SILVICULTURAL REQUIREMENTS FOR CONSERVATION OF *Plukenetia conophora* (MULL ARG) IN SOUTHWESTERN NIGERIA

By

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ABSTRACT

Plukenetia conophora, a liana with economic potentials is threatened by extinction. Ex-situ conservation of the species which requires knowledge of its early growth and vegetative propagation has the potentials to enhance its sustainability. However information on the silvicultural requirements for the early growth of this species is scanty. Hence, in this study, seed germination, seedling growth, macro and micropropagation of *Plukenetia conophora* in Southwestern Nigeria were investigated.

Fruits of *P. conophora* from Oyo (Ibadan), Ogun (Ijebu-Ode), Osun (Igbajo) and Ondo (Akure) states were collected and used for the study. Using completely randomised design, variations in fruit size, growth of seedlings and biomass production of *P. conophora* were assessed. Seeds stored for twelve months at room temperature (28^{o}C), in a freezer (-5^{o}C), refrigerator (7^{o}C), dry soil (29^{o}C) and freshly extracted seeds (control) were tested for viability using 1% tetrazolium salt. Effect of seed size: small (<9g/wt), medium (9-11g/wt) and large (12-15g/wt) on germination was investigated. Also, the influence of five watering regimes: submerging in 10 litres of water, watering daily, at 4 and 7 days interval with 250ml/seedling and no watering (control), on seedling growth were assessed. Effects of three organic fertilisers (poultry droppings, cow dung and compost) at 10, 20, 30 and 40g/2kg soil on seedling growth were investigated. Seedlings were decapitated at heights 10, 15 and 20cm above root collar and their coppicing potentials were investigated. Macro propagation of double node cuttings using Indole-Butyric Acid (IBA), Naphthalene Acetic Acid (NAA) and IBA + NAA hormones at 50ppm, 100ppm, 200ppm and their powdery forms at 8g/kg was carried out. Micropropagation using embryo explant on modified Murashige and Skoog (MS) media was studied. Data were analysed using descriptive statistics and ANOVA at p = 0.05.

Sources significantly affected the fruit weight and collar diameter while the length of liana was not significantly different. Fruit weight ranged between 96.7±30.2g and 62.4±15.1g. Collar diameter was between 0.9±0.1cm and 0.8±0.2cm and length of liana ranged between 224.8±15.8cm and 182.4±6.7cm. Biomass production ranged between 45.8±6.2g and 33.6±7.1g. Seeds stored at 7^{o}C gave the highest viability of 75% after 30 weeks. Seeds size had no significant effect on early growth. Watering regimes significantly affected growth with daily

watering producing the longest liana (140.8±7.3cm). Submerging in water and no watering liana wilted after the 8th week. Compost fertilizer at 40g/2kg soil produced the longest liana (242.5±14.1cm) while the control had the shortest (164±8.4cm). Decapitation at height 20cm produced the highest number of coppices (6.0±2.2). Double node cuttings using IBA + NAA in their powdery form produced 90% rooting over the control (no rooting). Embryo culture using MS medium alone produced the highest plantlets (9.0±1.8) while MS + 0.5mg benzyl aminopurine produced none.

Viability was maintained in the seed of *Plukenetia conophora* for 30 weeks when stored at 7^{o}C. Seedling performed best when watered daily. Rooting of double node cuttings was possible using powdery hormone and plantlets of *Plukenetia conophora* were produced using the embryo as explant.

Keywords: *Plukenetia conophora*, Liana, Coppicing, Vegetative Propagation.

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DEDICATION

I dedicate this thesis to God Almighty, in the name of Jesus Christ our Lord who by the power of the Holy Spirit, made this work a reality.

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Above all, to God Almighty my Creator, our Lord Jesus Christ my Saviour, the Holy Spirit my Strength and Mother Mary my Intercessor, I am eternally grateful for this privilege.

CERTIFICATION

I certify that this work was carried out by Joyce Okama AMADI in the Department of Forest Resources Management, University of Ibadan, Nigeria.

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ACRONYMS

AGR – Absolute Growth Rate

BAP – Benzyl Amino Purine

CBD – Convention on Biological Diversity

CNN – Cable Network News

2, 4 D – 2, 4 Dichlorophenoxyacetic acid

FAO – Food and Agricultural Organisation

FRIN – Forestry Research Institute of Nigeria

GBO – Global Biodiversity Outlook

IAR&T – Institute of Agriculture Research and Training

IAA – Indole Acetic Acid

IBA – Indole Butyric Acid

MS – Murashige and Skoog

NAA – Naphthalene Acetic Acid

NAR – Net Assimilation Rate

NRC – National Research Council

NACGRAB – National Centre for Genetic Research and Biotechnology

OMFs – Organomineral Fertilizers

RGR – Relative Growth Rate

UNCBD – United Nations Convention on Biological Diversity

UV – Ultra-violet light.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

The tropical forests of Nigeria are rich in plant species with high potentials to meet the fundamental needs of man which are basically food, good health, services and raw materials. Indeed all aspects of rural life is affected by the forest, (Falconer, 1992). These plant resources are vital to continuous human existence, because most of them have high nutritional values, such as protein, carbohydrate, vitamins, fat, minerals and fibre, while others are appreciated for their medicinal values, cultural symbols and fuel. Their dietary contribution is increased because, they are available during most seasons including strategic period of the year, when the conventional staples and vegetables are scarce, (Okafor, 1991). Edible, non-timber forest resources also generate substantial cash income, for rural dwellers thereby contributing to their welfare and means of livelihood. On the other hand, their ecological contributions cannot be overemphasized, as they provide a micro-climate which makes life conducive to man and a steady habitat for wildlife, (Science Daily, 2010). Surprisingly, a large percentage of the populace could not make use of the enormous potentials of the forest, because they do not have access to them, being in the wild. A few of these plants species that enjoy little domestication are not established in commercial plantations. They are found in isolated stands in farmlands, home gardens or existing agroforestry and cash crop plantation. For those growing in their natural habitat, little or no comprehensive in-situ regeneration study has been carried out in the forest, nor ex-situ conservation for plantation establishment of these species. However, sustainable management options for most of these products are necessary to curb over exploitation that may lead to extinction. These can only be possible if research effort is focused on improvement strategies, with insight on the structure and functioning of the tropical ecosystems, which include the silvicultural processes involved in regeneration, improvement and conservation of these species, (Philips *et al.*, 2002).

Most neglected are lianas (climbers), which due to their morphological characteristic as climbers are difficult to establish in orchards thereby discouraging research efforts on them. However, lianas are a vital part of the tropical rain forest ecosystem. Most of them have vital potentials that are yet to be exploited. A very important tropical liana in this category is *Plukenetia conophora* (Mull Arg) also known as African walnut, a multipurpose liana of high economic importance with vital nutritional, medicinal and industrial values.

1.2 Statement of problem

With the increasing trend of urbanization, bush burning and timber exploitation, a lot of indigenous non-timber forest plant species among which is *Plukenetia conophora*, are fast disappearing. Also as demand for fruits and other non-wood forest product is increasing in the highly populated farming areas of Nigeria, the supply of fruits from forests are threatened by increasing deforestation and unsustainable farming practices, (Anegbe *et al.*, 2003). Plant resources are thus continuously depleted through the subsequent changes in land use pattern. Oracle (2010) stated that biological resources are degraded and lost through many developmental activities. For centuries humans have altered landscapes through deforestation, fire and over-use. He explained further that already about half of the world's original forests have disappeared and they are still being removed at a rate ten times higher than any possible re-growth. According to Cable Network News (CNN) report given by (Mathew, 2010), at least one, of every eight plant species in the world is under threat of extinction. More threatened are useful plant species that are yet to be established in plantations and their conservation processes are yet to be ascertained owning to scanty knowledge on their silvicultural requirements.

Plukenetia conophora is one of such species, which despite its usefulness has not been comprehensively studied and no detail information exists on its improvement and conservation capabilities (Awodoyin *et al.*, 2000). Its distribution pattern and variability among and within its area of endemism is yet to be ascertained. The seeds of *Plukenetia conophora* are recalcitrant, with inadequate storage facilities; they disappear almost immediately after the fruiting season, due to post harvest damages. Germination of *Plukenetia conophora* under natural condition is plagued by human activities, animal, environmental and disease influences which make its large scale production difficult. On the other hand, plantations of *P. conophora* are not easily established due to inadequate knowledge about suitable silvicultural techniques, nutrient requirement and water use efficiency. The seeds of the species are eaten by man and rodents with little left for regeneration, thus, the need for its vegetative propagation through ex-vitro and in-vitro methods in accordance with the current trend in biological research.

1.3 Justification

Plukenetia conophora Mull Arg. (Walnut) is a multipurpose liana with high potentials of nutritional, medicinal, economic and other significant products in the land use system. (Awodoyin *et al.*, 2000) identified that the plant has great prospect as food, medicine and industrial raw material. Walnut is an oil rich food and a great source of Omega 3 fatty acids. It contains nutritional minerals such as calcium, iron, magnesium, and phosphorus. Its health benefit can hardly be over

emphasized particularly in area of cholesterol control, blood sugar and improvement of cardiovascular functions, (Onyema, 2010). Fresh seeds are antidote to snake bite, while roots are used for gynaecological problems. The oil is used as a massage, and industrially in the paints and vanish industries. Hence there is need to conserve this important plant to prevent it from going into extinction.

Ex-situ conservation of this species requires knowledge of the variation that exists within and among the different provenances of its endemism. Variation is the mainstay of biological stability. It enables species to adapt to changing environment and to survive, hence in establishing plantations of *Plukenetia conophora*, variation study is necessary to select an elite ecotype that will adapt best to the new environment. Silvicultural requirements for growths and conservation need to be considered when establishing plantations, these include ideal storage temperature for sustainable viability, since seed germination is a vital aspect in the life of every plant in determining the final yield. Response to water deficit is another requirement for conservation because it is an indicator of the level of stress at which the plant can survive. Hence, the water use efficiency of *Plukenetia conophora* was determined in this study to know how best it could survive in water logged and dry zones of Nigeria.

Before the advent of chemical fertilizer, the use of organic fertilizer has been in place, the earth and animals enhanced fertility of soil through decomposition. Enriching the soil with organic matter improves the soil's capacity to hold water and nutrients, (Gaballah *et al*., 2010). Organic fertilizer is cheap, affordable and available to farmers, hence the application of poultry manure, cowdung and compost in this study to determine the most suitable in enhancing the growth and development of *P. conophora* on the field.

Seeds of *Plukenetia conophora* are not regularly available for sowing due to their recalcitrant nature, irregularity in flowering and for anthropogenic reason hence, there is the need for alternative planting stock for regeneration. Vegetative propagation has been discovered to be an alternative to seed propagation, thus, juvenile double node cuttings produced from decapitated seedlings were used in this study to produce rooted cuttings as planting stock. With current development in biological sciences, tissue culture has been identified as being capable of producing true- to- type micro plants as clones for rapid multiplication. Therefore, micro- propagation using embryo culture and nodal cuttings with modified Murashige and Skoog media were applied to *P. conophora* for rapid multiplication of planting stock for plantation establishment.

1.4 Objective of the study

The broad objective of this research is to determine the silvicultural requirements for propagation and conservation of *P. conophora*.

The specific objectives are to:

(a) identify the variation that exists in *P. conophora* from four sources in Southwestern Nigeria.

(b) identify the best temperature for its seeds sustainable viability.

(c) enhance the growth of the species using organic manure.

(d) investigate the survival ability of *P. conophora* in low moisture soils and waterlogged areas.

(e) examine the effect of different heights of decapitation on the coppicing potential of the species as an ortet base; and

(f) investigate the possibility of mass producing *P. conophora* through micro and macro vegetative propagation.

1.5 Scope of the study

The studies were carried out on fruits and seedlings of *Plukenetia conophora* procured from existing stands in four sources, within the Southwestern Nigeria, where the species is endemic – Ibadan (Oyo state), Igbajo (Osun state), Ijebu Ode (Ogun state) and Akure (Ondo State).

Variations in fruit morphology and number of seeds per fruit within the four sources were investigated. Effect of different storage temperatures on viability of seeds of *P. conophora* was investigated. Silvicultural requirements for growth and development of seedlings were examined. The possibility of rapid multiplication of propagules through macro and micro propagation techniques were also investigated

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The study plant *Plukenetia conophora* Mull Arg. (Syn. *Tetracarpidium conophorum*).

Plukenetia conophora belongs to the plant family *Euphobiaceae*. It is a wild woody perennial climber (liana) whose length ranges between 12 and 30 metres (Hutchinson and Dalziel, 1958; Okafor, 1975; Awodoyin, *et al.,* 2000). The common name is at variance depending on cultural differences. However, it is referred to as African walnut, "conophor nut", "Awusa" (Sierra Leone), "Asala" (Yoruba), "Ukpa" (Ibo). It is a non-timber forest species protected on farmlands and sparingly cultivated for its oil rich fruits that serve as good source of income to the rural populace (Awodoyin *et al.,* 2000).

Plukenetia conophora originated from Sierra Leone where it is known as 'Awusa' and it spread to other parts of West Africa. Information from the herbarium (F.H.I) of the Forestry Research Institute of Nigeria (FRIN) revealed that the species is abundant in Oyo State, around Ibadan district (F.H.I. 19474, F.H.I. 2709). In Osun State, *Plukenetia conophora* is prevalent in Aba-Panu located on kilometer 8, Ikirun-Igbajo road (F.H.I. 47505). In Ogun State, it is found in Ijebu-Ode distrsict (F.H.I. 31106), especially in Akilla waterside. In Ondo province, it is abundant in Akure district (Apomu village) (F.H.I. 3608). It is endemic in Benin province, Evbonogbo Usonigbe Forest Reserve (F.H.I. 35697). The species is also endemic in Cross River State on Calabar Manfe road, (F.H.I. 105502).

Plukenetia conophora has a panicle inflorescence. The flowers are unisexual and monoecious. Each inflorescence carries one postulate flower near the base and a number of staminate flowers way up. The flowers are whitish in colour, petalous and have reduced greenish sepals. Male flowers have numerous stamens that are free. Style is stout with four spreading stigmas while the ovary is superior. Flowers are pollinated by visiting moths that are probably enticed by the fragrance. The fruit is a capsule with 1 – 4 chambers, based on the number of cells in the ovary. One seed unit occupies a chamber and it is completely secluded from other seed units. The seed unit which is also the planting unit is made up of an outer stony testa that is black in colour when fully mature and cream white at immature stage. The roundish seed unit has a diameter of 2.8 ± 0.2cm and weighs 6.86 ± 0.2g. The size of ripe fruit is 5 – 7cm^2 and about 4. 5cm thick when four seeded and weighs about 105 ± 0.2g, (Awodoyin *et al.,* 2000)

In testa cracked seedlot, germination commenced at 20 days after sowing while in seeds with intact testa, germination was first recorded on the 30th day of sowing. (Awodoyin *et al.*, 2000) Studies carried out by (Oduwaiye, 1991) on germination consistency in seeds of different size classes of *P. conophora* indicated that seeds from the small size class had the best germination value in the open (90.8%) than when planted under humid propagator (83.3%), indicating that the seeds do not really require humid propagator to germinate. Nevertheless, field establishment of the seedlings showed a survival percentage of 70%. Awodoyin *et al.*, (2000) reported that seedlings commenced flowering and fruit production at 18 months of growth.

2.2 Provenance variation in tree species

Variation is an important aspect of breeding in bringing about improvement to any particular plant species. According to (Hettasch *et al*., 2009) variation is the observable differences between and within individuals of a species. It is the mainstay of biological diversity. For all wood that have been adequately studied to date, variation among trees of the same age, growing on the same site has always been found to be large (Zobel *et al.*, 1987). Trees usually vary in their properties and appearances. Some of the variation is caused by differences in the genetic make-up of the individual tree; other variation is as a result of the different environments impacting on the tree, (Hettasch *et al.*, 2009). There can also be an interaction between the genetic make-up of the tree and the environment. It enables species to adapt to changing environments and to survive. Species which are unable to change their genetic make-up to suit a changed environment become extinct and species that are able to pioneer new environments spread over the globe. According to Hettasch *et al*., (2009), genetic variation is the foundation on which tree breeding is based. Tree breeding is an artificial form of evolution which requires genetic variation to be successful. The importance of an understanding of variation to tree improvement cannot be overemphasized. The tree breeder needs to be able to distinguish between genetic and environmental effects on trees. He needs to produce highly specialized products (trees) with possibly limited variation in some characteristics and at the same time with a maximum amount of genetic variation in order to make a good breeding progress. Abercomber *et al.*, (1980) stated that a breeding programme involves the regulation of variation through control over the reproductive system. He reported that the success of a breeding programme is contingent upon information about the amount and pattern of variation and the reproductive biology of the species involved. Also, that a tree breeding programme typically consists of: Selection of superior phenotype, variability of the species, its biology, the specific objectives of conservation, local conditions, perceived value of the species and germplasm.

Uguru, (1998) maintained that the variation existing among different varieties of plant species is the bedrock of any meaningful plant breeding and improvement programme. Many authors have reported that variation among individuals of any population is based on environmental modification, genetic recombination and mutation, (Otegbeye, 1986; Harten, 1988; Adewusi, 1997 and Gbadamosi, 1998). Oni and Gbadamosi, (1998) noted a significant variation in all quantitative characters of fruits and early growth characters of seedlings in *Dacryodes edulis* from four sources in Nigeria.

2.3 Conservation of genetic diversity

The world's forests are declining at unprecedented rates. Losses are resulting directly from clearing land for agriculture, roads, settlements, logging timber and cutting for fuel. As the number of individuals and populations that comprise a species are reduced and its gene pool eroded, the species can be pushed to extinction. This according to Hettasch *et al.,* (2009) is a major concern of breeders world-wide. Once the genes of populations or species are extinct, they can never be brought back to aid their adaption to changing environment or be used in developing improved varieties. Guarino *et al.,* (1995) explained that threats to genetic diversity can be classified into four main categories: external socio economic factors, direct human threats or local impact, regional/global impact and natural hazards.

Henne and Thies,(2001) reported that of the original forest cover, half is gone and only one-fifth remains as large tracts of ancient forest – that is forest ecosystems shaped mainly by nature where human impacts have been comparatively small. Science Daily, (September 29, 2010) reported that a global analysis of extinction risk for the world plant conducted by the Royal Botanic Gardens, Kew, together with the Natural History Museum London and the International Union for Conservation of Nature (IUCN), have revealed that one in five of the world's plants are threatened with extinction. This presents a baseline for future plant conservation. Despite the pressure of economic development, which elicited the unrestrained exploitation of the forest, these resources must be conserved for sustainability and posterity purposes. Allaby, (1994) defined conservation as the planning and management of resources so as to secure their wide use and continuity of supply while maintaining and possibly enhancing their quality, value and diversity. It is described as the wise use of nature's bounty on a sustained yield basis, (Elliott, 1996).

Forest resources can either be conserved *in-situ* or *ex-situ*, (Ola-Adams, 2000).

***In-situ* conservation** – involves maintaining plants and animals in their original habitat, it is highly desirable in cases of tree species with danger of over-exploitation and where the appropriate silvicultural or propagation techniques for their perpetuation are unknown, (Ola-Adams, 2000). In-

situ conservation areas include, Strict Nature Reserve, Games Reserve, National Park, Managed Nature Reserve, Botanical Gardens, Arboreta and Anthropogenic Reserves (Sacred grove).

***Ex-situ* Conservation -** refers to maintaining organisms outside their original habitat; in facilities such as plantations, orchard, seed gene banks, field gene banks, in-vitro gene banks and clone bank. A combination of in-situ techniques can be used to conserve and manage the genetic resources of trees. In-situ and ex-situ methods are taken as complementary, not opposing methodologies. The selection of one or the other of these methods will depend on factors such as the variability of the species, its biology, the specified objectives of conservation, local conditions, the perceived value of the species and germplasm, (Hettasch *et al.*,2009).

