fat and 53 percent carbohydrate. It contains calcium of 160 mg and iron of 70 mg per 100 g fresh weight (FAO, 2003). The seeds are fermented to produce food condiments eaten by more than 15 million people (Oyeyiola, 1999), while its gum is used in the medical industry (Adikwu et al., 2001). In toxicological studies, the gum's polysaccharides are found to be safe on mice which suggest their safety for human use. The seeds are fermented to produce dietary condiments eaten by over 15 million people (Oyeyiola, 1999), while their gum is used in the medical industry (Adikwu et al., 2001). Polysaccharides of the gum are found to be safe on mice in toxicological studies which suggest their safety for human use.

In Benue State of Nigeria, Ankyambe-Akue et al. (2004) worked on the possible of powdered *Prosopis africana* as a protective against cowpea seeds. We noticed that *Prosopis africana* powder could be adjudicated as a potential botanical substitute for synthetic insecticides. Very few studies on the physical and engineering properties of seed and pod *Prosopis africana* have been published. A few publications, however, describe the species without highlighting the properties which may be of engineering importance. *Prosopis africana*'s ripe and dry pods are dark brown, cylindrical (about 15 cm long and 3 cm in diameter), compartmentalized, thick, rough and brilliant with woody walls. With a thin, inter-marginal line across, there are about 10 loose, rattling seeds per pod. Seed can be extracted from the hard pod by crushing it. The seeds are preheated in boiling water for 15 min for propagation to soften the seed coat, allow for cooling, and are soaked overnight (Le Houerou, 2003). Processing the seed into condiments for food requires boiling the seed in water for 10–12 h and breaking the seed coat to release the cotyledons. The cotyledons are then washed with water and boiled for 2 h, allowed to cool and wrapped in fermentation leaves (Omofuvbe et al., 1999).

In Mali the leaves, bark, twigs and roots are used to treat and relieve bronchitis, dermatitis, decay of the tooth, dysentery, malaria and cramps of the stomach. In Ghana, boiled roots serve as poultice for sore throat, root decoction for toothache and bark as a to treat dressing or lotion for wounds or cuts (WAC, 2008). According to Kolapo 2009, the plant parts of *Prosopis africana* had an inhibitory effect on the growth of *Streptococcus mutans* and *Staphylococcus saprophyticus* and this means that the stem and root of *Prosopis africana* has potential candidate plant parts in the development of dentifrice.

Table 1: Economic potential of *Prosopis africana* in the Guinea Savanna regions of Nigeria.

Soil improvement	Environmental	Socioeconomic	Nutritional and food	Miscellaneous products
Aricultural-sylva pastoral systems	Alternative source of fuel	Job opportunities	Food for humans and Animals	Timber
Nutrient recycling	Positive modification of microclimate	Source of food for humans and animals	Source of forage	Wood for furniture
Nodulation and fixing of nitrogen	For wildlife	Cool shade for humans and animals		Honey production
Check soil erosion	Aesthetic value	Source of firewood		Charcoal Wooden implements Medicinal products Gums and resins

Source: Agboola, (2004)

Prosopis africana bark was say to have the best inhibitory effect on mycelial growth, sporulation and spore length also according to the report of Oluma *et al.* (2008), bark of *Parkia africana* inhibited radial mycelial growth of *Macrophomina phaseolina*.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant materials

3.1.1 Collection of seed samples.

Parkia biglobosa and *Prosopis africana* seeds used for this research were collected from Savannah region of Nigeria. Mature fruits containing the seeds were harvested during the fruiting period in 2015. *Prosopis africana* was collected between December–February while *Parkia biglobosa* was between April–June. *Parkia biglobosa* were collected from Jigawa State (PBJG), Kaduna State (PBKD), Katsina State (PBKT) and Kwara State (PBKW). *Prosopis africana* on the other hand were collected from Kebbi State (PAKB), Kwara State (PAKW), Kaduna State (PAKD) and Niger State (PANG).

3.2 Preparation of samples

3.2.1 *Parkia biglobosa*

The collected *Parkia biglobosa* fruits were subjected to fruit and seed characteristic studies. The pulp and seeds were removed manually from the dry brown indehiscent pods of matured fruits (Shelling); de-pulping was carried out by washing in water to remove the seeds from the pulp covering them. The seeds were then air dried, weighed, counted and packed after which the seed colour, texture, shape and size were determined (Olorunmaiye *et al.*, 2011).

3.2.2 *Prosopis africana*

The pods of *Prosopis africana* were threshed manually and winnowed to obtain the clean seeds. The immature seeds were carefully sorted out by handpicking and then packed in polyethylene bags and labeled.

The viability of the seeds was determined using floatation method (Ojo *et al.*, 2017)

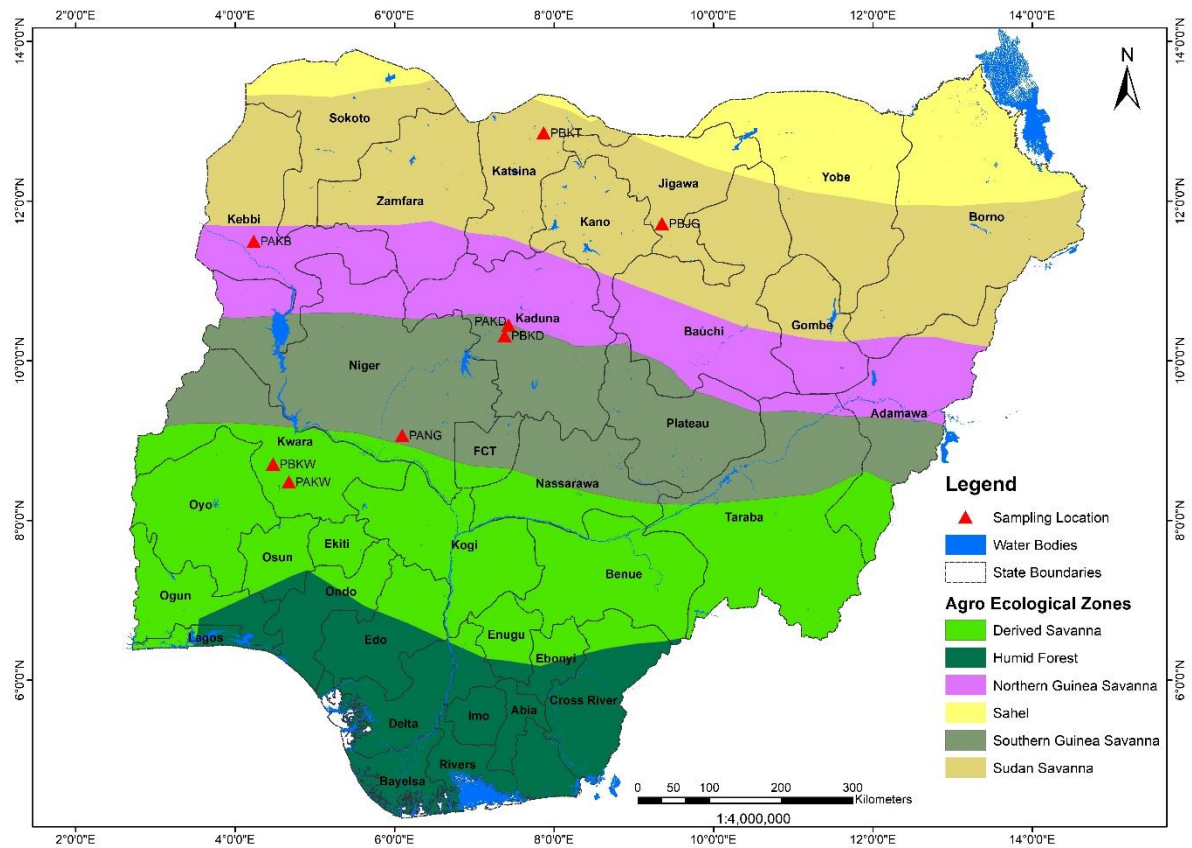


Fig. 3. Geographical locations of *Parkia biglobosa* and *Prosopis africana* seed samples used for study

3.3. Characteristic assessment of fruits and seeds of *Parkia biglobosa* and *Prosopis africana*

The fruits and seeds morphometric studies was carried out using the method of Olorunmaiye *et al* (2011). Five bunches and five pods were randomly collected from each tree sample of *Parkia biglobosa* and *Prosopis africana* respectively. Pod length, pod breadth, weight of 100 seeds, seed diameter at midpoint were measured while the seed coat colour, seed texture, seed size and shape were determined for each plant sample. Number of pod per bunch was counted in *Parkia biglobosa*.

3.3.1 Size and shape

Electronic Digital caliper (Titan 23175 model) was used to measure the axial dimensions of the seeds; length, width and thickness. From the axial dimensions, the geometric mean diameter (D_p) was determined in mm as described elsewhere (Frontezak and Metzgen, 1985; Joshi *et al.*, 1993; Akaaimo and Raji 2006) using the formula:

$$D_p = (abc)^{1/3}$$

where: a = the length (the dimension along the longest axis in mm); b = the width (the dimension along the longest axis perpendicular to a in mm) and c = the thickness (the dimension along the longest axis perpendicular to both a and b in mm).

The sphericity of the seed was determined using:

$$\psi = \frac{(abc)^{1/3}}{a}$$

The average values obtained for 10 trials were taken as the representative values of the axial dimensions a, b and c used in calculating the geometric mean diameter and sphericity for both the pods and seeds.

3.4. Germination studies

The germination studies experiments were laid out in completely randomized designs (CRD) with four pre-germination treatments (including the control) in three replicated. The four pre-germination treatments are: untreated seeds (control), chemical scarification using 98% concentrated H_2SO_4 , HNO_3 and HCl acids. The seeds were also treated with hormone at different concentrations.

3.4.1 Acid Scarification

Sixty (60) plastic petri dishes lined with two sheets of Whatman No. 1 filter paper with 10 plated seeds were allocated for each acid scarification for the study species.. The acids used were Concentrated Tetraoxosulphate (VI) acid (H_2SO_4), Trioxonitrate (V) acid (HNO_3) and Hydrochloric acid (HCl). The seeds were soaked in Concentrated H_2SO_4 (98%) in a beaker for 5, 10, 15 and 20 minutes respectively after which the seeds were removed, thoroughly washed and rinsed in tap and distilled water to remove the residual acid on the seeds. Ten seeds were placed in already prepared and labelled petri dishes for each of the treatments in three replicate (Emongor *et al.*, 2004). The experiment was repeated for HCl and HNO_3 . The experimental setups were kept moistened with distilled water whenever necessary for a period of 10 days.

3.4.2. Mechanical Scarification

Seeds of *Parkia biglobosa* and *Prosopis africana* were mechanically scarified by testa abrasion with sand paper for five minutes and then sowed for germination. Untreated seeds were used as control.

3.4.3 Germination count

Emergence of radicles was taken as visible sign of successful germination. Germination count was taken and germination capacity was calculated. The number of germinated seeds was

observed daily and the final germination percentage was determined after 10 days. The germination indices test (Table 2) were determined according to Khamassi *et al.* (2013).

3.4.4 Hormone Priming

Hormonal priming pre-treatment of seeds was also considered a viable option to solve the problem of seed germination. Seeds were primed with an aqueous solution of Indole acetic acid (IAA) and Gibberellic acid (GA). Treatment of IAA and GA were attained at concentration of 150, 200 and 250 mg/l using modified method of Sarwar *et al.* (2017). Seeds from each sample were then soaked in each of the prepared solutions of IAA and GA (150-250 mg/l respectively) for 12, 24 and 36 hours. Ten (10) seeds were plated for germination test and were replicated thrice.

Table 2: Germination indices, formula and definition used in this study

Germination index	Formula	Definition	References
GC Germination capacity	$G_t = [NT \times 100]/N$	NT: proportion of total germinated seeds in each treatment N: Number of seeds used in the bioassay	Khamassi <i>et al.</i> , (2013)
T50	Days required for 50% germination of the total number of seeds		Orchard (1977); Joseph and Maria (2002)
AGSD Average number of seeds germinated per day	$ASGD = NT/T$	N: Total number of germinated seeds T: total number of days required for final germination	Khamassi <i>et al.</i> , (2013)
M-days mean germination time (in days)	$M\text{-days} = [(N_1 \times T_1) + \dots + (N_n \times T_n)] / [N_1 + N_2 + N_3 + \dots + N_n]$	Tn: proportion of total germinated seeds in each treatment N: Number of seeds used in the bioassay	Edmond and Drapala (1958); Czabator (1962); Rana and Santana (2006)
S Speed of germination	$S = (N_1 \times 1) + (N_2 - N_1) \times 1/2 + (N_3 - N_2) \times 1/3 + \dots + (N_n - N_{n-1}) \times 1/n$	N ₁ , N ₂ , N ₃ , N _{n-1} , N _n : Proportion of germinated seeds observed at first, second, third ... (n - 1), (n) days or hours	Bradbeer (1988); Wardle <i>et al.</i> (1991)

AS Speed of accumulated germination	$AS = [N1/1 + N2/2 + N3/3 \dots + Nn/n]$	N1, N2, N3, Nn: Cumulative number of seeds which germinate at time 1, 2, 3, ... N	Bradbeer (1988); Wardle <i>et al.</i> (1991); Haugland and Brandsaeter (1996)
CRG Coefficient of the of velocity or rate of germination	$CRG = 100 \times ([N1 + N2 + N3 + \dots + Nn] / [(N1 \times T1) + \dots + (Nn \times Tn)])$	N1: number of germinated seeds at time T1 N2: number of germinated seeds at time T2 Nn: number of germinated seeds at time Tn	Bewley and Black (1985)

Adopted from Khamassi *et al.* (2013)

3.5 Qualitative secondary metabolites analysis

The seeds of *Parkia biglobosa* and *Prosopis africana* were milled using an electric blender (Qlink, Japan). The milled samples were extracted using methanol for 72 hours, concentrated and stored for phytochemical analysis.

3.5.1 Test of Alkaloids (Wager's reagent)

A fraction of extract was treated with 3-5 drops of Wager's reagent and formation of reddish brown precipitate was taken as the presence of alkaloids (Sofowora, 1993).

3.5.2 Test of Terpenoids

One (1) millilitre (ml) of extract was added to 1ml of acetic anhydride and conc. HCl. Formation of blue, green ring indicated the presence of terpenoids (Harborne, 1973).

3.5.3 Test of Phenols

A fraction of extract was treated with aqueous 5% ferric chloride and formation of deep blue or black colour indicated the presence of phenols (Sofowora, 1993).

3.5.4 Test of Flavonoids

One (1) millilitre (ml) of extract was added to 1 ml of 10% lead acetate solution. The formation of a yellow precipitate indicated the presence of flavonoids.

3.5.5 Test of Saponins

About 1 ml of extract was shaken vigorously with 1 ml of distilled water in a test tube and warmed. The formation of a stable foam was taken as an indication of the presence of saponins (Wall *et al.*, 1952).

3.5.6 Test of Steroids

One (1) millilitre (ml) of extract was treated with 2-3 drops of chloroform, acetic anhydride and conc. H_2SO_4 and observation of the formation of dark pink or red colour showed the presence of steroids (Sofowora, 1993).

3.5.7 Test of Glycosides

About 2 ml of extract with 2ml of dilute HCl and 2 ml Sodium nitroprusside in pyridine and sodium hydroxide solution were added. Formation of pink to blood red colour was regarded as the presence of glycosides (Sofowora, 1993).

3.5.8 Test of Tannins

Approximately 2 ml of the extract was combined with 2 ml of distilled water, adding a few drops of ferric chloride (FeCl_3) solution. Green precipitate formation was indicator of tannin presence.

3.6 Quantitative secondary metabolites analysis

3.6.1 Determination of total phenol content

The total phenol content (TPC) of the methanolic extracts was determined as reported by Chan et al. (2007) using the Folin-Ciocalteu method. Briefly, 300 μl of extract was dispensed into (triplicate) test tube. Then, 1.5 ml of Folin-Ciocalteu reagent (diluted 10 times with distilled water) was applied to the medium, followed by 1.2 ml of Na_2CO_3 solution (7.5 percent w / v). The reaction mixture was shaken, allowed to stand at room temperature for 30 minutes before the absorbance was measured at 765 nm against a blank prepared by dispensing 300 μl of distilled instead of sample extract. TPC was expressed as gallic acid equivalent (GAE) of mg/g material.

3.6.1 Determination of Tannins Content

The tannin content of the extract was measured using the Siddhuraj and Manian methods (2007). In total 500 μl of the extracts were taken separately into the test tube and treated with 100 mg polyvinyl pyrrolidone and 500 μl distilled water. This solution was incubated at 4h, then the sample was

centrifuged for 5 min at 5000r / min, and the supernatant was taken at 20 μ l. This supernatant has only basic phenolics free of tannins (the tannins along with the polyvinyl polypyrrolidone would have been precipitated). The supernatant's phenolic content was estimated at 725 nm and represented on a dry matter base as the amount of free phenolics. From the findings above, the tannins content of the extract was calculated as follows:

Tannins (mg GAE/g extract) = Total phenolics (mg GAE/g extract)-Free phenolics (mg GAE /g extract)

3.6.2 Determination of Total Flavonoid Content

As stated by Kale *et al.* (2010), total flavonoid content was measured using the aluminum chloride test. Methanol extract (0.5 ml) was dispensed into the test tube, followed by 1.5 ml of methanol, 0.1 ml of aluminum chloride (10 per cent), 0.1 ml of 1M of potassium acetate and 2.8 ml of distilled water. The reaction mixture was shaken and allowed to stand at room temperature for 30 minutes, before the absorbance was read at 514 nm. Total Flavonoid Content (TFC) was expressed as quercetin equivalent (QE) in mg/g material.

3.6.3 Determination of Total Saponin Content

The method defined by Makkar *et al.* (2007) had measured total saponin. Using 25 ml of 80 percent aqueous methanol, about 0.5 g of sample was collected after shaking on a mechanical shaker for 2 hours, after which the tube contents were centrifuged at 3,000 rpm for 10mins. An aliquot of 0.25 ml of the supernatant was taken into a test tube, to which 0.25 ml of vanillin reagent (8% vanillin in ethanol) and 2.5 ml of 72% aqueous H₂SO₄ were added. The reaction mixtures in the tubes were then in a water bath heated for 10 min at 60°C. Then tubes were refrigerated for 4 minutes in ice and then allowed to acclimatize to room temperature. The absorbance was subsequently measured at 544 nm

on a UV / Visible Spectrophotometer. Diosgenin was used as a baseline, and the results obtained were expressed as equivalent mg diosgenin per g of dry sample content.

3.6.4 Determination of Terpenoids

Approximately 1 g of the sample was macerated with 50 ml of ethanol and filtered. Then 2.5 ml of the filtrate was pipetted into 5 percent aqueous phosphomolybic acid solution with 2.5 ml of concentrated H₂SO₄ gradually added and mixed well. The solution was allowed to stand with ethanol for 30 minutes, refrigerated and brought up to 12.5 ml. The absorbance was measured at 700 nm.

3.6.5 Determination of Total Alkaloids

Each plant sample (1.25g) was weighed into 250 ml beaker and 50 ml of 100 percent acetic acid was added, covered and allowed to stand for 4 hr. This was then filtered, and condensed to one fifth of the original volume using a water bath. Once the precipitate was complete, concentrated ammonium hydroxide was applied to the extract. The entire solution was allowed to settle, and the precipitate was collected, washed and filtered with diluted ammonium hydroxide. Residues have been reported to be alkaloid. It was dried and then measured (Krishanaial *et al.*, 2009).

3.6.6 Determination of Steroids

About 1 ml of steroid solution test extract was transferred into 10 ml volumetric flasks. Sulphuric acid (4N, 2 ml) and iron (III) chloride (0.5% w / v, 2 ml), followed by a potassium hexacyanoferrate (III) solution (0.5%w / v, 0.5 ml). The mixture was heated with intermittent shaking in a water-bath held at 70±2°C for 30 minutes, and diluted further with distilled water. The absorbance was measured at 780 nm against the reagent blank (Madhu *et al.*, 2016)

3.6.7 Determination of Glycosides

Using 10 ml of 70 percent alcohol, about one gram (1 g) of the extract was removed, and the mixture filtered. Eight (8) millilitres ml was moved from the filtrate into a 100 ml volumetric flask and the

volume was made with distilled water to the limit. Approximately 8 ml of the mixture was added to 12.5 per cent lead acetate (for precipitating resins, tannins and pigments) in 8 ml. The mixture was well shaken, completed with distilled water to volume of 100 ml, and filtered. Approximately 50 ml of the filtrate was piped into another 100 ml volumetric flask, and a solution of 4.7 percent disodium hydrogen phosphate (Na_2HPO_4) was applied to precipitate excess lead. The mixture was made with distilled water and mixed up to volume. This was filtered over filter paper twice. Baljets reagent (10 ml) was added to the purified filtrate in 10 ml. A blank sample of 10 ml of distilled water had also been added to 10 ml reagent baljets. The two solutions were allowed to stand for an hour (time required to develop maximum colour). A blank of 20 ml distilled water was used. The intensity of the colour was read at 495 nm using Spectrophotometer. The colour was stable for several hours (Evans and Trease, 1999).

The percentage of glycosides = $A \times \frac{100g}{17} \%$

Where A = the absorbance of the colour at 495 nm

3.7 Proximate Analysis

For crude protein, crude fat / oil, fibre, mineral ash, moisture and carbohydrate content, 30 g of ground seed from each loacation was centrifuged with 0.5 sieves and analyzed in triplicate. All analyses were done according to the Official Methods of Associations of Analytical Chemist (AOAC, 1990).

3.7.1 Protein determination

Two (2) grams of each seed sample is weighed into a digestion tube, adding 15 ml of tetraoxosulphate (VI) acid. Kjedahl tablets were added and the digestion cycle was conducted for 45 minutes in a preset digestion fume cupboard at 41°C, until the solution was clear. Then, the solution containing 0.5 g of $\text{CuSO}_4 \cdot 7\text{H}_2\text{O}$ and 10 g of NaSO_4 were all poured into the digestive flask. The flask and its content were clamped in an inclined position and then heated gently for 15 min. It was then vigorously heated

for further 45 mins to digest in a fume cupboard until the mixture was clear or oxidation was complete and a green colour was observed indicating complete digestion. To prevent it from solidifying after digestion, seventy-five (75) ml of distilled water were added. The tube was placed in the distillery unit and 50 ml of 40 per cent sodium hydroxide (NaOH) was dispensed into the diluted solution and distilled for 5 minutes into 25 ml of boric acid. Afterwards, the distillate was titrated against 0.47 M HCL until the first grey color was seen. A blank was run at first and the titre value was recorded (AOAC, 1995).

Calculation:

$$\% \text{ Total Nitrogen} = \frac{\text{Titre value} \times 14.01 \times 0.47}{\text{Weight of sample}} \times 100$$

$$\% \text{Protein} = \% \text{ total Nitrogen} \times \text{conversion factor (6.25)}$$

14.01 = Molecular weight of Nitrogen

0.47 Molarity of HCl

Conversion factor = 6.25

3.7.2 Ash Content Determination

Two (2) grams of the sample of each of the samples was weighed into an already weighed crucible. The crucible was then placed into the muffle furnace which was set at 600 °C for six hours. After ashing, the crucible was placed in the desiccator to cool and weighed immediately after cooling. This was done in triplicate. Calculation:

$$\% \text{Ash} = (\text{weight of crucible plus Ash}) - (\text{weight of empty crucible}) \times 100 / \text{Weight of sample}$$

3.7.3 Moisture Content Determination

A clean crucible was first dried in an oven for 30 minutes, and then cooled in a dessicator. The cooled crucible was weighed as W_1 . Two (2) grams of the sample of each was introduced into the crucible

and weighed as W_2 before drying. This was placed in an oven set at 105 °C for an interval of two hours, cooled in a desiccator and weighed as W_3 . This procedure was repeated until we obtained a constant weight. The moisture content which was represented by weight lost was calculated as follows:

$$\% \text{ moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

3.7.4 Crude Fibre Determination

Two (2) grams of the sample each was weighed into the fibre flask and 100 ml of 0.255 Tetraoxosulphate (VI) acid was added. The mixture was heated under flux for one (1) hour with heating mantle and hot mixture filtered through a fibre filter cloth. The filtrate obtained was discarded and the residue was returned to the fibre flask to which 100 ml of 0.313M Sodium hydroxide (NaOH) was added and heated under reflux for another one (1) hour. The mixture was filtered through a sieve fibre, and 100 ml of acetone was added to dissolve the present organic component. The residue was washed twice on the sieve cloth with 350 ml of hot water, before being finally transferred to the crucible. At 105°C, the crucible and the residue were dried on oven. The oven dried crucible containing the residue was cooled in the desiccator and weighed (W_1). The crucible with its contents was transferred to the muffle furnace for ashing for 4 hours at 50°C. The crucible containing dry ash was refrigerated in the desiccator and measured to get the W_2 . The percentage fibre was expressed as below:

$$\% \text{ crude fibre} = \frac{W_1 - W_2}{\text{Weight of sample}}$$

W_1 = Weight of filtered sample before incineration in a muffle furnace.

W_2 = Weight of filtered sample after incineration in a muffle furnace.

3.7.5 Fat Content Determination

Extraction flask (250 ml) was washed, dried and weighed. The Soxhlet extractor with reflux condenser was fitted up and allowed through the condenser. Two (2) grams of sample of each of the populations

were weighed into a clean filter paper, folded and transferred into a fat free extraction thimble and plunged lightly with cotton wool. The thimble was put in the barrel of extraction, and petroleum was added until it siphons directly below it into the tank, ensuring all points were secure. The sample of flask and reflux was removed and dried from the barrel for extraction. The barrel was then removed and drained off the solvent until it was almost dried up in the extraction flask. The flask containing fat was detached and dried in the oven at a low temperature to evaporate the solvent completely. The flask plus fat was then weighed.

Calculation:

$$\% \text{ Fat} = \frac{W_2 - W_1}{\text{Weight of sample}} \times 100$$

W_2 = weight of flask and fat

W_1 = weight of empty flask

3.7.6 Carbohydrate Content Determination

The total carbohydrate content of each of the sample (population) was derived by difference. That is;

$$\% \text{ Total carbohydrate} = 100 - (\% \text{ water} + \% \text{ Protein} + \% \text{ Lipids} + \% \text{ Ash} + \% \text{ Crude fibre})$$

3.8 Economic value Determination

3.8.1 Determination of Acid Value

About 25 cm³ of 5% ethanol was boiled on a water bath the heating was to ensure the removal of dissolved gases. Extracted sample (2.5g) was added to 25 cm³ of hot ethanol and the mixture was heated to boil. Then few drops of 1% phenolphthalein indicator was added and titrated against 0.1M KOH with constantly shaking until a permanent pink colour was obtained, the acid value was expressed as

$$\text{Acid value} = \frac{56.1 \times M \times V}{W} \quad (\text{AOAC, 1998}).$$

Where M = Concentration of KOH

V = Titre value

56.1 = Molecular weight of KOH

W = weight of sample

3.8.2 Determination of Iodine value

About 0.4 g of the garlic oil sample was weighed into a conical flask and 20 cm³ of carbon tetrachloride was added to dissolve the oil. Then 25 cm³ of wij's reagent was added to the flask using a safety pipette in fume chamber. Stopper was then inserted and the content of the flask was vigorously swirled. The flask was then placed in the dark for 2 hours 30 minutes. At the end of this period, 20 cm³ of 10% aqueous potassium iodide and 125 ml of water were added using a measuring cylinder. The content was titrated with 0.1M sodium-thiosulphate solutions until the yellow colour almost disappeared. Few drops of 1% starch indicator was added and the titration continued by adding thiosulphate drop wise until blue coloration disappeared after vigorous shaking. The same procedure was used for blank test and other samples (Akpan *et. al.*, 2006). The iodine value (I.V) was given by the expression

$$\text{Iodine value} = \frac{12.69 \times (V_1 - V_2)}{M}$$

Where C = Concentration of sodium

V₁ = Volume of sodium thiosulphate used for blank

V₂ = Volume of sodium thiosulphate used for determination

M = Weight of the sample.

3.8.3 Determination of Free Fatty Acid

About 2 g of oil was measured into 250 cm³ Erlenmeyer flask, 100 cm³ of ethanol was added and followed by 2 cm³ of phenolphthalein indicator. The mixture was thoroughly shaken and titrated

against 0.1M NaOH with continuously shaking until the endpoint was reached, which was indicated by a slight pink colour that persists for 30 seconds, the free fatty acid is expressed as

$$\% \text{ FFA} = \frac{V \times N \times 282}{W} \times 100 \quad (\text{Chopra and Kanwar, 1991})$$

Where %FFA = Percent free fatty acid (g/100g)

V = Volume of NaOH (cm³)

N = Normality of NaOH

282 = Molecular weight of oleic acid

W = weight of sample

3.8.4 Determination of Saponification Value

About 2 g of the extract was added to a flask with 30 ml of ethanolic KOH and was then attached to a condenser for 30 minutes to ensure the sample was fully dissolved. After sample had cooled, 1 ml of phenolphthalein was added and titrated with 0.5M HCl until a pink endpoint has reached. Saponification value was calculated from the equation

$$\text{Saponification value} = \frac{(S - B) \times M \times 56.1}{\text{Sample weight (g)}} \quad (\text{Akpan et al., 2006}).$$

Where S = sample titre value

B = blank titre value

M = Molarity of HCl

56.1 = molecular weight of KOH

3.9 Reactive Oxygen Species (ROS) determination

Parkia biglobosa and *Prosopis africana* seeds {twenty (20)} each from each location were soaked in water for 24 hours and prepared for determination of the following:

3.9.1 Superoxide determination

The generation of Superoxide was determined according to the method of Oracz *et al.* (2007), using sulfanilamide and 2-naphtylamine. Fresh weight (0.2g) of seed were crushed in 4 mL of sodium phosphate buffer (pH 7.8, 50 mM) at 4 °C. The extracts were centrifuged at 16,000 g for 15 min, and the resulting supernatants were used for superoxide determination. The supernatant (1 ml) was first incubated at 25 °C for 30 min in the presence of 1ml hydroxylamine hydrochloride (1 mM) in 50 mM sodium phosphate buffer (pH 7.8). A volume (0.5 ml) of this reaction mixture was then incubated with 0.5 ml of 17 mM sulfanilamide and 0.5 ml of 7 mM 2-naphtylamine at 25 °C for 30 min. The absorbance was measured at 540 nm after centrifugation at 13,000 g for 30 min, and a calibration curve was established using sodium nitrite.

3.9.2 Hydrogen peroxide determination

The Hydrogen peroxide (H₂O₂) content of excised axes was determined spectrophotometrically following the protocol described by Oracz *et al.* (2007) using a peroxidase-based assay with 3-dimethylaminobenzoic acid and 1.3 mM 3-methyl-2-benzothiazolidone hydrazone (O’Kane *et al.*, 1996).

3.10 Enzyme Activities Determination

3.10.1 Enzyme extraction

The seeds were crushed and 1 g was weighed into a conical flask, and 100 ml of sterile distilled water was added and then placed on the rotary shaker at 200 rpm for 30 mins. After shaking, it was then filtered with Whatman no1 filter paper to obtain the crude enzyme (Ali, 1992).

3.10.2 Assay for Amylase

Estimation of amylase activity was carried out according to the 3, 5 dinitro salicylic acid (DNSA) method. One millilitre of 1% starch was incubated with different dilutions of the enzyme extract and 1ml of citrate- phosphate buffer (pH 6.0). The reaction mixture was incubated at 50 °C for 30 mins. The reaction was stopped by adding 2 ml of DNSA and kept in boiling water bath for 10 mins. The absorbance was read at 540 nm using a Spectrophotometer (Shimadzu, Thermoelectric cell holder, S-1700), against glucose as the standard.

3.10.3 Assay for Amylase Activity

One unit of enzyme activity is defined as the amount of enzyme which releases one mole of reducing sugar as glucose per minute, under the assay conditions (U/ml/min). The experiments were carried out in triplicates and standard error was calculated.

3.10.4 Assay for Protease

Protease assay was done by adding 2 ml of 0.1M phosphate buffer of pH 7 containing 1% of casein into 2 ml of the crude enzyme inside a test tube which was then incubated at 3 °C for 30 mins. After incubation, it was filtered again with whatman no 1 filter paper. Thereafter, 2 ml of reagent C [containing mixture of reagent A (1g of anhydrous Na_2CO_3 in 100 ml of 0.1M NaOH) and reagent B (0.25 g of Cupric sulphate in 50 ml of 0.5 g of Sodium tartate)] was added and left for 10 mins followed by addition of 0.2 ml of reagent D (1:3 of Folin-ciocalteu reagent and distilled water) then left for 30 mins. The absorbance was measured using a Spectrophotometer at wavelength 650 nm (Tremacoldi *et al.*, 2004)

3.10.5 Assay for Protease activity

The values of absorbance obtained from the protease assay was used to calculate the amount of protease produced (protease activity). One unit of enzyme activity is defined as the amount of enzyme required to liberate 1mg of tyrosine under assay condition.

3.10.6 Assay for Lipase

Lipase activity was measured with Spectrophotometric assay method with p-nitrophenyl laurate (p-NPL) as substrate. The reaction buffer with 12.5 mM p-NPL was prepared by dissolving substrate in acetonitrile with Tris-HCl buffer with (pH 8.0) containing 10 mM CaCl₂. Enzyme solution (0.2 ml) was added to the reaction buffer (0.8 ml). The reaction progress was monitored and Spectrophotometer was used to read the absorbance of 410nm over a period of 5 mins at 45 °C using. The molar extinction coefficient of p-nitrophenol (p-NP) $\epsilon=1.5 \times 10^4 \text{ cm}^2/\text{mol}$ (Sulyman *et al.*, 2017).