2.4 Seed storage and viability

The success of any afforestation or reforestation project depends largely on regular availability of good quality seeds in adequate quantity for seedling production. Thus, the importance of seeds in forestry cannot be overemphasized, (Oni, 1992). Seeds are grouped according to their physiological storage potential as recalcitrant and orthodox, (Bonner *et al.,* 1994). Recalcitrant seeds are those that lose their viability easily. Such seeds cannot survive desiccation, they germinate instantly and their storage life is usually only a few days. Most climax tropical rainforest tree species produce recalcitrant seeds and thus must be stored as growing seedlings rather than seeds, (Allaby, 2010). On the other hand, the orthodox seeds are those that can conveniently be stored by different means until when required for propagation. The seed is the beginning and the end of the life cycle of many higher plants. Seeds are extremophiles and can tolerate very severe stresses, including heat, cold, desiccation and high pressure. These attributes make seeds the ultimate means of survival of species and their population. Seed deterioration during storage is an inevitable and irreversible process. The rate of seed deterioration depends on external factors, (relative humidity and temperature) and internal factors (genetics and seed quality), (Research Interests, 2010). Studies carried out by Michael and Pritchard, (2002) revealed a large variability in Neem (*Azadirachta indica*) seeds from ten different mother trees in East Africa. There was a decline in viability after 4 months of storage from 78% - 60%. Water relations, desiccation tolerance and longevity of *Taxus brevifolia* (Nutt) seeds were studied by Christinal *et al.*, (2005). It was concluded that the species has poor storage characteristics even with optimum water content and low temperatures. Odiaka and Akoroda, (2009) studied the effect of storage duration on *Telferia occidentalis* seeds and concluded that the seeds are recalcitrant and lose viability easily. Thus storing the seeds for only sixty days could be a better option than storing for a longer period. Studies on the effect of seed maturity, temperature and storage vigor of *Picrasma javanica* carried out by Ninik, (2009)

indicated that storage under lower temperature 5°C – 20°C was able to maintain seed vigor for 3 months storage.

Studies on effect of seed storage conditions on the growth and yield of barley, broad beans and peas which was carried out by Abdalla and Roberts (2010) showed that a combination of factors such as time, temperature, moisture content and seed deterioration during storage reduced viability below 50 percent, while the final yields of crops produced from surviving seeds were significantly decreased. Ehiagboare and Onyebi, (2009) examined the optimum method of seed storage, enhancement of imbibitions capacity and pre-sowing treatment of *Newbouldia laevis*. (P.Beaav Ex bureau) and concluded that a maximum storage period of one month, imbibitions period of 24 hours and soaking in water at room temperature are optimum for germinating and raising *N. laevis*. Rao and Priya, (2008) studied the effect of duration of storage period on viability of seed and seedling vigour of *Entada purseatha* and concluded that although the seed maintained the same viability up to one year, the viability progressively reduced with prolonged storage period to 39% .

2.5 Influence of seed size on seedling growth

In any regeneration programme, the physiological properties of seed are most valuable and should not be ignored. Such properties include the effect of seed size on germination, seedling vigour, and growth morphology, (Festus *et al.*, 2003 and Tomoaki *et al.*, 2001).

A large variability in seed size is common in woody species and could affect seedling quality. Large seeds have traditionally been viewed as advantageous in closed communities, such as forest, whereas small seeds would be more suitable for open succession communities, (Gross, 1984). Seedlings resulting from large seeds, rich in food reserves confer competitive advantages on seedlings, (Fenner, 1985). According to Desai *et al.*, (1997) large seed size is an indication of vigour; seeds may be embryonic alone or may also contain endosperm, ready to be absorbed into the developing environment conditions over a long period of time, while small seeds under the same conditions deplete their reserves in the process of respiration and physiological rearrangements. When conditions improve, the seedlings emerging from smaller seeds are either unable to change over to autotrophic feeding and die, or their growth and development are strongly retarded, (Khera *et al.*, 2004). The bigger seeds on the other hand produce vigorous seedlings with a larger area of green leaves capable of photosynthesis, (Boot, 1996). In the investigation by Oni and Bada (1991) on the effect of seed size on seedling vigour of *Terminalia ivorensis* (Idigbo), seed size significantly affected all the growth parameters considered. Seedlings from large seeds showed better growth variables (height, diameter, leaf production, leaf area, biomass production and a higher relative growth rate), while seedlings from small seeds had higher values for net assimilation rate. Junquiang and Romo, (1998) investigated the relationships and seedling growth in *Ceratodes*

lanata (winter pursh) and *Artemisia canapursh* (silver sagebrush). Total germination for silver sagebush initially increased with seed weight but declined at weights greater than 0.57mg. Studies carried out by Ekta and Singh, (2000) on the influence of seed size on seedling growth of *Albizia procera* under different soil water levels inferred that seedlings from large seeds had a higher biomass and leaf area and were more tolerant of long term extreme water stress compared with those from small seeds. Although Khera *et al.,* (2004) and Navarro *et al.,* (2006) mentioned that seed size may increase germination and seedling growth of some species, Indira *et al.*, (2009) and Tilki and Alptekin, (2005) pointed out that, occasionally the large seeds performed less well than medium or small seeds. Studies that were conducted by Cicek and Tilki, (2006) to evaluate how acorn size (small, medium and large) influenced *Quercus patraea* (Maltoschka) moisture content and germination, showed a maximum germination percentage in large, and medium size, while the small size recorded the least. Seedling emergence and survival was the lowest in small seed size class in the nursery, although within one year they increased up to the size of the large seed size class. However, in the study on Samara size of *Ailanthus altissima*, large seed did not produce the highest germination and seedling survival (Delgado *et al.*, 2009).

2.6 Responses of young seedlings to different watering regimes

Water constitutes a major part of the tissue mass and is required for growth and development. When it is scarce in the soil, the plants become stressed due to an imbalance in the water potential. Understanding of the response of plants to water deficit is a vital indicator of the level of stress at which plants can survive and their coping mechanism, (Luvaha *et al.*, 2004). These authors investigated the effect of different watering regime on growth of rootstock seedlings of mango (*Mangifera indica*) which were subjected to four different watering regimes, viz, daily, once a week, twice a week and once in two weeks. The result showed that increasing water deficit affected growth variables, the root to shoot ratio also increased, concluding that water deficit reduces plant growth. Studies carried out by Ruiz – Sanches *et al.*, (2007) on daily variations in water relations of Apricot trees, under different irrigation regimes, indicated that the deficit irrigated trees showed among other effects, a pronounced decrease in leaf water potential and leaf conductance (g . The effect of short term flooding was also investigated by the same authors in two-year old Apricot trees (*Prunus ameriaca* C. V. Bulida). The young trees were subjected to fifty hour flooding by submerging the pots in plastic water tanks; some were irrigated daily, while the control was drained of water. A decrease in transpiration in the flooded trees with respect to the control plants was evident. The daily pattern of soil oxygen concentration and plant hydraulic resistance followed a similar trend during the flooding. Polomski and Rigling, (2010) also investigated the pathogenicity of *Bursa phelenchus* and *B. mucronatus*, isolated from declining scors pine (*Pinus sylvestris*) in

relation to drought stress of different watering treatments 50, 100, 150 and 250 ml of water per pot bi- weekly. Result showed that with decreasing water supply there was faster disease progress and pine mortality for both *Bursa phelenchus* species.

Changes in morphological,physical and biochemical indicators were observed in the studies on drought tolerance carried out by Xiao – (Yunchuo *et al.*, 2010),using three hybrid poplar clones grown under four watering regimes; control, well watered (100%) field capacity (fc), 50% fc, 40% fc, and 30% fc. The result showed a decrease in net photosynthetic stomata conductance, shoot height, total biomass and chlorophyll content in all the three clones. Studies have shown that annual growth of wetland trees are related to variations in hydrologic regimes, (Bobby and Sharits, 1997). The study also showed no significant relationships between changing water levels and tree growth in areas with permanent flooding or soil saturation. Further changes in growth of *Sylvatica*, *aquatic* and *Distichum* species were significantly cross-corrected with weekly changes in the atmospheric water balance at sites with either periodic or permanent flooding. Khalil, (1993) pointed out that the successful establishment and growth of woody plants in drought prone environments depends to a large extent on their capacity to adjust their forms and functions to offset the detrimental effects of both edaphic and atmospheric water deficit, He explained further that the ability of plants to function under conditions of low soil moisture depends on their capacity to adjust form and function to offset the damaging impact of negative water potentials in the soil and in the atmosphere.

2.7 Organics – An alternative to synthetic fertilizers.

Before the use of chemical fertilizers, the fertility of the soil was enhanced through the decomposition of raw natural proteins of its fauna and flora. Hence the soil received the nutrients needed to maximize fertility from only organic humus and that was sufficient to sustain life, (Rob, 2000). According to Rob, (2000) chemical fertilizers can neither add to the humus content of soil nor replace it. When chemical fertilizers are put into the soil, they dissolve and seek natural combination with minerals already present. Wolf, (2000) explained that chemical fertilizers do not work on the soil but are enforcedly imbibed by plants, the plants may look lush, but lush growth produces watery tissues which are more susceptible to disease. He further emphasized that organic matter supplies raw materials to earthworms and naturally occurring bacteria, fungi and other micro organisms in the soil. These organisms digest organic matter in a process known as the decay cycle. This cycle breaks down the complex compounds in the plant materials into forms that can be absorbed by plant roots, creating a natural recycling process.

Enriching the soil with organic matter also improves soil structure. This in turn improves the soil's capacity to hold water and nutrients and to release them to plant roots as needed. Studies carried out

by Gaballah *et al.*, (2010) on growth of *Geranium* plant under saline condition using bio-fertilization with *Rhizobium*, increased leaves content of NPK, Ca, Mg and Fe, while it decreased Na content. Also the use of bio-fertilizers under saline conditions increased the active principal contents in the geranium plant, which will be helpful for the cultivation of the plant under saline condition. Mahmound *et al.*, (2009) evaluated the effect of three compost types, plant residue, animal residue and when combined with mineral nitrogen fertilizers on cucumber, the result showed that the combination of organic and inorganic fertilizers could increase plant growth, yield quality and soil fertility. The study also confirmed that composted organic wastes can be used to substitute for around 25% of chemical nitrogen fertilizer. Sridhar, (2006) narrated his experience in organic agriculture in Nigeria, whereby community waste is recycled and converted to organic manure; he concluded that from the laboratory, the technology was able to produce up to 25 tons of finished organic fertilizer. A variety of crops such as cereals, leafy and fruit plants were field tested and agronomic data were collected showing increase in growth. The fertilizer produced was also found useful in remediation of soils contaminated with heavy metals such as lead, calcium and copper when used in phytoremediation technique. Ojo *et al.*, (2006) studied the effect of different organic mineral fertilizers (OMF) on maize sorghum and groundnut growth. The research concluded that application of (OMFs) significantly increased yields of the test crops compared with inorganic fertilizer and control plots. Studies carried out by Babalola, (2006) on fertilizer quality of composts made from various animal manures on the yield of okra and tomato showed that the growth of tomato consistently increased due to amendment with composted animal manure. The yield of okra also increased with application of swine manure (dung). The study on the effect of organic manures on the growth and dry matter yield of *Amaranthus cruentus* (tete) investigated by Daramola *et al.*, (2006) indicated that application of the organic Nitrogen sources gave taller plants, more profuse leaves and branches and shoot dry matter yield compared with the control.

2.8 Vegetative propagation

Vegetative propagation is the means of obtaining a new plant from vegetative parts of the original plant, which is possible because of the totipotency of the cell. Totipotency means that every cell of the plant contains the genetic information necessary to regenerate the entire organism, (Hettasch *et al.*, 2009). Vegetative reproduction comprises a broad range of techniques involving the manipulation of plant tissue samples, (section of stem, leaves, roots, seed or even cell cultures) which ultimately allows for complete vegetative propagation of the whole plant. The end results are genetically identical individuals, clones, varieties or lines, (Yanchuk, 2001). Mudge and Brennan, (1999) noted that the development of vegetative propagation technique represents the first step in the process of domestication of plant species for large scale afforestation materials. Since

seeds of most tropical forest trees are not regularly available either due to irregular flowering and fruiting phenologies or recalcitrant nature of the seed, it follows that effective method(s) of stock plant management must be devised. This is necessary to ensure all year round production of cuttings in nursery for large scale plantation programmes, (Oni, 1995). Vegetative propagation potentials of stem cuttings of different physiological ages of *Gongronema latifolia* were studied by Agbo and Obi, (2007) and the results showed a significant variability in rooting and shoot development and growth of shoot of the species. Oni and Ojo, (2002) investigated the germination, growth and cloning of the popular chewing stick, *Massularia acuminata* and reported that the species is amenable to cloning with or without auxin treatment. Likewise Mumtaz *et al.*, (2009) studied the cottage propagation of *Aesculus indica* tested by using different concentrations of indole-acetic acid (IAA) at 200, 400 ppm, indolebutyric acid (IBA) at 200, 400 (ppm) and naphthalene acetic acid (NAA) at 200, 400 (ppm). It was concluded that IBA at 400 ppm was best for vegetative propagation of the species. Vegetative propagation of *Pawsinystalia johimbe* carried out by Ngo *et al.*, (2003) showed that the ornamental plant conveniently rooted without any hormone treatment. Also, another study by Tiwari and Das, (2010) on in- vivo experiment of two ethnomedicinal shrub species, *Embelia tsjeriam* and *Caesalpinia boncluc*, using exogenous hormones in powder formulation,(IAA, IBA and NAA) concluded that maximum percentage of rooting and sprouting was obtained from NAA and IBA treatments at four nodal lengths, for the two species. Traditionally, vegetative propagation has been used to ensure genetic uniformity research and tree improvement programs. Vegetative propagation by stem cutting is becoming an important tool for forestry improvement activities and for the establishment of clonal plantations (Chaturvedi *et al.*, 1992). The authors report on the effect of stock age, number of leaves left on shoot, and the use of epicormic shoots on rooting of *Acacia auriculiformis* stem cuttings showed that cuttings from epicormic shoots developed from coppiced trees had superior rooting ability. Propagation by vegetative means is often the best way to preserve selected traits in trees when seeds are in short supply, unavailable, difficult to germinate or sterile, Shi and Brewbaker, (2006).

In line with this assertion, Anegbeh *et al.*, (2006) studied the vegetative propagation of *Allanblackia floribunda*, a highly economic tree species using stem cuttings; the success of the study by rooting the species early shows that vegetative propagation by leafy stem cuttings can increase the production of *Allanblackia floribunda* planting methods. Effect of propagation media on the rooting of leafy stem cuttings of *Irvingia wombolu* (*vermoesen*) was examined by Dolor *et al.*, (2009). Fine sand was recommended as an appropriate medium for optimum rooting, while, coarse sand – sawdust mixture may be an alternative. An extensive study was carried out by Dolor *et al.*, (2010) on the effect of pawpaw seed extracts on the rooting of leafy stem cuttings of *Irvingia*

wombolu. It was noted that rooting success of many species by leafy stem cutting in terms of rooting percentage and the quality of roots produced are directly influenced by many interacting factors, both pre-severance and post- severance. Their study aimed at developing a protocol for replacing expensive rooting hormones with pawpaw seed extracts which they found enhanced the rooting potentials of *Irivingia wombolu.*

2.9 Tissue culture (Micropropagation)

With current development in biological sciences, new methods of reproducing and conserving genetic resources have been identified; among these is Biotechnology. This according to the United Nations Convention on Biological Diversity (UNCBD) is any technological application that uses biological systems, living organisms or derivatives to modify product or processes for specific use. Yanchuk (2001) indicated that biotechnologies are categorized into three main parts viz: The use of vegetative reproduction methods, the use of molecular genetic markers and the production of genetically modified organisms (GMOs) or transgenic trees. Plant tissue culture (Micropropagation) is a sub-set in the area of biotechnology, (Michael and Great-Jan, 2007) defined tissue culture as the science of growing plant cells, tissues or organs isolated from the mother plant, on artificial media. Yanchuk (2001) and Hettasch *et al* (2009) opined that micropropagation is the development of clonal lines from small tissue samples such as buds, roots and embryos extracted from seeds. This organized growth whereby plant organs are transferred to culture and continue to grow in- vitro either directly upon an organ or a piece of tissue placed in culture (an explant) is called organogenesis or morphogenesis. Hettasch (2009) explained further that tissue culture is the propagation of pieces of plant material in an aseptic nutrient medium with a regulated environment. The donor plant is vegetatively propagated in order to produce large numbers of genetically identical individuals (clones). The explants for cloning selected genotypes could come from seeds, buds, leaves, cotyledons, stem sections and embryos. However, many forest and fruit trees species still remain recalcitrant to in-vitro culture and require highly specific culture conditions for plant growth and development (Jain, *et al.*, 2007). Micropropagation protocols for cloning of mature trees of *Balanites aegyptiaca*; (*Balanitaceae*), the Nimbu (*Rutaceae*) and *Syzygium cuminir*, the jamun *(myrtaceae*) were developed by Rathor *et al.,* (2005), fresh shoots sprouts derived from the trees were used as explants. The nodal explants produced multiple shoots in- vitro by activation of auxiliary meristems on modified Murashige and Skoog medium (MS + 0.45ml BAP). This medium was also used for rapid multiplication of elite plantlets of cotton *(Codiaum variegation*). Asma and Saifullah, (2008) worked on in- vitro propagation of the same species, (*Codiaum variegation*), roots were efficiently induced by using MS media supplemented with 20 mg/l of IBA. For shoot induction, MS supplemented with 0.5 mg/l of BAP. 6 benzel amino purine, shoots were effiiciently

induced in the study carried out by Soulange *et al.*, (2007). Molecular techniques and tissue culture were used to validate the morphological differences in two species of the *Lauraceae* family namely *Cinnamoumum camphora* and *Cinnamimum verum*. Shoot tip and leaf explants of these species obtained from field grown trees were cultured on MS medium supplemented with various levels of BAP and 2, 4-dichlorophenoxyacetic acid (24D). Hundred percent multiple shoot formation was observed in shoot tip explants of *C. camphora* cultured on MS medium containing 1.0mgl^{-1} BAP supplemental with 2.5mg L^{-1} 24D. Leaf explants of *C. camphora* responded only to MS medium containing 1.0 mgl^{-1} BAP supplemented with 24D (0.005 – 5.0 mgl^{-1}) by forming callus. *C. verum* explants reacted to the treatment by sprouting. All shoots (100%) produced were transferred to the basic MS medium and transplanted to polypots containing topsoil plus compost combined at 2: 1. In vitro propagation of *Jasminum officnale* L, a woody ornamental vine, was experimented by (Bhattacharya and Bhattacharya, 2010). The research was on protocols developed for shoot regeneration from existing as well as newly developed adventitious auxiliary buds via proper phytohormonal stimulation using MS medium containing 4.0mg/l BAP, 0.1 mg/l NAA and 40g/l sucrose over a period of 8 weeks. Successful results were obtained with 70% shoot regeneration. Simon *et al.*, (2007), also investigated the efficient preconditioning methods for in-vitro multiplication of *Uapaca kirkiana*. Plant material from mature stock plants were compared with lateral shoots (New shoots). Sodium hypochlorite (NaOCl) and mercuric chloride ($HgCl_2$) were used as surface sterilant. Lateral shoots responded positively to shoot multiplication on 0.75 strength MS medium supplemented with a combination of 0.1 mg/l.BAP, 0.04 mg/l, NAA and 0.3 mg/l casein hydrolysaite. (36% rooting) was achieved with 0.5 strength MS medium supplemented with 2.5mg/l indole- 3 butyric acid (IBA). Also studies conducted by Obembe, (2000) on protocols for establishing *Cola nitida* tissue in- vitro produced successful results. He worked on micro-propagation of *Cola nitida* (vent) Schott and Endlicher using modified MS medium. The best sterilization procedure was established in the step-wise treatment with 70% ethanol for 20 seconds and 10% CaOCl for 10 minutes. The use of modified MS medium without Zn and Cu elements as basal medium was found appropriate. The appropriate antioxidant technique was also established with 10 mgl^{-1} ascorbic acid.