3.11 Ultrastructure examination using Scanning Electron Microscope (SEM)

The seeds sample were crushed and seed coat was separated from the cotyledon. Dry materials were prepared and mounted directly onto clean stubs using double-sided adhesive tape. They were then coated with gold in a JOEL JFC1100E ion-sputtering device and examined with a Aspex 3020 scanning electron microscope, operated at accelerating voltage of 16 kV and displaying magnification of 250 at the Scanning Electron Microscope Unit of the Department of Material Science and Engineering, Kwara State University, Malete. Kwara State

3.12 Statistical Analysis

Data collected were analysed by analysis of variance (ANOVA) using SPSS package (version 16.0) at 5% level of significance. The Duncan Multiple Range Test (DMRT) was used to separate the means.

CHAPTER FOUR

RESULTS

4.1 Fruit and Seed Characteristics

The results of fruit and seed characteristics of *Parkia biglobosa* and *Prosopis africana* collected from different locations are presented below.

The quantitative characteristics result showed PBKD has the longest (28.14 cm) and the broadest (3.17 cm) pods as well as the highest value (36.94 g) of 100 seeds weighed (Table 3). The pod breadth length was highest in PBKD in terms of apex, middle and base with the values 2.49cm, 3.17cm and 2.43cm respectively. Meanwhile, PBJG, PBKT and PBKW were not significantly different in pod length. PBKT and PBKW had the same number of seeds per pod (Table 3). Thus, seeds of *Parkia biglobosa* collected from Kaduna had the biggest pod length with less number of pod/bunch.

The measurable character results showed the pod length of *Prosopis africana* varies between 12-17 cm with the longest length (17.34 cm) in PANG and the lowest (12.38 cm) length in PAKB (Table 3). Pod breadth (apex and middle) in both PAKW and PAKB was not significantly different from each other. PANG has the highest value of seed number /pod (18.40), the seeds number per /pod in PAKW and PAKB were not significantly different. PANG recorded the highest weight value of 100 seeds while PAKD and PAKB were not significantly different from one another in term of 100 seed weight (Table 3).

In *Parkia biglobosa*, there was no correlation between the pods length and the numbers of seeds number/pods as well as seeds number/bunch. This could be observed in PBJG with average number of 19 pods and 251 seeds/bunch while PBKT has average of 10 pods/bunch with 256 seeds/bunch respectively. There was correlation at 0.01 level of significance in pod breadth (middle-apex, and base-middle) and at 0.05 level of significance pod breadth (base-apex) (Table 4). There was no correlation in the fruit characteristics among the different sample of *Prosopis africana* collected from different

locations except the pod breadth (base) that has a correlation significant at 0.05 with pod breadth (middle and base) (Table 4).

Table 3: Quantitative Characteristics of *Parkia biglobosa* and *Prosopis africana* fruits

Samples	Pod	Pod Breadth (cm)			No of	Weight of 100 Seeds	No of	No of
	Length (cm)	Apex	Middle	Base	Seed/ Pod		Pod/ Bunch	Seed/ Bunch
<i>Parkia biglobosa</i>								
PBKD	28.14 ^a	2.49 ^a	3.17 ^a	2.43 ^a	17.73 ^a	36.94 ^a	8.40 ^c	119.40 ^b
PBJG	23.36 ^b	2.29 ^b	3.09 ^a	2.37 ^a	15.07 ^b	28.45 ^c	19.60 ^a	251.00 ^a
PBKT	22.94 ^b	1.41 ^c	2.03 ^c	1.03 ^b	17.80 ^a	30.80 ^b	10.80 ^b	256.80 ^a
PBKW	22.54 ^b	1.55 ^c	2.18 ^b	1.10 ^b	17.80 ^a	30.53 ^b	11.60 ^b	277.80 ^a
<i>Prosopis africana</i>								
PAKD	15.00 ^b	3.02 ^{ab}	3.72 ^a	3.36 ^{ab}	16.20 ^{ab}	14.57 ^c		
PAKW	13.26 ^{bc}	2.66 ^b	3.06 ^b	2.76 ^c	14.00 ^b	15.07 ^{bc}		
PANG	17.34 ^a	2.84 ^{ab}	3.52 ^{ab}	3.04 ^{a-c}	18.40 ^a	15.66 ^b		
PAKB	12.38 ^c	2.56 ^b	3.48 ^b	2.84 ^{bc}	14.40 ^b	14.66 ^c		

Values (mean) with the same superscript(s) along the column are not significantly different at $p \leq 0.05$.

Parkia biglobosa = PB: Kaduna = PBKD; Jigawa = PBJG; Katsina = PBKT; Kwara = PBKW

Prosopis africana = PA: Kaduna = PAKD; Kwara = PAKW; Niger = PANG; Kebbi = PAKB

Table 4: Correlation matrix of the fruit characteristics of *Parkia biglobosa* and *Prosopis africana*.

Characteristics		Pod length	Pod Breadth			No of Seeds/ Pod	No of Pods/ bunch	No of Seeds/ bunch
			Apex	Middle	Base			
<i>Parkia biglobosa</i>								
Pod Length		1						
<i>Pod Breadth</i>	Apex	0.8281	1					
	Middle	0.7656	0.9745**	1				
	Base	0.7337	0.9408*	0.9916**	1			
Seeds/Pod		0.4919	0.2282	0.0116	- 0.0950	1		
Pods/Bunch		-0.2899	0.2881	0.3522	0.3592	-0.4638	1	
Seeds/Bunch		-0.3937	-0.0853	-0.2126	- 0.2946	0.3195	0.5196	1
<i>Prosopis africana</i>								
Characteristics		Pod Length (cm)	Pod Breadth (cm)			No of Seed/ Pod		
			Apex	Middle	Base			
Pod Length (cm)		1						
Pod Breadth(cm)	Apex	0.3740	1					
	Middle	0.3791	0.7731	1				
	Base	0.4047	0.9532*	0.9000*	1			
Seeds/Pod		0.7799	0.8035	0.7418	0.7783	1		

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

All the seed samples of *Parkia biglobosa* have smooth seed coat texture. The seed coat colour varied from chocolate brown (PBKD), blackish brown (PBJG) and chocolate (PBKT and PBKW) (Table 5). The shape of PBKD, PBJG and PBKT were ovate while PBKW was round ovate. The pod colour of PBKD was brown, PBJG and PBKT had pinkish brown colour and PBKW had deep brown colour. The fruits of *Prosopis africana* occurred as pods containing brown-coated seeds within a fibrous pulp. The pods had varying colour. It was cylindrical, thick and hard, shiny, with woody walls. *Prosopis africana* seeds from the different location had smooth seed texture with coffee brown seed colour. The seed shape included ovate (PAKD and PAKW), oblong (PANG) and oblong/ovate (PAKB) (Table 5).

Table 5: Qualitative Characteristics of *Parkia biglobosa* and *Prosopis africana* Seeds

Seed samples	Texture	Seed Colour	Seed Shape	Pod Colour
<i>Parkia biglobosa</i>				
PBKD	smooth	chocolate brown	Ovate	Brown
PBJG	smooth	blackish brown	Ovate	Pink Brown
PBKT	smooth	chocolate	Ovate	Pink Brown
PBKW	smooth	chocolate	Round ovate	Deep Brown
<i>Prosopis africana</i>				
PAKD	smooth	coffee brown	Ovate	Dark Red
PAKW	smooth	coffee brown	Ovate	Dark Brown
PANG	smooth	coffee brown	Oblong	Dark Brown
PAKB	smooth	coffee brown	oblong/ovate	Dark Red

Parkia biglobosa = PB: Kaduna = PBKD; Jigawa = PBJG; Katsina = PBKT; Kwara = PBKW

Prosopis africana = PA: Kaduna = PAKD; Kwara = PAKW; Niger = PANG; Kebbi = PAKB

The frequency distribution for the axial dimensions of the seeds of *Parkia biglobosa* samples is presented in Fig. 4. The longest seed length (11.29 mm) was found in PBJG, followed by PBKD (10.86 mm) while the lowest length 9.57 mm occurred in PBKT. Furthermore, the highest seed width was recorded in PBKW (9.31 mm) followed by 9.18 mm in PBKT and the lowest (7.87 mm) in PBKD. The Geometric mean diameter of all seeds from the sample were similar and ranged between 7.18 mm-7.73 mm. The sphericity of all sample followed the same trend with Geometric mean diameter. Fig. 5 shows the frequency distribution of the axial dimension of the seeds of *Prosopis africana* sample, the seed length is almost the same at all the samples. PANG had the longest seed length (9.82 mm) while PAKW had broadest seeds (5.83 mm). PAKD had thickness seeds average at 4.34 mm. However, PAKD and PAKB had the same sphericity (0.65)

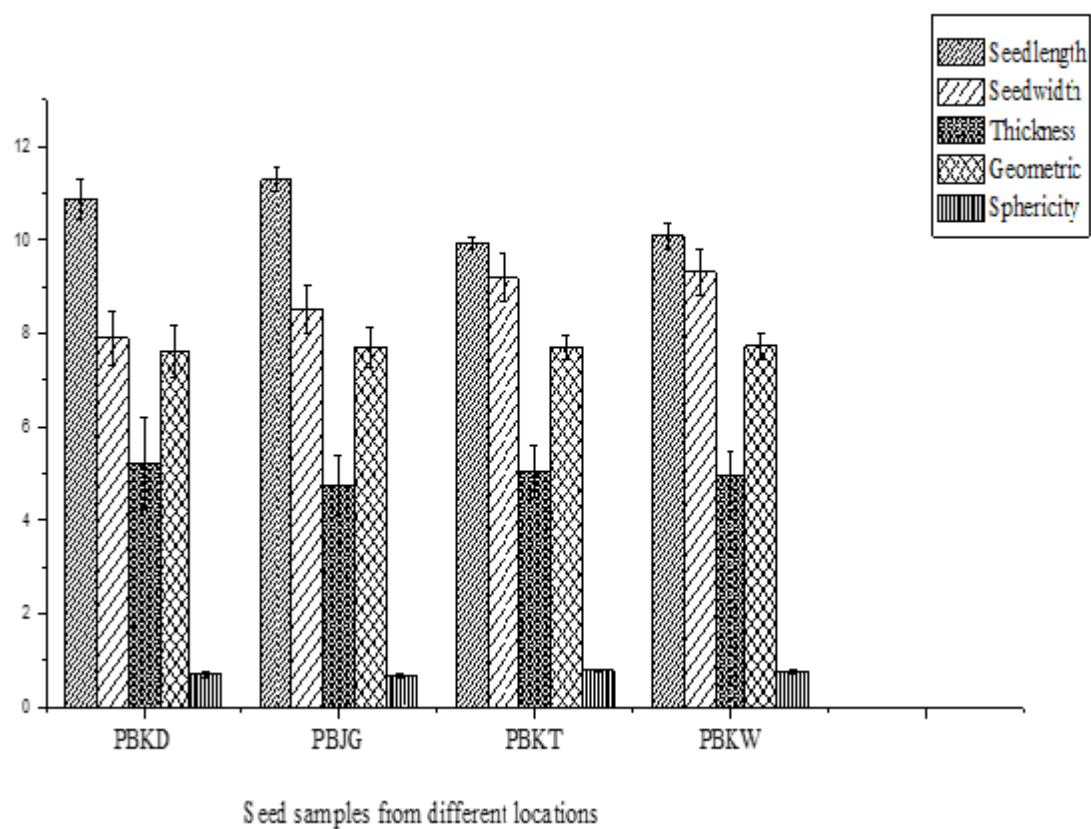


Fig. 4: Axial dimensions of the seeds of *Parkia biglobosa*

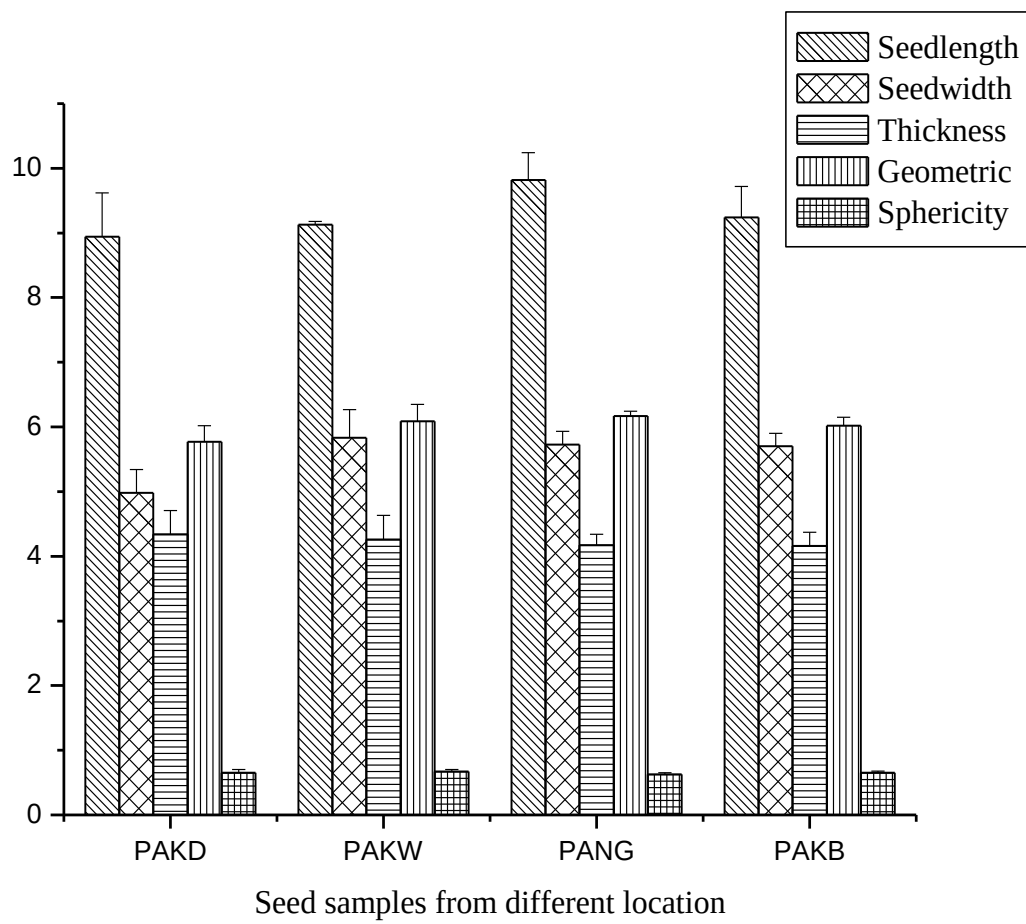


Fig. 5: Axial dimensions of the seeds of *Prosopis africana*

4.2 Germination Test

There were significant differences ($p \leq 0.05$) among the sampled seeds of *Parkia biglobosa* and *Prosopis africana* treated with different method of dormancy breaking. The results showed that the treatments had great effect on *Parkia biglobosa* and *Prosopis africana* seed germination. Dormancy in seeds of *Parkia biglobosa* and *Prosopis africana* are usually associated with the factors of deep exogenous (the protective covering the seeds), the seed coat or enclosed embryo. The cumulative germination of the seeds of *Parkia biglobosa* and *Prosopis africana* for 10 days treated with 98% concentrations of H_2SO_4 , HCl and HNO_3 respectively and were presented in Figures 6-29. The seeds treated with H_2SO_4 had better response to germination than HCl and HNO_3 . PBKW and PBJG seeds recorded 90% germinated respectively (Fig. 6). At 10 mins of acid scarification, 100% germination were observed in PBKW, PBKD, and PBJG (Fig. 7). Germination at 15 mins scarification and PB\KW was seen to be most responsive to the treatment with H_2SO_4 (Fig 8). The least germinated seed sample was PBKT. H_2SO_4 recorded 100% germination followed by HNO_3 showing 90% seeds germination at the 10th day (Figs 6-17).

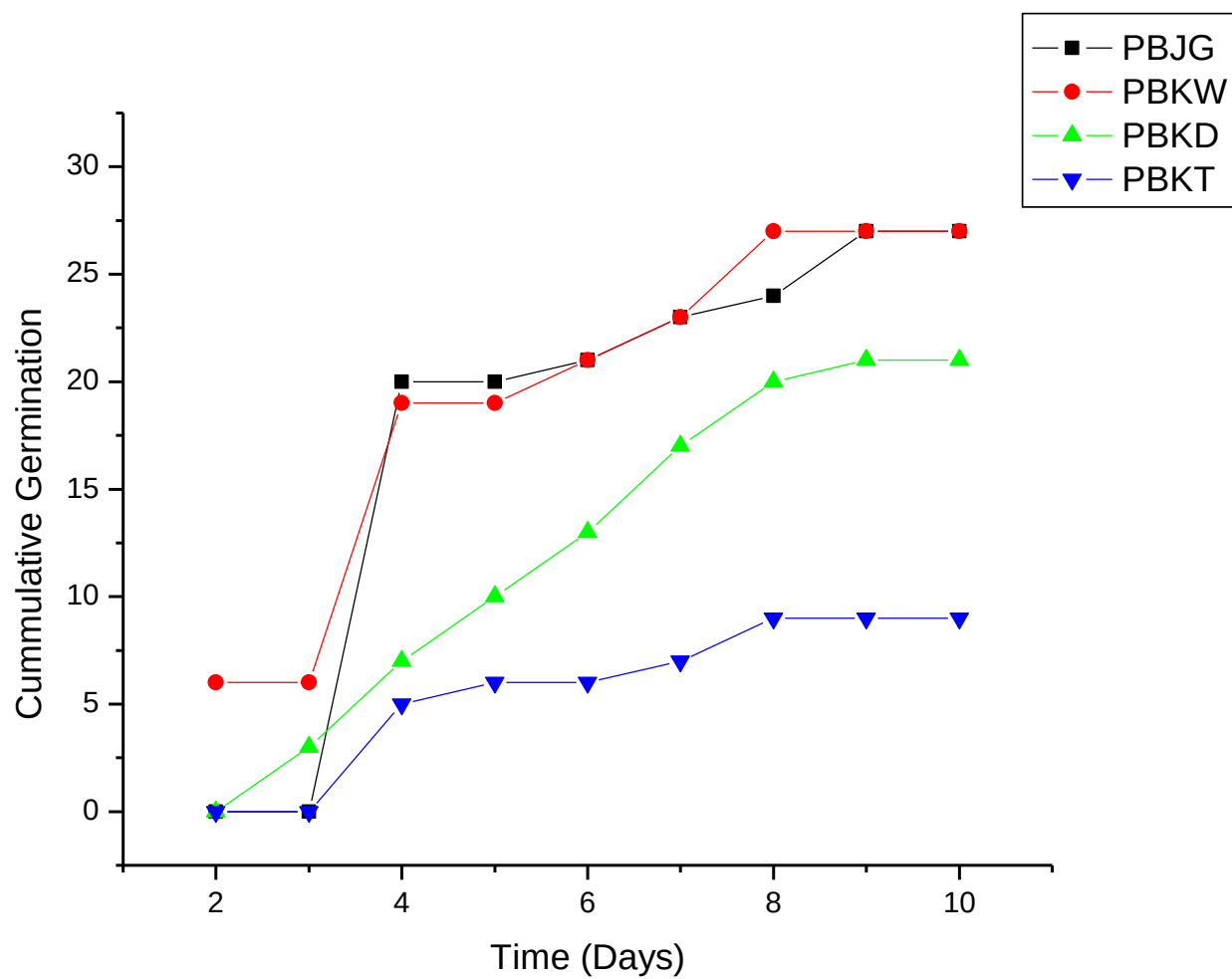


Fig. 6: Effects of H_2SO_4 on germination of *Parkia biglobosa* seeds at 5 mins scarification period

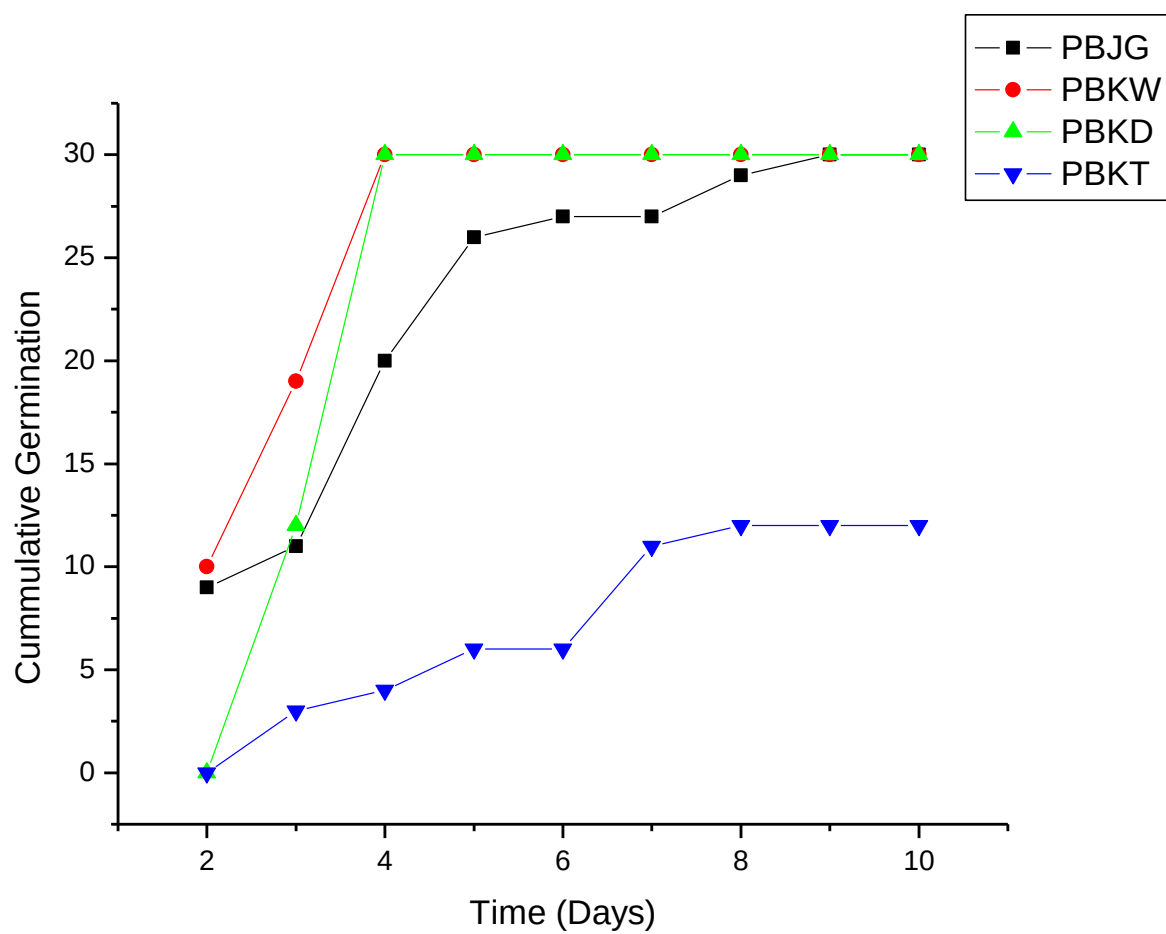


Fig. 7: Effects of H_2SO_4 on germination of *Parkia biglobosa* seeds at 10 mins scarification period

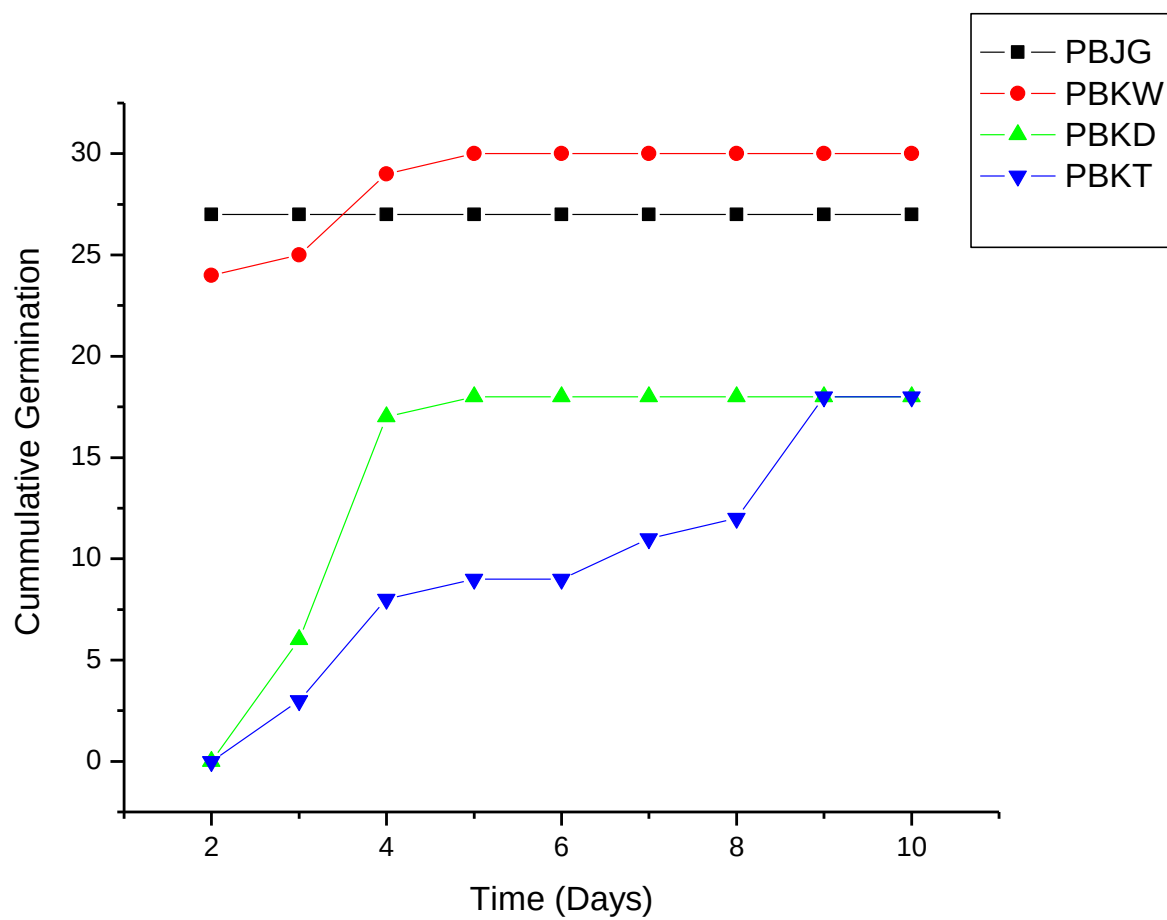


Fig. 8: Effects of H_2SO_4 on germination of *Parkia biglobosa* seeds at 15 mins scarification period

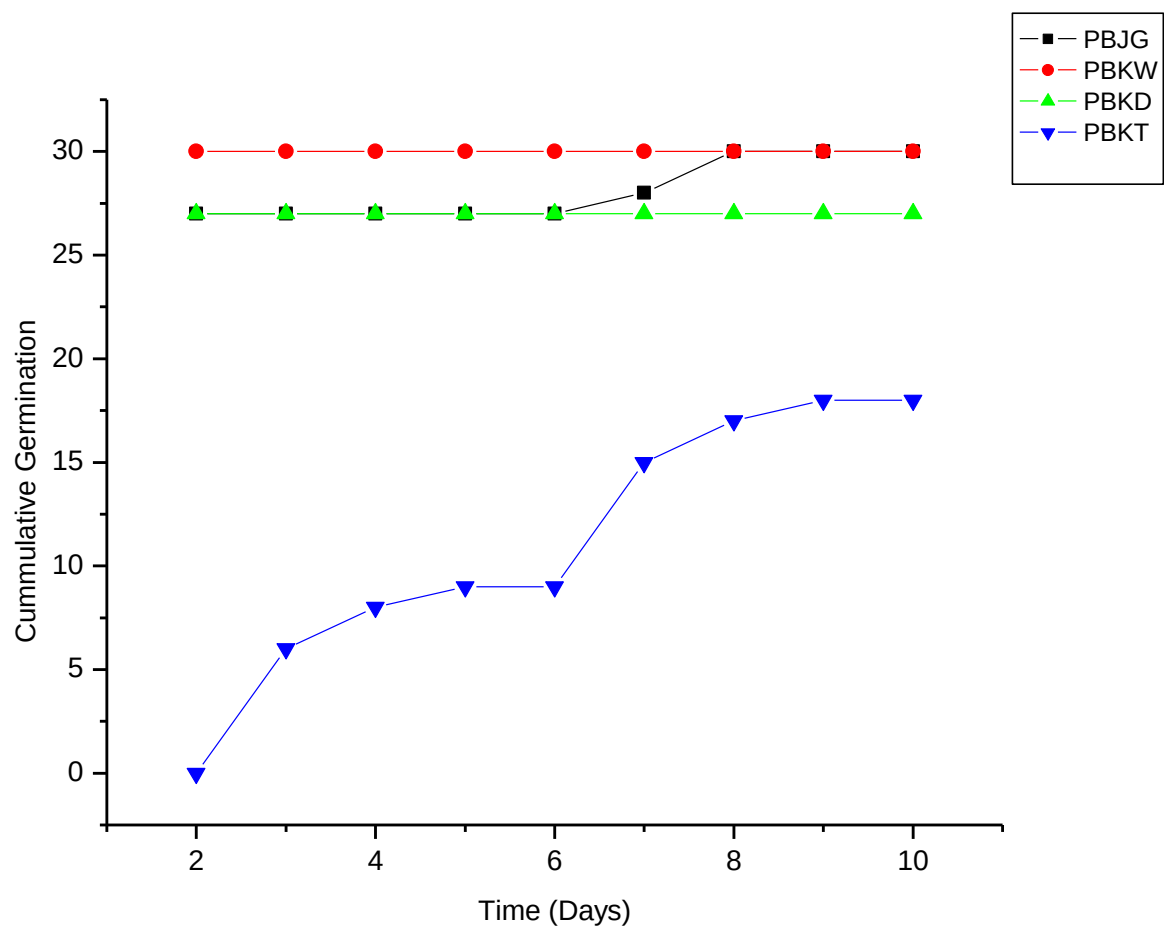


Fig. 9: Effects of H_2SO_4 on germination of *Parkia biglobosa* seeds at 20 mins scarification period

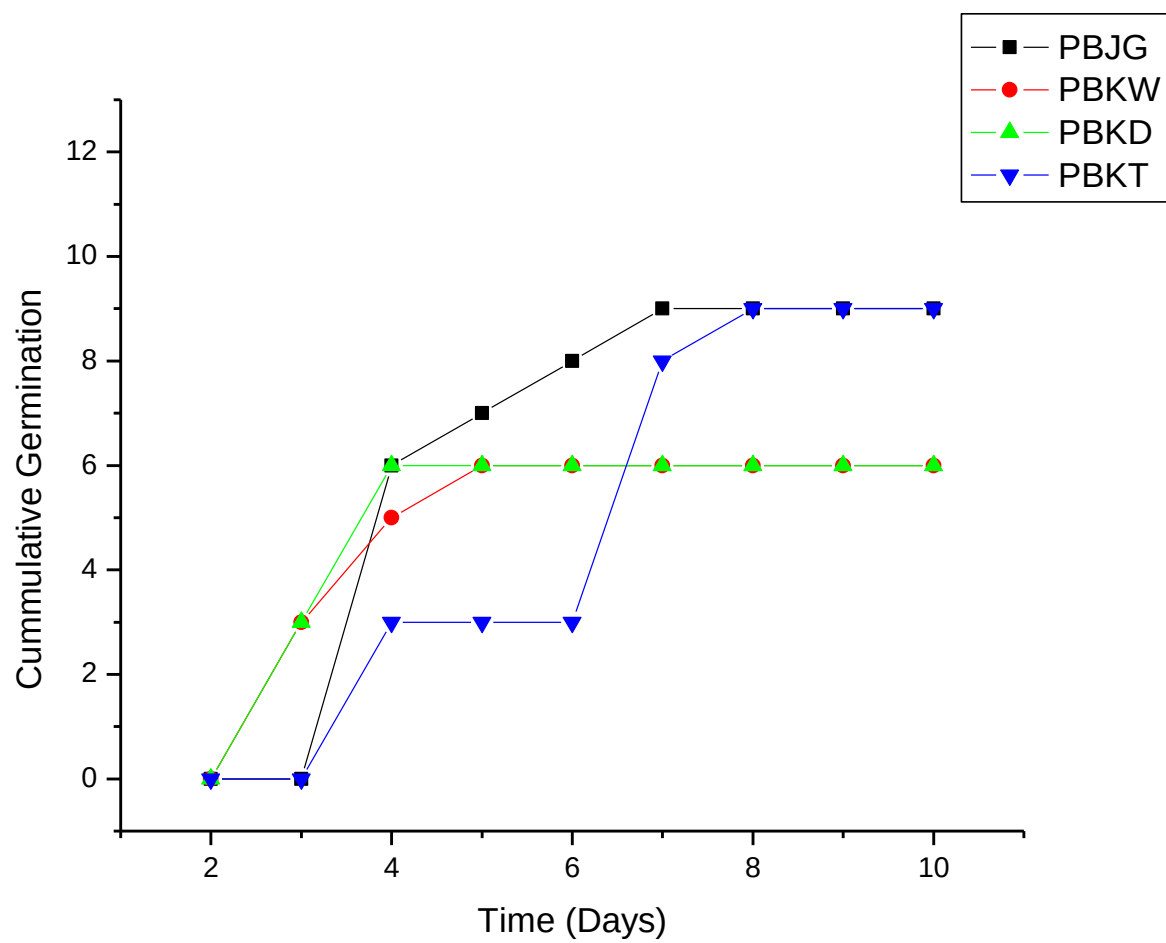


Fig. 10: Effects of HCl on germination of *Parkia biglobosa* seeds at 5 mins scarification period