According to Jain *et al* (2007) the main advantage of using tissue culture as a tool in breeding programs and mass propagation operations is the potential that exists for the multiplication of a genotype.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Reconnaissance survey

Based on information obtained from Forest Herbarium Ibadan, (FHI) on preliminary survey of the endemic areas of *Plukenetia conophora*, four sources were selected: Ibadan, (Oyo State), Igbajo (Osun State), Ijebu-Ode, (Ogun State) and Akure, (Ondo State) as seed sources in the study area (Fig 3.1). A reconnaissance survey of the distribution of the species in these locations within the Southwestern part of Nigeria was carried out prior to flowering and fruiting to ascertain stands with desirable traits and appropriate period for fruit procurement.

Location of experiments

Experiments on silvicultural requirements were carried out in the nursery at the Forestry Research Institute of Nigeria, Jericho, Ibadan, while those on micropropagation were carried out in the tissue culture laboratory of National Centre for Genetic Resources and Biotechnology (NACGRAB), Moor Plantation, Ibadan, Nigeria.

3.2 Description of seed sources

Mature seeds of *Plukenetia conophora* used for this study were obtained from four (4) sources along its natural spread in Nigeria. Fig 3.1 shows location of seed sources in Nigeria. This information was obtained from Forest herbarium Ibadan (FHI)

3.2.1 Ibadan (Oyo State)

Ibadan is the capital of Oyo State in Nigeria, GPS information indicates that Ibadan lies between Latitude 7°24´N and Longitude 3°55´E. The climate is wet and dry season with relatively high humidity. The rainfall distribution is bimodal between June, July, August and September, while the dry season is from end of October to early March. The annual rainfall range is between 2150-2,600mm.The mean minimum and maximum temperature range is between 23°C to 33°C respectively. The soil type is classified as Ferric luvisols and the vegetation is the rain forest type with few derived savanna species.

3.2.2 Ijebu Ode (Ogun State)

GPS information indicates that Ijebu Ode lies along latitude 6°47´ N and longitude 3°58´ E at an altitude of about 200m above sea level. The climate is tropical type with the rainy and dry seasons. The annual rainfall ranges between 2100mm and 2500mm with June and July as the peak. The dry season is between October and March. The mean minimum and maximum temperatures are 24°C and 34°C respectively. The soil is predominantly ferric luvisol. The natural vegetation is a mixed rain forest and derived savannah.

3.2.3 Igbajo (Osun State)

Igbajo is in Osun State, GPS information indicates that it is located at 7°56´N and 4°41'E. It is at an altitude of 200m above sea level. The climate is the West African monsoon type with the dry and wet seasons. The vegetation of Igbajo is more of guinea savannah. Annual rainfall range is between 1,500mm to 1900mm with the peak in June. The dry season is from October to March. The climate is warm humid with temperature of 25°C to 35°C. The soil type is ferric luvisol while the vegetation is more of derived savannah.

3.2.4 Akure (Ondo State)

Akure is the capital of Ondo state of Nigeria. GPS indicates that It is located at 7°57¹34N and 8°45¹E, with an altitude of 396m above sea level. The rainfall distribution is bi-modal with the peak in June and July. Annual rainfall ranges from 1524mm-2100mm while the dry season is from end of October to February. Akure has a warm humid climate characterized by low temperature range of 22°C to 31°C with a relative humidity of 86.72%. The soil is dystic regosols. The vegetation type is the tropical rain forest. The altitude is approximately 30 m above sea level. The topsoil is sandy – loam with clay deposits.

Source: Geographical information system unit of Forestry Research Institute of Nigeria Ibadan

Internet:Map –GPS –coordinates.com

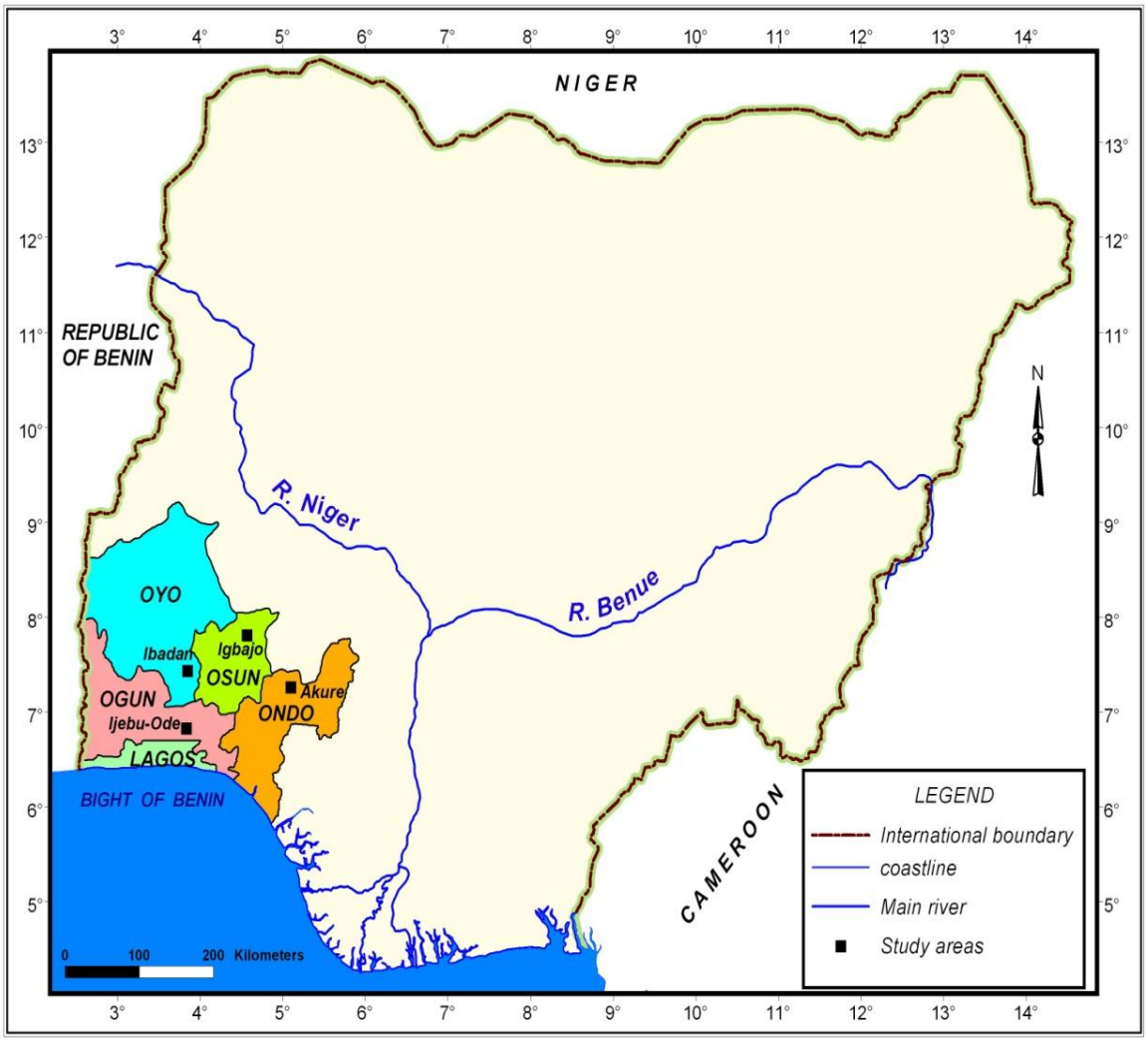

Fig. 3.1: Map of Nigeria showing the sources of *P. conophora* seeds used for the study

3.3 Materials

All the experiments were carried out in the Laboratories, Nurseries and experimental plots of Forestry Research Institute of Nigeria, (FRIN), Ibadan and National Centre for Genetic Resources Conservation and Biotechnology (NACGRAB), Ibadan, Nigeria.

Materials used for this study include:

Mature seeds of *P. conophora* from the selected study sites, Metler sensitive electronic weighing balance,(model E300), laboratory equipments – pH meter, autoclave, test tubes, fume cupboard, nursery tools , poly pots, hand trowels, watering can, propagators and nursery consumables such as washed and sterilized river sand, top soil and growth hormones.

3.4 Study 1: Morphological variation in fruits and early growth of *P. conophora.*

i. Fruit procurement and quantitative seed characterization;

ii. Experimental seedlings production

iii. Early growth and biomass assessment of seedlings.

i. Fruit procurement and quantitative seed characterization

One hundred (100) mature fruits were procured from each of the four provenances, Ibadan, Igbajo, Ijebu and Akure and assessed for wet weight, number of seeds per fruit, average weight of seeds per fruit, and average volume of fruits.

Weight measurement was carried out using a sensitive Metler electronic weighing balance (model E300).Volume measurements were done through the displacement method. Fruits were tied with string and suspended in a 250 ml measuring cylinder filled with 100ml of water. The amount of water displaced was equal to the volume of the fruit.

ii. Experimental seedlings production

Seven hundred seeds from each provenance were sown directly in 16 x 14 x 12 cm poly pots (One seed per pot) in order to produce the seedlings used for this study,(Plate 3.1). The medium used for raising the seedlings consisted of a mixture of top soil and river sand in ratio 2:1. Watering was done daily to field capacity.

iii. Early growth and biomass assessment of seedlings

At the two leaf stage, fifty-five uniformly growing seedlings from each provenance were transferred to the field and laid out in five replicates of ten seedlings each with 5 buffer seedlings in Complete randomized blocked design (Plates 3.2 & 3.3). Daily watering was done to field capacity, growth parameters such as the length of liana, collar diameter, leaf area (using the Grid method) and number of leaves per seedling was assessed fortnightly for 24 weeks.

Biomass assessment of seedlings was carried out bi-monthly for 12 months using two hundred and forty seedlings from each provenance. Ten seedlings were randomly selected from each provenance; the polypots were removed by opening the sides with a blade. The seedlings were placed in a bowl containing water to wash off the soil around the roots. Seedlings were then separated into root and shoot components, each placed in separate sample bag and labeled for ease of identification. The sample bags containing the components were weighed to determine the fresh weight of the samples, after which they were oven dried at 70°C to a constant weight. The leaf area was calculated using the Grid method. The dry weight and leaf area were then used to calculate the relative growth rate (RGR), net assimilation rate (NAR) and absolute growth rate (AGR) using equations 1, 2 and 3 (Adewusi, 1997).

Data collected were subjected to analysis of variance (ANOVA) and correlation analysis. Significant means were separated using least significant difference (LSD) at $p<0.05$.

1. Relative Growth Rate (RGR) = $\frac{lnW_2 - lnW_1}{T_2 - T_1}$ (g/*month*)……1

2. Net Assimilation Rate (NAR) = $\frac{W_2 - W_1}{A_2 - A_1} \times \frac{lnA_2 - lnA_1}{T_2 - T_1}$ (g/month)……...2

3. Absolute Growth Rate (AGR) = $\frac{W_2 - W_1}{T_2 - T_1}$ (g/*month)* ……3

A_1 = Initial Leaf Area

A_2 = Final leaf Area

W_1 = Initial Dry Weight

W_2 = Final Dry Weight

T_1 = Initial Harvest Time

T_2 = Final Harvest Time

In = Natural Logarithm (log l)

Plate 3.1: Experimental seedlings of *P. conophora* germinating in the nursery

Plate 3.2: 4 weeks old experimental seedlings of *P. conophora* arranged on the field

Plate 3.3 Seedlings *of P. conophora* from the four sources: Ibadan (Oyo State), Ijebu-Ode (Ogun State), Akure (Ondo State), Igbajo (Osun State)

3.5 Study 2: Effect of storage temperature and duration on viability of *P. conophora* seeds using *Tetrazolium* test:

The experiment was carried out in the seed storage unit of the National Centre for Genetic Research and Biotechnology (NACGRAB) Ibadan, where a regulated standard facility with varying storage temperatures and constant power supply was used.

Four thousand (4000) freshly extracted seeds of *P. conophora* procured from a farmer in Ibadan were used in this experiment. (Plate 3.4) The seeds were subjected to five different storage conditions viz:

- Stored at room temperature 28°C
- Stored in a freezer at -5°C
- Refrigerator condition 7°C
- Buried in a plastic container filled with dry soil 29°C
- Control (tested immediately after extraction).

A total of 340 seeds were randomly allocated to each treatment using complete randomized design. Each seed lot was put inside polythene as 20 seeds/ bag and stored at the various treatments. Assessment of viable seeds was carried out fortnightly. At each occasion, 20 seeds were tested with one percent Tetrazolium salt (T_2C). Seeds were cracked using a small pestle and mortar to remove the hard testa, the endosperm was opened with a knife to expose the embryo. The endosperm with the embryo was placed in each Petri dish, covered with the T_2C and observed after 24 hours. The seeds were examined for colour change: viable seeds turned red while non viable seeds remained unchanged (whitish). At the end of the study period, the data collected were subjected to Analysis of variance. Where significant difference occurred, LSD test was used to separate the means (Gomez and Gomez 1984).

3.6 Study 3: Germination consistency and early growth parameters in seeds of different size classes of *P. conophora*

A composite sample of freshly extracted seeds of *P. conophora* was obtained from the four provenances used for this study. The seeds were sorted into large, medium and small using their weight classes based on Chase *et al.*, (2009). Twenty (20) seeds from each size class were sown in medium sized poly pots filled with a mixture of top soil and river sand (ratio 2:1). The experiment was replicated five (5) times in a completely randomized design. Germination consistency was observed from the first week to the twenty fourth weeks. Two weeks after germination, the length of liana, collar diameter and number of leaves were measured fortnightly for 24 weeks. The data collected were presented in descriptive statistics like graph and subjected to analysis of variance and correlation analysis.

Plate 3.4: Freshly extracted seeds of *P. conophora* used for the studies

3.7 Study 4: Effect of watering regimes on growth of *Plukenetia conophora* seedlings

This study was carried out in a screen house of FRIN. 375 four weeks old uniformly growing seedlings of *P. conophora* were selected from the seedlings raised in Experiment1(ii) They were transplanted into medium sized poly pots of dimension (16x14x12cm) filled with 2kg top soil. The seedlings were subjected to five watering regimes viz.

1. Daily watering with 250ml per pot of seedling.
2. Watering at 4 days interval with 250ml per seedling
3. Watering at 7days interval with 250ml per pot of seedling
4. Submerging in a bowl containing 10 litres of water and watered with 250ml daily.
5. No watering at all after transplanting for 24 weeks.

The plants were arranged in a complete randomized design with 5 replicates of 15 seedlings per treatment to make a total of 375 seedlings. Assessment of growth variables such as length of liana, seedling collar diameter and number of leaves were carried out fortnightly for 24 weeks. The data were subjected to analysis of variance.

3.8 Study 5: Influence of decapitation height on the coppicing potentials of *P. conophora* juvenile seedlings

Two hundred, 10 weeks old uniformly growing seedlings were selected from the seedling lot raised in the nursery. They were arranged in a complete randomized design with five replicates of 10 seedlings per treatment. The seedlings were watered daily to field capacity and allowed to establish for 12 weeks, before their main stems were decapitated with secateurs at 10cm, 15cm and 20cm from the collar and the control was not decapitated. The length of coppice, diameter and number of leaves on coppices were measured at 2 weekly intervals for 12 weeks. Data were analyzed using analysis of variance.

3.9 Study 6: Effect of different types of organic fertilizer and their dosage on early growth of *P. conophora*

In this study the effect of three different organic fertilizers at different dosages were investigated on the early growth of *P.conophora* seedlings.

The poultry droppings were collected from Federal College of Forestry Ibadan, cow dung manure was collected from the abattoir of Bodija market Ibadan. The compost manure was obtained from Institute of Agriculture Research and Training (IAR&T), Ibadan. They were left to cure for one month. Three hundred and twenty seedlings were selected from four weeks old stock-seedlings (Experiment 1, ii), 10g, 20g, 30g, 40g of each organic fertilizer was applied per seedling. The

layout used was complete randomized blocked design with four replicates of five seedlings per replicate. The control had no fertilizer addition. Additional 20 seedlings were added to each treatment to take care of the monthly biomass assessment. The seedlings were allowed to adjust to the fertilizer for two weeks, after which data on height of seedlings, stem diameter and number of leaves were collected forthnightly for 24 weeks. Biomass assessment was carried out monthly for a period of six months. The dry weight of seedlings was calculated as in Study1,(iii) The data collected on dry weight of seedlings was used to calculate Relative Growth Rate (RGR), Absolute Growth Rate (AGR), and Net Assimilation Rate (NAR) using equations 1, 2 and 3 based on the method described by Adewusi, (1997).

3.10 Study 7: Vegetative propagation of *P. conophora* (macro propagation)

One hundred ten months old uniformly growing seedlings from those raised in study 3 were maintained as ortet base in the Physiology nursery of FRIN to produce juvenile stem cuttings for this experiment. These young shoots were severed from the ortets with secateurs, into a bucket containing water to prevent excessive transpiration. The shoots were further reduced to double node cuttings (A total of four hundred) using a sterilized surgical blade fixed unto a feather. The leaves on them were reduced to half, to prevent excessive transpiration and at the same time, aid photosynthesis (Plate 3.5). The double node cuttings were subjected to four hormonal treatments which include:-

i. Indole 3 - Butyric Acid (IBA)
ii. Naphthalene Acetic Acid (NAA)
iii. A combination of IBA + NAA.

The hormones were applied at three levels of concentration, 100ppm, 150ppm, 200ppm and a control (no hormone) using the quick dip method, (Oni, 1987).The experiment was repeated using the same type of hormone, (IBA, NAA and IBA + NAA) in powder form at a rate of 8g/kg applied to the base of the cutting, (Hettasch *et al.*, 2009). The cuttings were set in plastic germination trays containing sterilized river sand (Plate 3.6) and saw dust at the rate of 20 cuttings per tray with five replicates per treatment. Trays were placed under a humid propagator in a complete randomized blocked design (Plate 3.7) Watering was done daily to field capacity using a knapsack sprayer and the following variables were assessed after 60 days.

i.) Percentage survival
ii.) Percentage rooted cuttings
iii.) Percentage calloused cuttings
iv.) Number of roots and longest root per cutting

Data were subjected to analysis of variance.

Plate 3.5: Newly set double node cuttings of *P. conophora* under the humid propagator showing their leaves trimmed to reduce transpiration.

Plate 3.6: Three days old double node cuttings of *P. conophora* in rooting tray, using sterilized river sand

Plate 3.7: Ten days old double node cuttings of *P. conophora* under the humid propagation

3.11 Study 8: Protocols for micropropagation of *P. conophora.*

3.11.1 Node culture (Explants)

Nodal explants were collected from three months old seedlings of *Plukenetia conophora* raised in the Physiology nursery of Forestry Research Institute of Nigeria, Ibadan (FRIN), and transferred to the green house of National Centre for Genetic Research and Biotechnology (NACGRAB) where the study took place. The nodal explants were extracted using a surgical blade.

3.11.2 Embryo culture (Explants)

Fruits were harvested from the existing stands of *P. conophora* in the Physiology Nursery of FRIN and taken to NACGRAB. 600 seeds were extracted carefully in the laboratory to release the embryo (Explant).

300 explants were disinfected using each of the following:

i. 10% Sodium Hypochlorite (NaOCl)
ii. 0.05% Mercuric chloride.

3.11.3 Sterilization protocols

- Explants were collected from the seedlings using sterilized surgical blade and transferred to the tissue culture green house of NACGRAB.
- Explants were washed in running tap water to remove dust particles.
- Explants were washed in liquid detergent solution, Teepol/ Ranklean 5 – 10 drops.
- Explants were again washed with distill water to remove excess detergent.
- Explants were transferred to sterile empty flasks under aseptic conditions given a quick absolute alcohol (ethanol) for 20 seconds and subsequently washed in distilled water.
- Explants were washed in:-

10% sodium hypochlorite for 15 minutes

0.05% Mercuric Chloride for 10 minutes.

- Explants were rinsed again and dissected using sterilized surgical blade fixed on a scalpel holder. This was carried out under a laminar air flow hood to maintain an aseptic condition.
- The tools i.e. Forceps and scalpel holder were inserted in a conical flask containing 95% ethanol.
- Forceps were flamed in spirit lamp and allowed to cool prior to using them to pick explants.
- Sterilized explants were trimmed at proximal and distal ends and placed aseptically in test-tubes containing prepared nutrient media, Murashige and Skooge (MS) supplemented with varying concentrations of cytokinin and auxins/indole butyric acid (IBA), Naphtalene acetic acid (NAA), Benzylaminopurine (BAP), Kinetin, cysteine and coconut milk. (Table 3.1)
- Samples were replicated 3 times with 5 samples per replicate arranged in a complete randomized design in a test tube rack.

- Samples were sealed with parafilm and carefully labeled with date and placed in the growth room for observation such as:

i.) Percentage explants established
ii.) Number of buds per explants
iii.) Number of shoots
iv.) Days taken for root initiation
v.) Percentage micro shoots rooted
vi.) Average numbers of roots per micro shoot.

Table 3.1: Chemical concentration of media for micro propagation of *Plukenetia conophora*

20 Media

A	MS + 30% coconut milk
B	MS pure sample
C	MS + 2mg IBA +2mg kinetin
D	MS + 0.5mg IBA + 0.5mg BAP
E	MS + 1m1 NAA + 5ml BAP
F	MS + 0.1mg kinetin + 0.3mg 24D
G	MS + 5ml kinetin + 20mg cysteine
H	MS + 2.5mg NAA
I	MS +3.5mg NAA
J	MS +0.3mg kinetin + 0.1mg 24D
K	MS + 0.05mg BAP + 0.01mg NAA
L	MS + 0.05mg IBA + 0.01mg kinetin
M	MS + 0.01mg IBA +0.01mg kinetin
N	MS + 0.5mg BAP only
O	MS +2mg BAP + 1mg NAA
P	MS + 1mg BAP + o.o5mg NAA
Q	MS + 0.1mg BAP + 0.5mg NAA
R	MS + 1ml kinetin + 0.04 cystine
S	MS + 2ml kinetin + 0.002 cystine
T	MS + 20% coconut milk

*NOTE:

MS = Murashige and Skoog

IBA = Indole 3 – butyric acid

NAA = Naphtalene acetic acid

BAP = Benzyl amino purine

24D = 2, 4-dichlorophenoxyacetic acid

CHAPTER FOUR

4.0 RESULTS

4.1.0: Provenance variation in fruits and early growth of *Plukenetia conophora* from four sources.

4.1.1 Phenology of *Plukenetia conophora*

Based on observation during reconnaissance survey, the liana, *Plukenetia conophora* is a woody, perennial climber with oval shaped leaves. It undergoes active vegetative growth from September to early November (Plate 4.1). Flowers were observed in November to December. The flowers are whitish in colour with greenish sepals (Plate 4.2). The fruits set in from early February to March, (Plate 4.3). Fruits mature and are ready for collection by the end of April through August (Plate 4.4). However, some plants produce few fruits off-season in December through February. Fruits are greenish in colour at immature stage, but yellowish to brownish when fully mature and ripe (Plate 4.4). The number of ridges in a fruit indicates the number of seeds embedded in it.Plates 4.5 and 4.6 show three and four seeded fruits with three and four prominent ridges respectively. It takes a period of 30 months from germination to first fruiting. As the vine matures, it changes from its greenish succulent form to a brownish woody liana.

4.1.2 Effect of fruit sources on fruit weight of *Plukenetia conophora*

Fruits from Ibadan (Oyo state) had the highest total mean weight of 96.65g, followed by fruits from Ijebu Ode (Ogun) with 81.47g; Akure (Ondo) had 65.34g while Igbajo (Osun) had the least fruit weight of 62.39g (Table 4.1a).Table of means showed significant difference between the fruit weight from the four sources at $p \leq 0.05$ (Table 4.1b). There was also a significant difference at $p \leq 0.05$ in weight between fruits containing one, two, three and four seeds respectively from the four sources (Tables 4.2, 4.3, 4.4 and 4.5).

4.1.3 Early growth parameters of *Plukenetia conophora* as affected by seed source

The seedlings with the longest length were from Ibadan, (Oyo State) with a mean value of 224.8cm at 24 weeks. This was followed by Ijebu Ode, (Ogun state) with 199.5cm, Igbajo, (Osun state) had 188.3cm and the least length of vine was recorded in seedlings from Akure (Ondo State) with 182.4cm (Table 4.6) There was no significant difference in seedling lengths among the four sources.

Variations were observed in the collar diameter of the seedlings from the four sources. At week two, Ibadan source had the highest mean collar diameter of 1.82cm after germination while

Igbajo had the least, 1.28cm. The mean collar diameter reduced with age. At the 24th week after germination, Ibadan had the highest diameter value of 0.89cm while Igbajo had the least of 0.79cm (Table 4.7). Sources of seed significantly ($p<0.05$) affected the collar diameter but had no significant effect on other variables measured.

The highest mean number of leaves (16.8) was recorded in the seedlings from Igbajo at the 24th week of germination. Ibadan had the lowest mean number of leaves of 12.2 at 24th week. However, the means were not significantly different from those of Ijebu Ode and Akure (Table 4.8). The seed sources did not have any significant effect on the number of leaves,

The initial highest leaf dry weight of 0.75g was recorded by Ibadan at 4 weeks of germination, while Akure had the least value of 0.69g. At 20 weeks after germination Ibadan still recorded the highest value of 3.93g; this was followed by Igbajo 3.88g, Ijebu Ode and Akure had 3.32g and 3.13g respectively. However, at the end of the 24th week of germination, the highest leaf dry weight had reduced to 3.17 and this was recorded by Ibadan, Igbajo came next with 3.11g while Ijebu Ode and Akure had 2.12g and 2.09g respectively.

Ibadan source recorded the initial highest stem dry weight of 0.50g at 4 weeks after germination while Ijebu-Ode had the least stem dry weight of 0.49g (fig 4.1). At the end of the experimental period of 24 weeks, Ibadan had the highest stem dry weight of 6.21g, while Akure had the lowest stem dry weight of 5.11g, Ijebu Ode and Igbajo had 5.16g and 6.14g respectively (Table 4.5). However, the difference in means was not significant among the four sources.

The highest root dry weight was observed in Ibadan with 6.59g at 4 weeks after germination. Ijebu Ode had 6.23g while Akure and Igbajo had 5.60g and 6.06g respectively. At the end of the experimental period of 24 weeks Ibadan maintained the highest root dry weight of 36.39g, Ijebu Ode had 30.26g while Akure and Igbajo had 26.35g and 29.36g respectively**.**

Akure recorded the least leaf area of 85.04cm^2 at 24 weeks of germination. While Ibadan had a total of 90.01cm^2, Ijebu Ode and, Igbajo had 86.51cm^2 and 86.21cm^2 respectively (fig 4.3). There was no significant difference in leaf area among the sources.

At the end of the experimental period of 24 weeks, the Ibadan source had the total biomass accumulation of 45.77g next to this was Igbajo with 38.61g while Ijebu Ode and Akure had 37.64g and 33.55g respectively (fig 4.4). Analysis of Variance showed no significant difference in biomass from the four sources.

At 4 weeks, Oyo (Ibadan) had a relative growth rate of 0.088g; Ogun (Ijebu Ode) had 0.081g while Ondo (Akure) and Osun (Igbajo) had 0.080g and 0.084g respectively. There was no significant difference between the sources. At 8 weeks of germination, Ibadan source recorded the best relative growth rate with 0.095g. This was followed by Igbajo with 0.092g, Ijebu Ode and

Akure had 0.089g and 0.084g respectively. The least relative growth rate of – 0.00325 was recorded in Akure source at 20 weeks of germination. However the relative growth rate (RGR) showed a diminishing trend from the 8th week to the 20th week of germination (Table 4.10).

At the 4th week of germination, Ibadan had 0.021g, followed by Ijebu-Ode with 0.017g while Akure and Igbajo had 0.016g and 0.015g respectively. The highest Net Assimilation Rate (NAR) of 0.026g was recorded in Ibadan source at the 8th week of germination. This was followed by the other three sources Ijebu Ode, Akure and Igbajo with 0.022g each. However, the Net Assimilation Rate gradually reduced with time, with Akure recording the least value of – 0.001g at the 20th week of sowing (Table 4.11). The NAR from the four sources did not differ significantly from each other.

At the 4th week of germination the highest AGR was recorded by Ibadan with 1.898g, this was followed by Igbajo with 1.567g, while Ijebu-Ode and Akure had 1.511g and 1.242g respectively. At week 8, the highest AGR of 2.237g was recorded by Ibadan source. This was followed by Igbajo with 1.856g, Ijebu Ode had 1.788g and Akure had 1.242g. The AGR gradually reduced with increasing period of months. At week 20, Akure source had the least AGR of -0.108, Ibadan had the highest with 1.450g, Igbajo and Ijebu Ode had 0.343g and 0.245g respectively (Table 4.12).

Plate 4. 1: The study plant *P.conophora* (liana) undergoing active vegetative growth in October

Plate 4.2: Flowering branch of *P.conophora*

Plate 4.3: Fruiting branch of *P. conophora*

Plate 4.4: Mature fruits of *P. conophora*

Plate 4.5: Mature 3 seeded fruits of *P. conophora*

Plate 4.6: Matured 4 seeded fruit of *P.conophora*

Table 4.1a: LSD Table for seed/fruit source on fruit weight (g) of *Plukenetia conophora*

Fruit Sources	Seeds/fruit	Mean fruit wt.(g)	Std. Deviation	N
Ibadan (Oyo)	4	133.97	12.31242	10
	3	111.40	15.58386	10
	2	81.02	4.99236	10
	1	60.19	2.70860	10
	Total	96.65	30.24653	40
Akure (Ondo)	4	87.60	5.23967	10
	3	67.29	16.33889	10
	2	59.78	4.63611	10
	1	46.70	10.55913	10
		65.34	17.99035	40
Ijebu-Ode (Ogun)	4	118.57	14.07892	10
	3	90.84	1474822	10
	2	66. 38	7.83057	10
	1	50.08	4.56157	10
	Total	81.47	28.30807	40
Igbajo (Osun)	4	76.11	16.23261	10
	3	66.23	13.09384	10
	2	56.88	6.17315	10
	1	50.32	9.49440	10
	Total	62.39	15.06990	40
Total	4	104.06	26.47864	40
	3	83.94	23.75836	40
	2	66.01	11.08066	40
	1	51.82	8.89284	40
	Total	76.46	27.30935	160

Table 4.1b: LSD Table for effect of fruit source on fruit weight (g) of *Plukenetia conophora*

	Variation	Mean Diff	Std. Error	Sig.
Ibadan	Akure	-18.69750 NS	36.54173	0.611
	Ijebu-Ode	15.17750 NS	36.54173	0.680
	Igbajo	34.26000 NS	36.54173	0.354
Akure	Ibadan	18.69750 NS	36.54173	0.611
	Ijebu-Ode	33.87500 NS	36.54173	0.359
	Igbajo	52.95750 NS	36.54173	0.154
Ijebu-Ode	Ibadan	-15.17750 NS	36.54173	0.680
	Akure	-33.87500 NS	36.54173	0.359
	Igbajo	19.08250 NS	36.54173	0.604
Igbajo	Ibadan	-34.26000 NS	36.54173	0.354
	Akure	-52.95750 NS	36.54173	0.154
	Ijebu-Ode	-9.08250 NS	36.54173	0.604

NS= Not Significant

Table 4.2: LSD Table for effect of number of seeds on weight of fruits (g) from Ibadan

variation	variation	Mean Diff.	Std. Error	Sig.
4seed fruit	3 seed fruit	22.61900*	4.58372	.000
	2 seed fruit	52.99200*	4.58372	.000
	1 seed fruit	73.81900*	4.58372	.000
3seed fruit	4 seed fruit	-22.61900*	4.58372	.000
	2 seed fruit	30.37300*	4.58372	.000
	1 seed fruit	51.20000*	4.58372	.000
2 seed fruit	4 seed fruit	-52.99200*	4.58372	.000
	3 seed fruit	-30.37300*	4.58372	.000
	1 seed fruit	20.82700*	4.58372	.000
1 fruit seed	4 seed fruit	-73.81900*	4.58372	.000
	3 seed fruit	-51.20000*	4.58372	.000
	2 seed fruit	-20.82700*	4.58372	.000

*. = Significant at $p<0.05$

Table 4.3:LSD Table for effect of number of seeds on weight of fruit (g) from Akure

variation	variation	Mean Diff.	Std. Error	Sig.
4seed fruit	3 seed fruit	20.30700*	4.62202	.000
	2 seed fruit	27.81600*	4.62202	.000
	1 seed fruit	40.90400*	4.62202	.000
3 seed fruit	4 seed fruit	-20.30700*	4.62202	.000
	2 seed fruit	7.50900	4.62202	.113
	1 seed fruit	20.59700*	4.62202	.000
2 seed fruit	4 seed fruit	-27.81600*	4.62202	.000
	3 seed fruit	-7.50900	4.62202	.113
	1 fruit seed	13.08800*	4.62202	.008
1 seed fruit	4 seed fruit	-40.90400*	4.62202	.000
	3 seed fruit	-20.59700*	4.62202	.000
	2 seed fruit	-13.08800*	4.62202	.008

*. Significant at $p<0.05$

Table 4.4: LSD Table for effect of number of seeds on weight of fruits (g) from Ijebu-Ode

variation	variation in	Mean Diff.	Std. Error	Sig.
4 seed fruit	3 seed fruit	27.93300*	5.20002	.000
	2 seed fruit	52.39600*	5.20002	.000
	1 seed fruit	69.69400*	5.20002	.000
3 seed fruit	4seed fruit	-27.93300*	5.20002	.000
	2 seed fruit	24.46300*	5.20002	.000
	1 seed fruit	41.76100*	5.20002	.000
2 seed fruit	4seed fruit	-52.39600*	5.20002	.000
	3 seed fruit	-24.46300*	5.20002	.000
	1 seed fruit	17.29800*	5.20002	.002
1 seed fruit	4seed fruit	-69.69400*	5.20002	.000
	3 seed fruit	-41.76100*	5.20002	.000
	2 seed fruit	-17.29800*	5.20002	.002

*. = Significant at $p<0.05$

Table 4.5: LSD Table for effect of number of seeds on weight of fruits (g) from Igbajo

variation	variation	Mean Diff.	Std. Error	Sig.
4 seed fruit	3 seed fruit	9.88800	5.39350	0.075
	2 seed fruit	19.22600*	5.39350	0.001
	1 seed fruit	27.78300*	5.39350	0.000
3 seed fruit	4seed fruit	-9.88800	5.39350	0.075
	2 seed fruit	9.33800	5.39350	0.092
	1 seed fruit	17.89500*	5.39350	0.002
2 seed fruit	4seed fruit	-19.22600*	5.39350	0.001
	3 seed fruit	-9.33800	5.39350	0.092
	1 seed fruit	8.55700	5.39350	0.121
1 seed fruit	4seed fruit	-27.78300*	5.39350	0.000
	3 seed fruit	-17.89500*	5.39350	0.002
	2 seed fruit	-8.55700	5.39350	0.121

*. Significant at $p < 0.05$

Table 4.6: Length of vine (cm) of *Plukenetia conophora* seedlings as affected by seed sources.

Source	Length of seedlings in weeks											
	2	4	6	8	10	12	14	16	18	20	22	24
Ibadan	22.10	71.9	117.8	133.4	146.2	151.6	153	158.5	161.4	177.7	190.1	224.8
Ijebu-Ode	20.30	66.1	98.3	111.9	132.8	134.8	141.0	147.2	153.9	160.8	183.4	199.5
Akure	20.80	54.2	91.2	121.2	130.7	132.0	142.4	144.5	151.6	159.1	171.0	182.4
Igbajo	17.70	66.9	116.5	117.1	135.1	137.7	142.5	145.9	153.7	158.3	174.2	188.3

Table 4.7: Mean collar diameter (cm) of *Plukenetia conophora* as affected by seed sources

Source	Collar diameter of seedlings in weeks											
	2	4	6	8	10	12	14	16	18	20	22	24
Ibadan	1.82	1.72	1.26	1.12	1,11	1.1	0.97	0.97	0.95	0.95	0.89	0.89
Ijebu-Ode	1.37	1.28	0.92	0.91	0.87	0.86	0.85	0.85	0.85	0.84	0.81	0.80
Akure	1.39	1.31	1.09	0.93	0.91	0.88	0.86	0.83	0.82	0.82	0.81	0.86
Igbajo	1.28	1.08	0.97	0.87	0.85	0.84	0.81	0.81	0.81	0.82	0.79	0.79

Table 4.8: Effect of seed sources on mean number of leaves of *Plukenetia conophora* seedlings

Source	Number of leaves in weeks											
	2	4	6	8	10	12	14	16	18	20	22	24
Ibadan	4.00	6.50	8.00	10.75	10.45	13.60	10.36	8.89	10.58	11.50	12.00	12.20
Ijebu-Ode	3.60	7.00	8.80	10.00	10.00	12.63	12.81	11.51	12.55	13.10	14.10	16.10
Akure	4.00	6.00	8.50	11.45	11.45	13.42	13.55	14.30	13.50	13.63	14.30	14.80
Igbajo	4.00	7.00	9.00	12.75	12.75	14.00	13.80	13.30	13.80	13.90	14.72	16.80

Table 4.9: LSD table showing mean effect of fruit sources on early growth of *Plukenetia conophora*

Source	Collar diameter	Liana length	No of leaf
Ijebu-Ode	0.934	129.2	11.02
Akure	0.957	125.1	11.58
Igbajo	0.894	129.5	12.15
Ibadan	1.146	142.4	9.90
L.S.D.	*0.182	NS	NS

*Significant at P< 0.05

NS = Not significant

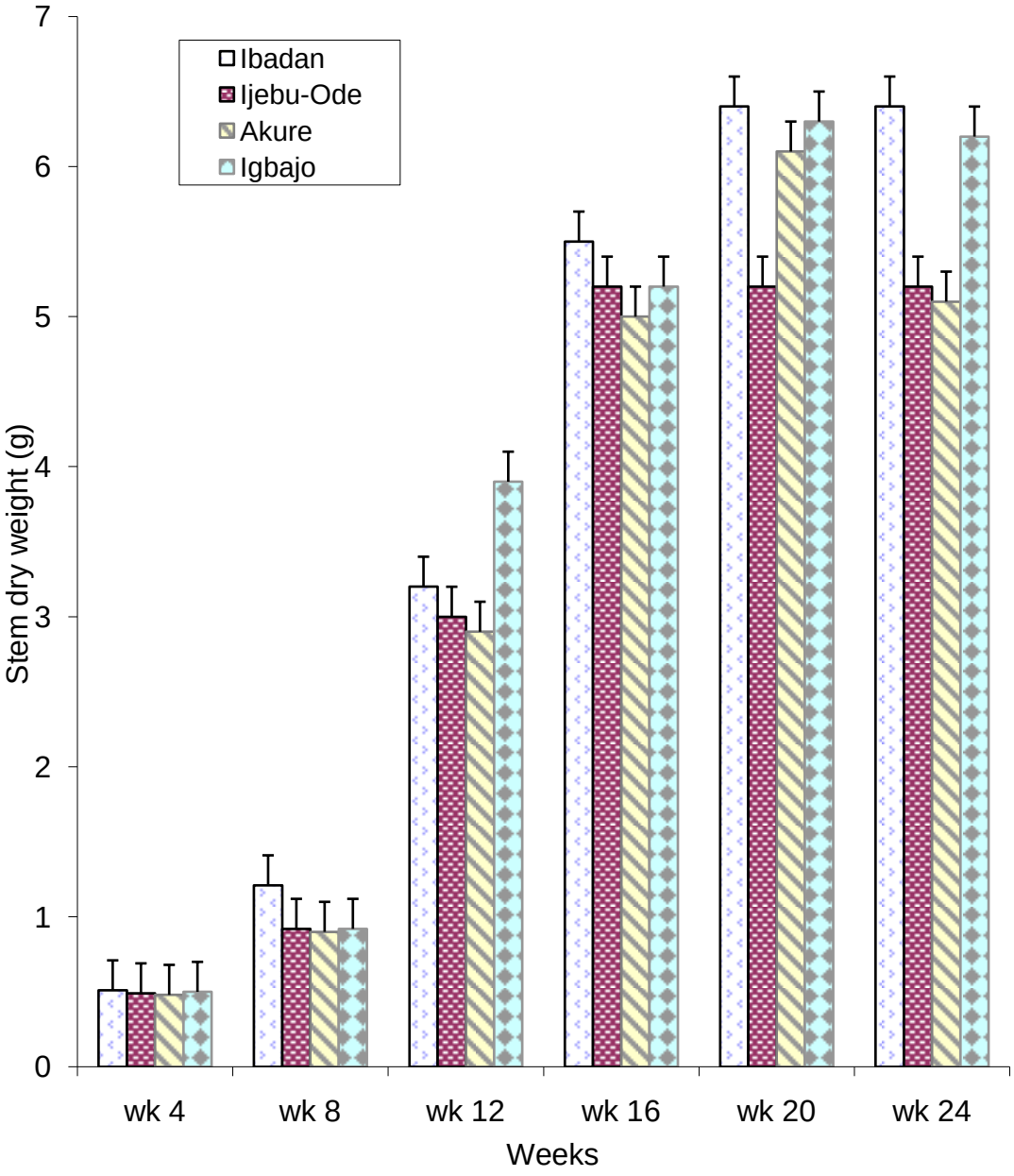

Figure 4.1: Effect of seed sources on Stem Dry Weight (g) of *P. conophora* seedlings.