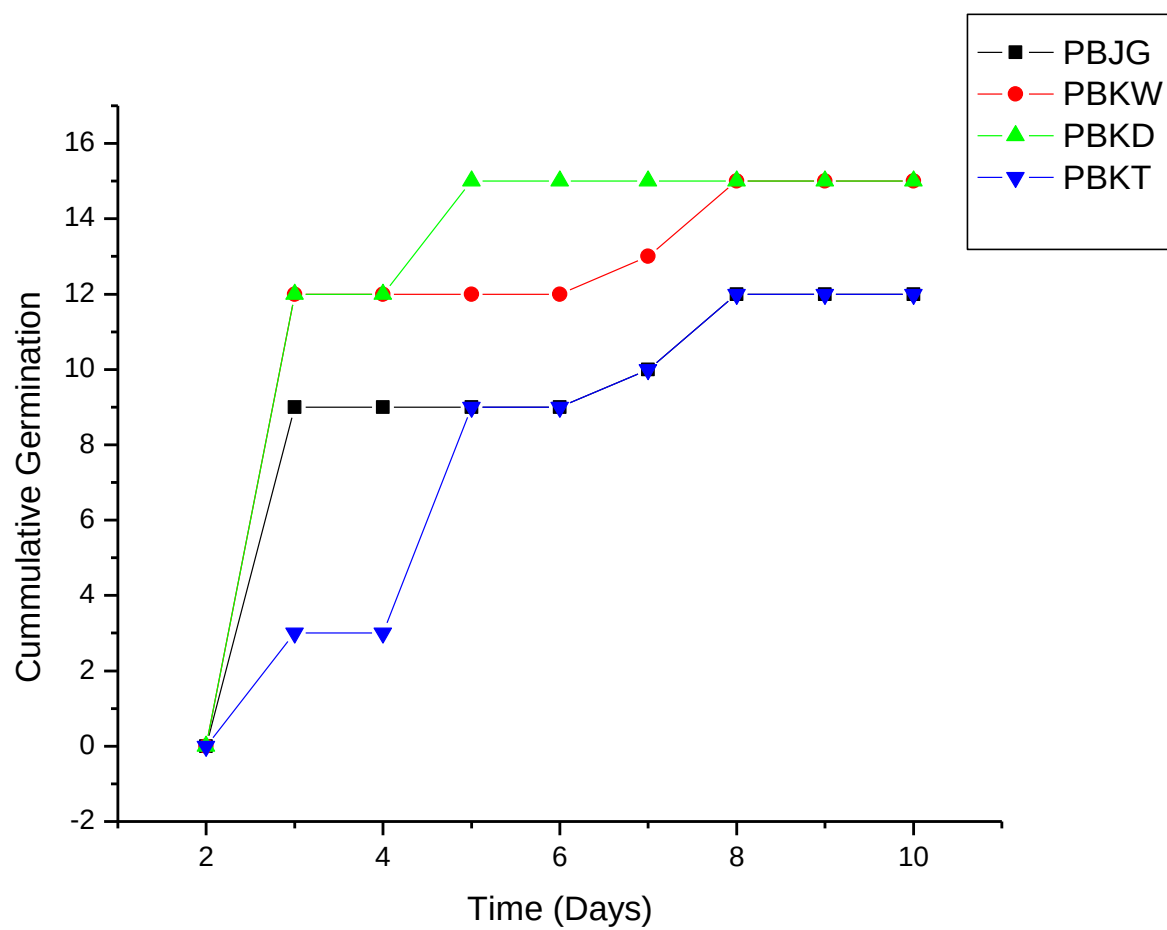


Fig. 11: Effects of HCl on germination of *Parkia biglobosa* seeds at 10 mins scarification period

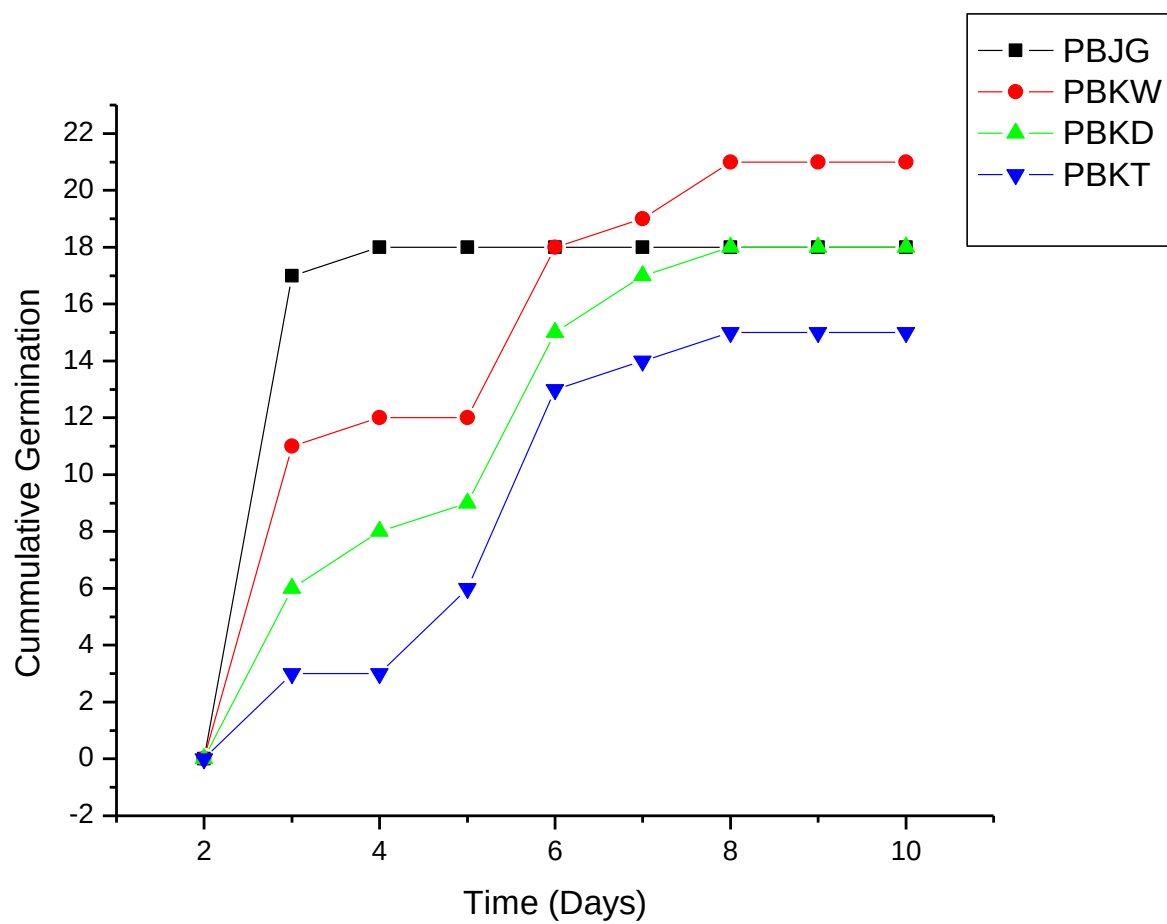


Fig. 12: Effects of HCl on germination of *Parkia biglobosa* seeds at 15 mins scarification period

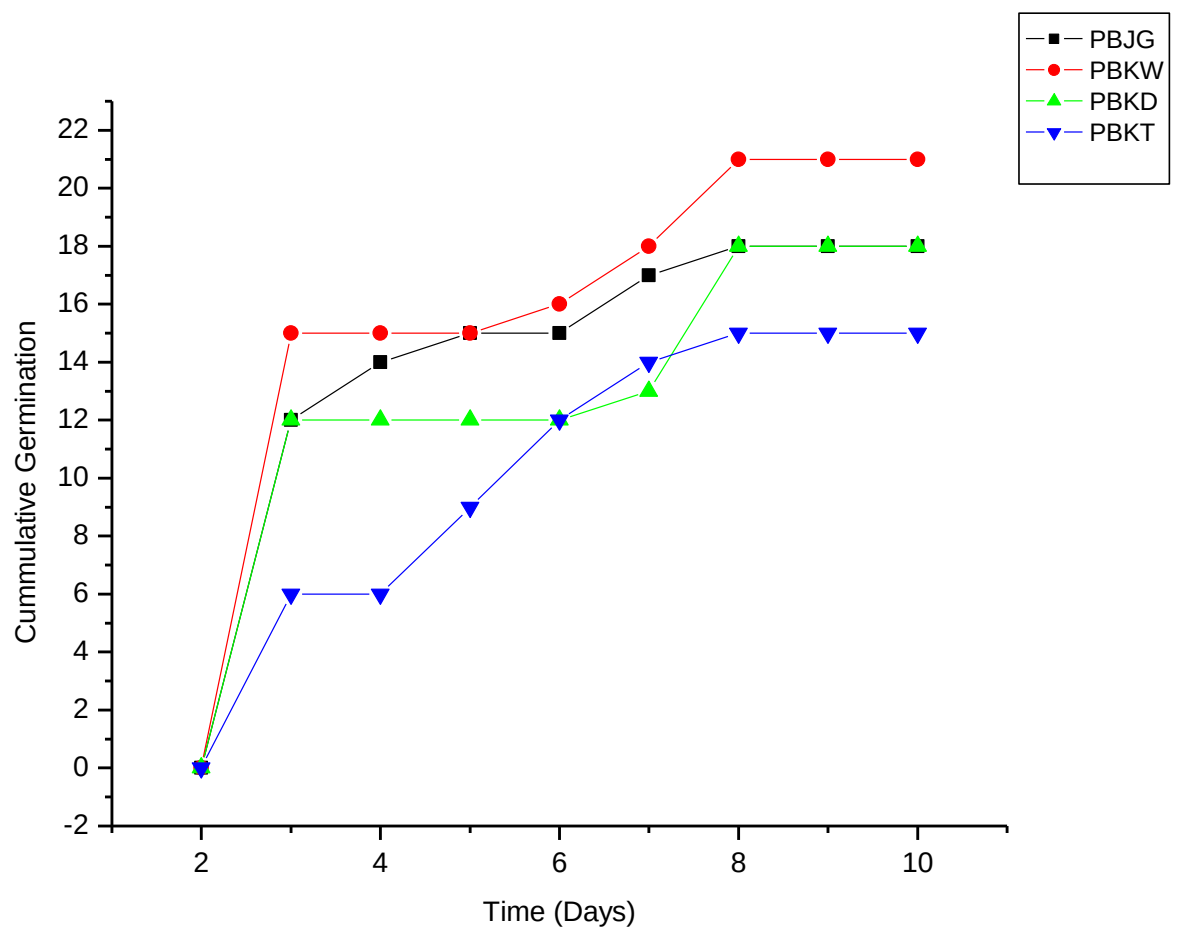


Fig. 13: Effects of HCl on germination of *Parkia biglobosa* seeds at 20 mins scarification period

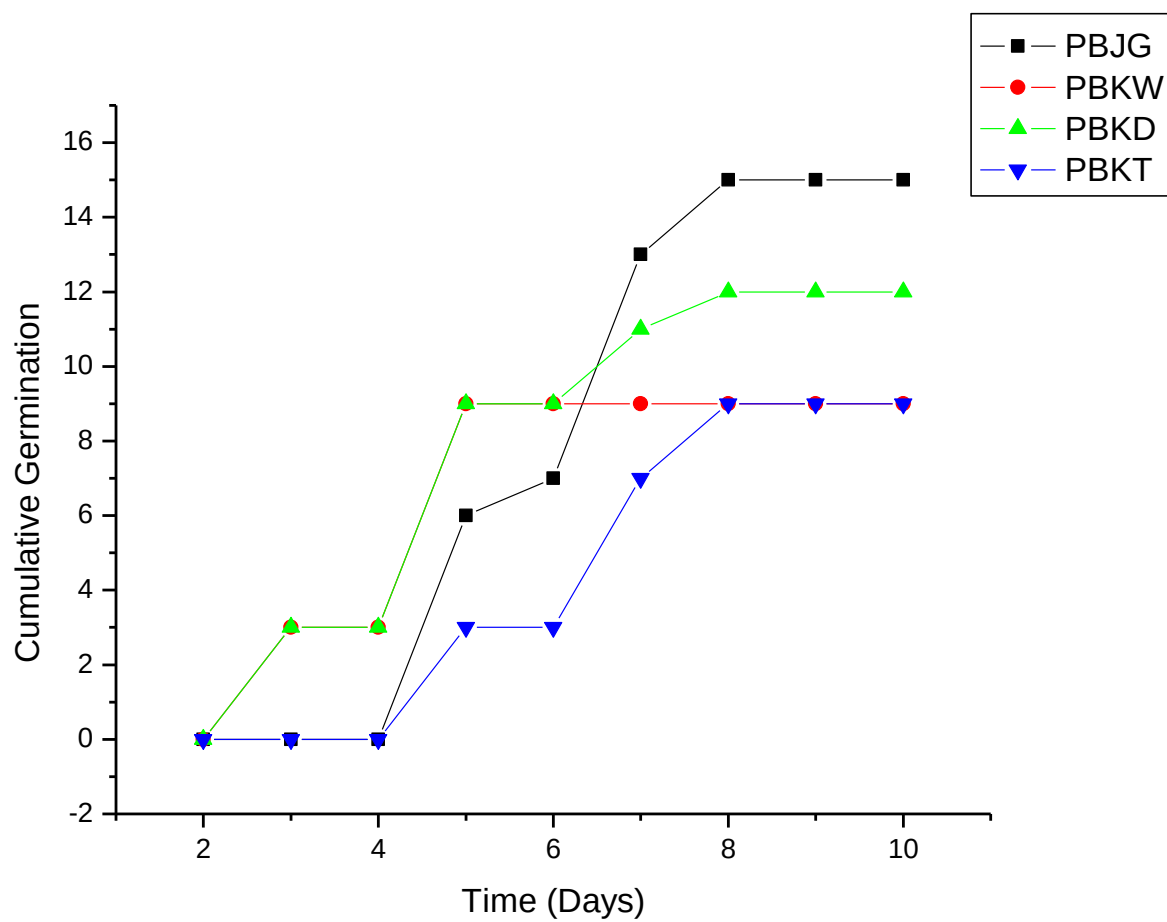


Fig.14: Effects of HNO_3 on germination of *Parkia biglobosa* seeds at 5 mins scarification period

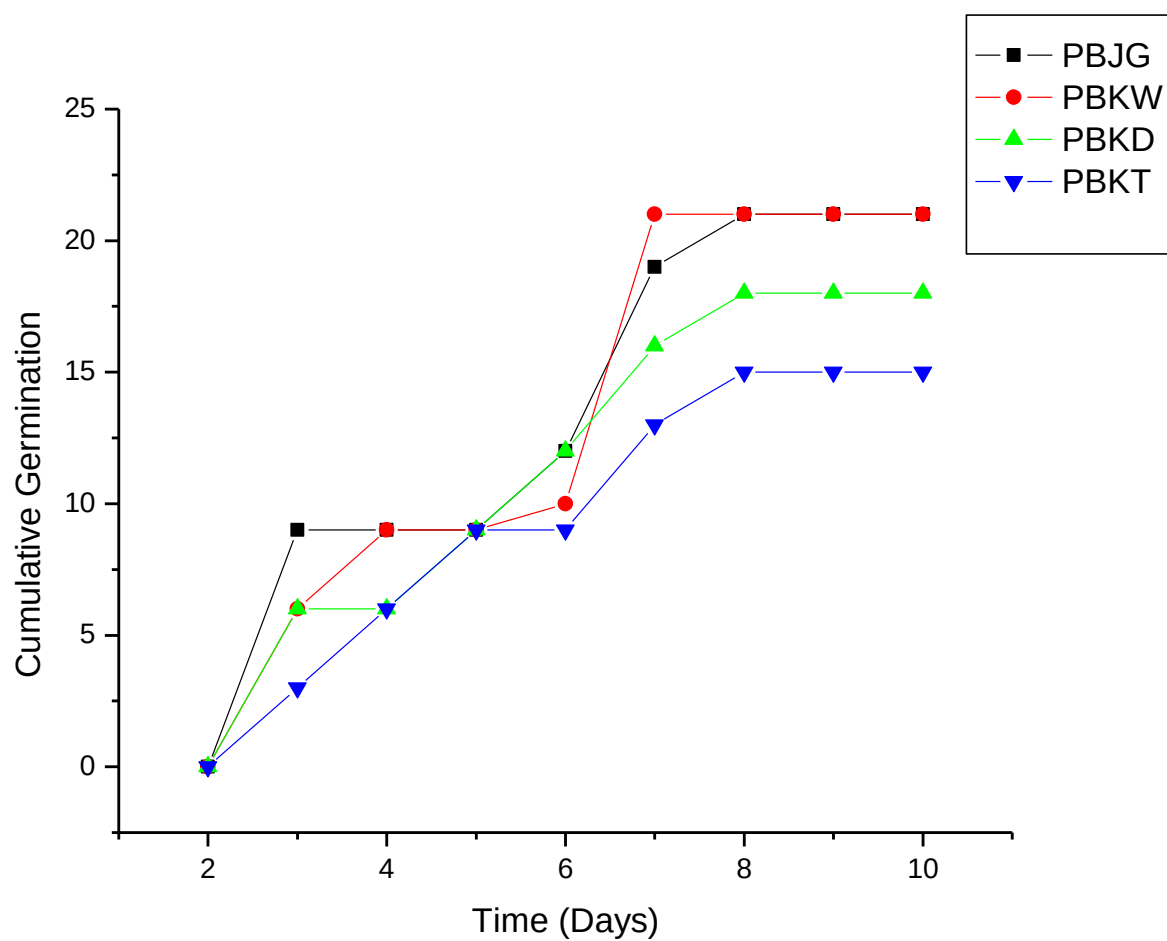


Fig. 15: Effects of HNO_3 on germination of *Parkia biglobosa* seeds at 10 mins scarification period

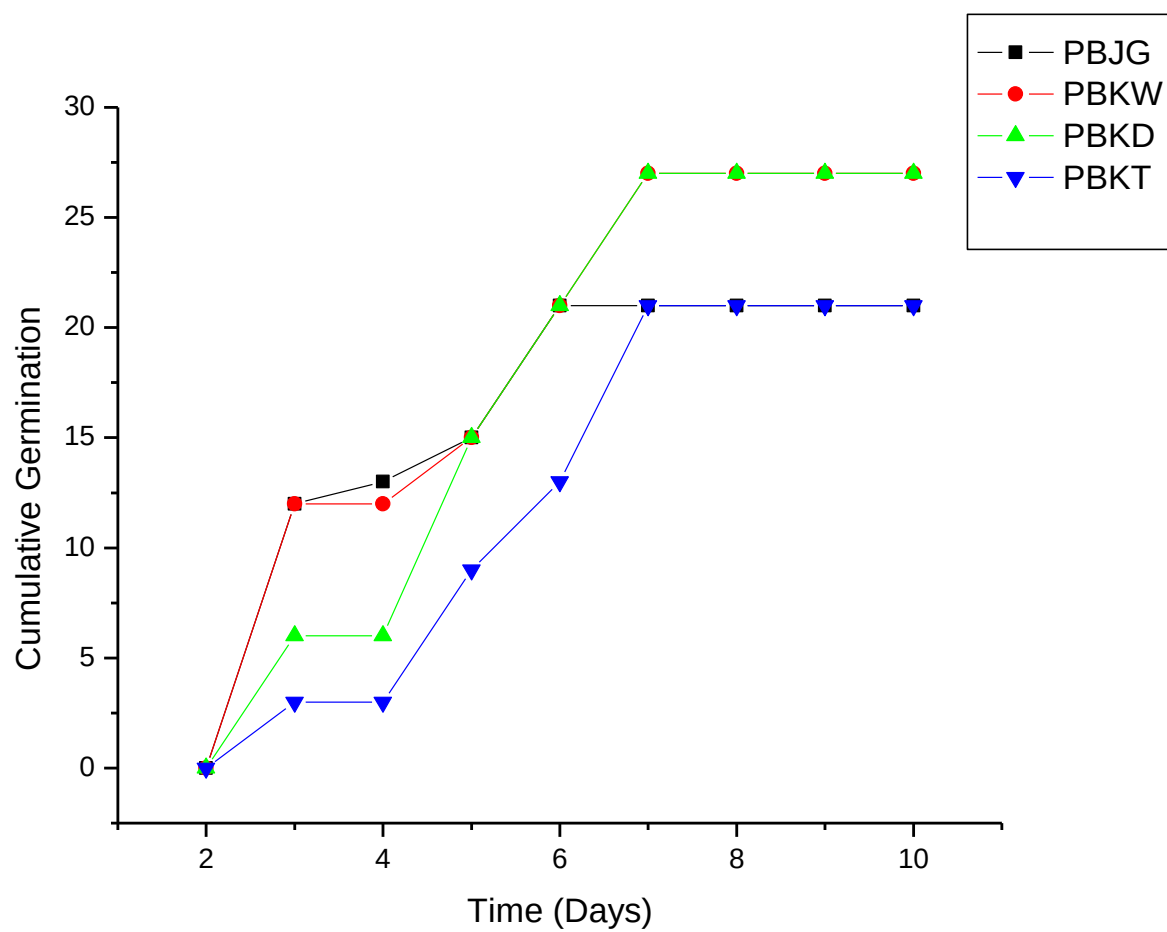


Fig. 16: Effects of HNO_3 on germination of *Parkia biglobosa* seeds at 15 mins scarification period

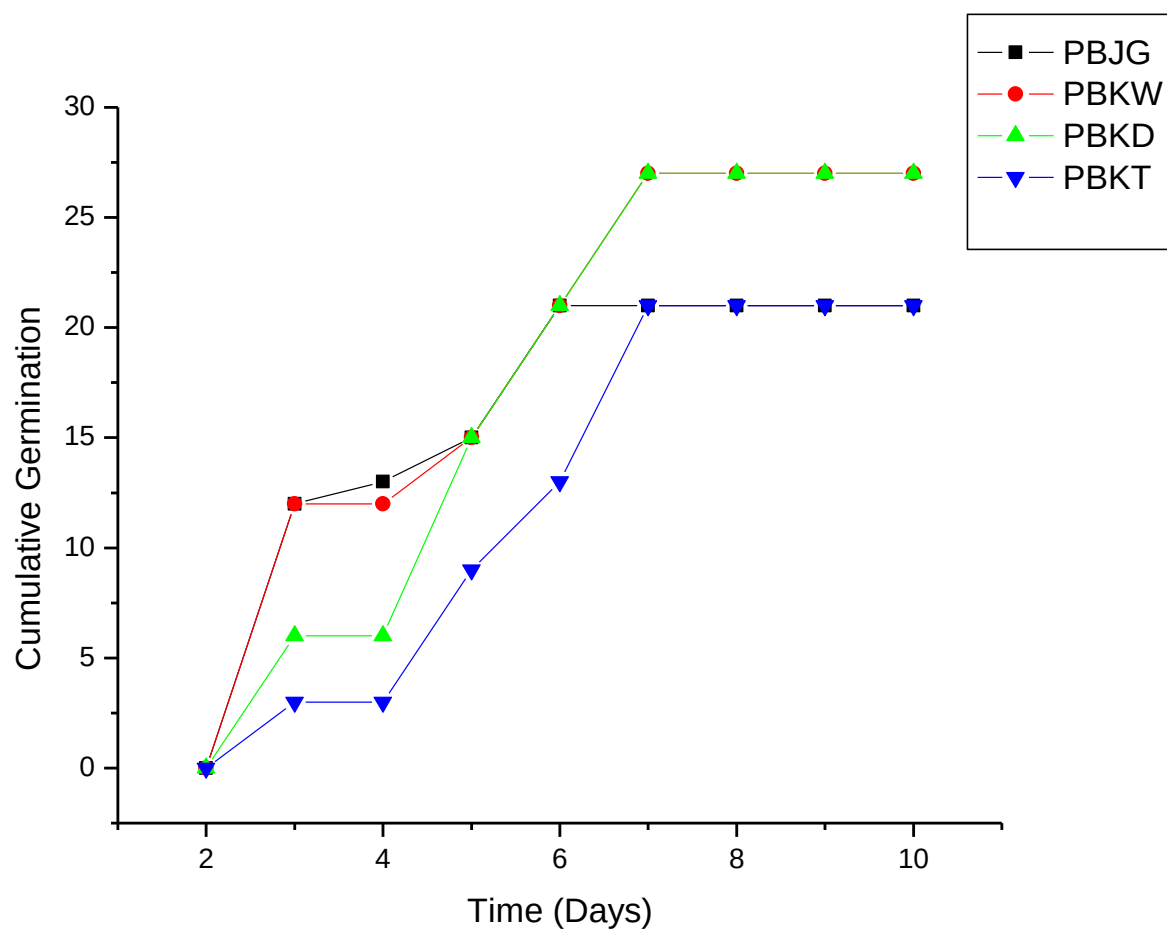


Fig. 17: Effects of HNO_3 on germination of *Parkia biglobosa* seeds at 20 mins scarification period

The response of *Prosopis africana* to the acid scarification was not as effective in breaking the seed dormancy as *Parkia biglobosa*. This might be due to the thickness of the seed coat of *Prosopis africana*. Although, Scarification with H_2SO_4 resulted in germination of 80-93.33% seeds at 20 mins of scarification in all the seed sample (Fig. 21). The acid that is least effective for breaking the dormancy of *Prosopis africana* was HNO_3 which has maximum of 66.66% seeds germination in 20mins especially in PANG.

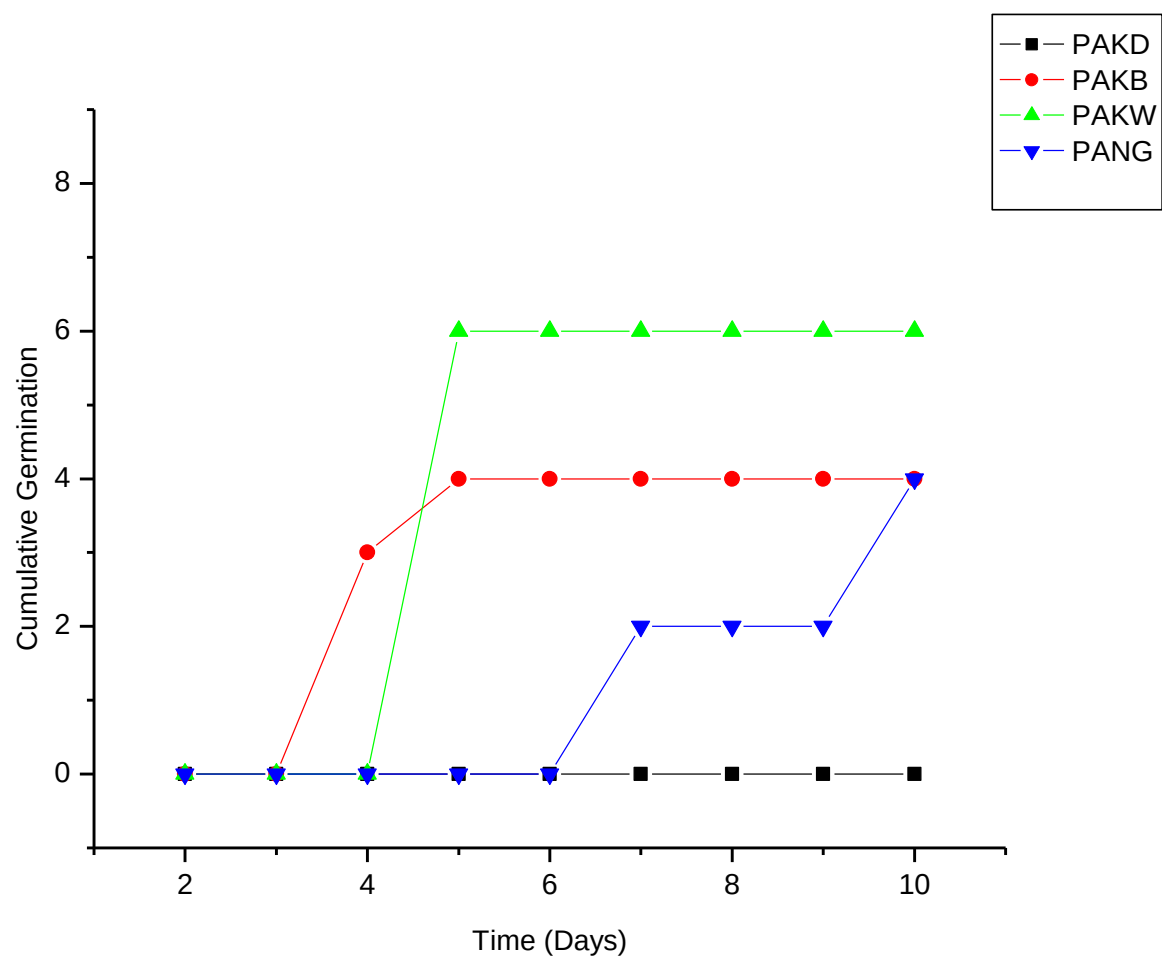


Fig. 18: Effects of H_2SO_4 on germination of *Prosopis africana* seeds at 5 mins scarification period

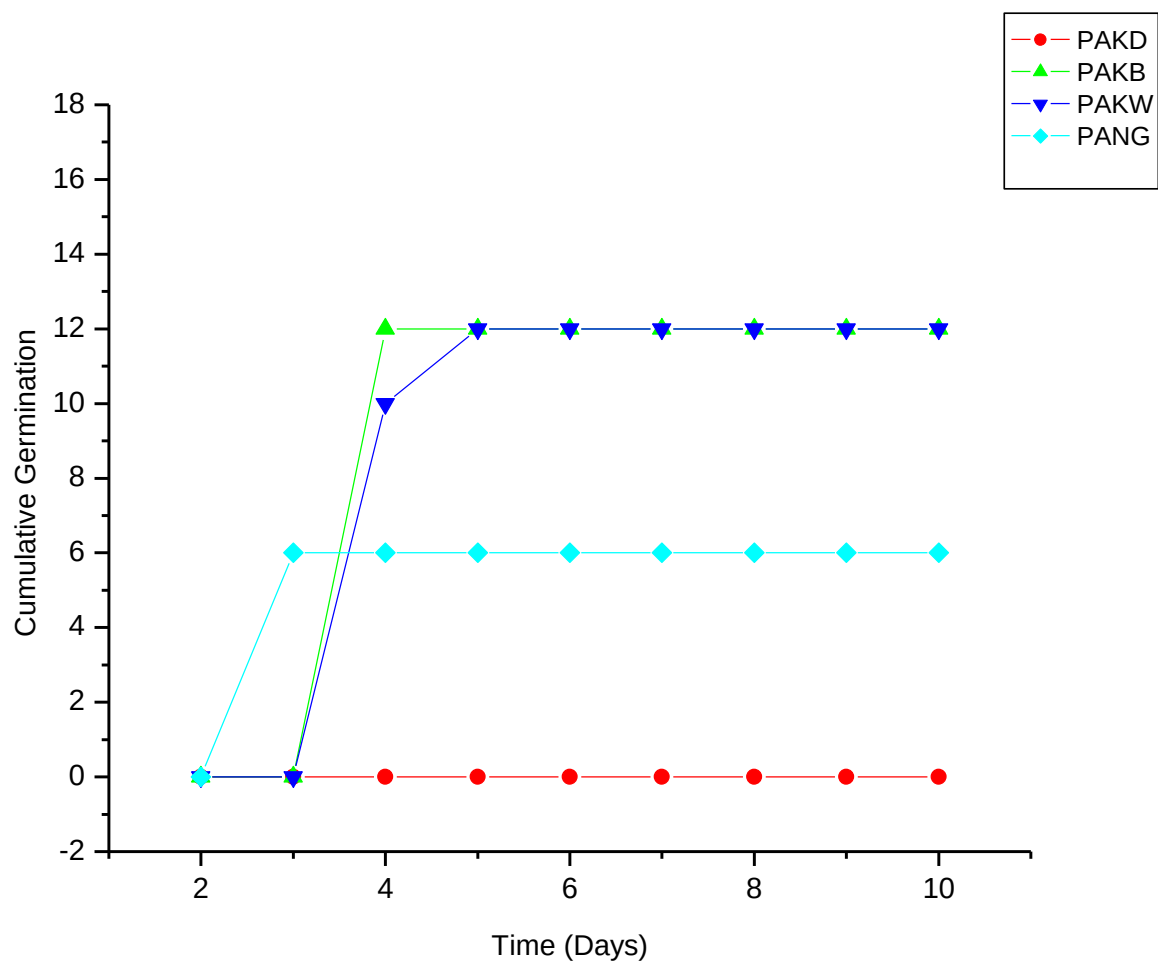


Fig. 19: Effects of H_2SO_4 on germination of *Prosopis africana* seeds at 10 mins scarification period