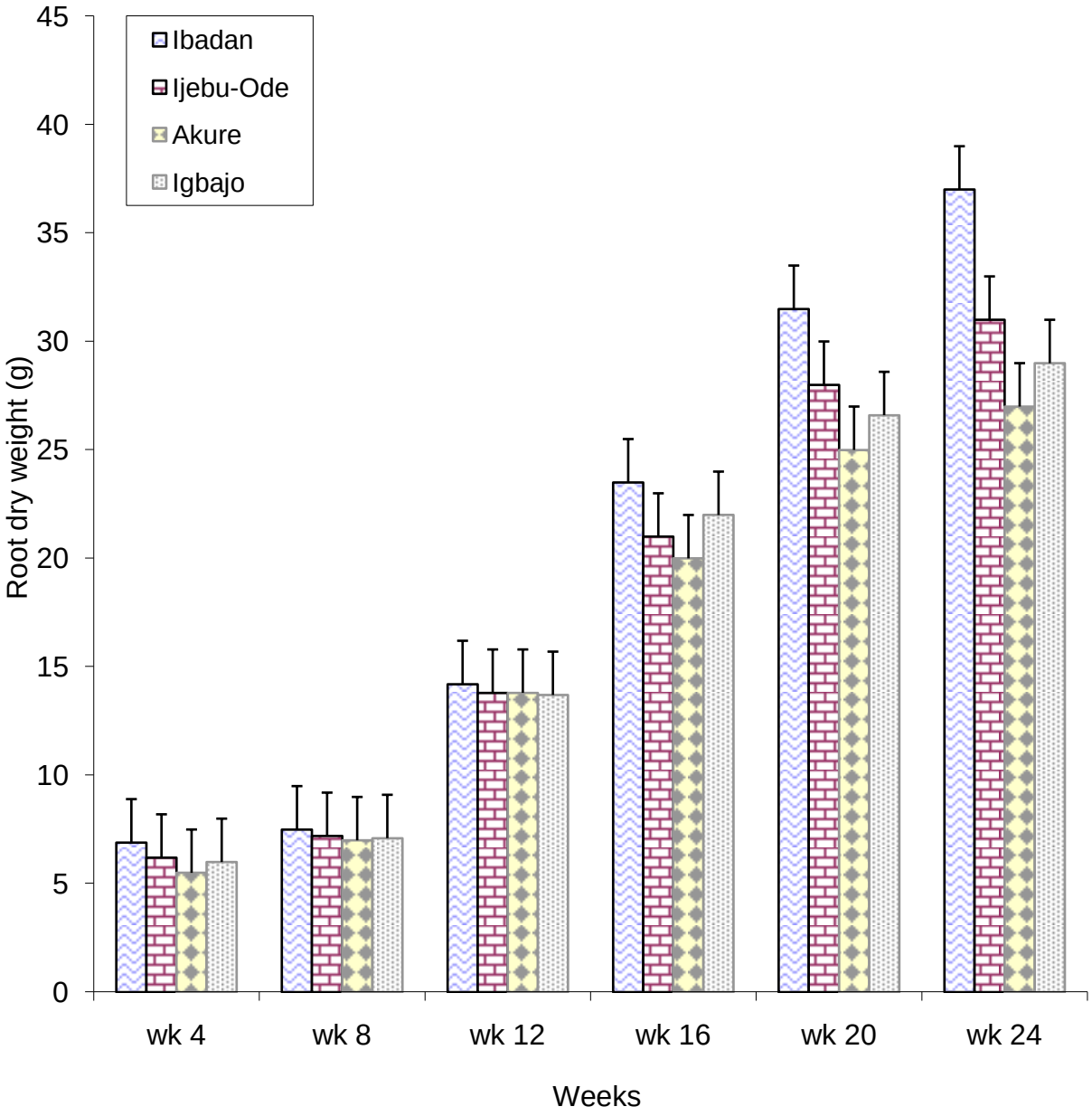

Figure 4.2: Effect of seed sources on root dry weight (g) of *P. conophora* seedlings

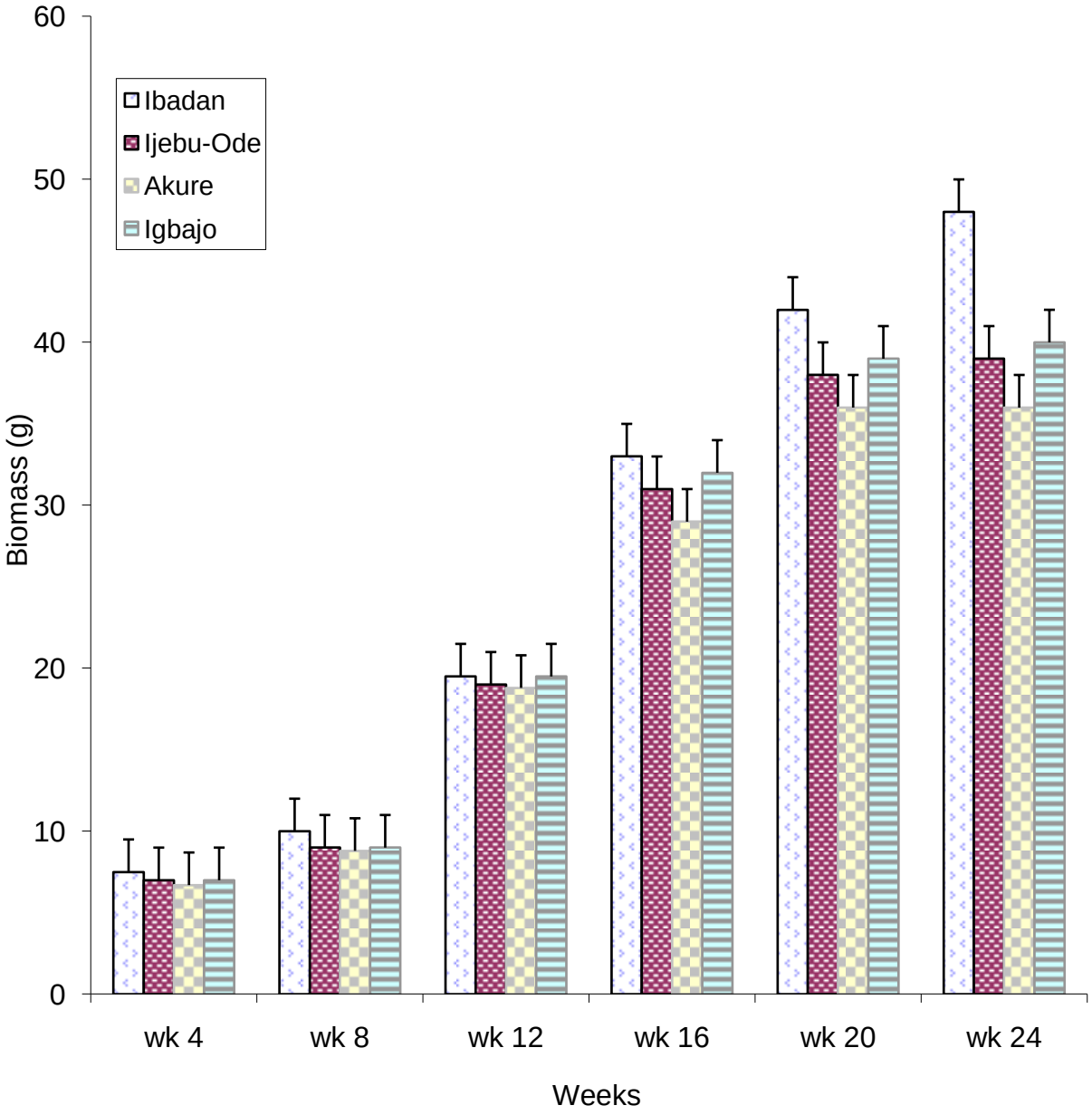

Figure 4.3: Effect of Seed Sources on Total Biomass (g) of *P.conophora* seedlings

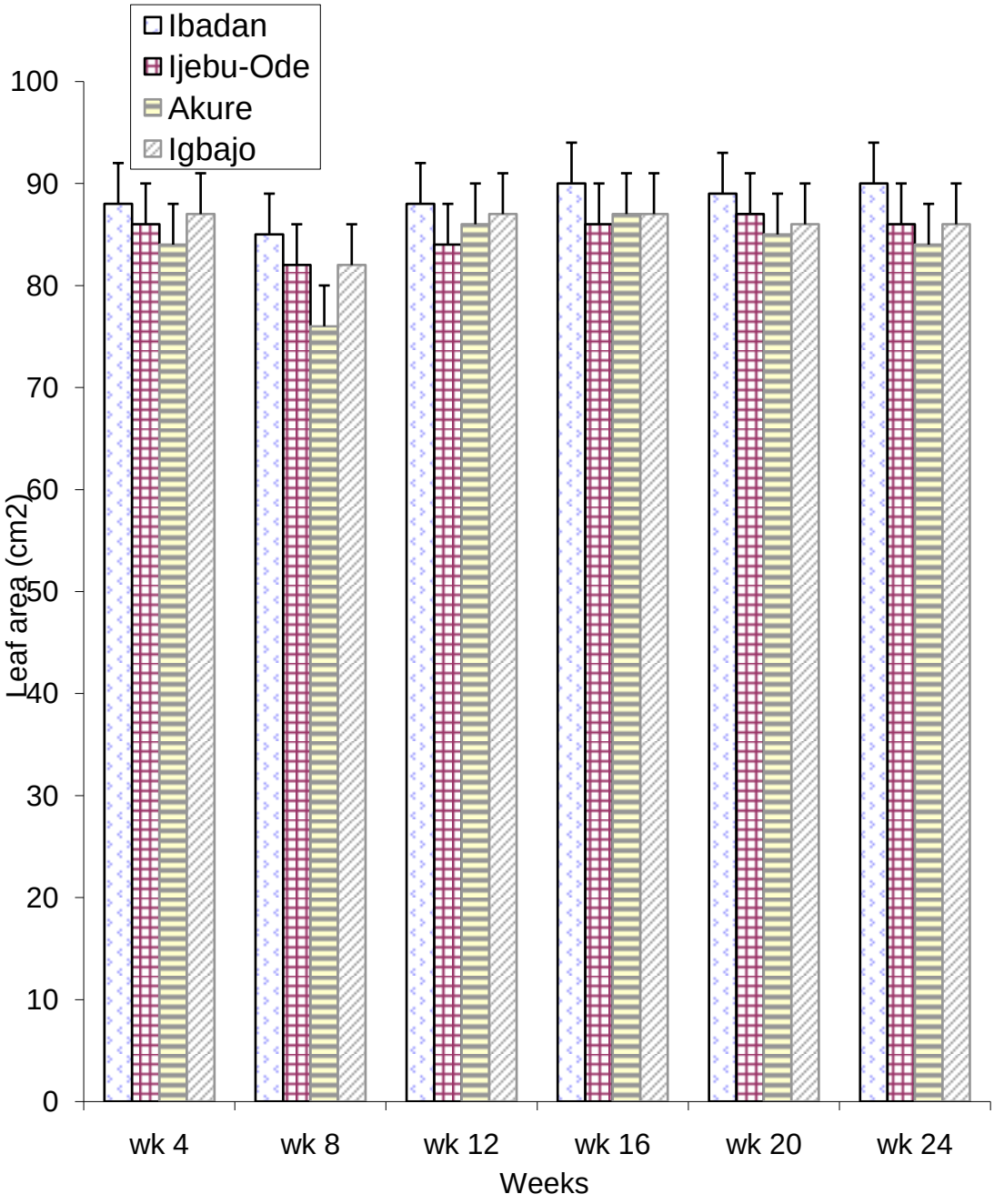

Figure 4.4: Effect of seed sources on Leaf Area of *P. conophora* seedlings

Table 4.10: Relative Growth Rate (RGR) g/months of seedling as affected by seed source

Source	wk 4 - 7	wk 8 – 11	wk 12 - 15	wk 16 – 19	wk 20 – 24
	1	2	3	4	5
Ibadan	0.088	0.095	0.075	0.049	0.034
Ijebu-Ode	0.081	0.089	0.061	0.032	0.00675
Akure	0.080	0.084	0.053	0.020	-0.00325
Igbajo	0.084	0.092	0.060	0.031	0.00925

Table 4.11: Net Assimilation Rate (NAR) g/month of seedlings as affected by seed source

Source	wk 4 – 7 1	wk 8 – 11 2	wk 12 - 15 3	wk 16 – 19 4	wk 20 – 24 5
Ibadan	0.021	0.026	0.026	0.025	0.015
Ijebu-Ode	0.017	0.022	0.019	0.012	0.003
Akure	0.016	0.022	0.015	0.007	-0.001
Igbajo	0.015	0.022	0.020	0.012	0.004

Table 4.12: Absolute Growth Rate (AGR) g/month of seedling as affected by seed source

Source	wk 4 – 7	wk 8 – 11	wk 12 - 15	wk 16 – 19	wk 20 – 24
	1	2	3	4	5
Ibadan	1.898	2.237	2.266	1.854	1.450
Ijebu-Ode	1.511	1.788	1.636	1.047	0.245
Akure	1.242	1.242	1.308	0.625	-0.108
Igbajo	1.567	1.856	1.651	1.060	0.343

4.2.0: Effect of temperature, storage medium and duration on viability of *Plukenetia conophora* seeds using Tetrazolim test

4.2.1: Effect of storage condition on viability

The control, (seeds extracted and tested immediately) recorded the highest viability percentage of 100%, as the 20 embryo tested with Tetrazolium salt (T_2C) overnight changed from cream colour to red, (Plate 4.7 and 4.8).

Seeds stored at room temperature (28^{o}C) recorded the highest viability percentage of 85% at the second week of storage, this gradually decreased until the 18th week of storage when only 10% of the seeds were viable, after that all the left over seeds showed no signs of viability, (Fig. 4.5)

Seeds stored in Refrigerator 7^{o}C had the highest viability percentage of 100% at the 2nd week through 4th week. At the sixth week the viability percentage reduced to 95% and gradually decreased to 40% at the 30th week of storage, after which no signs of viability were observed, (Fig.4.6)

Seeds stored under freezer condition (at-5^{o}c) had a viability percentage of 80% at the 2nd week of storage, this gradually reduced to 25% at the 10th week of storage, after which no more signs of viability were observed, (Fig. 4.7)

Seeds stored in a plastic container filled with dry soil at 29^{o}C had a viability percentage of 70% at the 2nd week of storage which gradually reduced to 15% at the 10th week of storage, after which the seeds lost their viability (Fig. 4.8).

4.2.2: Interaction effect of storage medium and viability

Analysis of variance showed that the storage medium used has significant effect on the number of seeds that changed to red (viable) at ($P < 0.01$).

Interactive effect of storage temperature and viability showed a significant difference at $P<0.01$. Table of means and LSD also indicated that the storage temperature used had significant effect on the number of seeds that changed to red.

Plate 4.7: Fresh seeds of *P. conophora* showing the endosperm with the embryo, before the tetrazolium solution test

Plate 4.8: Viable embryo of *P. conophora* turned red after applying Tetrazolium salt solution for 24 hours

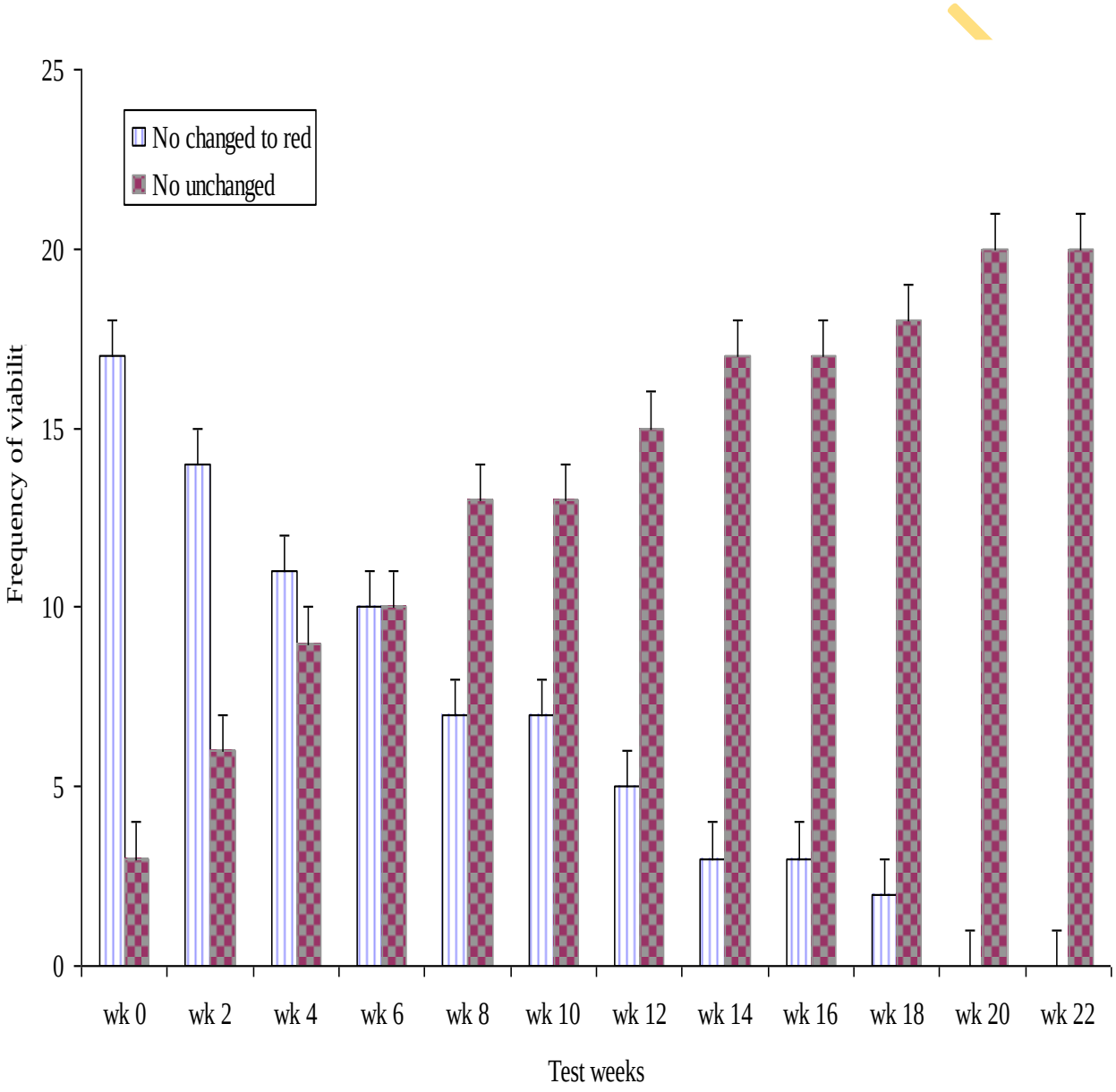

Fig. 4.5: Effect of room temperature (28^0C) on viability of *P.conophora* seeds.

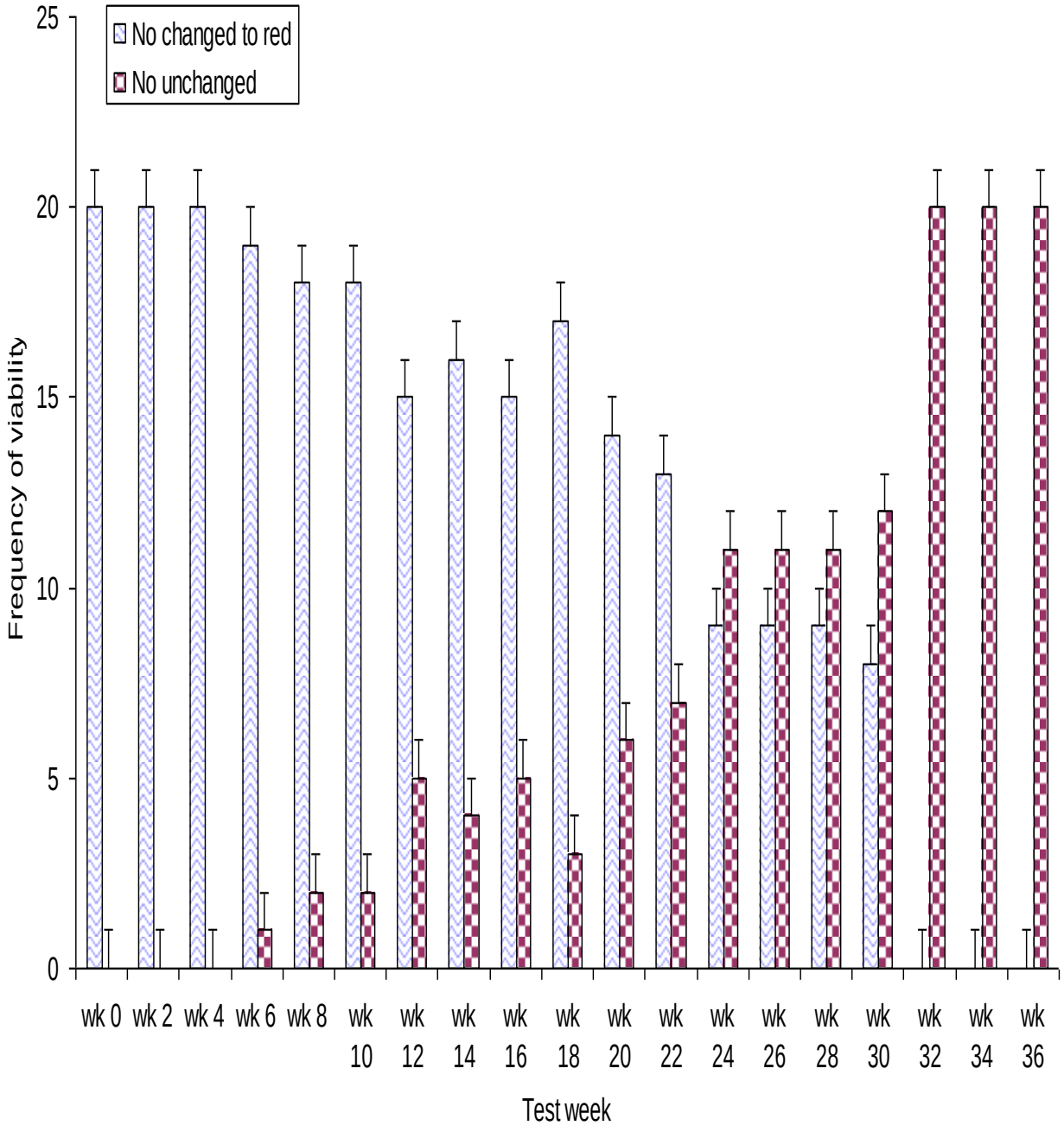

Fig 4.6: Effect of refrigerator storage (7^{O}c) on viability of *P. conophora* seeds

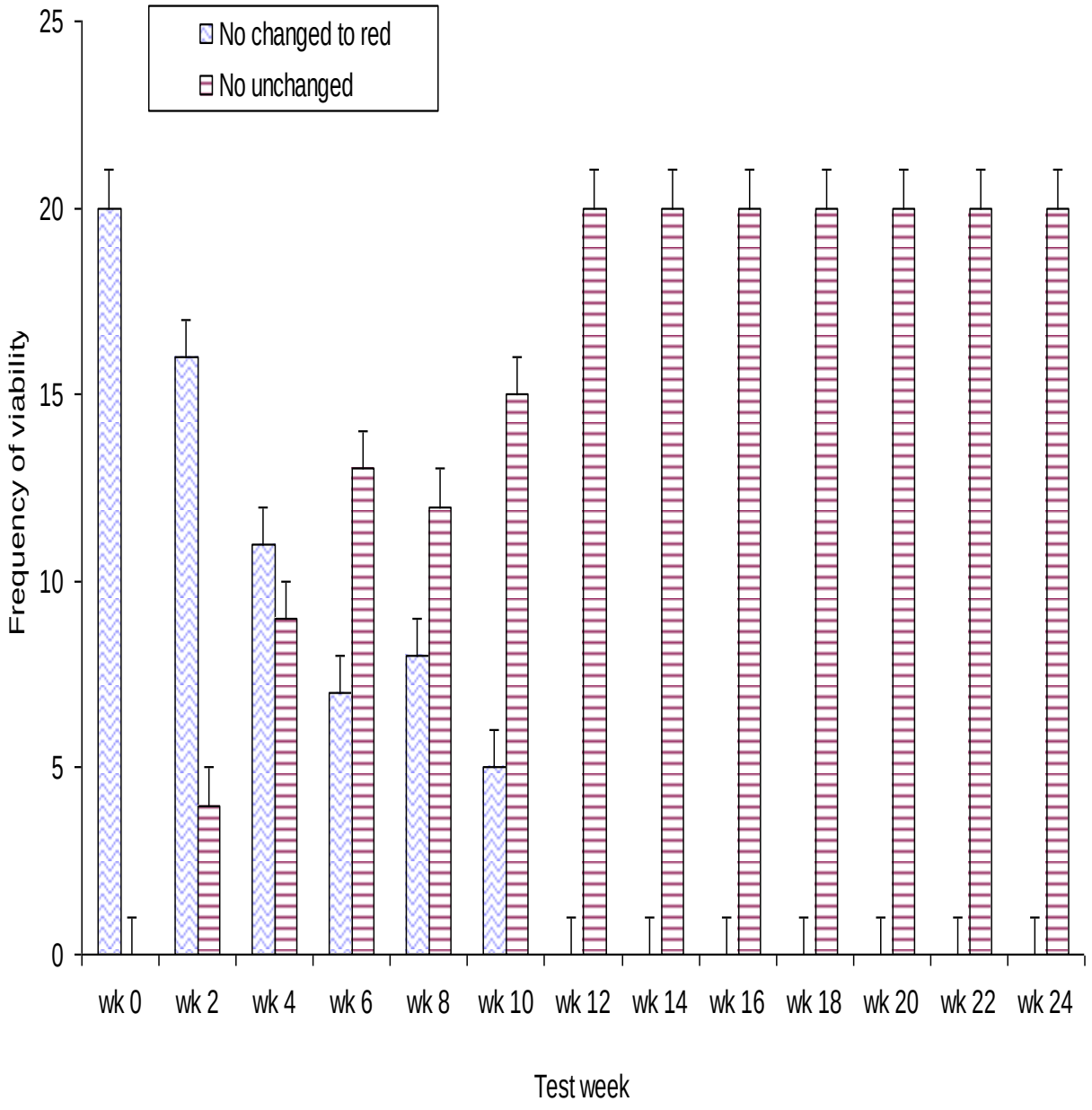

Fig 4.7: Effect of storage in freezer (-5^{O}C) on viability of *P. conophora* seeds

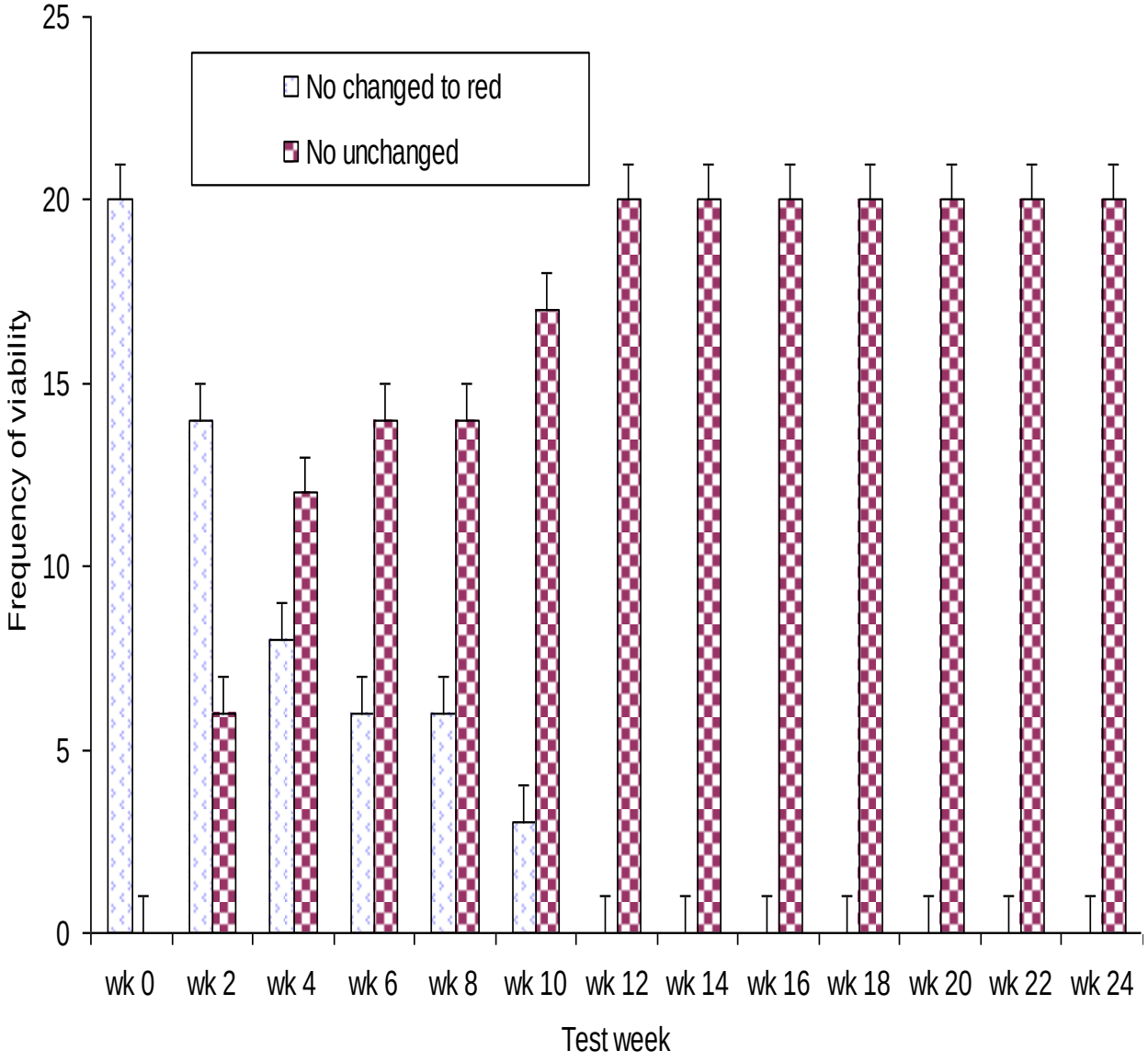

Fig 4.8: Effect of storage of seeds in dry soil ($29^{O}C$) on viability of *P. conophora* seeds

4.3.0: Germination and early growth parameters in different size of *P. conophora* seeds

The large seeds had a mean weight of 13.21 ±0.02g, the medium size class had 10.36 ±0.02g while the small size class had 7.25 ±0.07 (Table 4.13) and (Plate 4.9). LSD shows significant difference in the weight of the different size classes (Table 4.14).

Result showed that at the onset of germination, the large seeds had the highest germination of 52% at the 4th week of sowing. This was followed by the medium sized seeds with 40% while the small seed size had 36% (Table 4.15). By the 24th week of sowing germination percentage had increased, the large seeds had 92%. This was followed by the small size seeds with 80%, while the medium seeds had 76% (Table 4.15). LSD shows a significant difference in germination from the three size classes (Table 4.16).

Seedlings from large size class had the highest length of 97.42cm at 10 weeks of germination the medium size class had 82.27cm while the small size had 94.27cm. By the 24th week, the length of vine had reduced to 54.73cm for seedlings from the large size, 47.25cm for medium and 46.5cm for small size, due to die back of tips by the hot sun of dry season. Seed size showed no significant effect on length of vine of *P. conophora* (Table 4.17).

The highest collar diameter (1.23cm) was recorded in the seedlings from the large size class on the 14th week of planting while the least collar diameter of (0.43cm) was recorded in seedlings with small size. At the 24th week of planting, the highest collar diameter of 1.08cm was recorded in seedlings from the large size class. This was followed by the middle class size with 0.71cm, while the least, 0.55cm was recorded in seedling from the small size class. Size classes of seeds had no significant effect on seedling collar diameter. Also, interaction of size class and age of seedling had no significant effect on collar diameter (Table 4.17).

At the 24th week of planting, the highest mean leaf count of 11±6 was recorded in the small size seed class; this was followed by the medium size class with 11±2, while the large class had 11±3. Seed size did not have any significant effect on leaf count (Table 4.17).

The highest mean leaf dry weight (LDW) of 0.72±0.1g was recorded among the seedlings in the large size class. This was followed by the small size class with a mean (LDW) of 0.69±0.2g and the medium size class had 0.69±0.2g at the end of the experimental period of 24 week (Table 4.18)

The highest stem dry weight of 0.52g was recorded among seedlings from the large size class. This was followed by the medium size class with a stem dry weight of 0.51g. The small size class had 0.51g at the end of the 24 week experimental period (Table 4.18).

At the first month of assessment (4 weeks old seedlings) the highest root dry weight of 7.12g was recorded among the seedlings from the large size class, next to this was the medium size with 6.85g while the least root dry weight of 6.60g was observed in the seedlings from the small size seeds. At the end of the experimental period of 24 weeks, the seedlings from the big size seeds had the highest root dry weight of 31.66g though not significantly different from the medium and small size seedlings with 28.6g and 27.9g respectively (Table 4.18 and 4.19).

At the end of experimental period of 24 weeks total biomass produced by the large seed size was 32.9g while the medium and small size class had 29.8g and 29.1g respectively, (Table 4.18 and 4.19).

Plate 4.9. Seeds of *P.conophora* sorted into large (L), medium (M) and small (S) size classes

Table 4.13: Mean weight of different size classes of *Plukenetia conophora* seeds (g)

Large 12 – 15g	Medium 9 – 11	Small < 9
13.82	9.93	7.79
13.57	9.37	6.03
12.00	9.21	6.92
12.47	11.47	8.22
14.23	11.53	7.35
13.86	11.15	6.51
12.86	9.23	7.56
12.86	10.42	6.94
14.12	11.66	7.38
13.00	11.74	8.36
13.75	9.60	4.93
12.26	9.18	8.00
13.43	9.92	6.83
12.25	10.13	7.52
13.42	11.85	6.58
12.65	9.85	7.71
13.51	9.45	8.06
14.10	9.41	6.44
12.41	11.61	7.41
13.51	10.55	8.49
Total weight 264.08g	217.26g	145.03g
Mean = 13.204g	10.363g	7.25g

Table 4.14: LSD table for weight of different seed classes of *P. conophora*

sizes	sizes	Mean Diff.	Std. Error	Sig.
large	medium	2.84100*	.27273	.000
	small	5.95250*	.27273	.000
medium	large	-2.84100*	.27273	.000
	small	3.11150*	.27273	.000
small	large	-5.95250*	.27273	.000
	medium	-3.11150*	.27273	.000

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

Table 4.15: Mean germination percentage of different size classes of *Plukenetia conophora*

Seed size	Weeks after sowing												
	0	2	4	6	8	10	12	14	16	18	20	22	24
Large	0	0	52	72	88	92	92	92	92	92	92	92	92
Medium	0	0	40	52	68	72	76	76	76	76	76	76	76
Small	0	0	36	56	72	72	80	80	80	80	80	80	80

Table 4.16: LSD for effect of different seed size on germination of *Plukenetia conophora*

	seed sizes	seed sizes	Mean Difference	Std. Error	Sig.
	large	medium	2.84100*	.27273	.000
		small	5.95250*	.27273	.000
	medium	large	-2.84100*	.27273	.000
		small	3.11150*	.27273	.000
	small	large	-5.95250*	.27273	.000
		medium	-3.11150*	.27273	.000

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

Table 4.17: Correlation analysis for effect of seed size on early growth of *P. conophora*

Length of vine

		Large	Medium	Small
Pearson Correlation	Large	1.000	0.987	0.944
	Medium	0.987	1.000	0.978
	Small	0.944	0.978	1.000

Collar Diameter

		Large	Medium	Small
Pearson Correlation	Large	1.000	0.469	0.771
	Medium	0.469	1.000	0.664
	Small	0.771	0.664	1.000

Leaf count

		Large	Medium	Small
Pearson Correlation	Large	1.000	0.942	0.775
	Medium	0.942	1.000	0.869
	Small	0.775	0.869	1.000

Table 4.18: Effect of Seed Size on Biomass of *Plukenetia conophora*

4th Week	Leaf Dry weight (g)	Stem Dry weight (g)	Root Dry weight (g)	Total Dry weight (g)	Leaf Area (cm^2)
Large	0.70	0.42	7.12	8.24	82.57
Medium	0.64	0.41	6.85	7.90	80.22
Small	0.69	0.40	6.60	7.69	79.59
24th Week					
Large	0.72	0.52	31.66	32.90	89.64
Medium	0.69	0.51	28.60	29.80	86.52
Small	0.69	0.51	27.90	29.10	86.41

Table 4.19: Analysis of variance for biomass in seeds of different size classes of *Plukenetia conophora*

Source of variation		df	ss	ms	f	fpr
Leaf dry weight	Treatment	2	477.3	238.6	0.48	0.623Ns
	Error	33	16390.8	496.7		
	Total	35	16868.1			
Stem dry weight	Treatment	2	65.03	32.52	2.44	0.103Ns
	Error	33	440.39	13.35		
	Total	35	505.42			
Root dry weight	Treatment	2	18.335	9.168	1.42	0.256Ns
	Error	33	213.220	6.461		
	Total	35	231.555			

Ns = not significant

4.4.0: Effect of watering regimes on growth of *Plukenetia conophora* seedlings

Watering daily had the highest mean vine length of 140.8±0.6cm at the 24th week of experimental period (Fig.4.9). Watering once in four days had a mean vine length of 126±0.2cm while, once in seven days had a mean length of 42.1±0.2cm. Seedlings that were sub-merged in bowl of water wilted after the 12th week with a length of 29.7±0.7cm. The leaves turned yellowish and wilted indicating that *P. conophora* needs adequate water to thrive, but it is not necessarily a hydrophyte. The control, which was not watered for 24 weeks, had the least length of 14.4cm, the seedling length gradually reduced from initial length of 19.8cm at 6th week to 14.4cm at the 10th week, before wilting totally indicating that it is neither a xerophyte. The effect of watering regimes, age of seedling, and their interactions between treatment and age were significant ($p<0.05$) on length of vine, among the seedlings of *Plukenetia conophora* (Table 4.20b).