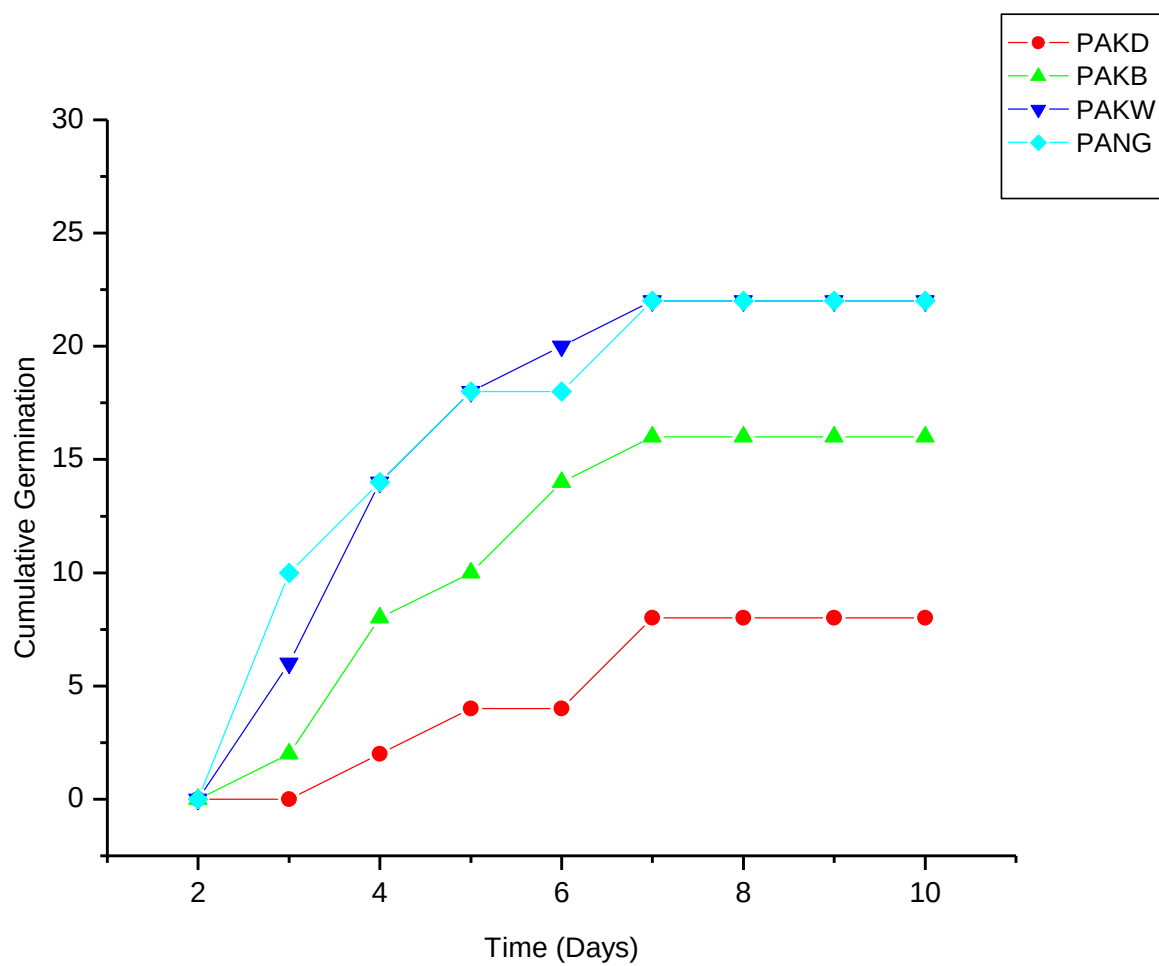


Fig. 20: Effects of H_2SO_4 on germination of *Prosopis africana* seeds at 15 mins scarification period

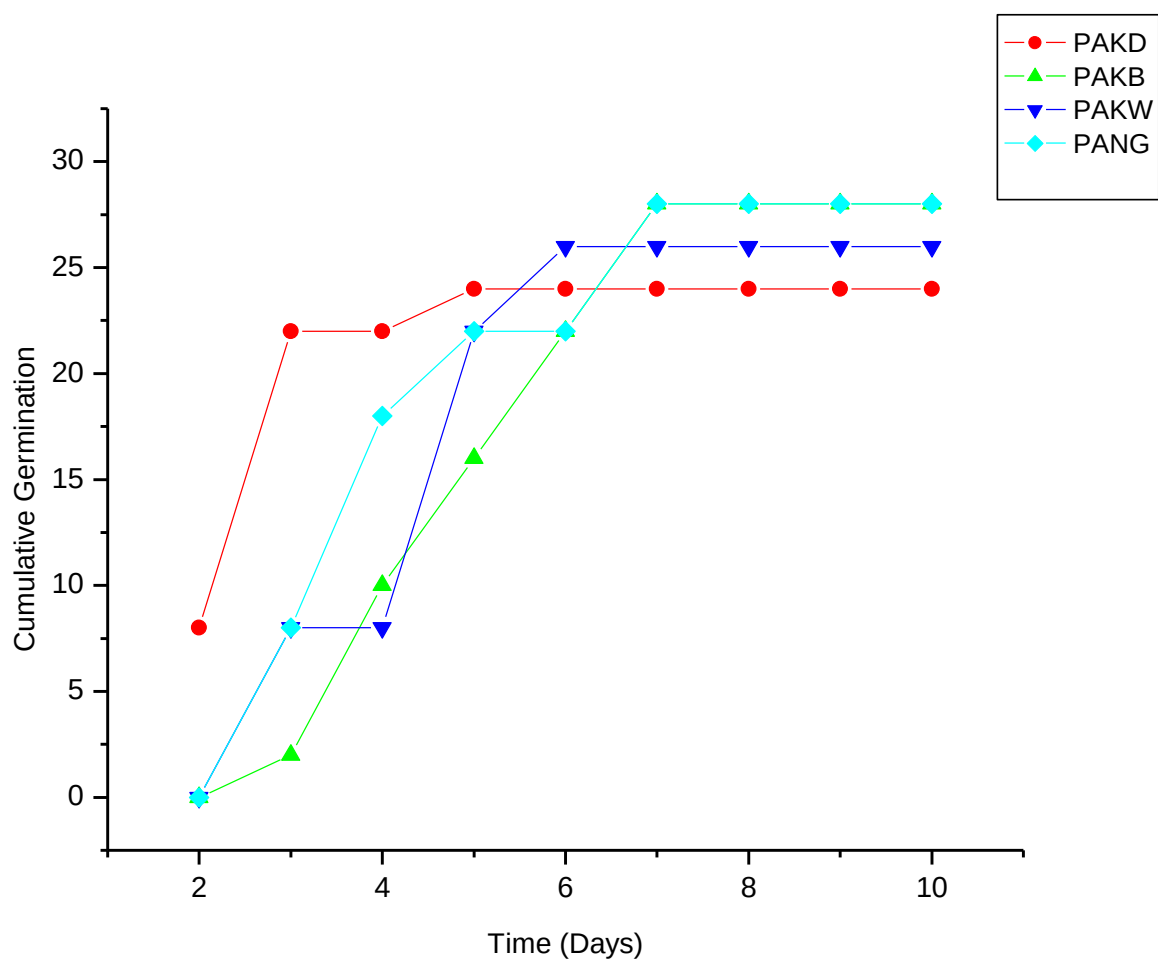


Fig. 21: Effects of H_2SO_4 on germination of *Prosopis africana* seeds at 20 mins scarification period

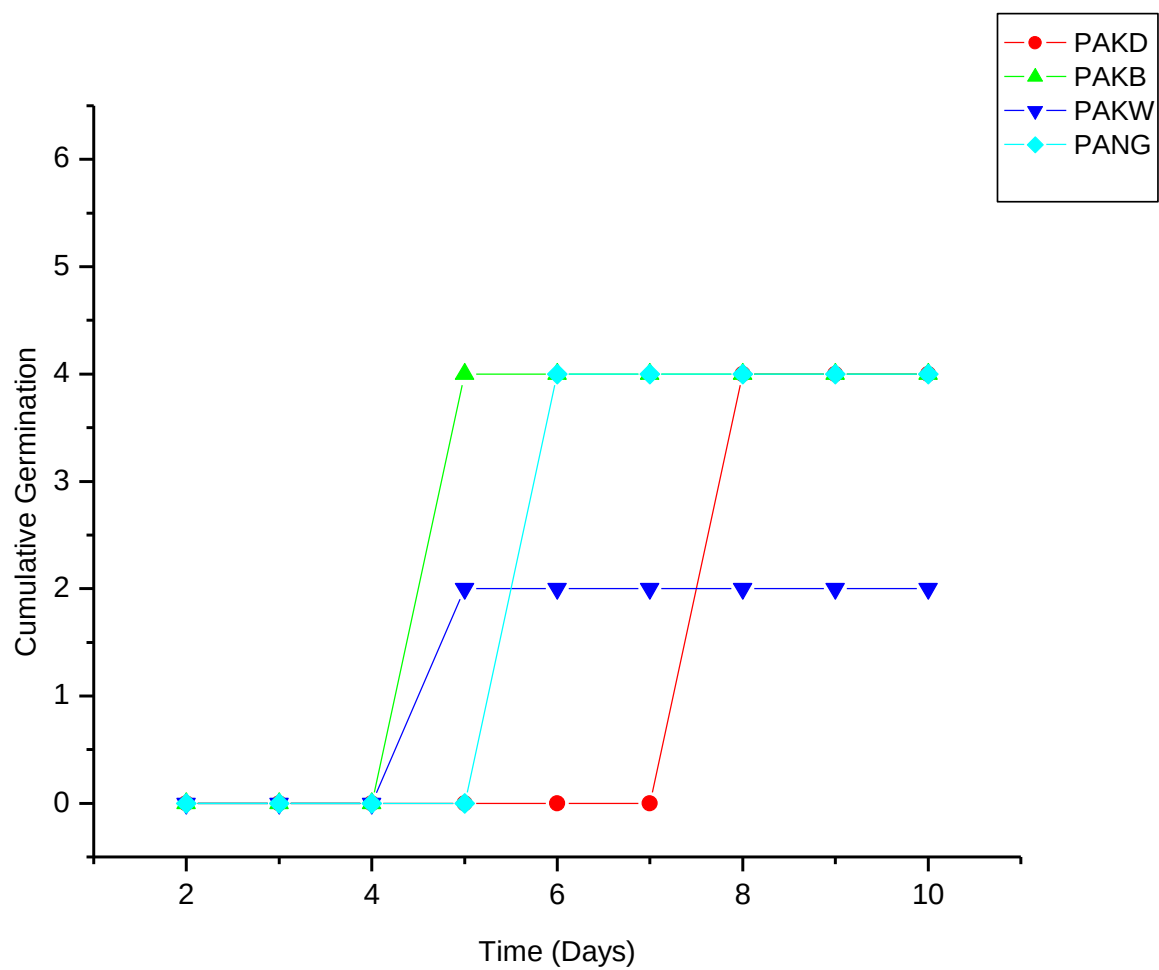


Fig. 22: Effects of HCl on germination of *Prosopis africana* seeds at 5 mins scarification period

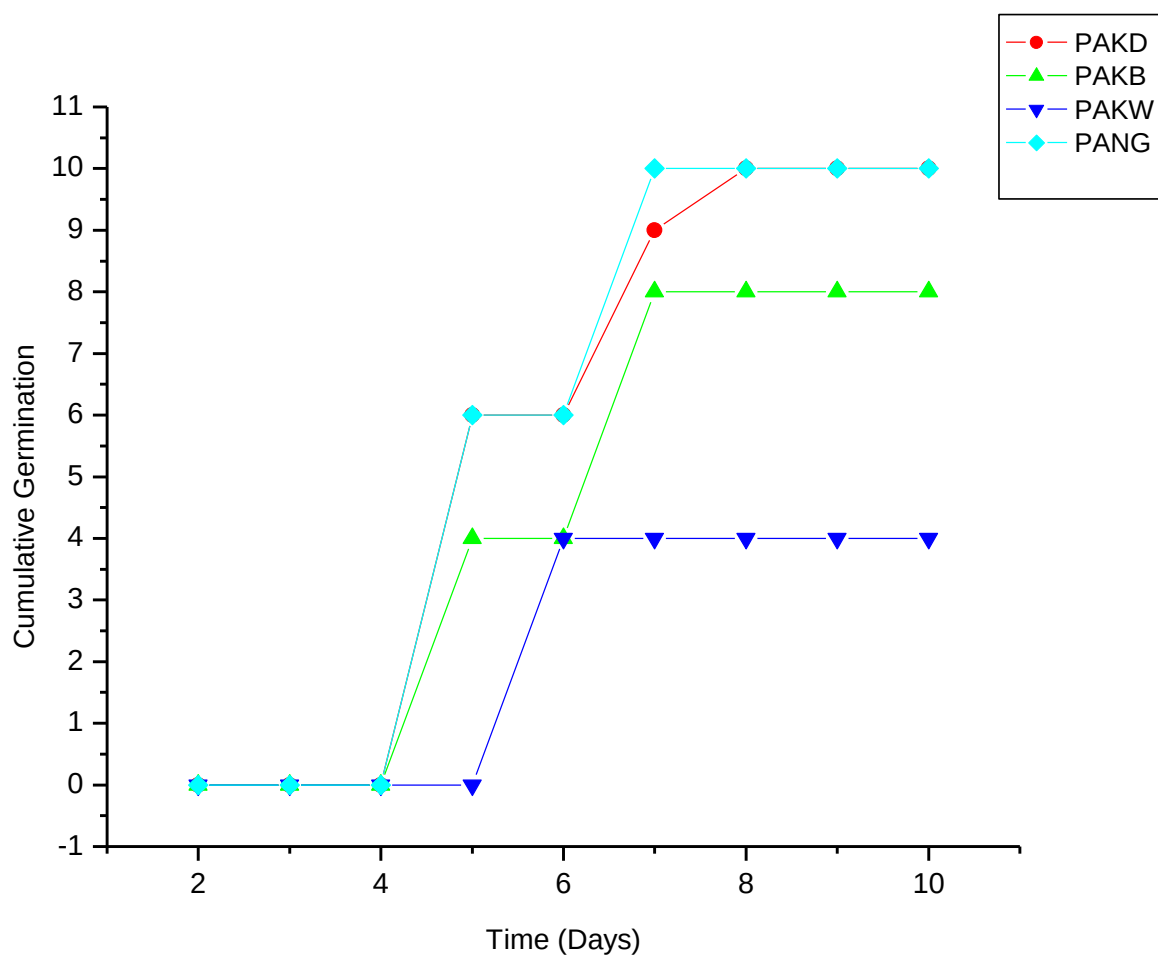


Fig. 23: Effects of HCl on germination of *Prosopis africana* seeds at 10 mins scarification period

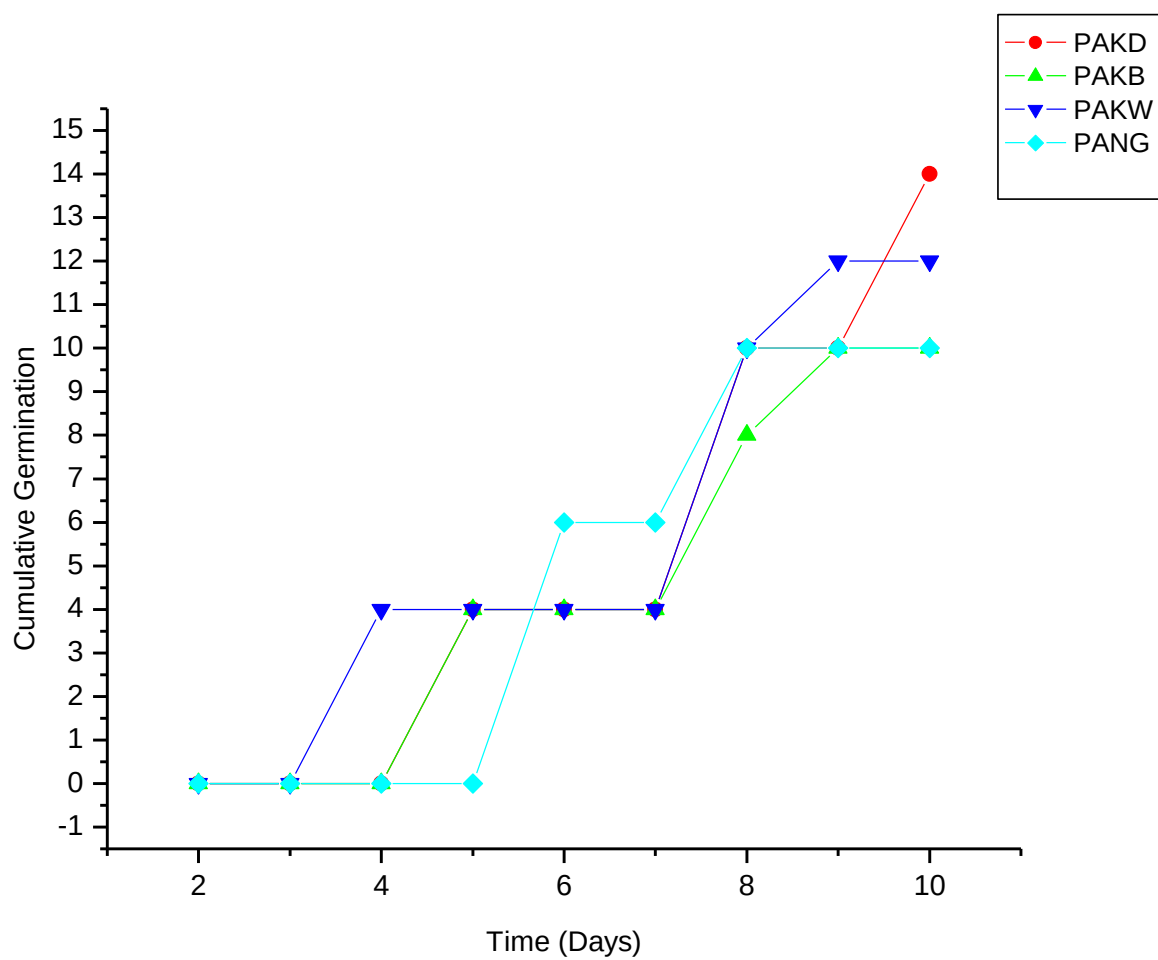


Fig. 24: Effects of HCl on germination of *Prosopis africana* seeds at 15 mins scarification period

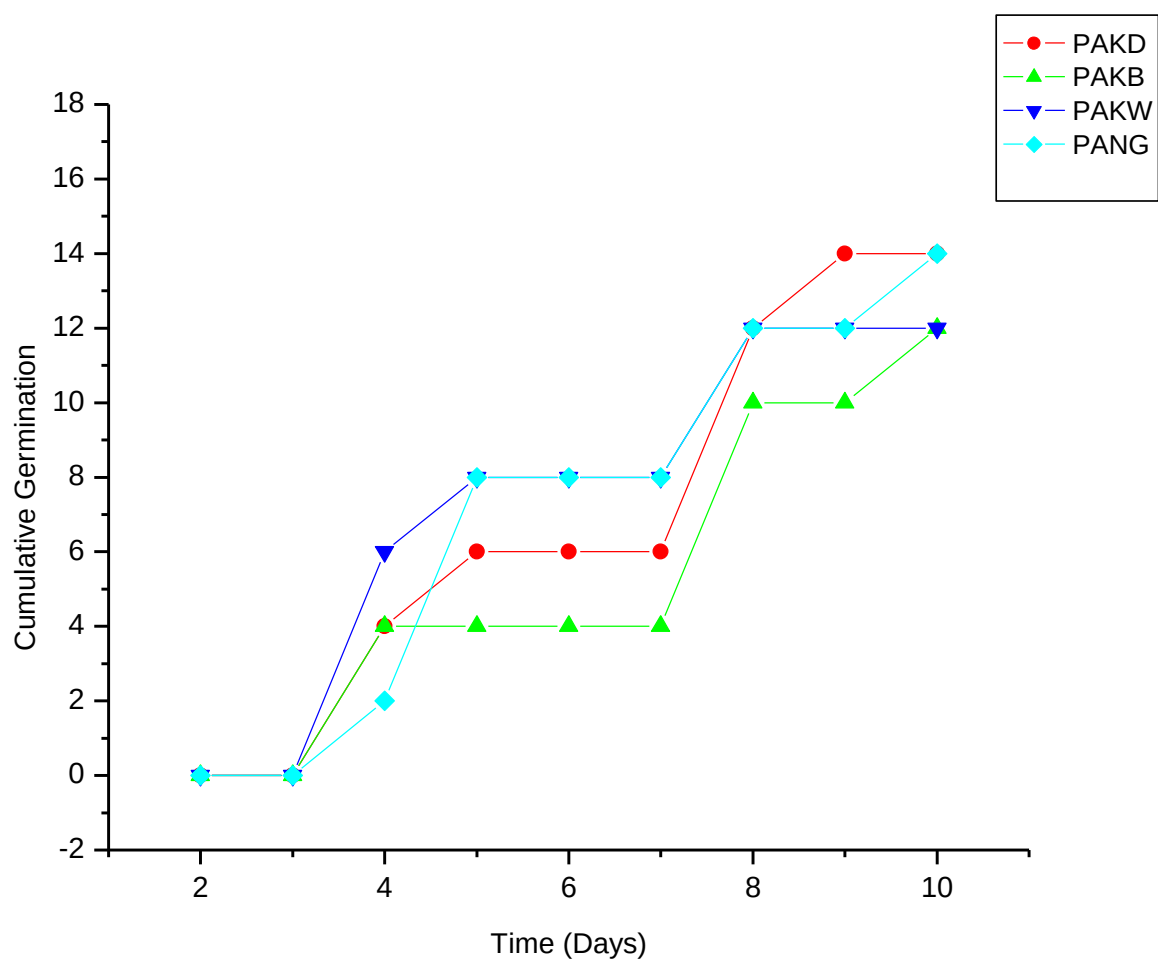


Fig. 25: Effects of HCl on germination of *Prosopis africana* seeds at 20 mins scarification period

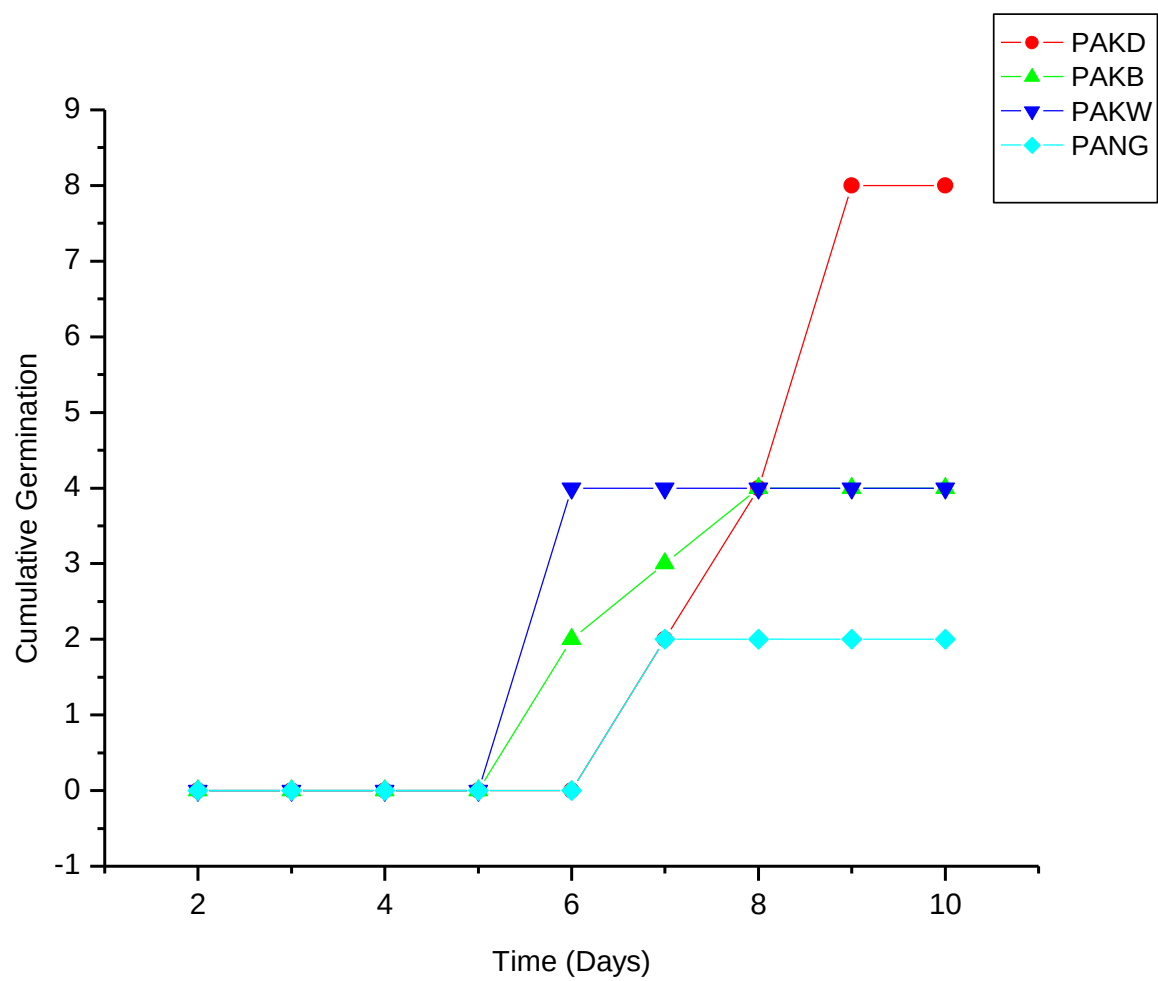


Fig. 26: Effects of HNO_3 on germination of *Prosopis africana* seeds at 5 mins scarification period

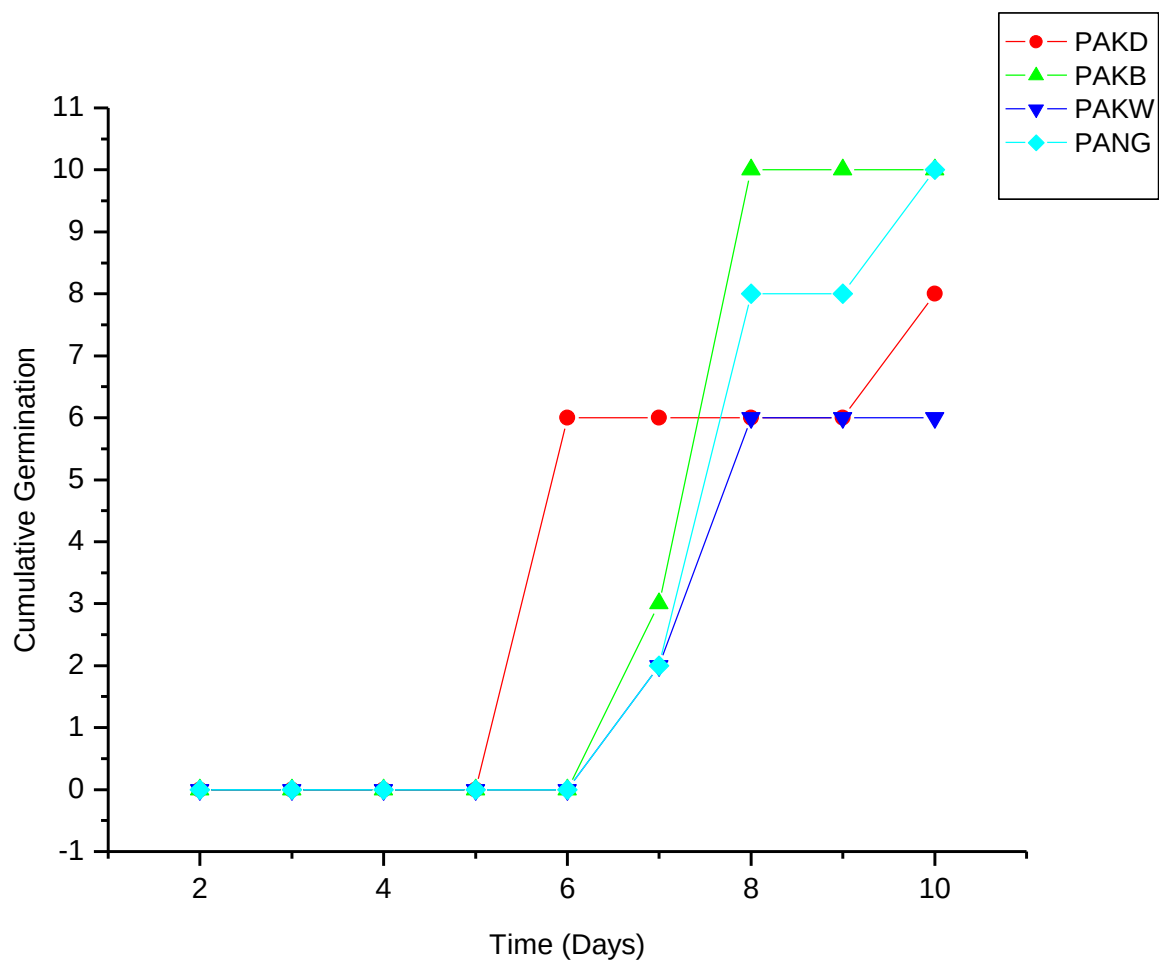


Fig. 27: Effects of HNO_3 on germination of *Prosopis africana* seeds at 10 mins scarification period

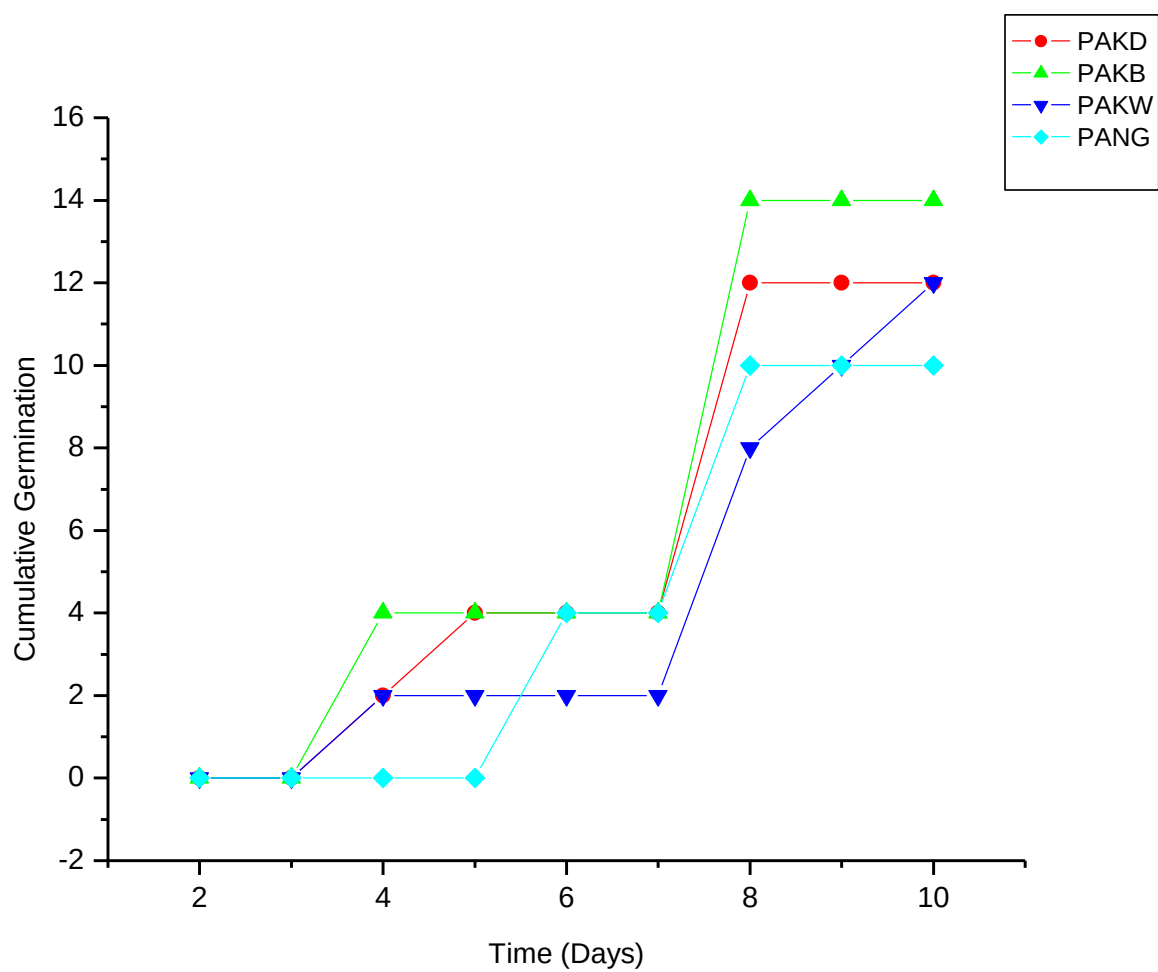


Fig. 28: Effects of HNO₃ on germination of *Prosopis africana* seeds at 15 mins scarification period

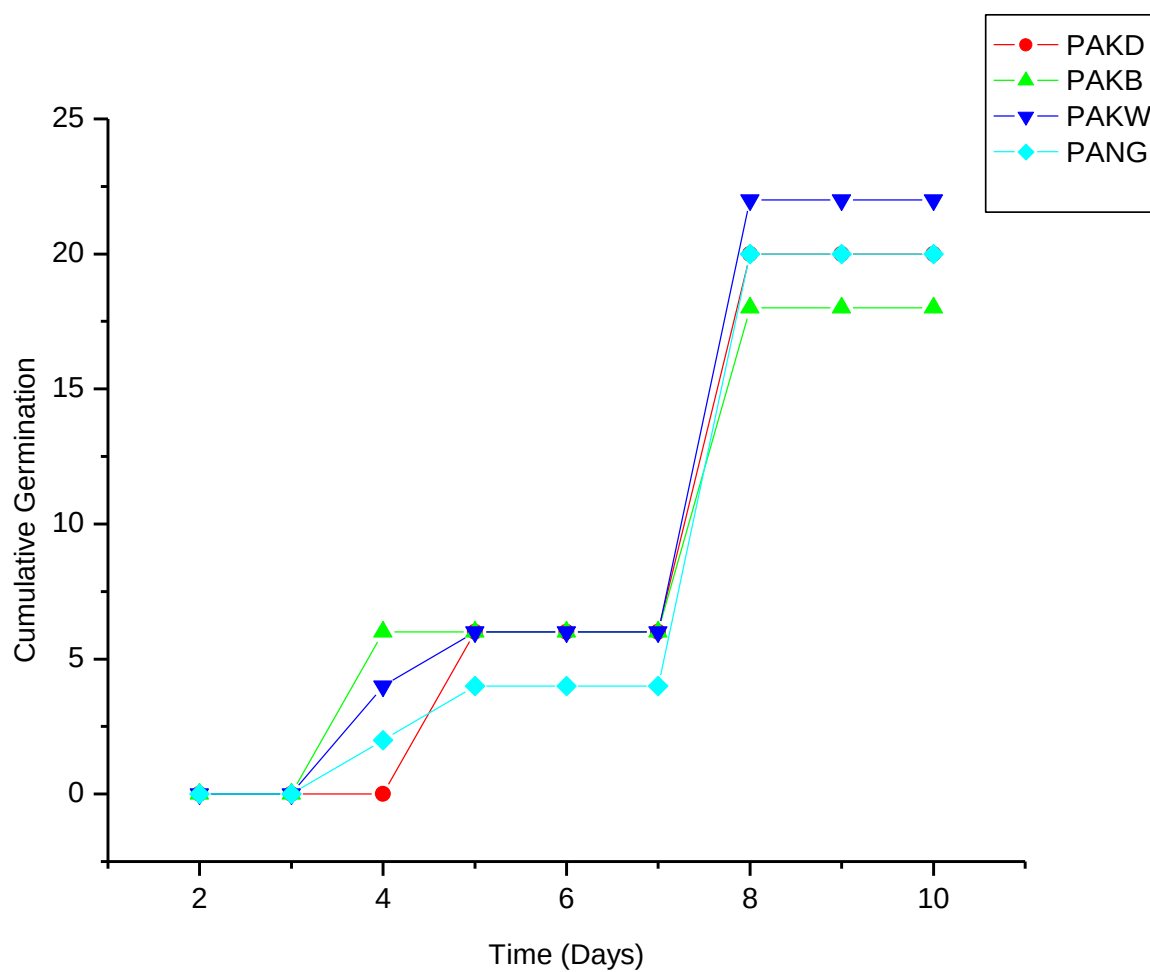


Fig. 29: Effects of HNO₃ on germination of *Prosopis africana* seeds at 20 mins scarification period.

4.3 Germination indices

The results of six germination indices determined for the *Parkia biglobosa* and *Prosopis africana* seeds used for the study are represented in Tables 6-11.

Germination capacity (GC) above 50% was acceptable as fairly good for the seed samples. The coefficient of the germination (CRG) index varies within the seed samples as the calculation was based on the number of seeds germinated and it is the reciprocal of M-days in percentage. Based on this, the shortest M-days were considered effective. This can be observed in PBKD and PBKW at 20 mins scarification with H_2SO_4 acid treatment when compared with HCl and HNO_3 treatments (Tables 6-8). The treatment of *Parkia biglobosa* seeds with H_2SO_4 recorded 100% germination (Table 6). More than 50% germination were recorded with HCl and HNO_3 (Tables 7 and 8) in almost all the seed samples at different duration of treatment. Less than 50% germination occurred in seeds scarified for 5 mins. From the indices, the treatments were effective in breaking the seed dormancy of *Parkia* unlike *Prosopis*. The longest mean germination time (6.33) was observed in PBKT at 5 mins for HCl and HNO_3 respectively. The CRG was highest (50%) in PBKW scarified with H_2SO_4 for 20 mins and lowest (15.79%) in PBKT with 5 mins HNO_3 scarification. The average seed germinated per day was high in PBKW 20 mins scarification with H_2SO_4 where, germination of the seeds occurred within the first three days of planting. Low germination of seeds per day were noted in seeds scarified with HNO_3 and some in HCl (Tables 10 and 11).

All T50 with zero value indicated that either there was no germination or the number of seeds germinated were not up to 50% of the total seeds sown. M-days means the mean germination time for each of the treatment at different time. For *Prosopis africana*, the day for 50% seed germination was best at treatment with H_2SO_4 compared with HCl and HNO_3 in 15 and 20 minutes with all seed samples at values ranged between 1-3 and with corresponding T50 of 1-5 (Tables 9-11).

Table 6: Germination indices of *Parkia biglobosa* at different time of Scarification with H₂SO₄

Seed Samples	Time	TG	M- days	T50	GC (%)	S	AS	ASGD	CRG (%)
PBJG	5	27	4.56	4	90	6.33	26.93	3.00	21.95
	10	30	3.80	3	100	9.63	36.74	3.75	26.32
	15	27	2.67	2	90	12.38	47.30	3.38	37.50
	20	30	2.60	2	100	13.88	53.09	3.75	38.46
PBKW	5	27	4.11	4	90	7.83	32.73	3.86	24.32
	10	30	2.90	3	100	11.25	45.87	7.50	34.48
	15	30	2.20	2	100	14.25	55.37	7.50	45.45
	20	30	2.00	2	100	15.00	57.87	10.00	50.00
PBKD	5	21	4.14	4	70	5.25	22.66	3.50	24.14
	10	30	3.60	4	100	8.50	36.87	7.50	27.78
	15	18	3.67	4	60	5.00	21.72	4.50	27.27
	20	27	2.20	2	90	12.50	49.08	9.00	45.00
PBKT	5	9	5.33	0	30	1.88	7.58	1.29	18.75
	10	12	5.25	0	40	2.61	10.45	1.71	19.05
	15	18	5.50	7	60	3.73	14.74	3.00	18.18
	20	18	5.33	7	60	3.98	15.74	2.25	18.75

Key: Germination capacity (GC), 50% germination (T50), mean germination time (M-days), coefficient of the velocity or rate of germination (CRG), speed of germination (S), accumulated germination speed (AS). To better understand AS, the average germinated seed per day (AGSD) was calculated.

Parkia biglobosa = PB: Kaduna = PBKD; Jigawa = PBJG; Katsina = PBKT; Kwara = PBKW

Table 7: Germination indices of *Parkia biglobosa* at different time of Scarification with HCl

Seed Samples	Time	TG	M- days	T50	GC (%)	S	AS	ASGD	CRG (%)
PBJG	5	9	4.00	0	30	2.25	9.86	2.25	25.00
	10	12	4.25	0	40	3.38	13.87	3.00	23.53
	15	18	3.00	3	60	6.00	25.72	6.00	33.33
	20	18	3.83	4	60	5.00	21.81	2.57	26.09
PBKW	5	6	3.50	0	20	1.75	7.57	1.50	28.57
	10	15	4.00	8	50	4.38	18.16	1.25	25.00
	15	21	4.57	6	70	5.38	22.03	2.63	21.88
	20	21	4.29	3	70	5.80	23.88	2.63	23.33
PBKD	5	6	3.50	0	20	1.75	7.57	1.50	28.57
	10	15	3.40	5	50	4.60	13.68	3.00	29.41
	15	21	5.57	6	70	4.36	17.80	3.00	17.95
	20	18	4.67	8	60	4.75	19.16	2.25	21.43
PBKT	5	9	6.33	0	30	1.46	6.16	1.29	15.79
	10	12	5.25	0	40	2.98	10.37	3.00	19.05
	15	16	5.60	8	50	2.98	11.71	2.00	17.86
	20	17	5.00	8	50	3.48	14.06	2.13	20.00

Key: Germination capacity (GC), 50% germination (T50), mean germination time (M-days), coefficient of the velocity or rate of germination (CRG), speed of germination (S), accumulated germination speed (AS). To better understand AS, the average germinated seed per day (AGSD) was calculated.

Parkia biglobosa = PB: Kaduna = PBKD; Jigawa = PBJG; Katsina = PBKT; Kwara = PBKW

Table 8: Germination indices of *Parkia biglobosa* at different time of Scarification with HNO₃

Seed Samples	Time	TG	M-days	T50	GC (%)	S	AS	ASGD	CRG (%)
PBJG	5	15	6.2	7	50	2.49	9.38	2.14	16.13
	10	21	5.14	7	70	4.79	19.11	3.00	19.44
	15	21	4.14	5	70	5.60	23.56	3.50	24.14
	20	27	4.88	6	90	6.36	25.83	3.86	20.45
PBKW	5	9	4.33	0	30	2.20	9.36	1.80	23.08
	10	21	5.43	7	70	4.46	17.61	3.00	18.42
	15	27	3.56	5	90	6.46	26.43	3.86	28.13
	20	27	4.56	3	90	6.86	28.18	3.86	21.95
PBKD	5	12	5.00	7	40	2.63	10.80	1.71	20.00
	10	27	4.25	0	90	3.10	17.82	2.00	23.53
	15	27	5.33	6	90	5.56	22.33	3.86	18.75
	20	27	4.78	5	90	6.46	26.43	3.86	20.93
PBKT	5	9	6.33	0	30	1.46	5.41	1.29	15.79
	10	15	5.20	7	50	3.21	12.98	2.14	19.23
	15	21	5.71	7	70	3.99	15.61	3/00	17.50
	20	27	5.44	6	90	5.56	22.08	3.86	18.37

Key: Germination capacity (GC), 50% germination (T50), mean germination time (M-days), coefficient of the velocity or rate of germination (CRG), speed of germination (S), accumulated germination speed (AS). To better understand AS, the average germinated seed per day (AGSD) was calculated.

Parkia biglobosa = PB: Kaduna = PBKD; Jigawa = PBJG; Katsina = PBKT; Kwara = PBKW

Table 9: Germination indices of *Prosopis africana* at different time of Scarification with H₂SO₄

Seed Samples	Time	TG	M- days	T50	GC (%)	S	AS	ASGD	CRG (%)
PAKD	5	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0
	15	8	3.00	0	26.67	1.47	5.8	1.14	33.33
	20	24	1.87	3	80.00	8.63	35.30	3.43	53.33
PAKB	5	4	2.00	0	13.33	1.00	4.38	1.00	50.00
	10	12	2.00	0	40.00	3.00	13.15	3.00	50.00
	15	16	2.43	7	53.33	3.35	14.66	2.29	41.03
	20	28	2.10	5	93.33	6.12	23.44	3.50	47.46
PAKW	5	6	2.50	0	20.00	0.90	5.07	1.20	40.00
	10	12	2.08	0	40.00	2.83	12.97	2.40	48.00
	15	22	2.18	5	73.33	5.59	22.97	2.75	45.83
	20	26	2.57	5	86.67	6.13	25.85	4.33	38.81
PANG	5	4	3.74	0	13.33	0.69	0.96	0.40	26.67
	10	6	1.49	0	20.00	1.00	8.57	1.00	66.67
	15	22	2.13	5	73.33	5.99	23.97	3.14	46.81
	20	28	2.25	4	93.33	6.54	28.64	4.00	44.44

Key: Germination capacity (GC), 50% germination (T50), mean germination time (M-days), coefficient of the velocity or rate of germination (CRG), speed of germination (S), accumulated germination speed (AS). To better understand AS, the average germinated seed per day (AGSD) was calculated.

Prosopis africana = PA: Kaduna = PAKD; Kwara = PAKW; Niger = PANG; Kebbi = PAKB

Table 10: Germination indices of *Prosopis africana* at different time of Scarification with HCl

Seed Sample	Time	TG	M- days	T50	GC (%)	S	AS	ASGD	CRG (%)
PAKD	5	4	3.50	0	13.33	0.57	1.92	0.57	28.57
	10	10	2.90	0	33.33	1.77	6.99	1.43	34.48
	15	14	3.64	0	46.67	2.06	6.66	1.40	27.45
	20	14	3.36	0	46.67	2.48	9.37	1.56	29.78
PAKB	5	4	2.50	0	13.33	0.80	3.38	0.67	40.00
	10	10	2.60	0	33.33	1.62	5.97	1.25	38.46
	15	10	3.30	0	33.33	1.59	5.72	1.11	30.30
	20	12	3.58	0	40.00	2.06	7.46	1.20	27.91
PAKW	5	2	2.50	0	6.67	0.33	1.69	0.40	40.00
	10	4	3.00	0	13.33	0.67	2.58	0.66	33.33
	15	12	3.17	0	40.00	2.08	7.68	1.33	31.58
	20	12	2.58	0	40.00	2.47	10.18	1.71	38.71
PANG	5	4	3.00	0	13.33	0.67	4.05	0.66	33.33
	10	10	2.90	0	33.33	1.77	6.99	1.43	34.48
	15	10	3.20	0	33.33	1.86	5.79	1.43	31.25
	20	14	3.07	0	46.67	2.47	9.38	1.40	32.56

Key: Germination capacity (GC), 50% germination (T50), mean germination time (M-days), coefficient of the velocity or rate of germination (CRG), speed of germination (S), accumulated germination speed (AS). To better understand AS, we calculated the average germinated seed per day (AGSD).

Prosopis africana = PA: Kaduna = PAKD; Kwara = PAKW; Niger = PANG; Kebbi = PAKB

Table 11: Germination indices of *Prosopis africana* at different time of Scarification with HNO₃

Seed Samples	Time	TG	M-days	T50	GC (%)	S	AS	ASGD	CRG (%)
PAKD	5	8	4.00	0	26.67	1.00	2.67	1.00	25.00
	10	8	3.50	0	26.67	1.20	2.04	0.60	28.57
	15	12	3.58	0	40.00	1.90	6.57	1.50	27.91
	20	20	3.55	8	66.67	2.60	9.78	2.50	28.17
PAKB	5	4	3.50	0	13.33	0.69	2.65	0.50	28.57
	10	10	4.00	0	33.33	1.25	3.76	1.25	25.00
	15	14	2.67	0	46.67	2.25	7.74	1.75	37.50
	20	18	3.33	8	60.00	3.00	10.61	2.25	30.00
PAKW	5	4	3.00	0	13.33	0.66	2.58	0.67	33.33
	10	6	4.00	0	20.00	0.75	2.02	0.75	25.00
	15	12	3.83	0	40.00	1.70	5.08	1.20	26.09
	20	22	3.50	8	73.33	3.40	11.45	2.75	28.57
PANG	5	2	4.00	0	6.67	0.25	0.67	0.25	25.00
	10	10	4.20	0	33.33	1.20	2.89	1.00	23.81
	15	10	3.60	0	33.33	1.42	4.6	1.25	27.78
	20	20	3.70	8	66.67	2.90	8.07	2.25	27.03

Key: Germination capacity (GC), 50% germination (T50), mean germination time (M-days), coefficient of the velocity or rate of germination (CRG), speed of germination (S), accumulated germination speed (AS). To better understand AS, the average germinated seed per day (AGSD) was calculated.

Prosopis africana = PA: Kaduna = PAKD; Kwara = PAKW; Niger = PANG; Kebbi = PAKB

Seeds of *Parkia biglobosa* and *Prosopis africana* were mechanically scarified using sandpaper and the results indicated that average germinated seeds per day (AGSD) were very low for *Prosopis* throughout the period for germination test for all treatments and the index values ranged from 0.25-4.33. The germination indices of *Parkia biglobosa* and *Prosopis africana*, indicated that mechanical scarification supported the breaking of dormancy of the seeds. Total germination was highest in PBKW and lowest in PBKT. Although, germination capacity recorded was above 50%, the 50% germination was observed on the 4th day after planting except in PBKT. The seeds of PAKW and PANG germinated, the rate of germination was highest in PAKB with 38.46%. The value of T50 for all the seeds sample of *Prosopis africana* were zero (Table 12).

Table 12: Germination indices of *Parkia biglobosa* and *Prosopis africana* seeds with Mechanical scarification

Seed samples	TG	M-days	T50	GC (%)	S	AS	ASGD	CRG (%)
<i>Parkia biglobosa</i>								
PBJG	23	4.30	4	76.67	7.93	33.63	3.5	23.26
PBKW	30	3.10	4	100	14.62	57.37	7.51	32.26
PBKD	22	4.25	4	73.3	8.23	34.64	3.5	23.53
PBKT	17	5.57	6	56.67	4.03	21.55	4.3	17.93
<i>Prosopis africana</i>								
PAKD	4	3.5	0	13.33	0.57	1.92	0.57	28.57
PAKB	10	2.6	0	33.33	1.62	5.97	1.25	38.46
PAKW	4	3	0	13.33	0.67	2.58	0.66	33.33
PANG	10	3.6	0	33.33	1.42	4.6	1.25	27.78

Key: Germination capacity (GC), 50% germination (T50), mean germination time (M-days), coefficient of the velocity or rate of germination (CRG), speed of germination (S), accumulated germination speed (AS). To better understand AS, the average germinated seed per day (AGSD) was calculated.

Parkia biglobosa = PB: Kaduna = PBKD; Jigawa = PBJG; Katsina = PBKT; Kwara = PBKW
Prosopis africana = PA: Kaduna = PAKD; Kwara = PAKW; Niger = PANG; Kebbi = PAKB

4.3 Hormone priming

The results of germination percentage of seeds of *Parkia biglobosa* and *Prosopis africana* collected at different locations primed with Gibberellic acid (GA₃), Indole acetic acid (IAA) and tap water (H₂O) at different concentrations and time are represented in Tables 13-17.

The results showed that primed seed of PBJG with 150 mg/l GA₃ had highest germination percentage while the least was observed in PBKW. Seeds of *Parkia biglobosa* primed with 200 mg/l GA₃ are significantly different in germination % except PBKD and PBJG at priming intervals of 18 and 24 hours. Also PBKT and PBKW were not significantly different at 12, 18 and 24 hours priming at 200mg/g. PBKD recorded highest germination (86.67%) at 250mg/l concentration for 24 hours (Table 13).

There was no significant difference in germination of seeds of *Prosopis africana* primed with 150 mg/l GA₃. Germination was observed to progress with the period of priming except in 12 and 18 hours priming of PAKB at 150mg/l (Table 14). Seeds of PAKD and PAKW primed with 250 mg/l GA₃ germinated better at 24 hours although there was no significant difference in all the seeds of *Prosopis africana* primed (Table 14).

PBJG seeds primed with 150 and 200 mg/l of IAA had the highest germination % of 66.67 and 70.00 respectively at 24 hours of priming. The least germinated seed was observed at 150, 200 and 250 mg/l IAA on PBKT at all the period of priming (Table15).

The primed seed of *Prosopis* with 150 mg/l, 200 mg/l and 250 mg/l IAA shows no significant difference at the time interval of priming (Table 16). PAKW, PAKD and PANG, and PAKB had 70 percent germination at 24 hours of 250 mg/l IAA respectively. The germination % of seeds primed with IAA at different concentration does better than GA₃. *Parkia biglobosa* seeds primed with water recorded 70 % germination in PBJG while the lowest was found in PBKT. The seeds of *Prosopis africana* primed with water had no significant different with the time of priming. PAKD and PAKB

recorded 70% germination while PAKW and PANG recorded 66.67% germination respectively (Table 17).

Hormonal priming pre-treatment affect percentage seed germination of *Parkia biglobosa* and *Prosopis africana*. GA₃ at 200 mg/l and 250 mg/l have significant higher germination percentage than IAA and H₂O. The results also showed that there were a significant interaction between the species collected at different location and hormone pre-treatment, and also, between the species and concentration and / or time of priming on the total seed germination of *Parkia biglobosa* and *Prosopis africana*.

Table 13: Germination percentage of Primed seeds of *Parkia biglobosa* with Gibberellic acid (GA₃) concentrations at different priming time

Seed Samples	12 hrs	18 hrs	24 hrs
Conc. 150 mg/l			
PBKD	46.67 ^b	53.33 ^{ab}	63.33 ^a
PBJG	56.67 ^a	60.00 ^a	66.67 ^a
PBKT	43.33 ^b	46.67 ^{bc}	50.00 ^b
PBKW	40.00 ^b	43.33 ^c	56.67 ^b
Conc. 200 mg/l			
PBKD	53.33 ^{ab}	63.33 ^a	70.00 ^a
PBJG	63.33 ^a	66.67 ^a	86.67 ^a
PBKT	46.67 ^b	46.67 ^b	63.33 ^b
PBKW	40.00 ^b	46.67 ^b	70.00 ^b
Conc. 250 mg/l			
PBKD	56.67 ^{ab}	63.33 ^{ab}	86.67 ^a
PBJG	66.67 ^a	70.00 ^a	80.00 ^a
PBKT	56.67 ^a	56.67 ^{ab}	80.00 ^a
PBKW	53.33 ^b	53.33 ^a	80.00 ^a

Values (mean) with the same superscript along the column and concentration are not significantly different at $p \leq 0.05$.

Parkia biglobosa = PB: Kaduna = PBKD; Jigawa = PBJG; Katsina = PBKT; Kwara = PBKW

Table 14: Germination percentage of Primed seeds of *Prosopis africana* with Gibberellic acid (GA₃) concentrations at different priming time

Seed Samples	12 hrs	18 hrs	24 hrs
Conc. 150 mg/l			
PAKD	43.33 ^a	46.67 ^a	70.00 ^a
PAKB	46.67 ^a	46.67 ^a	70.00 ^a
PAKW	43.33 ^a	46.67 ^a	63.33 ^a
PANG	40.00 ^a	43.33 ^a	76.67 ^a
Conc. 200mg/l			
PAKD	40.67 ^a	50.00 ^a	73.33 ^a
PAKB	46.67 ^a	50.00 ^a	76.67 ^a
PAKW	46.67 ^a	50.00 ^a	63.33 ^a
PANG	40.00 ^a	46.67 ^a	76.67 ^a
Conc. 250mg/l			
PAKD	50.00 ^a	50.00 ^a	86.67 ^a
PAKB	50.00 ^a	63.33 ^a	80.00 ^a
PAKW	46.67 ^a	50.00 ^a	86.67 ^a
PANG	46.67 ^a	53.33 ^a	80.00 ^a

Values (mean) with the same superscript along the column and concentration are not significantly different at $p \leq 0.05$.

Prosopis africana = PA: Kaduna = PAKD; Kwara = PAKW; Niger = PANG; Kebbi = PAKB

Table 15: Germination percentage of Primed seeds of *Parkia biglobosa* with Indole Acetic Acid (IAA) concentrations at different priming time

Seeds	12 hrs	18 hrs	24 hrs
Conc. 150 mg/l			
PBKD	43.33 ^a	46.67 ^a	56.67 ^a
PBJG	56.67 ^a	63.33 ^a	66.67 ^a
PBKT	40.00 ^a	43.33 ^a	50.00 ^a
PBKW	50.00 ^a	53.33 ^a	63.33 ^a
Conc. 200 mg/l			
PBKD	46.67 ^a	50.00 ^a	53.33 ^a
PBJG	56.67 ^a	56.67 ^a	70.00 ^a
PBKT	43.33 ^a	50.00 ^a	53.33 ^a
PBKW	53.33 ^a	50.00 ^a	66.67 ^a
Conc. 250 mg/l			
PBKD	56.67 ^a	50.00 ^a	53.33 ^{ab}
PBJG	60.00 ^a	56.67 ^a	63.33 ^a
PBKT	46.67 ^a	46.67 ^a	46.67 ^b
PBKW	50.00 ^a	53.33 ^a	56.67 ^{ab}

Values (mean) with the same superscript along the column and concentration are not significantly different at $p \leq 0.05$.

Parkia biglobosa = PB: Kaduna = PBKD; Jigawa = PBJG; Katsina = PBKT; Kwara = PBKW

Table 16: Germination percentage of Primed seeds of *Prosopis africana* with Indole Acetic Acid (IAA) concentrations at different priming time

Seeds	12 hrs	18 hrs	24 hrs
Conc. 150 mg/l			
PAKD	50.00 ^a	53.33 ^a	60.00 ^a
PAKB	60.00 ^a	63.33 ^a	63.33 ^a
PAKW	63.33 ^a	66.67 ^a	70.00 ^a
PANG	56.67 ^a	63.33 ^a	66.67 ^a
Conc. 200 mg/l			
PAKD	53.33 ^a	56.67 ^a	70.00 ^a
PAKB	63.33 ^a	60.00 ^a	66.67 ^a
PAKW	60.00 ^a	63.33 ^a	66.67 ^a
PANG	60.00 ^a	60.00 ^a	70.00 ^a
Conc. 250 mg/l			
PAKD	53.33 ^a	53.33 ^a	60.00 ^a
PAKB	60.00 ^a	63.33 ^a	70.00 ^a
PAKW	63.33 ^a	63.33 ^a	66.67 ^a
PANG	56.67 ^a	60.00 ^a	66.67 ^a

Values (mean) with the same superscript along the column and concentration are not significantly different at $p \leq 0.05$.

Prosopis africana = PA: Kaduna = PAKD; Kwara = PAKW; Niger = PANG; Kebbi = PAKB

Table 17: Germination percentage of Primed seeds of *Parkia biglobosa* and *Prosopis africana* with water at different priming time

Seeds	12 hrs	18 hrs	24 hrs
<i>Parkia biglobosa</i>			
PBKD	53.33 ^a	60.00 ^a	76.67 ^a
PBJG	60.00 ^a	66.67 ^a	70.00 ^{ab}
PBKT	46.67 ^a	53.33 ^a	60.00 ^a
PBKW	53.33 ^a	60.00 ^a	70.00 ^{ab}
<i>Prosopis africana</i>			
PAKD	60.00 ^a	66.67 ^a	70.00 ^a
PAKB	56.67 ^a	60.00 ^a	70.00 ^a
PAKW	53.33 ^a	53.33 ^a	66.67 ^a
PANG	50.00 ^a	56.67 ^a	66.67 ^a

Values (mean) with the same superscript along the column and plants are not significantly different at $p \leq 0.05$.

Parkia biglobosa = PB: Kaduna = PBKD; Jigawa = PBJG; Katsina = PBKT; Kwara = PBKW

Prosopis africana = PA: Kaduna = PAKD; Kwara = PAKW; Niger = PANG; Kebbi = PAKB

4.4 Seedling growth

The result of shoot and root lengths of seeds primed with hormone indicated that all the hormonal priming techniques for pre-treatment of seeds of *Parkia biglobosa* and *Prosopis africana* improve the post emergent seedling growth. There was no significant difference in shoot length of *Parkia biglobosa* seedling primed with GA₃ (150, 200 and 250 mg/l) at different time interval. This indicated that concentration, location and time of priming does not have effect on the seed length (Table 18).

The root length showed no significant different among the seed samples of *Parkia* with priming with GA₃ (150, 200 and 250 mg/l) at time interval of 12, 18 and 24 hours except in RL24 where PBJG and PBKT was significantly different with values 12.03 cm and 10.23 cm respectively. It was also observed that the seedlings of PBKT had the shortest root (9.87 cm; 10.53 cm) and shoot lengths (9.53 cm; 10.03 cm) irrespective of the hormonal GA₃ and IAA) concentrations and time of seed priming (Table 18).

Seedlings of seed of *Parkia biglobosa* primed with IAA had no significant different in shoot and root lengths at different concentrations and time of priming (Table 19).

Prosopis africana seedlings also showed a positive response to hormonal priming pre-treatment as *Parkia biglobosa*. There was no significant different in almost all the shoot and root lengths across the different concentration of GA₃ and time of priming except in root length of seeds primed with 200 mg/l IAA for 18 hours where PAKB and PAKB were significantly different from PAKW and PANG (Table 20). Also, PAKD and PAKB were significantly different from PAKW and PANG in shoot lengths at time of priming of 12 and 18 hours respectively. At conc. 250 mg/l, PANG recorded the highest root length value of 11.98 cm at RL18 and shoot length of 11.07 cm at SL12 respectively (Table 20).

Like in *Parkia biglobosa*, the shoot and root lengths of seedlings of *Prosopis africana* seeds primed with IAA at different concentrations and time of priming showed no significant difference from one another (Table 21).

The time interval with water priming of *Parkia biglobosa* and *Prosopis africana* seeds did not have effect on the lengths of shoot and roots. The best root and shoot lengths were obtained by seedlings of PBJG and PANG seeds primed with GA₃, IAA and water at different concentrations and prime time.