After the experimental period of 24 weeks, the largest mean diameter was obtained among the seedlings watered daily, (0.79± 0.32cm). This was followed by seedlings subjected to four days interval of watering with a mean collar diameter of (0.72 ± 0.12cm). The seven days interval regime had (0.60± 0.02cm). However the seedlings submerged in a bowl of water withered at the 12th week with a mean collar diameter of 0.31 ± 0.02cm, while the control (Not watered at all wilted at the 8th week with a mean diameter of (0.31 ± 0.02cm) (Fig.4.10). There were significant differences ($P<0.01$) in collar diameter among the seedlings of *P. conophora* under different watering regimes. Age of seedlings as well as the interaction between watering regimes and replicates had significant effects on collar diameter among the seedlings (Table 4.20b).

The highest mean number of leaves, 16±0.5 was obtained from daily watering. This was followed by the four days watering interval with 10±0.2 and once in seven days watering with a mean value of 4.±0.5. The seedlings that were submerged in a bowl of water withered at the 12th week with a mean value of 4 leaves. The control (not watered) for 24 weeks wilted at the 8th week of germination with a mean value of 3.5±0.2 leaves, (Table 4.20a). Age of seedlings, watering regime, replicates and the interactions between them and the seedlings had significant effect on the number of leaves of *P. conophora* ($p<0.05$).

The highest mean leaf area of 95cm^2 was recorded in seedlings watered daily followed by seedlings watered at four days interval, with 91.67cm$^{2.}$ Seedlings watered at seven days interval had 81.5cm^2 while seedlings submerged had 61cm^2 before they wilted, (Table 4.20a).

Seedlings subjected to daily watering had a mean leaf dry weight of 3.07.The four days interval watering had 2.05g while seven days watering interval had 1.03. Seedlings sub-merged and those not watered at all (control) had 0.61g and 0.54g respectively at 8th weeks, (Table 4.21).

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The highest stem dry weight (3.18g) was recorded among seedlings with daily watering. This was followed by seedlings watered at four days interval, (2.12g). The sub-merged seedlings and control had the least stem dry weight of (0.46g) and (0.28g) respectively, at the 8th week before wilting (Table 4.21). They were significantly different from seedlings watered at seven days interval, 0.89

Seedlings watered daily gave the highest mean RDW of 20.59 $\pm$ 0.033g followed by seedlings watered at four days interval with 15.53g. Seedlings watered at seven days interval had a root dry weight of 12.50g. The seedlings that were sub-merged and the control wilted at the 8th week with a RDW of 5.22g and 5.01g respectively. (Table 4.21) Watering regimes had significant effect on the root dry weight (RDW) ($P < 0.01$) of the seedlings.

Seedlings subjected to daily watering recorded the highest total dry weight of 26.84g, watering at four days interval had a total dry weight of 19.42g while those watered at seven days had a total value of 14.12g, seedlings that were sub-merged and not watered at all (control) had a value of 0.46g and 0.28g respectively after 8th weeks of planting, (Table 4.21).

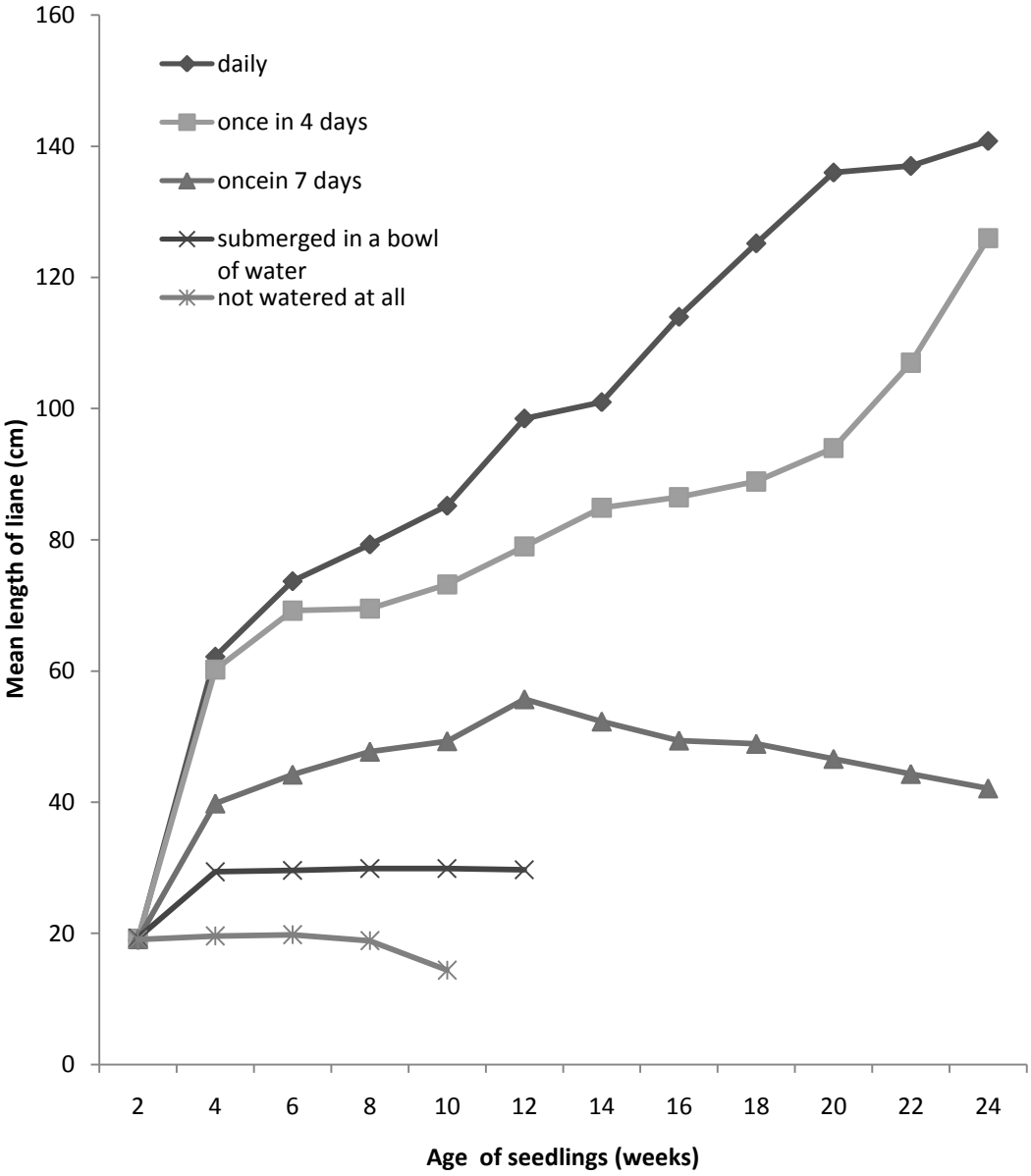


Fig. 4.9 : Mean length of vine of *P.conophora* seedlings as affected by watering regimes

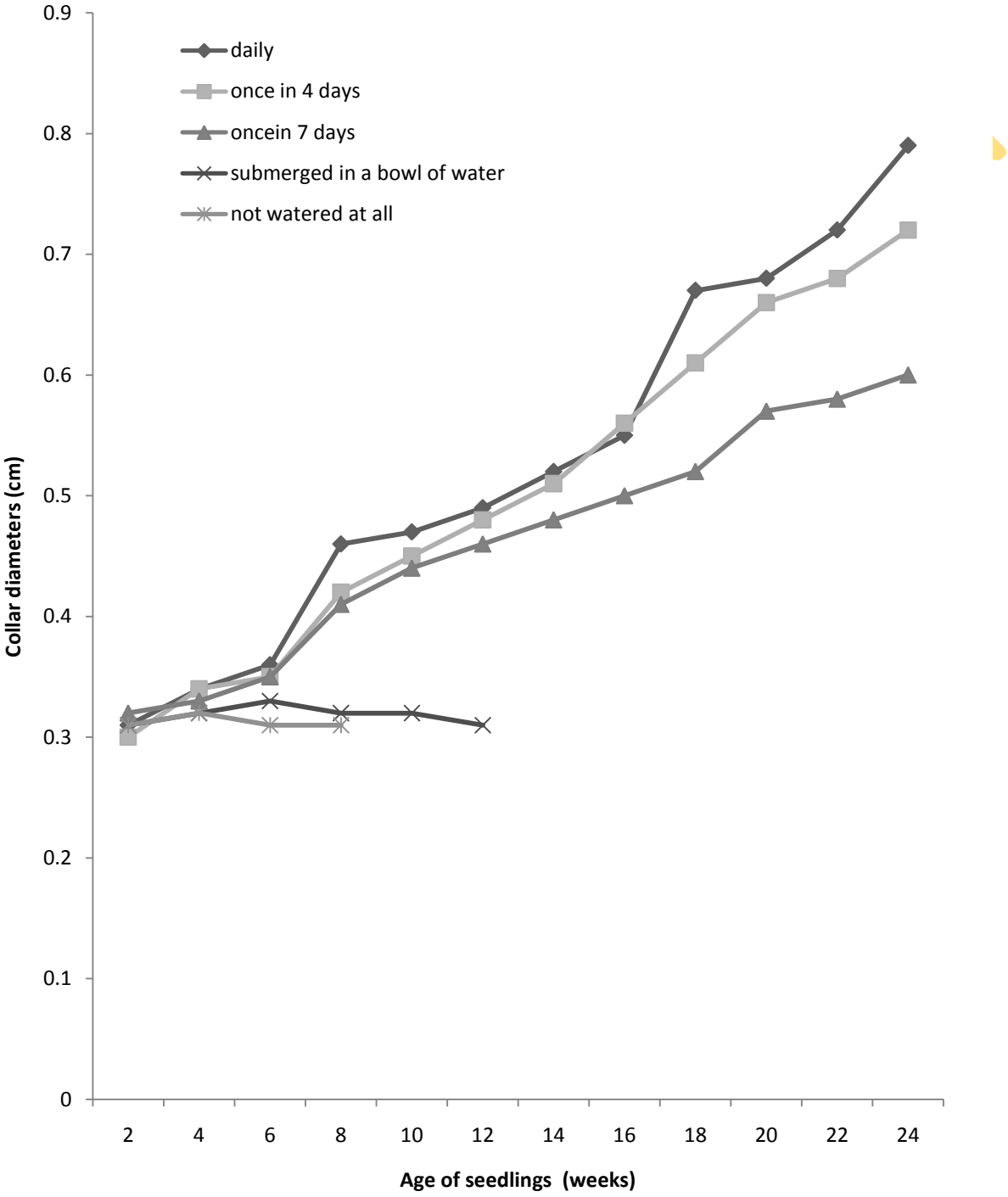


Fig. 4.10 : Effect of watering regimes on mean collar diameter of *P. conophora* seedlings

Table 4.20a: Mean Number of leaves of *Plukenetia conophora* over a period of 24 weeks as affected by watering regime

Watering regime	Number of leaves in weeks											
	2	4	6	8	10	12	14	16	18	20	22	24
	4.5	4.2	6.1	7.5	9.7	10.2	12.0	12.6	14.0	14.4	15.0	16.0
Daily												
Once in 4 days	4.0	4.4	4.5	5.1	5.7	6.8	7.6	7.9	8.3	9.0	9.0	10.4
Once in 7 days	4.0	4.5	4.5	4.7	3.8	3.8	0.48	4.1	4.5	4.2	4.3	4.4
Sub-merging in a bowl of water	4.5	4.5	4.0	4.0	4.0	4.0	0	0	0	0	0	0
Not watered at all (control)	4.4	4.2	4.0	3.5	0	0	0	0	0	0	0	0

Table 4.20b: Means for the Effect of Watering Regimes on Early Growth of *Plukenetia conophora*

Treatment	Parameter	Measured	
Watering regime	Collar diameter	Vine length	No. of leaf
Daily	0.530a	97.7a	9.68a
Once in 4 days	0.507a	79.8a	6.89a
Once in 7 days	0.463b	45.0bc	4.04bcd
Not watered	0.313c	18.4bcd	

LSD Significant at P< 0.01

Means with the same letter under each column are not significantly different from each other at P = 0.01

Table 4.21: Mean biomass production of *P. conophora* under different watering regime for 24 weeks

Watering regimes (Treatment)	Stem dry weight (g)	Root dry weight (g)	Leaf dry weight (g)	Total dry weight (g) (Biomass)	Leaf area (cm^2)
Daily	3.19	20.59	3.07	26.85	95cm^2
4 days	2.13	15.53	2.05	19.71	91.67cm^2
7 days	0.89	12.50	1.03	14.42	81.5
Sub-merged	0.46	0	0	0	61cm^{2*}
Not watered	0.28	0	0	0	49cm^{2*}

*- Data terminated at 8 weeks after sowing

4.5: Influence of height of decapitation on the coppicing potential of juvenile *Plukenetia conophora*

The longest coppice in mean length was 202.00±0.4cm, which was obtained in seedlings decapitated at 20cm above the collar. In the seedlings decapitated at 15cm above the collar, the highest mean length of coppice was 36±0.2cm while the seedlings decapitated at 10cm had a highest mean length of 9.9±0.22cm. (Table 4.22) The means of length growth of sprouts obtained by decapitating the seedlings at different heights differed significantly ($P<0.01$) (Table 4.23).

In seedlings decapitated at 20cm above the collar had the highest mean diameter of sprout was 0.77± 0.04cm while the lowest was 0.03±0.01cm This was followed by those decapitated at 15cm, with 0.16±0.02cm and 0.03±0.01cm as the highest and lowest mean collar diameter respectively. The seedlings decapitated at 10cm above the collar also recorded 0.07±0.02cm as the highest and 0.04±0.01cm as the lowest mean diameter of sprout at the 24th week of the experiment (Table 4.20). Heights at which seedlings were decapitated significantly ($P<0.01$.) affected the diameter growth of sprouts (Table 4.23).

The highest numbers of coppice shoots 6 were recorded among seedlings decapitated at 20cm above collar. These were significantly different from those decapitated at 15cm with highest number of coppice shoots of 5 and 3 for those decapitated at 10cm. The control (not decapitated) had no coppice shoot instead it developed a branch at height 25cm above the collar. (Table 4.20) The height of decapitation of seedlings from the collar significantly affected the number of coppices ($P<0.01$) (Table 4.23).

The highest mean number of leaves per sprout of 15±0.5 was recorded among seedlings decapitated at 20cm above collar. This was followed by seedlings decapitated at 15cm with a mean value of 9±0.3, while the least number of 5±0.5 was recorded among seedlings decapitated at 10cm above collar, at the end of experimental period of 24 weeks, (Table 4.22). Number of leaves per sprout differed significantly ($P< 0.01$) at the different heights of decapitation (Table 4.23).

Table 4.22: Influence of height of decapitation on coppicing of *Plukenetia conophora*

	Seedling No.	Mean No. of Coppice	Mean Length	Mean Diameter	Mean No. of Leaves/Coppice
	1	5	16.42	0.04	5.40
	2	4	95.0	0.40	9.25
20cm	3	1	202.0	0.77	15
	4	2	15.55	0.18	6.50
	5	6	53.15	0.13	8.00
	6	3	37.33	0.16	4.67
	7	3	132.37	0.52	12.00
	8	3	32.73	0.07	6.67
	9	2	55.60	0.14	10.50
	10	2	70.70	0.20	11.50
	1	4	18.83	0.03	7.25
	2	3	20.43	0.06	6.00
15cm	3	4	23.30	0.07	6.50
	4	2	25.65	0.08	5.50
	5	3	33.50	0.06	8.33
	6	4	36.00	0.11	9.75
	7	5	35.20	0.10	8.80
	8	3	16.23	0.05	8.33
	9	3	26.20	0.11	6.33
	10	2	30.25	0.16	9.00
	1	2	7.20	0.05	3.00
10cm	2	2	7.95	0.05	3.50
	3	1	8.50	0.04	5.00
	4	3	9.90	0.06	4.33
	5	2	9.85	0.07	4.50
	6	-	-	-	-
	7	1	9.80	0.06	4.00
	8	-	-	-	-
	9	-	-	-	-
	10	2	9.85	0.06	4.00
Control No.	1	-	-	-	8.4
Decapitation	2	-	-	-	8.1
	3	-	-	-	9.3
	4	1	69	0.52	8.0
	5	-	-	-	-
	6	1	58	0.41	7.0
	7	-	-	-	9
	8	-	-	-	7.6
	9	-	-	-	7.4
	10	-	-	-	6.2

Table 4.23: Table of Means and LSD for Effect of Height of decapitation on Coppicing

Height of decapitation	Mean diameter	Mean length of vine	Mean no of coppices	Mean no of leaf
10	0.038c	6.3f	1.50ab	2.83ba
15	0.081b	26.6e	3.30ac	7.58bc
20	0.259a	71.1a	3.10ac	8.95bd
LSD	0.1257	31.26	1.165	2.191

***Significant at P< 0.05**

Means with the same letter under each column are not significantly different from each other at P < 0.05

4.6.0: Effect of different types and dosage of organic fertilizer on early growth of *Plukenetia conophora*

The chemical properties of the organic fertilizer used and the physical and chemical properties of the soil used in this study are shown in Table 4.24a.

The highest mean length of vine (242.5cm) was recorded among seedlings treated with compost at 40g/2kg soil at the 24th week of application. The least length of vine (164cm) was obtained in the control at the 24th week of observation. The highest mean length in poultry manure was 198cm, obtained from 40g/2kg soil. While cowdung manure recorded 190cm from 40g/2kg soil (Table 4.25). However there was no significant difference between different rates of application in terms of length of vine

The highest collar diameter at the end of the experimental period of 24 weeks was 0.98cm which was recorded by compost manure at 30kg/2kg soil. Poultry manure recorded 0.84cm, obtained from 20kg/2kg soil. Cowdung manure had 0.80cm from 40g/2kg soil, while the highest collar diameter recorded in the control was 0.63cm (fig 4.11). Diameter growth of the seedlings differed significantly under different fertilizer sources at ($P< 0.05$), but the rate used did not have any effect on the variables measured (fig 4.11).

The highest mean number of leaves (25±0.02) leaves was obtained from compost at 24 weeks of application. Poultry manure recorded 22±0.02 while cowdung manure and the control had 21±0.03 and 17±0.05 respectively at the end of 24 weeks (Fig 4.12). Organic fertilizer type and dosage did not show any significant effect on the number of leaves produced by the seedlings,

Leaf area of seedlings treated with compost recorded the highest value of 100cm^2 with 40g/2kg soil at the 4th week of application. This was followed by poultry waste with 89.93cm^2 at 20g/2kg soil, while cowdung manure had 81.73cm^2 with 40g/2kg soil, and the control had 81.09cm2, (Table 4.26). At the 24th week of application, the highest leaf area of 127.01cm^2 was recorded in compost with 40g/2kg soil. Poultry waste had 119.82cm^2 with 30g/2kg soil while cowdung manure had 113.04cm^2 at 40g/2kg soil, and the control had 99.45cm2, (Table 4.27). Fertilizer sources and dosage were significant on the leaf area of seedlings at $P <0.01$. Table of means also showed a significant difference in means of the leaf Area (Table 4.28).