Table 18: Root and Shoot length (cm) of primed seeds of *Parkia biglobosa* with Gibberellic acid (GA3) at different time interval

Seed samples	RL12	RL18	RL24	SL12	SL18	SL24
Conc. 150 mg/l						
PBKD	11.20±1.22 ^a	10.17±0.69 ^a	10.77±0.82 ^{ab}	10.23±1.89 ^a	9.73±0.76 ^a	10.03±0.55 ^a
PBJG	11.57±0.78 ^a	11.53±1.22 ^a	12.03±1.16 ^a	10.13±0.50 ^a	9.97±0.40 ^a	10.23±0.35 ^a
PBKT	10.57±1.15 ^a	11.20±0.46 ^a	10.23±0.85 ^b	9.73±0.55 ^a	9.83±0.45 ^a	9.57±0.32 ^a
PBKW	10.90±0.62 ^a	11.47±1.30 ^a	11.33±0.50 ^{ab}	10.13±0.31 ^a	10.03±0.29 ^a	9.87±0.31 ^a
Conc. 200 mg/l						
PBKD	11.77±0.86 ^a	10.60±0.10 ^a	11.00±0.78 ^a	10.87±1.5 ^a	10.07±0.72 ^a	10.17±0.92 ^a
PBJG	11.90±0.92 ^a	11.87±1.05 ^a	12.17±1.10 ^a	10.37±0.21 ^a	10.20±0.56 ^a	10.37±0.45 ^a
PBKT	10.73±1.26 ^a	11.27±0.40 ^a	10.57±0.85 ^a	10.03±0.49 ^a	10.00±0.26 ^a	9.67±0.40 ^a
PBKW	11.43±0.57 ^a	11.60±1.11 ^a	11.47±0.58 ^a	10.43±0.46 ^a	10.20±0.30 ^a	10.20±0.61 ^a
Conc. 250 mg/l						
PBKD	10.67±1.39 ^a	10.20±0.61 ^a	10.40±0.78 ^a	10.50±0.98 ^a	9.93±0.23 ^a	10.37±0.29 ^a
PBJG	10.07±0.75 ^a	10.40±0.36 ^a	10.53±0.25 ^a	9.77±0.45 ^a	10.20±0.50 ^a	10.53±0.95 ^a
PBKT	10.03±0.81 ^a	10.20±0.96 ^a	9.87±0.75 ^a	9.70±0.10 ^a	9.87±0.31 ^a	9.53±0.21 ^a
PBKW	10.77±0.15 ^a	10.20±0.52 ^a	10.10±0.50 ^a	10.13±0.49 ^a	10.20±1.23 ^a	9.93±0.23 ^a

Table 19: Root and Shoot length (cm) of primed seeds of *Parkia biglobosa* with Indole acetic acid (IAA) at different time interval

Seed samples	RL12	RL18	RL24	SL12	SL18	SL24
Conc. 150 mg/l						
PBKD	10.83±1.10 ^a	10.20±0.10 ^b	10.90±0.53 ^a	11.50±1.04 ^a	11.97±0.51 ^a	11.17±0.90 ^a
PBJG	11.10±1.00 ^a	11.77±0.58 ^a	11.67±0.51 ^a	11.30±1.01 ^a	11.77±0.61 ^a	11.67±1.18 ^a
PBKT	10.53±0.67 ^a	11.30±1.10 ^{ab}	10.57±0.72 ^a	10.97±0.59 ^a	10.93±1.01 ^a	11.07±0.42 ^a
PBKW	11.47±0.81 ^a	10.70±0.53 ^{ab}	10.90±1.04 ^a	10.97±0.32 ^a	11.17±0.06 ^a	11.73±0.47 ^a
Conc. 200 mg/l						
PBKD	11.73±0.92 ^a	10.83±0.55 ^a	11.67±0.45 ^a	12.13±0.81 ^a	12.23±0.81 ^a	11.57±1.23 ^a
PBJG	11.87±0.68 ^a	12.43±0.35 ^a	12.23±0.51 ^a	12.30±0.50 ^a	11.63±0.38 ^a	12.10±0.82 ^a
PBKT	10.83±0.78 ^a	11.90±0.82 ^a	11.20±1.40 ^a	11.63±1.04 ^a	11.47±1.21 ^a	11.90±0.79 ^a
PBKW	11.90±1.01 ^a	11.17±0.81 ^a	11.47±0.83 ^a	11.27±0.35 ^a	12.13±0.76 ^a	11.90±0.66 ^a
Conc. 250 mg/l						
PBKD	9.87±0.64 ^a	10.17±0.90 ^a	10.17±0.81 ^a	10.27±0.58 ^a	11.33±0.87 ^a	8.07±5.74 ^a
PBJG	11.03±0.21 ^a	10.83±0.25 ^a	10.97±0.32 ^a	11.13±0.25 ^a	11.70±0.36 ^a	11.20±0.36 ^a
PBKT	10.03±0.49 ^a	10.20±1.06 ^a	10.57±0.85 ^a	11.17±0.99 ^a	10.53±0.06 ^a	10.83±0.42 ^a
PBKW	11.13±0.25 ^a	10.87±0.25 ^a	10.37±0.84 ^a	11.50±0.87 ^a	11.17±0.64 ^a	11.30±0.66 ^a

Table 20: Root and Shoot length (cm) of primed seeds of *Prosopis africana* with Gibberellic acid (GA3) at different time interval

Seed samples	RL12	RL18	RL24	SL12	SL18	SL24
Conc. 150 mg/l						
PAKD	11.00±1.31 ^a	9.97±1.10 ^a	10.40±1.14 ^a	8.80±1.31 ^a	8.97±2.29 ^a	9.67±2.28 ^a
PAKB	11.30±1.15 ^a	9.43±1.33 ^a	10.17±1.00 ^a	9.60±1.37 ^a	9.77±1.72 ^a	9.80±0.95 ^a
PAKW	9.97±2.01 ^a	10.13±0.87 ^a	10.70±1.05 ^a	9.97±1.10 ^a	10.60±1.74 ^a	10.70±1.31 ^a
PANG	11.27±1.86 ^a	11.07±1.58 ^a	11.27±1.23 ^a	10.23±1.59 ^a	11.03±0.015 ^a	10.30±1.02 ^a
Conc. 200 mg/l						
PAKD	11.23±1.06 ^a	9.53±0.40 ^b	10.67±1.80 ^a	8.67±0.93 ^b	9.30±0.85 ^b	9.70±2.25 ^a
PAKB	10.57±0.95 ^a	9.30±0.40 ^b	10.57±1.11 ^a	8.47±0.12 ^b	9.47±0.59 ^b	10.07±1.01 ^a
PAKW	10.57±1.10 ^a	11.20±0.56 ^a	10.70±1.10 ^a	10.30±0.76 ^a	10.70±0.90 ^a	10.70±1.10 ^a
PANG	10.97±1.63 ^a	11.20±1.47 ^a	11.07±0.76 ^a	11.17±0.06 ^a	12.17±0.75 ^a	11.07±0.76 ^a
Conc. 250 mg/l						
PAKD	11.23±1.71 ^a	10.23±0.57 ^{bc}	10.47±1.16 ^a	9.37±0.59 ^{bc}	9.70±1.23 ^a	8.67±1.21 ^a
PAKB	10.23±0.70 ^a	9.47±0.70 ^c	9.63±1.10 ^a	8.93±0.58 ^c	9.57±0.97 ^a	9.77±1.72 ^a
PAKW	10.30±0.56 ^a	11.20±0.72 ^{ab}	10.00±0.66 ^a	10.23±0.72 ^{ab}	10.67±1.90 ^a	10.90±1.61 ^a
PANG	11.33±1.08 ^a	11.97±0.64 ^a	10.73±2.27 ^a	11.07±0.23 ^a	10.77±0.60 ^a	10.70±2.46 ^a

Table 21: Root and Shoot length (cm) of primed seeds of *Prosopis africana* with of Indole Acetic Acid (IAA) at different time interval

Seed samples	RL12	RL18	RL24	SL12	SL18	SL24
Conc. 150 mg/l						
PAKD	9.47±0.12 ^a	9.77±0.40 ^a	7.43±5.11 ^a	11.57±0.91 ^a	10.40±0.53 ^a	11.43±0.85 ^a
PAKB	10.53±0.35 ^a	10.40±0.75 ^a	10.23±0.65 ^a	11.60±0.70 ^a	11.67±0.26 ^a	11.53±0.67 ^a
PAKW	9.97±0.55 ^a	10.33±0.38 ^a	10.27±0.49 ^a	11.20±1.45 ^a	11.80±0.95 ^a	10.53±0.67 ^a
PANG	10.70±0.35 ^a	10.67±0.21 ^a	10.30±0.35 ^a	10.97±0.38 ^a	11.33±0.68 ^a	11.70±0.82 ^a
Conc. 200 mg/l						
PAKD	11.00±0.44 ^a	10.83±0.55 ^a	11.30±0.75 ^a	11.73±1.44 ^a	12.23±0.47 ^a	12.23±0.81 ^a
PAKB	10.83±0.25 ^a	11.07±0.10 ^a	10.80±0.30 ^a	11.53±0.78 ^a	11.40±1.35 ^a	11.13±1.17 ^a
PAKW	10.57±0.35 ^a	10.90±0.36 ^a	10.63±0.38 ^a	11.50±0.20 ^a	12.10±0.82 ^a	12.00±0.70 ^a
PANG	11.10±0.88 ^a	11.03±0.32 ^a	11.10±0.63 ^a	12.53±0.31 ^a	11.67±0.86 ^a	12.07±0.69 ^a
Conc. 250 mg/l						
PAKD	10.43±0.25 ^a	10.17±0.06 ^a	10.50±0.53 ^a	11.20±1.01 ^a	11.83±0.46 ^a	11.97±0.51 ^a
PAKB	10.53±0.40 ^a	10.70±0.36 ^a	10.70±0.26 ^a	11.47±0.55 ^a	11.23±1.15 ^a	11.10±1.00 ^a
PAKW	10.33±0.32 ^a	10.37±0.31 ^a	10.40±0.26 ^a	11.40±0.10 ^a	11.33±0.11 ^a	11.47±0.25 ^a
PANG	10.60±0.44 ^a	10.67±0.25 ^a	10.57±0.15 ^a	12.07±0.76 ^a	11.73±0.78 ^a	11.73±0.76 ^a

Table 22: Root and Shoot length (cm) of primed seeds of *Parkia biglobosa* and *Prosopis africana* with water at different priming time

Seed samples	RL12	RL18	RL24	SL12	SL18	
<i>Parkia biglobosa</i>						
PBKD	10.87±1.07 ^a	11.53±0.98 ^a	10.50±0.61 ^b	10.83±1.10 ^{ab}	10.97±0.67 ^{ab}	11.03±1.21 ^a
PBJG	11.87±0.68 ^a	11.93±0.72 ^a	11.87±0.40 ^a	11.87±0.50 ^a	11.70±0.6 ^a	11.50±0.52 ^a
PBKT	11.17±0.90 ^a	11.70±0.53 ^a	10.80±0.52 ^b	10.23±0.12 ^b	10.40±0.17 ^a	10.77±0.51 ^a
PBKW	10.83±1.10 ^a	11.83±0.64 ^a	11.37±0.46 ^{ab}	11.17±0.12 ^{ab}	10.57±0.57 ^a	10.83±0.40 ^a
<i>Prosopis africana</i>						
PAKD	9.80±0.69 ^b	10.67±1.16 ^a	10.40±0.75 ^a	9.90±0.62 ^b	10.03±0.49 ^b	11.13±0.25 ^a
PAKB	9.93±0.58 ^b	10.53±0.35 ^a	10.17±0.55 ^a	10.27±0.50 ^b	10.60±0.10 ^{ab}	10.60±0.10 ^a
PAKW	10.80±0.60 ^{ab}	11.07±1.27 ^a	10.60±0.17 ^a	11.53±0.51 ^a	10.80±0.27 ^{ab}	11.13±1.25 ^a
PANG	11.13±0.55 ^a	11.53±0.67 ^a	10.97±1.36 ^a	11.87±0.84 ^a	11.83±1.17 ^a	11.50±0.87 ^a

All the phytochemicals analyzed in the seeds of *Parkia biglobosa* and *Prosopis africana* collected at different locations are presented in Tables 23 and 24. The result showed that alkaloids and flavonoids were more abundant in PBJG, Steroids was found to be more abundant in PAKD while glycosides were most abundant in PBJG and PBKW, terpenoid was abundant in all the seeds sampled; All the seeds of *Prosopis africana* collected at different locations had the most abundant of saponins (Table 23). Tannins was most abundance in PBKT, PBKW, PAKB, PAKW and PANG. Phenols was more abundance in all the sampled seeds of *Parkia* and *Prosopis* from all locations except in seed of PAKB.

For the quantitative phytochemical compositions in the seeds of *Parkia biglobosa* and *Prosopis africana*, there were significant difference at $P \leq 0.05$. Alkaloids value ranged from 0.91-1.02 in sampled seeds of *Parkia* and 0.85-0.89 in *Prosopis*. The highest value was recorded in PBJG while the lowest value was seen in PAKB. Steroids value was highest in PAKD and lowest in PANG. Flavonoids and Glycosides were highest in PBJG and the lowest values were found in PAKB and PAKW respectively. The highest value of terpenoids was recorded in PAKB and lowest value in PANG. Saponins value was highest in the seeds of PAKD and lowest in PBKT. PANG recorded the highest value in tannins and lowest value in PBKD. The phenols value was highest in PAKB and lowest in PAKD (Table 24).

Table 23: Qualitative Phytochemical compositions in the seeds of *Parkia biglobosa* and *Prosopis africana* from different locations

Samples	Akaloids	Steroids	Flavonoids	Glycosides	Terpenoids	Saponins	Tannins	Phenols
<i>Parkia biglobosa</i>								
PBKD	+	+	+	+	+	+++	++	++
PBJG	++	+	++	+++	+	+	++	++
PBKT	+	+	+	++	+	+	+++	++
PBKW	+	+	+	+++	+	+++	+++	++
<i>Prosopis Africana</i>								
PAKD	+	++	+	++	+	+++	++	++
PAKB	+	+	+	++	+	+++	+++	+++
PAKW	+	+	+	+	+	+++	+++	++
PANG	+	+	+	++	+	+++	+++	++

Key: + = abundance; ++ = more abundance; +++ = most abundance

Parkia biglobosa = PB: Kaduna = PBKD; Jigawa = PBJG; Katsina = PBKT; Kwara = PBKW

Prosopis africana = PA: Kaduna = PAKD; Kwara = PAKW; Niger = PANG; Kebbi = PAKB

Table 24: Quantitative Phytochemical compositions in the seeds of *Parkia biglobosa* and *Prosopis africana* from different locations

Sample	Alkaloids	Steroids	Flavonoids	Glycosides	Terpenoids	Saponins	Tanins	Phenols
<i>Parkia biglobosa</i>								
PBKD	0.91±0.02 ^{cd}	0.38±0.01 ^{ab}	0.37±0.04 ^{ab}	10.52±0.91 ^d	0.30±0.02 ^{ab}	8.54±0.07 ^a	3.54±0.02 ^g	11.01±0.01 ^c
PBJG	1.02±0.06 ^a	0.35±0.01 ^{b-e}	0.39±0.01 ^a	17.40±0.02 ^a	0.31±0.01 ^{ab}	6.39±0.02 ^d	3.63±0.04 ^f	12.12±0.02 ^c
PBKT	0.93±0.04 ^{b-d}	0.37±0.02 ^{a-c}	0.31±0.01 ^c	16.37±0.02 ^c	0.32±0.01 ^a	5.72±0.05 ^f	4.21±0.01 ^c	11.99±0.12 ^c
PBKW	0.94±0.03 ^{bc}	0.32±0.02 ^{ef}	0.28±0.01 ^d	17.00±0.02 ^{ab}	0.28±0.01 ^{bc}	8.22±0.04 ^b	4.31±0.06 ^b	11.09±0.01 ^d
<i>Prosopis africana</i>								
PAKD	0.87±0.02 ^{e-g}	0.39±0.01 ^a	0.36±0.03 ^{ab}	16.45±0.04 ^c	0.31±0.02 ^{ab}	8.56±0.03 ^a	3.71±0.04 ^e	10.18±0.03 ^f
PAKB	0.85±0.01 ^{fg}	0.35±0.03 ^{b-e}	0.26±0.02 ^d	16.51±0.01 ^{bc}	0.33±0.03 ^a	8.23±0.04 ^b	4.34±0.03 ^b	14.52±0.20 ^a
PAKW	0.96±0.01 ^b	0.34±0.04 ^{c-f}	0.35±0.04 ^{a-c}	10.16±0.07 ^d	0.33±0.03 ^a	8.09±0.02 ^c	4.27±0.02 ^{bc}	11.10±0.04 ^d
PANG	0.89±0.02 ^{d-f}	0.33±0.01 ^{d-f}	0.33±0.04 ^{bc}	16.43±0.04 ^c	0.27±0.01 ^c	8.19±0.02 ^b	4.83±0.04 ^a	12.50±0.02 ^b

Values (mean±standard deviation) with the same superscript along the column are not significantly different at p≤0.05.

Parkia biglobosa = PB: Kaduna = PBKD; Jigawa = PBJG; Katsina = PBKT; Kwara = PBKW

Prosopis africana = PA: Kaduna = PAKD; Kwara = PAKW; Niger = PANG; Kebbi = PAKB

Table 25 shows the results of the proximate compositions in the seeds of *Parkia* and *Prosopis* from different location. The results indicated that PBJG had the highest value of moisture while PAKW recorded the lowest value. There was no significant different in the ash content among the seeds of *Parkia* collected from different location. The *Prosopis* seed collected from Kaduna showed the highest value while seeds of *Prosopis* from other locations were not significantly different. The highest value of crude fat was recorded in PBKW while PANG recorded the lowest value. Crude protein was highest in the seeds of PBKW and lowest in PAKD. The crude fibre recorded the highest value in PBKT while PAKW recorded the lowest value. Carbohydrate value was observed to be higher than other proximate compositions analyzed in the seeds of *Parkia* and *Prosopis* from the different locations. The highest value of carbohydrate was observed in PAKD and lowest in PBKT.

Table 26 presents the Reactive Oxygen Species analyzed in the seeds of *Parkia* and *Prosopis*. All seeds sampled contained hydrogen peroxide (H_2O_2), superoxide anion and hydroxyl radicals with high values. The value of H_2O_2 was highest in PBJG and lowest in PBKD. The H_2O_2 content in PBKT, PBKW and PAKW were not significantly different from each other. Similarly, PBKD, PBJG and PAKD were not significantly different from one another in superoxide anion however, the highest value was observed in PBJG and PAKB had the lowest value. The hydroxyl radicals were highest in PBKD and lowest in PANG.

Table 25: Proximate analysis of the seeds of *Parkia biglobosa* and *Prosopis africana* collected at different locations

Sample	Moisture (%)	Ash (%)	Crude Fat (%)	Crude protein (%)	Crude fibre (%)	Carbohydrate (%)
<i>Parkia biglobosa</i>						
PBKD	9.25±0.01 ^{ab}	3.79±0.01 ^c	1.17±0.02 ^b	24.76±0.02 ^{bc}	2.94±0.01 ^b	58.22±0.10 ^{cd}
PBJG	9.29±0.01 ^a	3.76±0.01 ^c	1.26±0.02 ^{ab}	24.77±0.01 ^{bc}	2.92±0.05 ^b	58.09±0.06 ^d
PBKT	9.21±0.02 ^{bc}	3.81±0.01 ^c	1.22±0.01 ^{ab}	24.82±0.03 ^{ab}	3.17±0.38 ^a	58.11±0.16 ^d
PBKW	9.15±0.02 ^c	3.74±0.02 ^c	1.27±0.01 ^a	24.86±0.02 ^a	2.91±0.07 ^{bc}	58.18±0.06 ^{cd}
<i>Prosopis Africana</i>						
PAKD	8.83±0.05 ^{ef}	4.38±0.16 ^a	0.92±0.06 ^d	23.61±0.06 ^f	2.77±0.03 ^{b-d}	59.94±0.04 ^a
PAKB	8.93±0.05 ^d	4.18±0.06 ^b	1.22±0.10 ^{ab}	23.90±0.05 ^d	2.70±0.04 ^{b-d}	59.33±0.05 ^b
PAKW	8.76±0.03 ^g	4.24±0.10 ^b	0.99±0.03 ^{cd}	23.96±0.09 ^d	2.58±0.07 ^d	59.78±0.11 ^a
PANG	8.79±0.01 ^{fg}	4.22±0.02 ^b	0.92±0.04 ^d	23.78±0.03 ^e	2.67±0.06 ^{cd}	59.77±0.12 ^a

Values (mean±standard deviation) with the same superscript along the column are not significantly different at $p \leq 0.05$.

Parkia biglobosa = PB: Kaduna = PBKD; Jigawa = PBJG; Katsina = PBKT; Kwara = PBKW

Prosopis africana = PA: Kaduna = PAKD; Kwara = PAKW; Niger = PANG; Kebbi = PAKB

Table 26: Reactive Oxygen Species Properties in the seeds of *Parkia biglobosa* and *Prosopis africana*

Sample	Hydrogen Peroxide ($\mu\text{mol g/DW}$)	Superoxide Anion ($\mu\text{mol g/DW}$)	Hydroxyl Radicals
<i>Parkia biglobosa</i>			
PBKD	30.43 $\pm$.64 ^c	23.43 $\pm$.21 ^a	82.72 $\pm$.28 ^a
PBJG	32.72 $\pm$.12 ^a	23.66 $\pm$.03 ^a	81.80 $\pm$.83 ^b
PBKT	32.11 $\pm$.03 ^{ab}	22.83 $\pm$.06 ^b	80.88 $\pm$.44 ^e
PBKW	32.21 $\pm$.06 ^{ab}	22.30 $\pm$.30 ^d	80.69 $\pm$.50 ^{cd}
<i>Prosopis africana</i>			
PAKD	30.48 $\pm$.20 ^c	23.50 $\pm$.10 ^a	82.53 $\pm$.27 ^{ab}
PAKB	30.61 $\pm$.41 ^c	21.62 $\pm$.17 ^e	79.91 $\pm$.20 ^{de}
PAKW	32.36 $\pm$.39 ^{ab}	22.84 $\pm$.06 ^b	80.42 $\pm$.52 ^{c-e}
PANG	31.81 $\pm$.21 ^b	22.46 $\pm$.08 ^e	79.78 $\pm$.15 ^{de}

Value (mean $\pm$ standard deviation) with the same superscript along the column are not significantly different at $p \leq 0.05$.

Parkia biglobosa = PB: Kaduna = PBKD; Jigawa = PBJG; Katsina = PBKT; Kwara = PBKW

Prosopis africana = PA: Kaduna = PAKD; Kwara = PAKW; Niger = PANG; Kebbi = PAKB

The economic properties of the seeds of *Parkia* and *Prosopis* collected at different locations was shown in Table 27. These properties showed some economic values of the seed which included saponification value, free fatty acid, iodine value and acid value. The acid value was not significantly different in PBJG, PBKW, PAKD, PAKB, PAKW and PANG, the highest value (3.55) was observed in PBKT while the lowest value (3.32) was in PBKD. Iodine values of PBJG, PBKT, PBKW, PAKD, PAKB, PAKW and PANG were not significantly different one from another with the range of 14.08 – 14.35. The free fatty acid of the seeds of *Parkia* and *Prosopis* were not significantly different except in PBKD and PANG. The saponification value in PBJG was not significantly different from PAKB. PBKD, PBKT.

Table 27: Physicochemical properties of the seeds of *Parkia biglobosa* and *Prosopis africana* collected at different locations

Sample	Acid Value (mgKOH/g)	Iodine Value (mgKOH/g)	Free Fatty Acid (%)	Saponification Value (mgKOH/g)
<i>Parkia biglobosa</i>				
PBKD	3.32±0.11 ^c	14.35±0.30 ^a	1.77±0.12 ^a	199.60±1.02 ^{ab}
PBJG	3.38±0.04 ^{bc}	14.10±0.03 ^b	1.70±0.02 ^{ab}	203.03±4.68 ^a
PBKT	3.55±0.15 ^a	14.11±0.04 ^b	1.71±0.03 ^{ab}	198.30±4.87 ^{ab}
PBKW	3.44±0.14 ^{bc}	14.08±0.10 ^b	1.70±0.02 ^{ab}	195.47±2.05 ^{bc}
<i>Prosopis africana</i>				
PAKD	3.38±0.02 ^{bc}	14.10±0.11 ^b	1.71±0.02 ^{ab}	199.60±2.75 ^{ab}
PAKB	3.38±0.03 ^{bc}	14.16±0.04 ^b	1.71±0.02 ^{ab}	201.07±6.67 ^a
PAKW	3.47±0.11 ^{bc}	14.06±0.03 ^b	1.70±0.02 ^{ab}	195.50±4.89 ^{bc}
PANG	3.40±0.11 ^{bc}	14.05±0.04 ^b	1.68±0.02 ^b	186.70±2.33 ^d

Values (Mean±standard deviation) with the same superscript along the column are not significantly different at $p \leq 0.05$.

The enzyme activities concentrations monitored in planted seeds of *Parkia* and *Prosopis* are amylase, protease and lipase, they are presented in Tables 28- 20. The enzymes activities were observed for 7days. The results are significantly different at $P \leq 0.5$ for some of the seeds sampled and during the seeds germination period, a progressive amylase activity was observed although it was slow as seen in Table 28. The highest amylase activity was found at 7 DAP in PBJG (0.081 $\mu\text{mol/min}$) followed by PBKW (0.073 $\mu\text{mol/min}$) while the lowest value was seen in PANG (0.053 $\mu\text{mol/min}$).

In Table 29, the protease activity was seen to increase from 1 DAP to 2 DAP in all the seeds analyzed. Protease activity in PBKD, PBKD and PANG showed the highest increase at 2 DAP. Form 3DAP, decrease in the protease activity was observed till the 7 DAP except in the seed of PBKW which showed an increase at 7 DAP ($\mu\text{mol/min}$) although, the protease concentration was not significantly different at 7 DAP in all the sampled seeds at different locations. The result of enzyme concentrations reported in Table 30 shows an inconsistency in the lipase activity.

Table 28: Amylase enzyme concentration ($\mu\text{mol}/\text{min}$) in the seeds of *Parkia biglobosa* and *Prosopis africana* collected from different locations at different days after planting

Sample	1	2	3	4	5	6	7
DAYS AFTER PLANTING (DAP)							
<i>Parkia biglobosa</i>							
PBKD	0.022 $\pm$ 0.0005 ^c	0.101 $\pm$ 0.0002 ^a	0.018 $\pm$ 0.0000 ^g	0.046 $\pm$ 0.0000 ^c	0.053 $\pm$ 0.0000 ^d	0.061 $\pm$ 0.0000 ^c	0.072 $\pm$ 0.0000 ^c
PBJG	0.007 $\pm$ 0.0001 ^f	0.100 $\pm$ 0.0001 ^b	0.022 $\pm$ 0.0000 ^e	0.050 $\pm$ 0.0000 ^a	0.062 $\pm$ 0.0000 ^a	0.074 $\pm$ 0.0000 ^a	0.081 $\pm$ 0.0000 ^a
PBKT	0.040 $\pm$ 0.0006 ^a	0.021 $\pm$ 0.0001 ^f	0.042 $\pm$ 0.0000 ^a	0.047 $\pm$ 0.0000 ^b	0.054 $\pm$ 0.0000 ^c	0.061 $\pm$ 0.0000 ^c	0.063 $\pm$ 0.0000 ^d
PBKW	0.017 $\pm$ 0.0001 ^e	0.034 $\pm$ 0.0001 ^c	0.040 $\pm$ 0.0000 ^b	0.047 $\pm$ 0.0000 ^b	0.060 $\pm$ 0.0000 ^b	0.069 $\pm$ 0.0000 ^b	0.073 $\pm$ 0.0000 ^b
<i>Prosopis Africana</i>							
PAKB	0.021 $\pm$ 0.0001 ^d	0.023 $\pm$ 0.0001 ^e	0.033 $\pm$ 0.0000 ^d	0.037 $\pm$ 0.0000 ^f	0.040 $\pm$ 0.0000 ^f	0.046 $\pm$ 0.0000 ^g	0.055 $\pm$ 0.0000 ^g
PAKD	0.007 $\pm$ 0.0001 ^f	0.017 $\pm$ 0.0003 ^g	0.021 $\pm$ 0.0005 ^f	0.043 $\pm$ 0.0000 ^d	0.052 $\pm$ 0.0000 ^e	0.058 $\pm$ 0.0000 ^d	0.062 $\pm$ 0.0000 ^e
PAKW	0.007 $\pm$ 0.0002 ^f	0.012 $\pm$ 0.0001 ^h	0.022 $\pm$ 0.0001 ^e	0.040 $\pm$ 0.0000 ^e	0.040 $\pm$ 0.0000 ^f	0.051 $\pm$ 0.0001 ^e	0.060 $\pm$ 0.0000 ^f
PANG	0.023 $\pm$ 0.0010 ^b	0.023 $\pm$ 0.0001 ^d	0.034 $\pm$ 0.0000 ^c	0.040 $\pm$ 0.0000 ^e	0.040 $\pm$ 0.0000 ^f	0.050 $\pm$ 0.0000 ^f	0.053 $\pm$ 0.0000 ^h

Values (mean $\pm$ standard deviation) with the same superscript along the column are not significantly different at $p\leq 0.05$

Table 29: Protease enzyme concentration ($\mu\text{mol}/\text{min}$) in the seeds of *Parkia biglobosa* and *Prosopis africana* collected from different locations at different days after planting

Sample	1	2	3	4	5	6	7
DAYS AFTER PLANTING (DAP)							
<i>Parkia biglobosa</i>							
PBKD	0.31 $\pm$ 0.0012 ^a	0.37 $\pm$ 0.0013 ^a	0.21 $\pm$ 0.0010 ^c	0.11 $\pm$.0015 ^d	0.10 $\pm$ 0.0038 ^{ab}	0.09 $\pm$ 0.0060 ^{bc}	0.09 $\pm$ 0.0031 ^a
PBJG	0.25 $\pm$ 0.0003 ^e	0.29 $\pm$ 0.0018 ^{bc}	0.22 $\pm$ 0.0009 ^b	0.13 $\pm$.0010 ^c	0.12 $\pm$ 0.0162 ^a	0.10 $\pm$ 0.0001 ^b	0.09 $\pm$ 0.0004 ^a
PBKT	0.24 $\pm$ 0.0006 ^j	0.29 $\pm$ 0.0157 ^b	0.25 $\pm$ 0.0033 ^b	0.17 $\pm$.0047 ^b	0.10 $\pm$ 0.0547 ^{ab}	0.09 $\pm$ 0.0058 ^b	0.09 $\pm$ 0.0059 ^a
PBKW	0.24 $\pm$ 0.0003 ^f	0.28 $\pm$ 0.0149 ^{bc}	0.22 $\pm$ 0.0003 ^b	0.17 $\pm$.0015 ^b	0.14 $\pm$ 0.0154 ^a	0.09 $\pm$ 0.0001 ^{bc}	0.34 $\pm$ 0.4438 ^a
<i>Prosopis africana</i>							
PAKB	0.25 $\pm$ 0.0001 ^d	0.28 $\pm$ 0.0006 ^b	0.16 $\pm$ 0.0001 ^d	0.13 $\pm$.0002 ^c	0.09 $\pm$ 0.0003 ^{ab}	0.09 $\pm$ 0.0049 ^{bc}	0.09 $\pm$ 0.0059 ^a
PAKD	0.25 $\pm$ 0.0001 ^d	0.35 $\pm$ 0.0001 ^a	0.29 $\pm$ 0.0001 ^a	0.12 $\pm$.0161 ^{cd}	0.13 $\pm$ 0.0579 ^a	0.09 $\pm$ 0.0000 ^{bc}	0.08 $\pm$ 0.0204 ^a
PAKW	0.17 $\pm$ 0.0001 ^h	0.26 $\pm$ 0.0002 ^b	0.21 $\pm$ 0.0001 ^c	0.19 $\pm$.0002 ^a	0.10 $\pm$ 0.0020 ^{ab}	0.11 $\pm$ 0.0046 ^a	0.10 $\pm$ 0.0016 ^a
PANG	0.31 $\pm$ 0.0002 ^b	0.35 $\pm$ 0.0001 ^a	0.30 $\pm$ 0.0001 ^a	0.12 $\pm$.0001 ^d	0.09 $\pm$ 0.0058 ^{ab}	0.09 $\pm$ 0.0001 ^{bc}	0.08 $\pm$ 0.0025 ^a

Value (mean $\pm$ standard deviation) with the same superscript along the column are not significantly different at $p\leq 0.05$.

Table 30: Lipase enzyme concentration (U/mol) in the seeds of *Parkia biglobosa* and *Prosopis africana* collected from different locations at different days after planting (DAP)

Sample	1	2	3	4	5	6	7
DAYS AFTER PLANTING (DAP)							
<i>Parkia biglobosa</i>							
PBKD	0.8×10^{-5}	0.11×10^{-4}	0.66×10^{-5}	0.1×10^{-5}	0.87×10^{-4}	0.47×10^{-4}	0.6×10^{-5}
PBJG	0.99×10^{-5}	0.11×10^{-4}	0.58×10^{-5}	0.87×10^{-5}	0.95×10^{-4}	0.43×10^{-4}	0.85×10^{-5}
PBKT	0.3×10^{-5}	0.71×10^{-5}	0.91×10^{-5}	0.16×10^{-4}	0.94×10^{-4}	0.22×10^{-4}	0.25×10^{-5}
PBKW	0.59×10^{-5}	0.99×10^{-5}	0.14×10^{-4}	0.22×10^{-5}	0.93×10^{-4}	0.15×10^{-4}	0.52×10^{-5}
<i>Prosopis africana</i>							
PAKB	0.34×10^{-5}	0.81×10^{-5}	0.71×10^{-5}	0.2×10^{-4}	0.97×10^{-4}	0.47×10^{-4}	0.37×10^{-5}
PAKD	0.98×10^{-5}	0.15×10^{-4}	0.51×10^{-5}	0.51×10^{-5}	0.97×10^{-4}	0.45×10^{-4}	0.42×10^{-5}
PAKW	0.64×10^{-5}	0.12×10^{-4}	0.8×10^{-5}	0.93×10^{-5}	0.96×10^{-4}	0.48×10^{-4}	0.43×10^{-5}
PANG	0.38×10^{-5}	0.17×10^{-4}	0.12×10^{-4}	0.5×10^{-4}	0.92×10^{-4}	0.67×10^{-5}	0.18×10^{-5}

Value (mean±standard deviation) with the same superscript along the column are not significantly different at $p \leq 0.05$.

The electron dispersive x-rayon the elemental composition of the seed coat of *Parkia biglobosa* and *Prosopis africana* were presented in Figs 29-36 below. All spectral indicated that Carbon and Oxygen were abundant in the seed coat.

Other elements found in the seed coat of *Prosopis africana* and *Parkia biglobosa* seeds were summarized in Figs 37 and 38. Calcium had the highest value in all the sampled seed coats followed by Antimony, Tin, Potassium and Surphur.

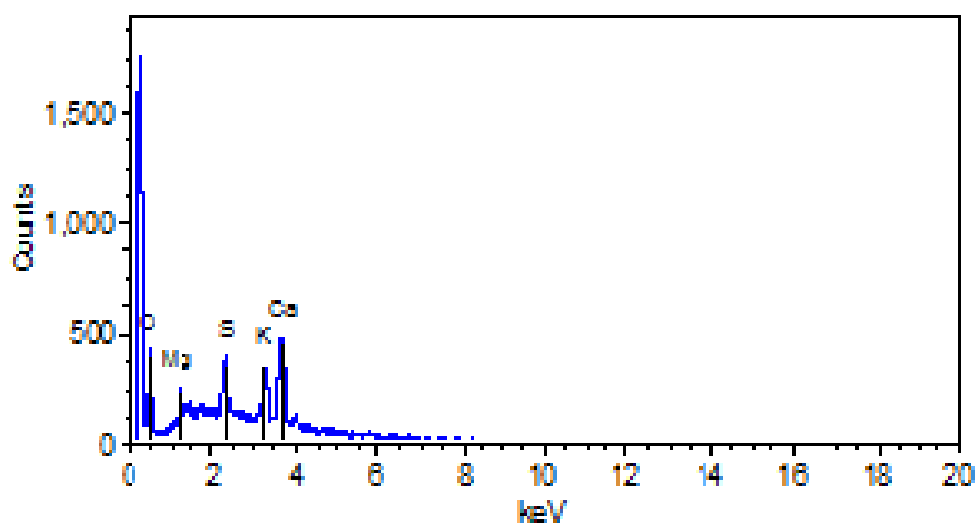


Fig 29: Electron Dispersive X-ray (EDX) on the element composition of PAKB

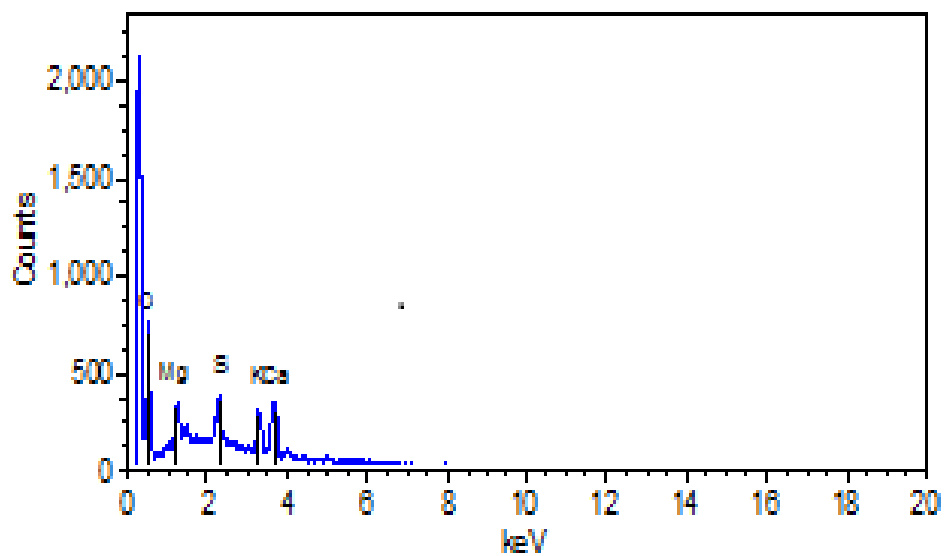


Fig 30: Electron Dispersive X-ray (EDX) on the element composition of PAKD

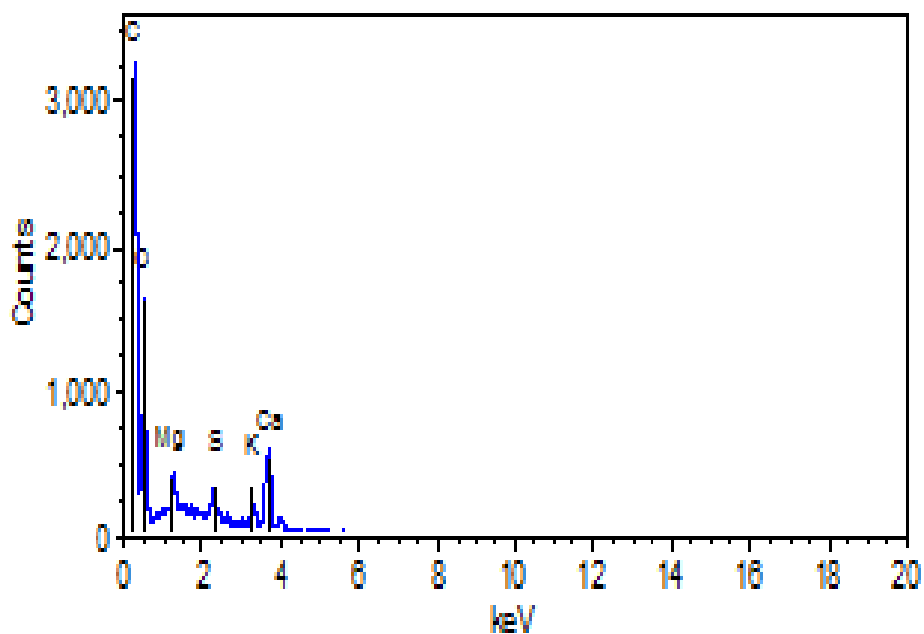


Fig 31: Electron Dispersive X-ray (EDX) on the element composition of PAKW

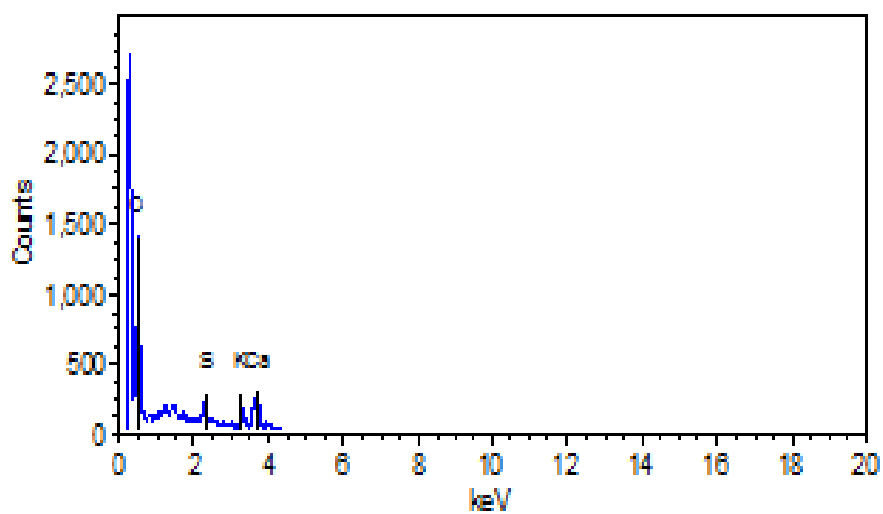


Fig 32: Electron Dispersive X-ray (EDX) on the element composition of PANG

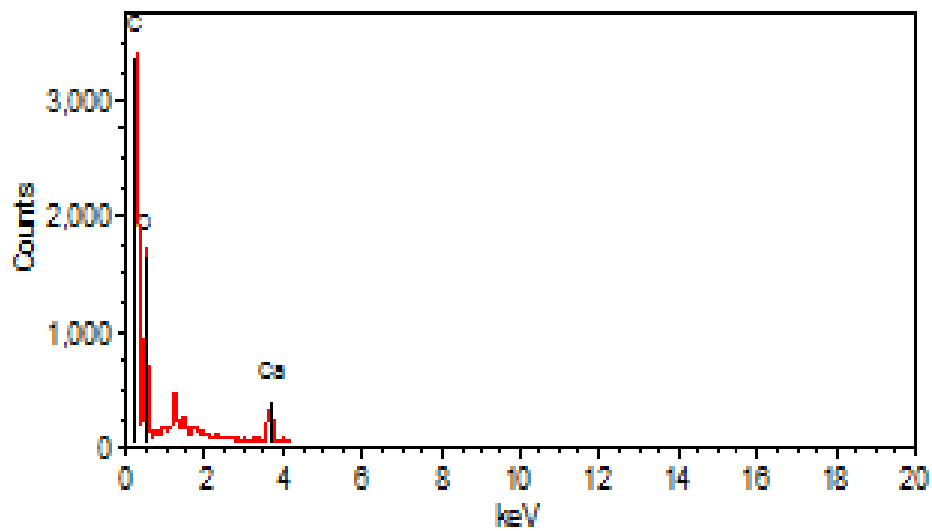


Fig 33: Electron Dispersive X-ray (EDX) on the element composition of PBJG

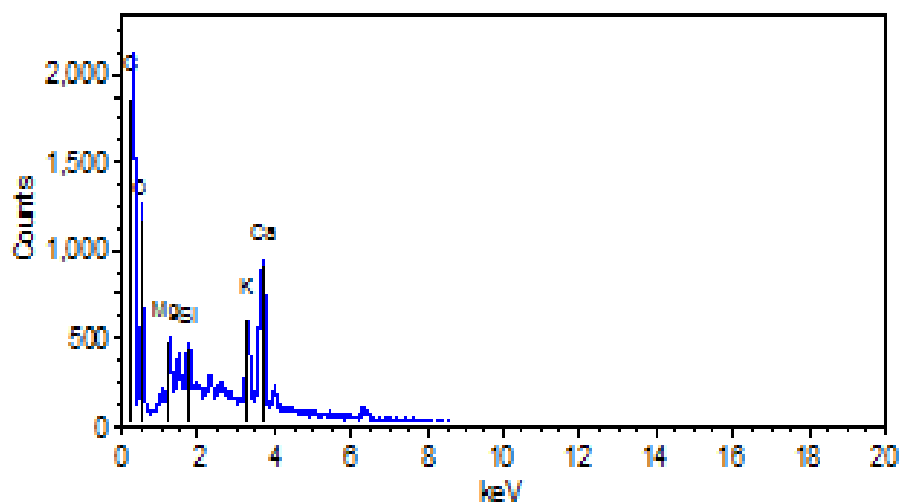


Fig 34: Electron Dispersive X-ray (EDX) on the element composition of PAKD

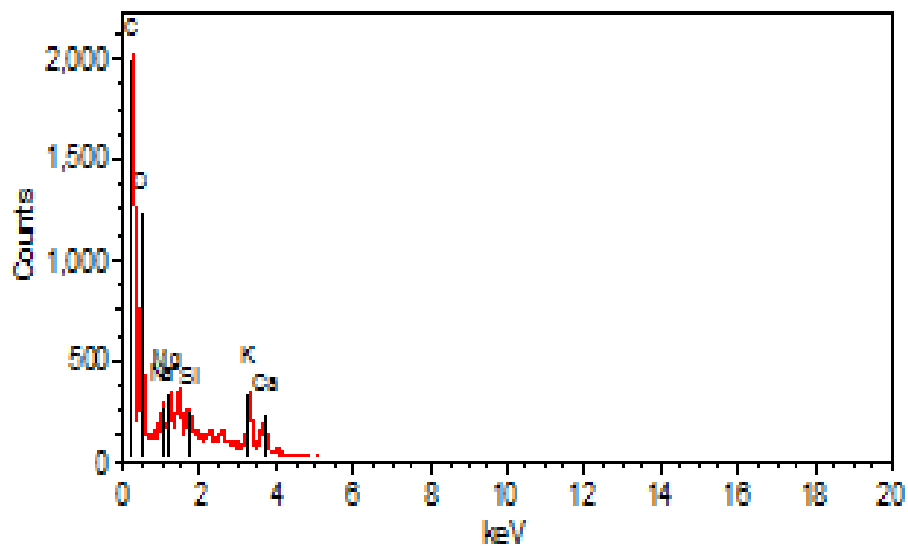


Fig 35: Electron Dispersive X-ray (EDX) on the element composition of PAKT

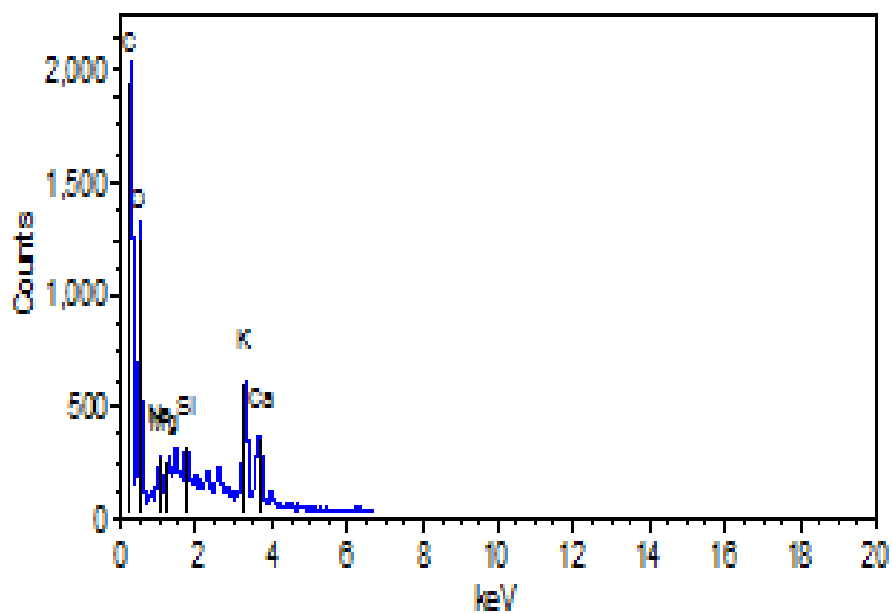


Fig 36: Electron Dispersive X-ray (EDX) on the element composition of PBKW

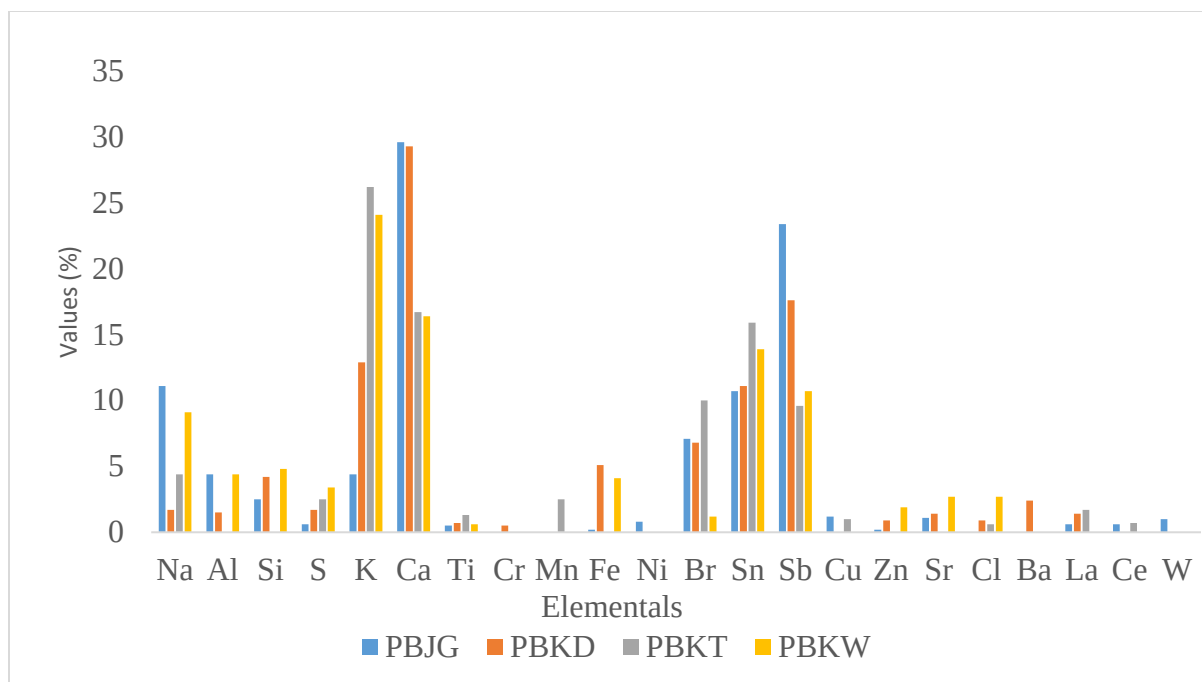


Fig. 37: Elemental concentrations (%) in the seed coat of *Parkia biglobosa* collected from different locations

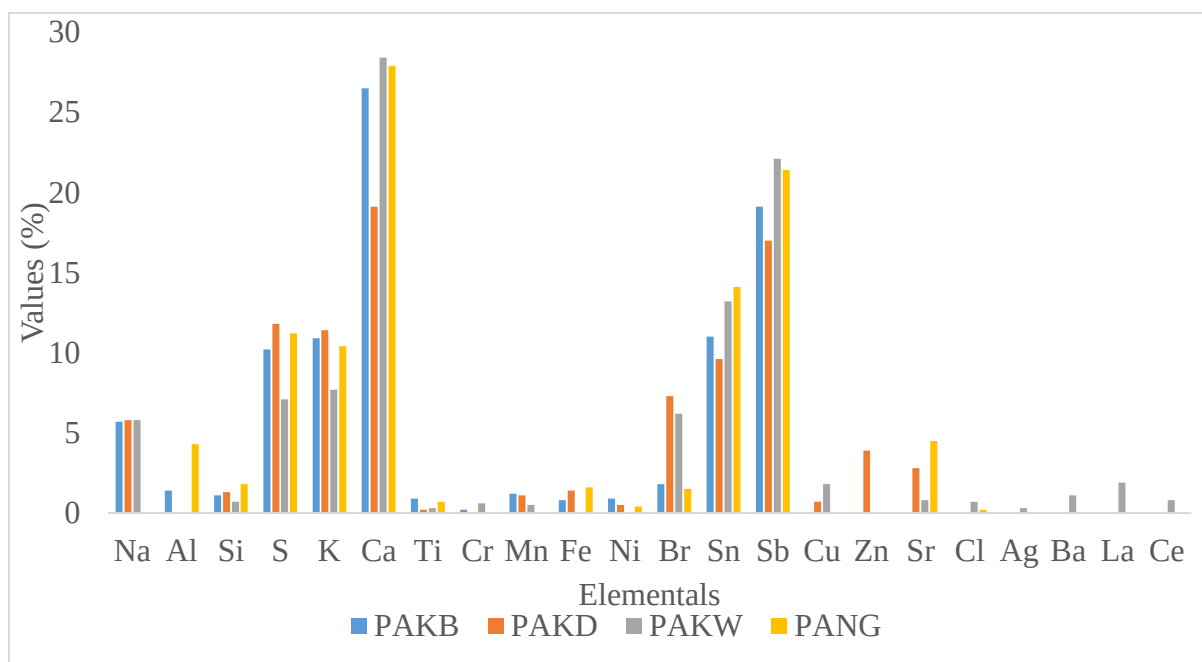


Fig. 38: Elements concentration (%) present in the seed coat of *Prosopis africana* collected from different locations

SEM micrographs of outer and inner seed coat of *Prosopis africana* and *Parkia biglobosa* were presented in plate 1- 16.

The SEM observation of the surface sculpture were focused on the arrangement of epidermal cells, there were curvature and fine cuticular ornamentation. The seed coats showed some degree of secondary thickening of some part of the cell wall which can be said to be sclerotic. They also varied in shape, size and orientation. There was extensive differentiation of the cell layers in the seed coat.

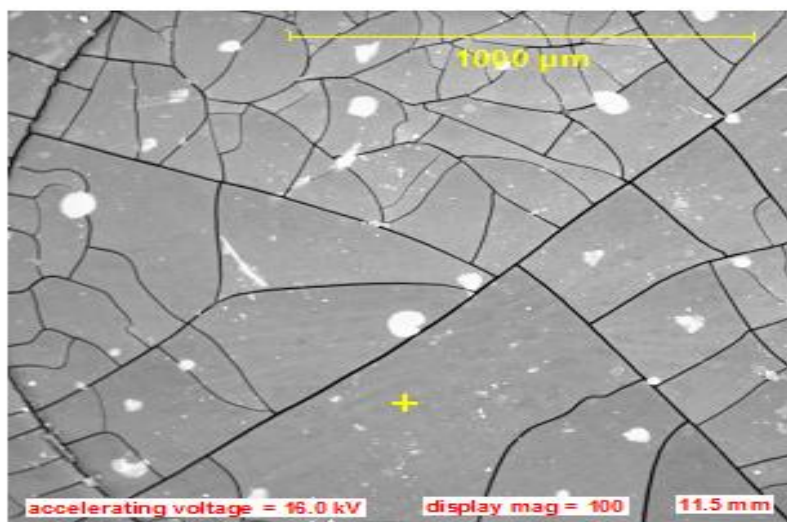


Plate 1: SEM micrographs of the outer seed coat of PAKB

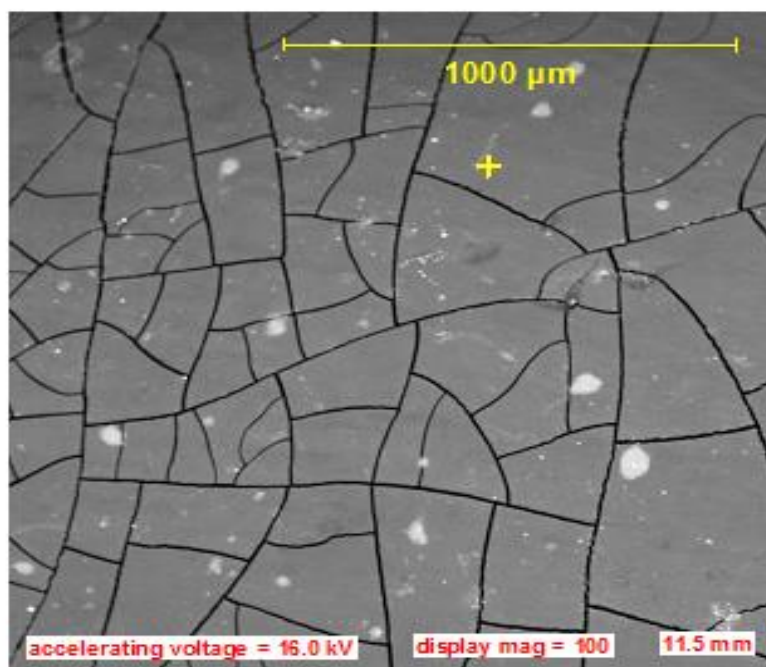


Plate 2: SEM micrographs of the outer seed coat of PAKD

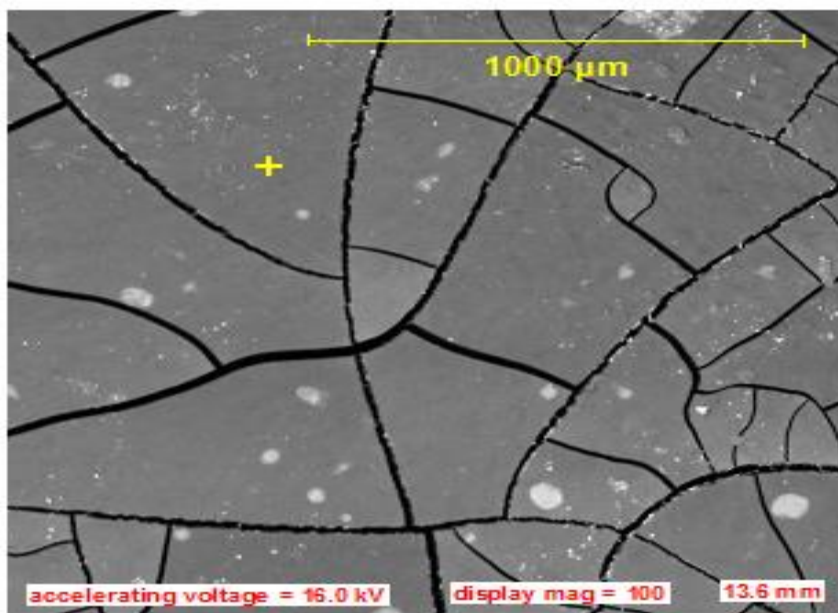


Plate 3: SEM micrographs of the outer seed coat of PAKW

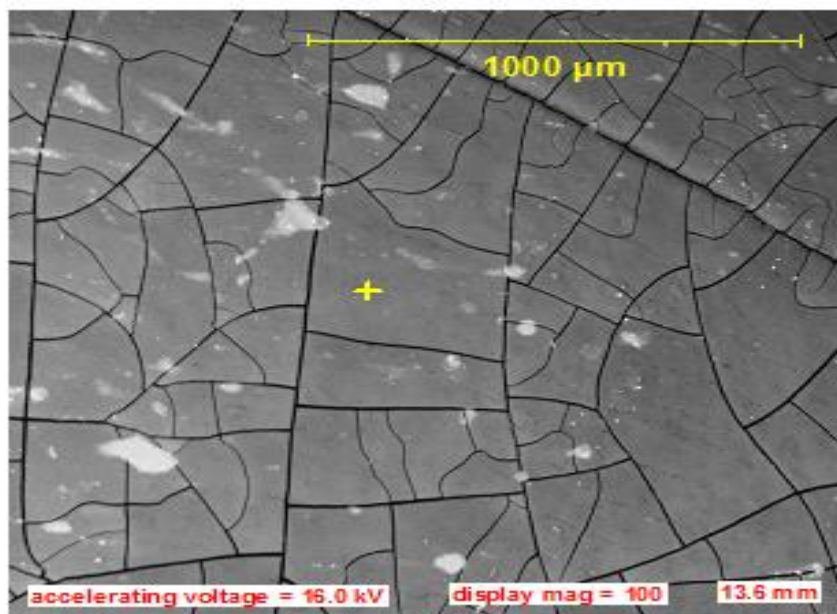


Plate 4: SEM micrographs of the outer seed coat of PANG

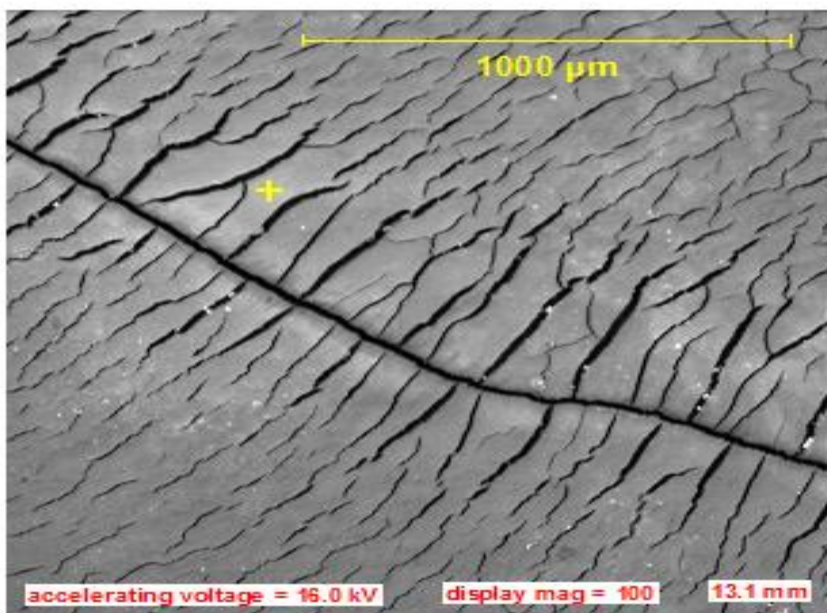


Plate 5: SEM micrographs of the outer seed coat of PBJG

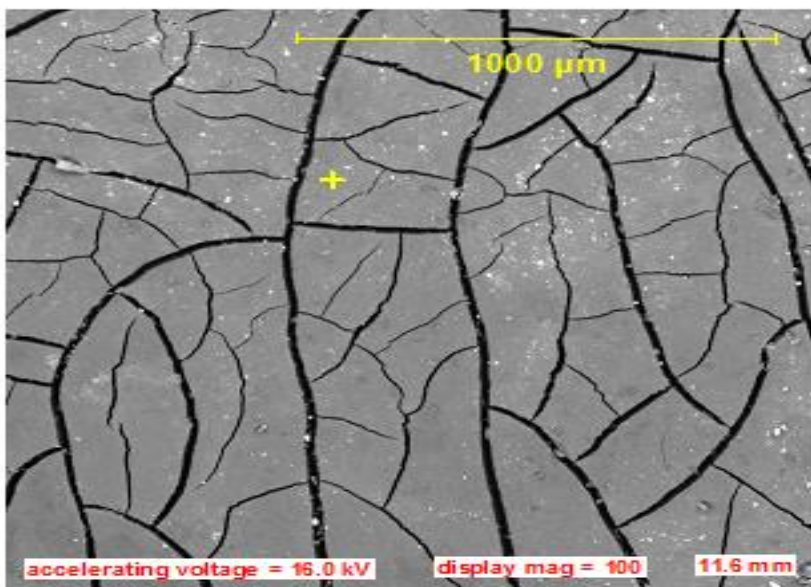


Plate 6: SEM micrographs of the outer seed coat of PBKD

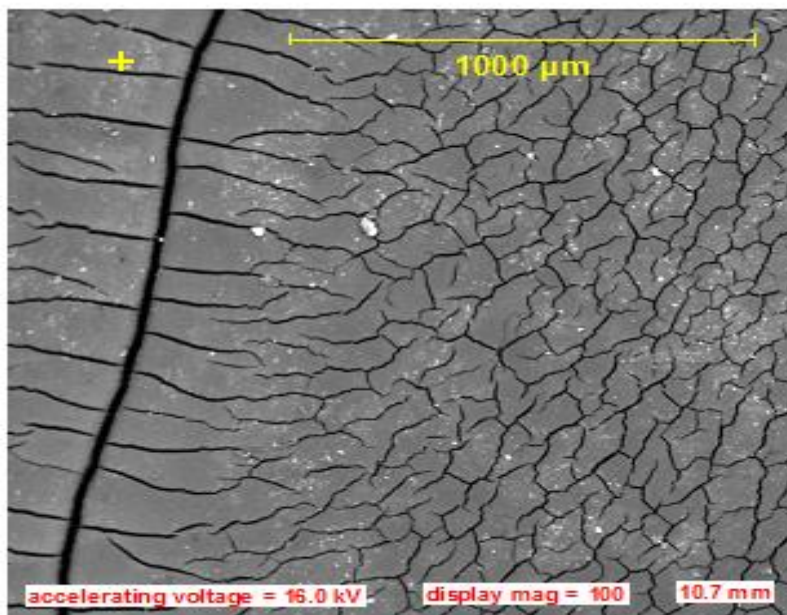


Plate 7: SEM micrographs of the outer seed coat of PBKT

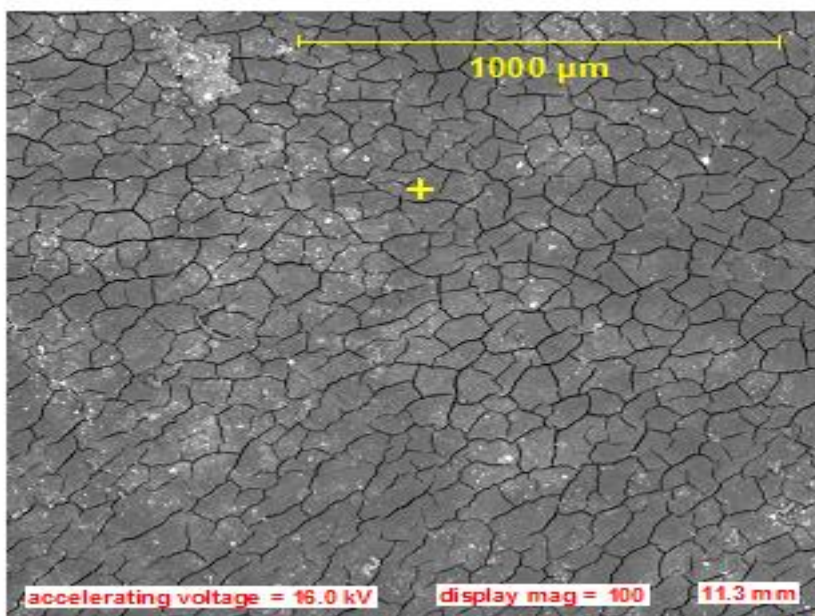


Plate 8: SEM micrographs of the outer seed coat of PBKW

It was observed that the outer part of the seed coat of both *Propopsi africana* and *Parkia biglobosa* show different degree and pattern of cracks.

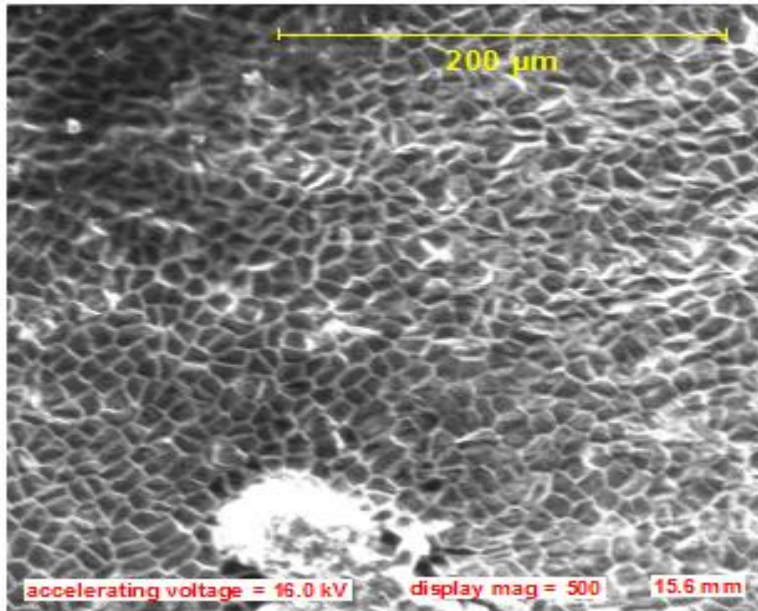


Plate 9: SEM micrographs of the inner seed coat of PAKB

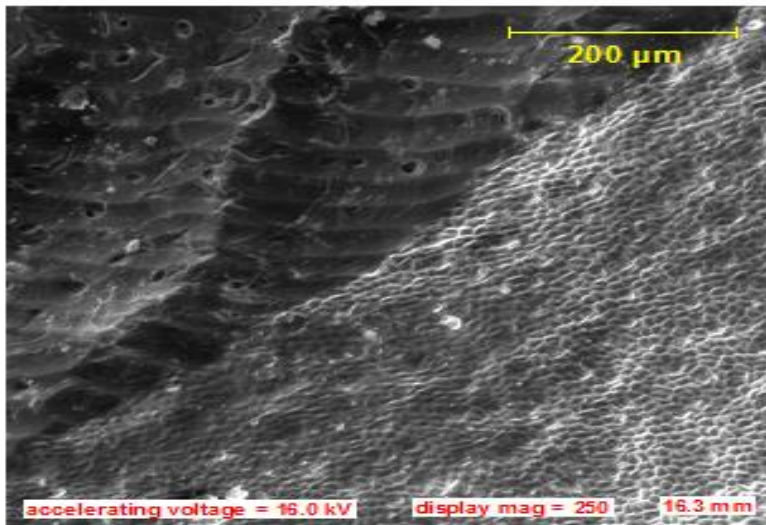


Plate 10: SEM micrographs of the inner seed coat of PAKD

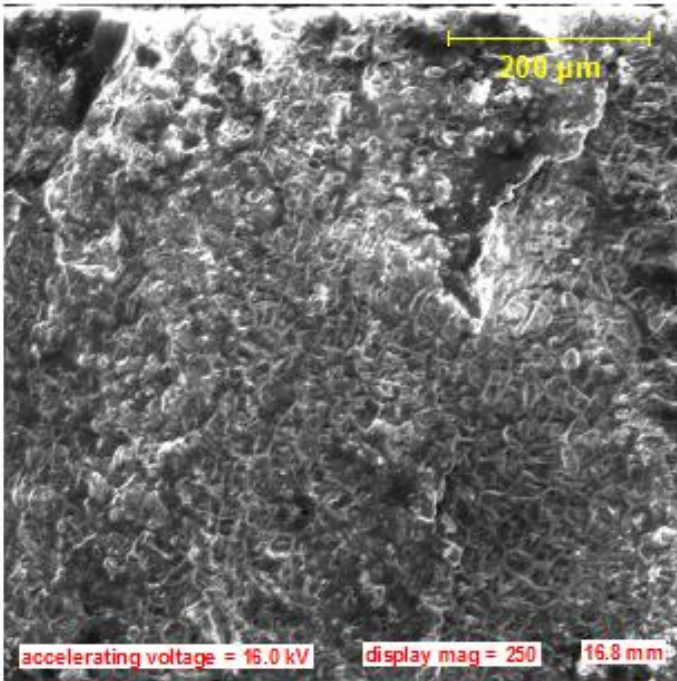


Plate 11: SEM micrographs of the inner seed coat of PAKW

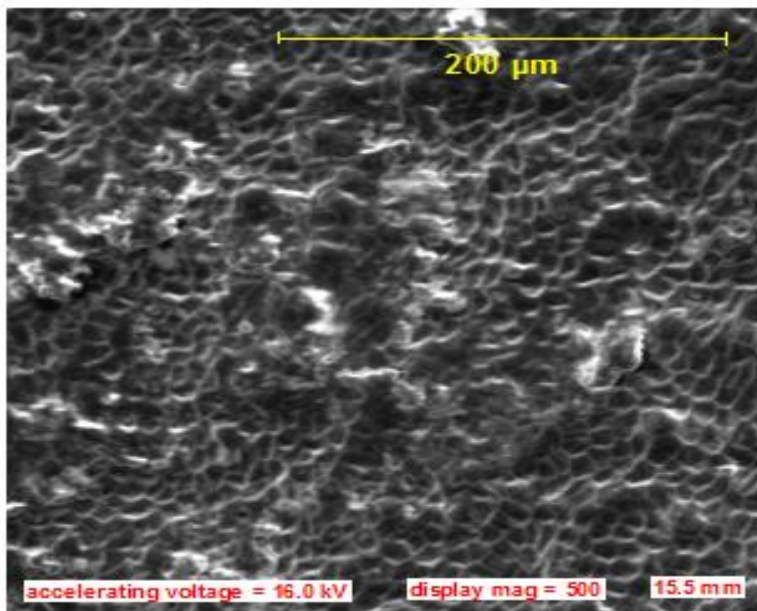


Plate 12: SEM micrographs of the inner seed coat of PANG

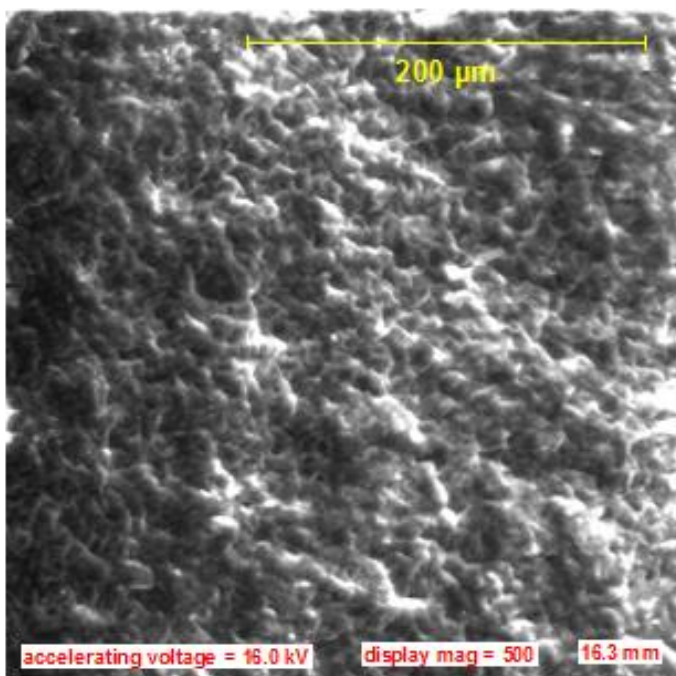


Plate 13: SEM micrographs of the inner seed coat of PBJG

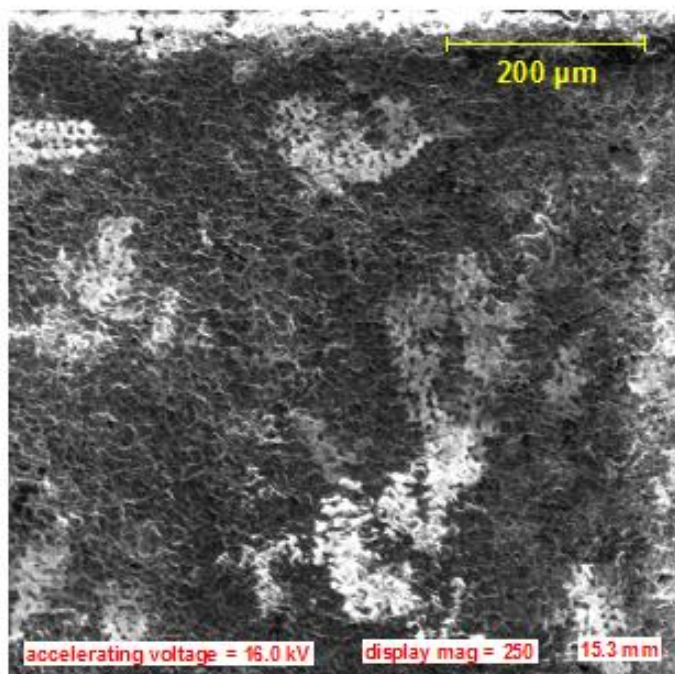


Plate 14: SEM micrographs of the inner seed coat of PBKD

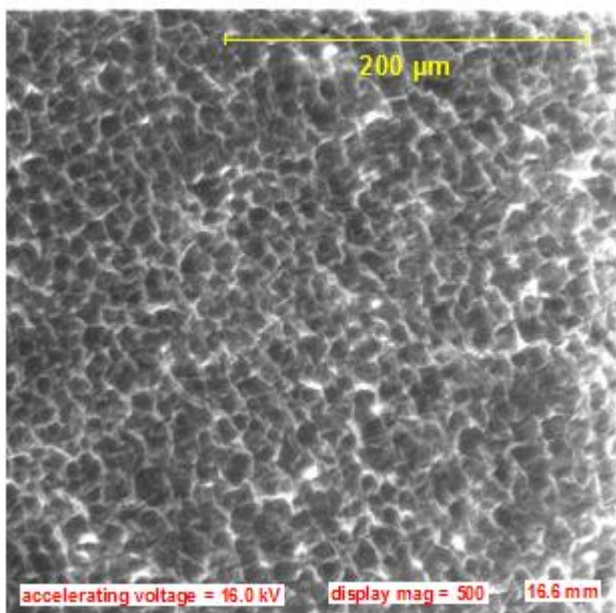


Plate 15: SEM micrographs of the inner seed coat of PBKT

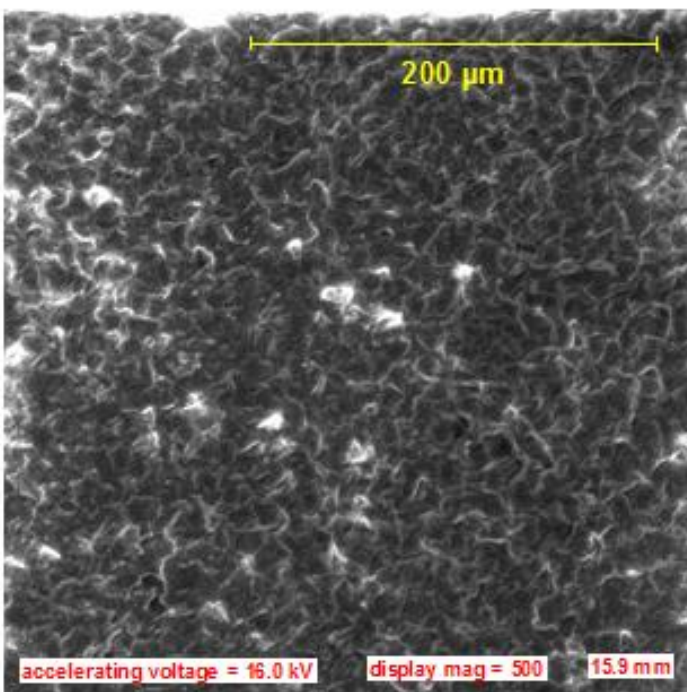


Plate 16: SEM micrographs of the inner seed coat of PBKW

The morphological features of the inner seed coat are summarized in Table 26.

The shape of the anticlinal wall of PBKD, PBJG and PBKT were undulate while PBKW was wavy.

The anticlinal walls were thin and raised in all *Parkia biglobosa* seeds sampled with exception in PBKW which had thick anticlinal wall.

All the seeds of *Prosopis africana* had undulate anticlinal wall shape except PAKW which had wavy anticlinal wall shape. The level of the anticlinal wall were all raised and thin in all *Prosopis africana* seeds sampled.

Table 39: Morphological characterization of the inner seed coat of *Parkia biglobosa* and *Prosopis africana*

Sample	Anticlinal wall shape	Anticlinal wall thickness	Anticlinal wall Level	Periclinal wall Level
<i>Parkia biglobosa</i>				
PBKD	Undulate	Thin	Raised	Convex
PBJG	Undulate	Thin	Raised	Convex
PBKT	Undulate	Thin	Raised	Convex
PBKW	Wavy	Thick	Raised	Convex
<i>Prosopis africana</i>				
PAKB	Undulate	Thin	Raised	Convex
PAKD	Undulate	Thin	Raised	Convex
PAKW	Wavy	Thin	Raised	Convex
PANG	Undulate	Thin	Raised	Convex

CHAPTER FIVE

DISCUSSION

The results of the quantitative seed characters of *Parkia biglobosa* in term of pod length and number of seeds per pod in all seed samples followed the report of Olorunmaiye *et al.* (2011) who reported varying pod length values ranging from 15 to 27 cm in 26 different *Parkia* populations. The seed breadth (2.03-3.17 mm) similarly concurs with the description of the millennium seed bank project Kew which put *Parkia biglobosa* pod to be 2-3 cm wide (Sacande and Clethero, 2007). In addition, the present results agreed with the work of Oje, (1993) on *Parkia biglobosa*. For *Prosopis*, the outcome of the quantitative characteristics of the pods was in line with the report of Orwa *et al.*, (2009) and Le Houerou, (2011) on pod length, pod breadth and number of seeds per pod with average pod length ranging of 15 cm, breadth 3 cm and about 10 loose seeds per pod.

Prosopis africana pod colour in this study agreed with Orwa *et al.*, (2009) and Ugwuara, (2010). The authors reported dark brown and brown colour respectively among their study population. Also, *Parkia* seed coat colour was similar to the earlier observations of Orwa *et al.* (2009), Olorunmaiye *et al.* (2011) and Simonyan, (2012).

Seed length, seed width and thickness geometric mean diameter and sphericity represent the physical and engineering properties of the seeds. These are important in the analysis of the behaviour of the product during agricultural processes such as handling, planting, harvesting, threshing, cleaning, sorting and drying (Akaaimo and Raji, 2010). This is also important in obtaining information required to ease the operations of seeds extraction from the pods. Results from the measurements taken for the axial dimensions, geometric mean diameter and sphericity for *Parkia biglobosa* and *Prosopis africana* seeds showed that the seed length, width and thickness for the seeds fall within the range reported by Mohsenin (1986) and Akaaimo and Raji (2006).

Furthermore, the result of these seed characteristics also agreed with Oje (1993), locust bean; Ige, (1977) cowpea; Dev *et al.* (1982) sorghum; Chowde-Gowda *et al.* (1991) pigeon pea; Oje and Ugbor, (1991) oil bean seeds; Orji, (2000) breadfruits seeds and Joshi *et al.* (1993) on pumpkin seeds and kernel.

The results on the seed dormancy breaking showed that germination was observed after 24 hours of planting the treated seeds. This is in agreement with the basic principle of seeds germination, during the imbibition phase there is no germination, it just a first step of seed germination involving rapid absorption of water by the seed (Tung and Serrano, 2011). Generally, all seeds treated with H₂SO₄ had the most positive impact on dormancy breaking and seed germination compared to the other acids treatment. The optimum period for acid scarification was 15 - 20 mins (100%) compared to 5 mins and 10 mins. This agreed with Sadeghi *et al.*, (2009) on common madder. In a similar study, Ailero (2004) discovered that treatment of the seeds of *Parkia biglobosa* with H₂SO₄ acid induced germination of the seeds. Reports of earlier works on *Enterolobium cyclocarpum* (Agboola and Adedire, 1998) *Piliostigma reticulatum* (Aduradola, 1999) and *Adansonia digitata* (Adio *et al.*, 2006) demonstrated that treatment with acid significantly promoted germination of the species seeds.

Mechanical scarification of the seeds supports the work of Abubakar and Maimuna, (2013) who reported that treatment by mechanical scarification (sand paper) was effective in the breaking seed dormancy of *Parkia biglobosa* (90%) among other treatment. In support of this statement, Sadeghi *et al.*, (2009) report that mechanical scarification turned out to be an excellent treatment to overcome seed dormancy with 83% in common madder. The result from the study showed that seeds mechanically scarified improved seed germination based on the percentage germination. It can be suggested that treatment of seeds *Parkia biglobosa* and *Prosopis africana* either with acids

or mechanical scarification may be effective for breaking dormancy and improving the seedling vigour which can enhance the domestication and cultivation of these valuable seeds in the environment

Germination rate is discussed in terms of coefficient of the velocity (CRG), speed of germination (S) and accumulated germination speed (AS). The highest CRG index was noted at 10 mins H_2SO_4 (PANG) and 15 mins HNO_3 (PAKB) in *Prosopis* and 20 mins H_2SO_4 in *Parkia*. This index explains precisely the velocity and spread of germination during the whole period of germination. Furthermore, CRG is estimated by the same formula suggested by Maguire (1962) and which is applicable in both field and laboratory experiments. The high value of this index provides information about the vigor of seed samples (Ranal and Santana, 2006). The speed germination (S index) indicates that the maximum speed of germination was at 20 mins of scarification. Thus this index implies that scarification time below 15 mins and above 20 mins decreased the speed of germination but did not halt it. Accumulated germination speed (AS) increased in proportion to the increase in scarification time. Consequently, this index gives an idea about the progress of cumulative germination during the planting period (10 days), except at 15 mins for some seed sample of *Prosopis* and 10 mins in *Parkia*. The difference between AGSD and AS is considered: the former represents the speed of germination taking into consideration the total incubation period and the total number of germinated seeds, while the latter represents the cumulative germination speed per day in which new germinated seeds are observed.

Seed priming is a technique which improve germination and seedling growth. In this research, hydropriming and hormonal priming with GA_3 and IAA improve the seed germination and seedling growth. The results show that the hormonal priming induced by GA_3 (250 mg/l) enhanced the germination of *Parkia biglobosa* and *Prosopis africana*. This may be ascribed to the fact that

at high concentration, enhanced and prompt expression of the enzymes responsible for germination which increased and affect both physiological and metabolic activities inside the seeds (Varier *et al.*, 2010; Shah *et al.*, 2011; Jyot *et al.*, 2016; InsafBankaji *et al.*, 2018) and thereby encourage embryo growth, reduce physical restrain imposed by the endosperm or seed coat and making way for the protrusion of radicle. According to Shinde *et al.* (1994), GA₃ at high concentration is effective in overcoming dormancy so causing rapid seed germination. Also, Kucera *et al.* (2005) and Linkies and Leubner-Metzger (2012) reported that GA play a major and role in regulating the processes of dormancy release and germination. The results of this present study agree with Ojo *et al.* (2017) on three Cucurbits; Ebofin *et al.* (2003) on savannah tree legumes and Joshi *et al.* (2010) on *Pyracantha crenulata* respectively where they all observed increased germination at higher concentration of GA₃. The seeds of *Parkia biglobosa* and *Prosopis africana* all responded to GA₃ treatment meaning that location does not have effect on the seed germination except for priming period, hence, there were no significant different in their germination %.

The effect of IAA also showed a great influence on the seed germination irrespective of the concentrations especially at 24 hours priming. This is contrary to the report of Ojo *et al.* (2017) who reported that germination % observed in three Cucurbits was at low concentration of IAA. This is probably due to the fact that IAA exercises its stimulatory effect on germination and plant growth within a narrow concentration range beyond which may made the seeds unresponsive and growth inhibited (Deyo and O'Malley, 2008).

The results of the qualitative and quantitative phytochemical analysis reported revealed that the seed of *Parkia* and *Prosopis* from the different locations sampled are rich in the phytochemical analyzed. The reports are similar to the work of Sani, (2014) on ethanol extract of *Parkia* seed. The presence of these secondary metabolites are evidence that the plants are involved in the

interaction of plants with other species. Alkaloids especially quinolizidine alkaloids are seen to be an inhibitor to germination of seeds like *Lactuca sativa* (Wink, 1983). Some phenolic compounds which act as an antioxidant suppressed seed germination (Ogawa and Iwabuchi, 2001). Flavonoids help in stress reduction by scavenging hydroxyl and lipid peroxy radical, superoxide and anions (Okwu, 2005) but the value of flavonoids recorded in the seeds analyzed were significantly low compared to those reported in literature.

The proximate analysis revealed that the seeds of *Parkia* and *Prosopis* are similar to the work of Boeteng *et al.* (2014) on processed and unprocessed seed of *Parkia biglobosa*. The seed have some appreciable amount of moisture. The moisture content is an important characteristic for oxidation and hydrolysis processes (Osawa *et al.*, 2007). The result of the moisture content is lower than the report of Elemo *et al.* (2011) on *Parkia biglobosa* (11.21%). According to Salisu *et al.* (2014), the low moisture content will give a long shelf life. This means that the high moisture content can be a reason for the short shelf life observed in the sampled seeds of *Parkia biglobosa* especially. The ash content of *Parkia* and *Prosopis* agreed with the reports of Aremu *et al.*, (2015) on processed and unprocessed seeds of *Parkia* and *Prosopis*; Olaofe *et al.* (2008) on the seeds of *Luffa cylindrica* and Aremu *et al.*, (2006) on the seeds of *Anarcadium occidentale*. The crude fat contents in this result are very low compared to the results of Aremu *et al.*, (2015). The seeds of *Parkia* and *Prosopis* from all the locations sampled are not oil-rich seed when compared with some legume oil-rich seeds like Soybean (Olaofe, 1994; Aremu, 2006). The protein value of the seeds was low compared with the report of Adebawale and Malik (2011) for Pigeon pea; Boeteng *et al.* (2014) for processed and unprocessed seed of *Parkia biglobosa*. The result also supports the result of Vadivel and Janardhanan (2001) on *Canavalia ensiformis* (Wild Jack bean); and Anhawange (2004) on *Baulinia nonander*. The processing of the seeds of fermentation of *Parkia* and *Prosopis*

seeds enhance crude protein. The result of the crude fibre for *Parkia* and *Prosopis* from all locations sampled was similar to earlier studies of Oshodi and Ekperigin (1989) and Adeyeye and Aye (1998) on Pigeon pea and Africa yam bean respectively. The result for carbohydrate favourably compared with the acceptable range mean value for legumes (Asp, 1996). The result supports the reports of Ajah and Madubuike (1997) and Hossain and Becker (2001) on Jack bean and *Sebania* seeds respectively. The carbohydrate content gave an indication that the seeds studied can be considered as a rich source of energy (Aremu, 2015) and possess a rich store of the energy required for the germinating seed and seedling.

Saponification values have been reported inversely related to the average molecular weight of the fatty acids in the oil parts (Abayeh *et al.*, 1998). According to Abayeh *et al.* (1998), any seed with saponification value of 200 mgKOH/g and above had been reported to possess low molecular weight fatty acid. Therefore, it is reviewed that the seeds of *Parkia biglobosa* and *Prosopis africana* with saponification value approximately 200 mgKOH/g and above possess very low molecular weight fatty acid except in PANG with a correspondent low free fatty acid.

Acid value represents free fatty acid content due to enzymatic activity (Afolabi, 2007). The maximum acceptable level of acid value is 4 mgKOH/g (CODEX, 1969). Therefore, all the seeds subjected to this research are acceptable for consumption. This is in support of the work of Afolabi, (2007) on Palm kernel seed, Almond seed and castor seed.

Reactive Oxygen Species (ROS) production is a continuous phenomenon which is initiated immediately after harvest, it was low at harvest time, and later increased again after seed dispersion from the mother plant (El-Maarouf *et al.* 2007). The results on ROS indicated that the the seeds are undergoing oxidative stress and this may be the reason while the seeds of *Parkia biglobosa* deterioration and became non-viable four months after harvesting.

The seed coats were said to be impermeable to water and gases (Baskin and Baskin, 1998) which has led to dormancy (Etejere *et al.*, 1982). The elemental constituents of the seeds coat of *Parkia* and *Prosopis* indicate that there was some element deposit on the seed coat and according to Argel and Paton (1999), the basic histological structure of the permeable and impermeable of seed coat result from a particular chemical composition of cells that are part of the thick seed coat which means that the thickness of seed coat alone does not account for impermeability since even in a thick seed coat, water may access the embryo through the hilum, lens or raphe. The seed coat impermeability has also been associated with waxy cuticle (Arechavaleta-Medina and Snider, 1981) (this can be seen in the result obtained for Saponification value), high Calcium and low phosphorus seed coat concentration (Saio, 1976). According to Hill *et al.* (1986), the proportion of hard seed produced in legume species varies according to year and geographic location. This may explain the variations in the elements present in the seeds coat of *Parkia biglobosa* and *Prosopis africana* collected at different locations. The colour of the seed coat characteristics was associated with water absorption. All the seeds used were chocolate (Dark brown) for *Parkia* and coffee brown for *Prosopis*. It has been reported by Asiedu and Powell (1998) that seed coat pigmentation correlate with slow rate of imbibition in several legume species. Browning of seed coat during maturation was found to be associated with its impermeabilization in *Phaseolus vulgaris*, *Cicer arietinum*, *Vigna. unguiculata* (Legesse and Powell, 1996), *Parkia biglobosa* (Etejere, *et al.*, 1982 and Adeyemi, *et al.*, 2013).

The morphology of the inner seed coat surface revealed the characteristic pattern which appear to be covered with small, pebble-like bumps. This agreed with the report of Hughes and Swanson, (1985). The seed coat shows reticulate pattern with different shaped cells. This is in agreement with the report of Karcz *et al.*, (2005) on the micrograph of seed coat of *Brassica* seeds.

CONCLUSION

Physical and engineering properties of the seeds of *Parkia* and *Prosopis* are important in the analysis of the behaviour of the product for agricultural process operations such as handling, planting, harvesting, threshing, cleaning, sorting and drying and also providing information to ease the operations of seeds extraction from the pods.

The hard seed coat of the plants used for this research are impermeable to water and gases or may mechanically constrain the embryo. These seeds are glossy due to the presence of wax in the seed coat which prevents easy penetration of water. The control treatment in the study had least performance of 18% which means that viable seeds of *Parkia biglobosa* and *Prosopis africana* will not germinate fairly well. The percentage germination increased by pre-treating the seeds before planting so as to reduce germination period and have higher percentage of germination. Therefore, in order to enhance optimum and uniform germination as well as, seedling production, Acid scarification of the seeds is recommended. This will definitely facilitate the availability of seedlings for reforestation and agroforestry projects. On the other hand, for farmers who may not have access to acids or afford the cost of procurement, mechanical scarification of the seed could be employed. The hydropriming and hormonal priming with GA₃ and IAA improved seed germination and seedling growth especially with GA₃ concentrations in *Parkia biglobosa* and *Prosopis africana*. This will equally hasten the seedling emergence rate and in turn, increase in seedling production for afforestation programmes on sustainable basis.

The results of this research show that *Parkia biglobosa* and *Prosopis africana* seeds exhibit physical dormancy- a known fact- but the failure of these seeds to germination is not only attributed to seed coat but could be as a result of ROS present in the seeds. This means that the seeds also have a physiological dormancy.

The low enzymes activities monitored in the germinating seeds of *Parkia biglobosa* and *Prosopis africana* collected at different locations could be as a result of the ROS especially, superoxide radicals which can attack susceptible biological targets such as lipids, proteins and nucleic acids and can also denature enzymes, oxidize lipids and fragment DNA.

The study of the micro-structure of the seed coat indicated that the seed coats are made up of sclerenchyma cells. This is an indication for the high concentration of calcium in the seed coat elemental composition - which are composed of lignin; causing hardness and impermeability of the seed coat.

RECOMMENDATION

Parkia biglobosa and *Prosopis africana* are two plants with great economic values such as source of protein from their condiments, income for rural women and so on but the seeds of these trees face with challenges of dormancy which pose a great danger to the future of these plants. It is therefore recommended that the dormancy of these seeds can be broken with different methods of scarification as described by many researchers and priming with hormones. This is also supported with study of seed coat morphology. Also, the studies on the Reactive Oxygen Species, Phytochemicals and Physicochemicals on the seeds show that the dormancy is not based on the seed coat only. The seed coat anatomy should be looked into for further studies

CONTRIBUTION TO KNOWLEDGE

This study contribute the followings to knowledge:

1. Dormancy of the seeds of *Parkia biglobosa* and *Prosopis africana* is not just physical but also morph-physiological.
2. Hormone are able to induce seeds germination and seedling growth of the of *Parkia biglobosa* and *Prosopis africana*
3. Reactive Oxygen Species may contribute to the inability of the seeds of *Parkia biglobosa* and *Prosopis africana* to germinate. The ROS are accumulated in the seeds during seed maturation under stress especially, drought stress.
4. The seeds of *Parkia biglobosa* especially undergoes quick aging, so it is advisable to plant the seeds immediately after crop processing or store unprocessed fruit that is storing the seeds with the pulp.

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APPENDIX

Legend: Location

OD=Oyo

KD=Kaduna

KT=Katsina

KW=Kwara

JG=Jigawa



OY



KD



KW



JG



KT

Legend: Location

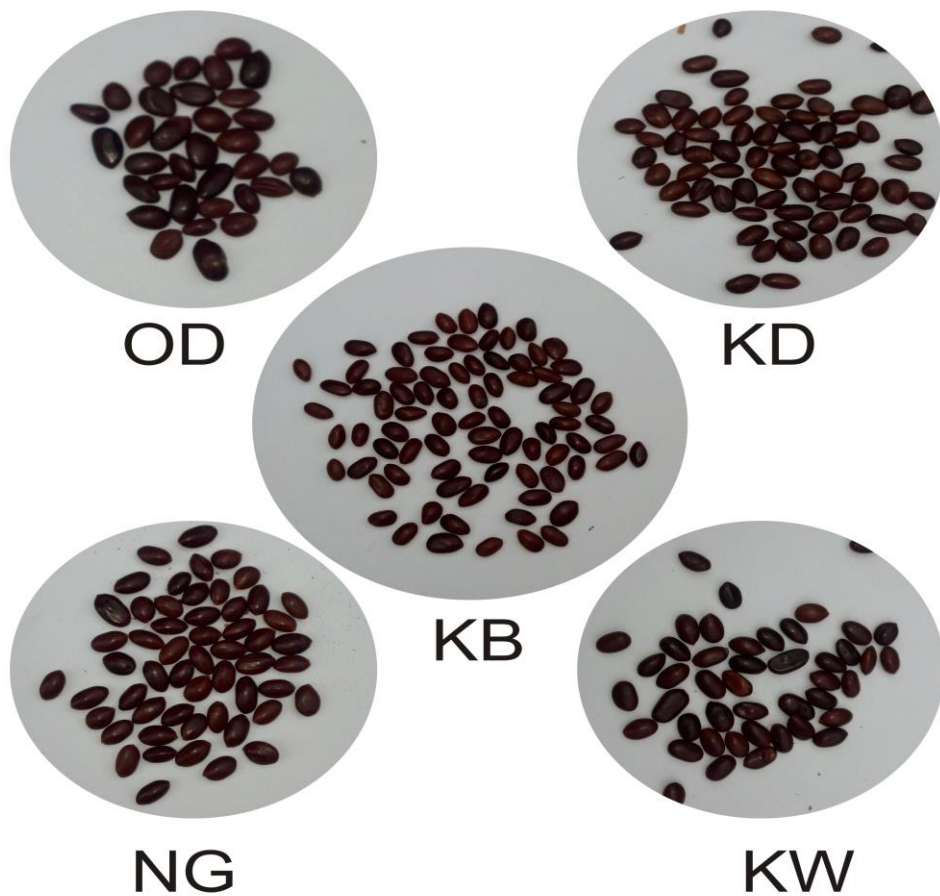
OD=Ondo

KD=Kaduna

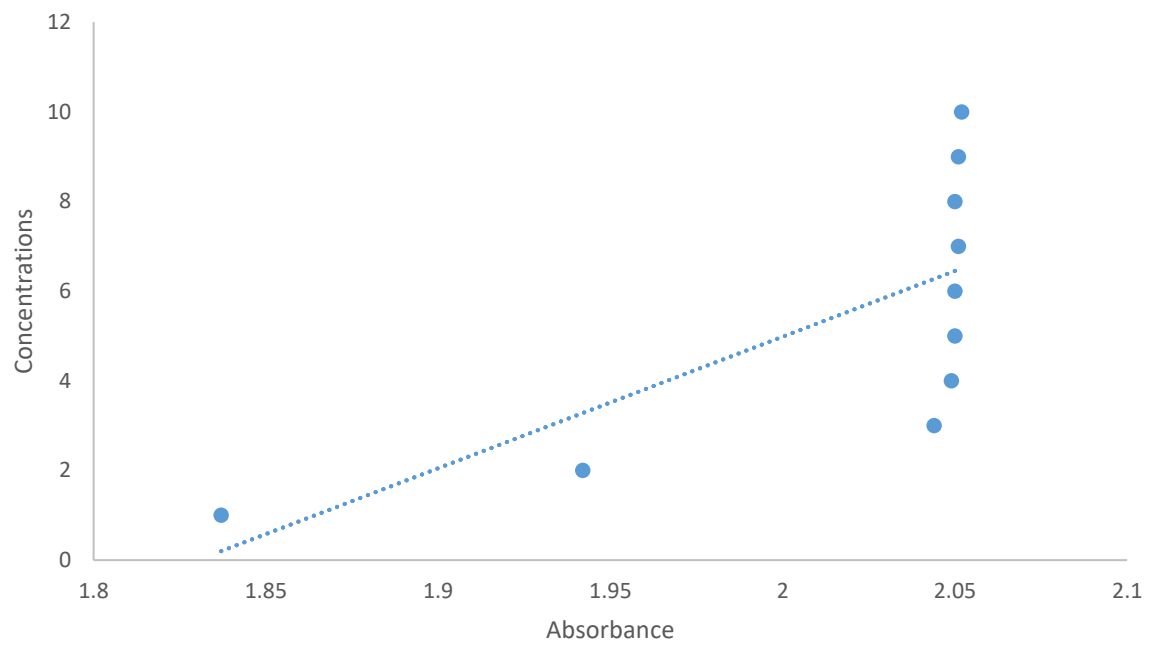
KB=Kebbi

KW=Kwara

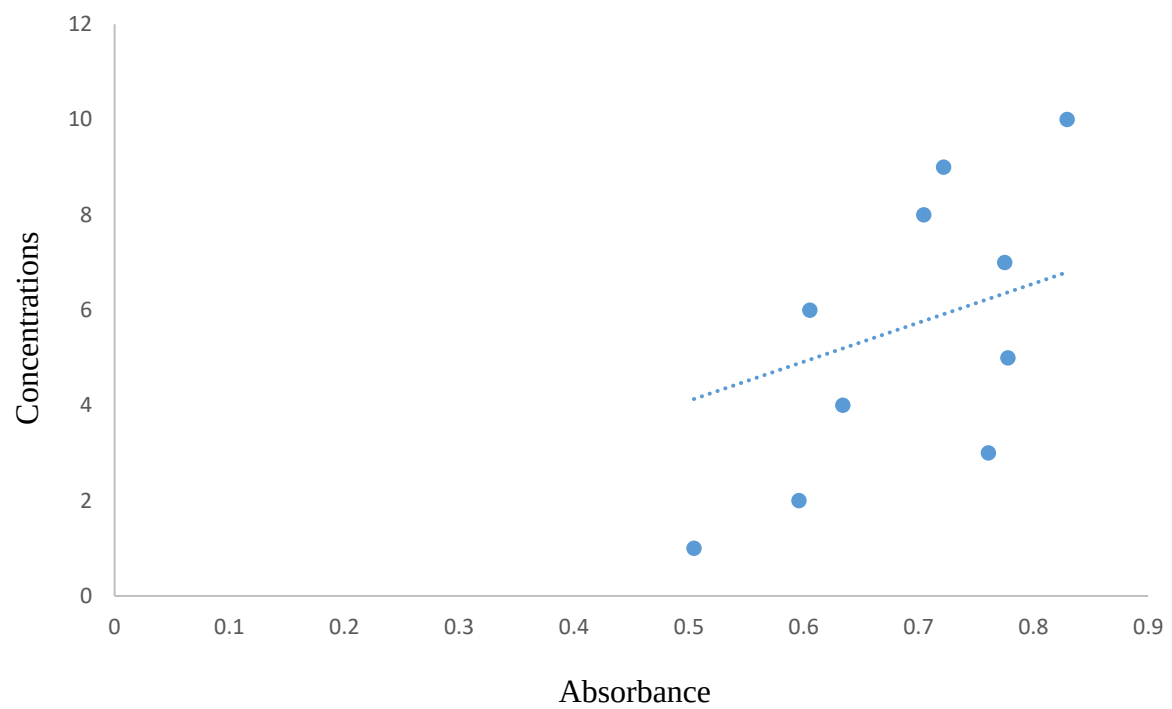
NG=Niger



Seeds of *Prosopis africana* accessions



Standard curve for amylase activity



Standard curve for Protease activity