At 4 weeks, the highest leaf dry weight of 1.10g was obtained in compost at 20g/2kg soil; the highest in cowdung was 0.99g while poultry manure had 0.89g, (Table 4.26). At 24 weeks, the highest LDW of 8.97g was obtained in compost at 30g/2kg soil. The highest leaf dry weight in poultry manure and cowdung manure were 8.62g obtained from 30g/2kg soil and 8.04g from 40g/2kg soil. The control had a leaf dry weight of 8.73g (Table 4.27). Types and dosage of organic fertilizer did not significantly affect the leaf dry weight of the seedlings.

At the 4th week of application, the highest stem dry weight of 0.91g was obtained with the poultry manure applied at 30g/2kg soil. This was followed by the compost with 0.90g SDW recorded in 30g/2kg soil. The cowdung manure had 0.70 at 40g/2kg soil. The control had a value of 0.69, (Table 4.26). At 24 weeks of application, the highest mean stem dry weight (SDW) of 18.54g was obtained with compost at 40g/2kg soil. This was followed by poultry manure with 13.87g, from 30g/2kg soil, the cowdung manure had 11.32g with 30g/2kg dosage and the control had 7.52g, (Table 4.27). Fertilizer source and dosage did not have any significant effect on the stem dry weight.

At the 4th week of application, the highest RDW of 8.72g was recorded with cowdung manure applied at 40g/2kg soil; 8.58g was obtained from poultry waste at a dosage of 40g/2kg soil while the control had 7.60g, (Table 4.26).

At the 24th week of application, the highest RDW of 48.12g was obtained from poultry manure applied at 40g/2kg soil, followed by 48.11g with compost applied at 40g/2kg soil, while cowdung manure had 43.22g at 40g/2kg soil; the control recorded 33.41g, (Table 4.27). Fertilizer source and dosage did not significantly affect the root dry weight.

At 4th week after application of organic fertilizer, the highest TDW, 11.30g was obtained from compost applied at 40g/2kg soil. This was followed by the cowdung manure with 10.42g recorded at 40g/2kg soil; the poultry manure had 10.37g at 40g/2kg soil, while the control had 9.19g/2kg soil, (Table 4.26). There was no significant difference between the source and dosage of fertilizer (Table 4.28). Highest TDW at the 24th week was 75.61g, obtained from compost at 40g/2kg soil, followed by 70.43g weight obtained from poultry manure at 40g/2kg soil, while Cowdung manure had 62.40g at a dosage of 40g/2kg soil. The control had 45.28g (Table 4.27)

Net assimilation rate (NAR) gm/month of *Plukenetia conophora* as affected by different organic fertilizer

The data for Net Assimilation Rate (NAR) did not show a discernible trend. The highest rate of 0.029gm in the 4th week of experiment was recorded in compost fertilizer while the least was

0.020gm/t from the control. At week 20, the highest rate of 0.039gm/t was obtained from cowdung manure at 10g/2kg soil, (Table 4.29).

Relative growth rate (RGR)

The highest relative growth rate (RGR) of 0.110g/t was obtained from poultry waste at week 8 under the treatment with 40g/2kg soil. However a mean RGR of 0.109g/t at week 8 was recorded in compost manure while the lowest RGR of 0.029g/t was recorded in the control at week 20, (Table 4. 30).

Absolute growth rate (AGR) of *P.conophora* seedlings as affected by different types and dosage of organic manure

Seedlings treated with compost at 40g/2kg soil recorded the highest (AGR) of 3.898 at weeks 8 to 12 of application. Poultry manure had 3.640, cowdung recorded 3.141, while the control had 2.176 at the same period. At weeks 20 to 24, cowdung manure had the highest AGR of 3.928 at 40g/2kg soil .Poultry manure had 3.908 at 30g/2kg soil while the control had 2.415g/2kg soil, (Table 4.31).

Table 4.24a: Physical and chemical properties of organic material

Properties	Compost manure	Poultry manure	Cowdung manure
Nitrogen	16.1	17.3	8.1
Organic carbon	38.3	26.0	16.2
Phosphorus	18.7	22.6	11.6
Potassium	29.2	20.9	2.2
Calcium	21.5	9.4	8.0
Magnesium	6.0	4.0	1.0
Iron	6.9	14.7	3.4
Zinc	155.3	181.0	142.0
Copper	29.2	37.0	23.0
Lead	0	0	0

Table 4.24b: Physical and chemical properties of sowing medium (soil)

Soil Physical and Chemical Properties	
Sand	53%
Silt	39%
Clay	13%
PH	6.1%
Nitrogen	0.012%
P	8.65%
K	0.10%
Ca	0.18%
Mg	0.31%
Organic Matter	1.42%

Table 4.25: Mean values of vine length of *Plukenetia conophora* as affected by organic fertilizer over a period of 24 weeks

Fertilizer/g/2kg soil	Length of seedlings over 24 weeks											
	2	4	6	8	10	12	14	16	18	20	22	24
Poultry manure												
0	19.3	66.3	91.3	108.5	141.5	149.1	151.8	156.0	159.3	163.0	167.0	173.2
10	19.0	68.5	100.5	103.5	129.5	139.0	152.0	160.0	170.5	174.2	176.0	183.5
20	20.0	67.3	97.8	100.0	119.5	141.0	150.6	158.8	168.2	175.8	180.1	188.0
30	19.8	65.4	105.2	103.6	120.5	140.8	147.5	155.9	168.5	178.7	182.2	192.7
40	19.6	63.0	106.1	105.0	120.7	144.0	150.9	160.4	170.0	179.0	183.5	198.6
Cow-dung												
0	18.4	54.5	110.4	111.8	132.7	140.0	144.7	147.2	153.9	158.2	163.0	168.5
10	19.2	63.6	90.7	102.1	111.1	135.0	143.4	157.0	165.0	173.7	175.5	180.5
20	20.3	68.4	93.5	107.8	125.4	133.9	150.0	154.2	160.7	169.0	171.6	179.2
30	20.3	68.6	99.3	101.5	119.7	130.0	150.6	155.5	163.5	166.5	171.0	185.7
40	19.6	71.3	99.6	103.4	117.9	128.8	156.1	162.0	177.9	183.0	188.0	190.7
Compost												
0	20.1	70.1	89.5	99.7	123.8	135.7	138.6	143.0	149.2	153.4	159.1	164.0
10	19.6	70.2	102.1	105.7	136.2	153.0	158.4	161.0	178.5	191.1	217.9	237.3
20	20.3	68.0	107.3	110.0	140.5	156.2	158.6	165.0	177.3	199.9	222.6	233.5
30	20.3	69.5	101.4	111.1	138.2	163.5	169.8	168.3	188.6	197.0	224.5	239.1
40	19.6	73.4	109.2	124.2	148.0	164.2	172.5	175.0	190.2	202.4	231.8	242.5

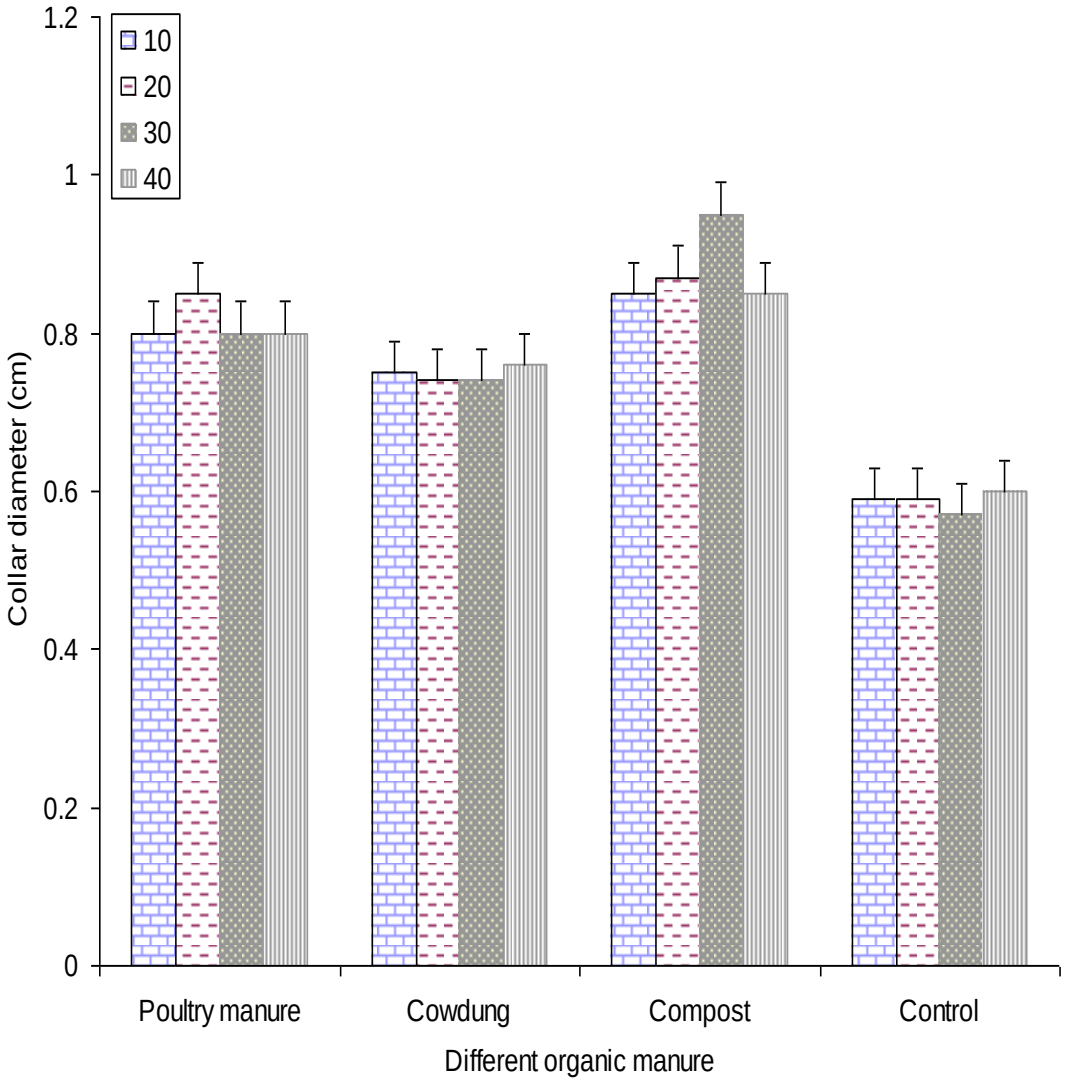


Fig. 4.11: Seedling collar diameter (cm) of *P. conophora* at 24 weeks using diferent organic manure.

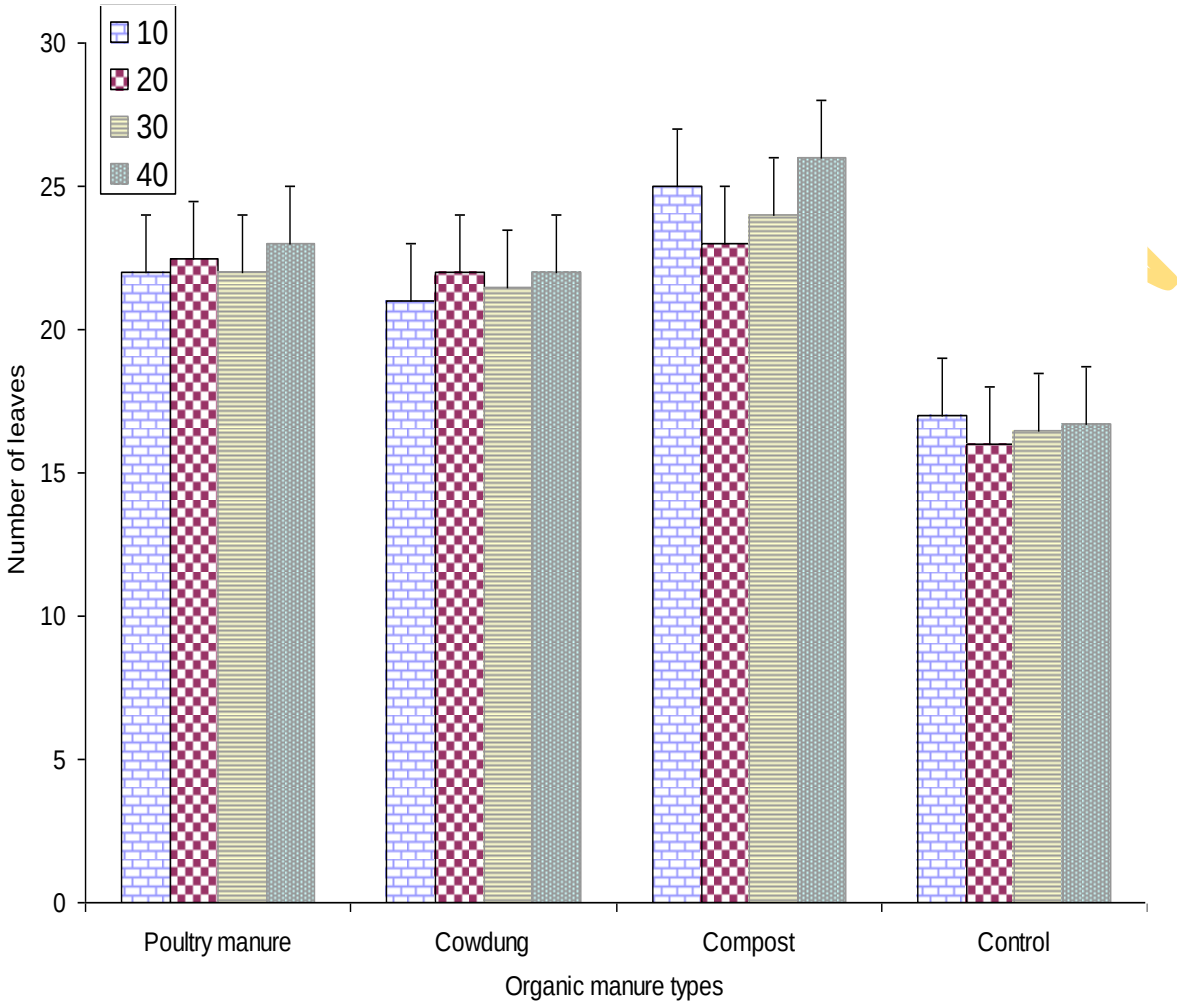


Fig. 4.12: Number of leaves of *Plukenetia conophora* as affected by different organic manure

Table 4.26: Effect of different organic fertilizer on seedling biomass and leaf area at 4 weeks

Organic fertilizer and rate	Root dry weight (g)	Stem dry weight (g)	Leaf dry weight (g)	Total dry weight (g)	Leaf area (cm^2)
Poultry manure					
0	7.35	0.61	0.82	8.78	79.00
10	7.15	0.69	0.97	8.81	89.72
20	8.22	0.72	0.99	9.92	89.93
30	7.87	0.91	0.96	9.74	89.84
40	8.58	0.82	0.98	10.37	89.74
Cow-dung					
0	7.21	0.68	0.89	8.78	79.52
10	8.15	0.62	0.99	9.77	81.26
20	8.20	0.69	0.97	9.86	81.25
30	7.58	0.69	0.99	9.27	81.38
40	8.72	0.70	0.99	10.42	81.73
Compost					
0	7.13	0.69	0.89	8.72	81.09
10	9.12	0.69	1.00	10.81	88.89
20	8.92	0.79	1.10	10.82	88.63
30	9.39	0.90	1.00	11.29	89.72
40	9.41	0.90	0.99	11.30	100.00

Table 4.27: Effect of different organic fertilizer on seedling biomass and leaf area at 24 weeks

Organic fertilizer and rate	Root dry weight (g)	Stem dry weight (g)	Leaf dry weight (g)	Total dry weight (g)	Leaf area (cm^2)
Poultry manure					
0	33.41	6.77	4.99	45.17	91.50
10	41.51	12.13	8.42	62.06	108.43
20	43.55	13.55	8.41	65.51	111.52
30	47.88	13.59	8.62	70.09	119.82
40	48.12	13.87	8.44	70.43	118.88
Cow-dung					
0	31.66	6.81	5.22	43.69	89.53
10	40.33	10.32	7.81	58.52	99.55
20	40.12	9.44	7.57	57.13	99.05
30	41.55	11.32	8.01	60.88	108.42
40	43.22	11.14	8.04	62.40	113.04
Compost					
0	32.42	7.52	4.89	44.83	98.02
10	46.73	15.72	8.91	71.36	118.42
20	46.13	16.83	8.83	71.79	122.53
30	47.01	17.01	8.97	72.99	126.44
40	48.11	18.54	8.96	75.61	127.01

Table 4.28: LSD table for the effect of fertilizer on Leaf area

Effect of fertilizer	Effect of fertilizer	Mean Diff.	Std. Error	Sig.
poultry manure	cow dung	3.7711*	1.90884	.049
	compost	-11.8528*	1.90884	.000
cow dung	poultry manure	-3.7711*	1.90884	.049
	compost	-15.6239*	1.90884	.000
compost	poultry manure	11.8528*	1.90884	.000
	cow dung	15.6239*	1.90884	.000

*. Significant at p< 0 .05 .

Table 4.29: Net Assimilation Rate (NAR) g/month of *Plukenetia conophora* as affected by different organic fertilizer

Organic Fertilizer gm/2kg soil		Week 4	Week 8	Week 12	Week 16	Week 20
Poultry manure	0	0.021	0.024	0.024	0.023	0.140
	10	0.027	0.032	0.028	0.025	0.024
	20	0.028	0.033	0.026	0.026	0.027
	30	0.029	0.033	0.025	0.030	0.033
	40	0.029	0.033	0.022	0.027	0.029
Cow-dung	0	0.021	0.024	0.021	0.018	0.140
	10	0.027	0.032	0.031	0.031	0.039
	20	0.026	0.030	0.270	0.028	0.030
	30	0.027	0.029	0.013	0.023	0.034
	40	0.027	0.028	0.014	0.023	0.036
Compost	0	0.020	0.021	0.023	0.020	0.021
	10	0.029	0.036	0.030	0.030	0.017
	20	0.029	0.035	0.028	0.028	0.030
	30	0.029	0.034	0.021	0.022	0.025
	40	0.029	0.034	0.020	0.022	0.026

Table 4.30: Relative Growth Rate (RGR) g/month of Seedlings as affected by different types and dosage of organic manure

Organic fertilizer gm/2kg soil		Week 4	Week 8	Week 12	Week 16	Week 20
Poultry manure	0	0.082	0.090	0.070	0.056	0.029
	10	0.098	0.100	0.063	0.049	0.044
	20	0.094	0.102	0.058	0.051	0.049
	30	0.099	0.105	0.052	0.057	0.063
	40	0.096	0.110	0.047	0.056	0.055
Cow-dung manure	0	0.080	0.086	0.062	0.044	0.030
	10	0.090	0.101	0.071	0.062	0.074
	20	0.088	0.099	0.067	0.058	0.056
	30	0.094	0.101	0.059	0.050	0.069
	40	0.090	0.102	0.056	0.050	0.073
Compost	0	0.082	0.085	0.068	0.053	0.041
	10	0.094	0.107	0.062	0.055	0.059
	20	0.095	0.106	0.060	0.054	0.052
	30	0.093	0.107	0.043	0.042	0.046
	40	0.095	0.109	0.039	0.042	0.047

Table 4.31: Absolute Growth Rate (AGR) of Seedlings as affected by different types and dosage of organic manure

Organic Manure(g)		Week 4	Week 8	Week 12	Week 16	Week 20
g/2kg soil						
Poultry manure g/2kg soil	0	1.820	2.159	2.138	2.041	1.255
	10	2.663	3.098	2.740	2.521	2.498
	20	2.779	3.296	2.737	2.735	2.933
	30	3.017	3.568	2.695	3.220	3.908
	40	3.003	3.640	2.546	3.161	3.458
Cow-dung manure	0	1.745	2.045	1.917	1.631	1.268
	10	2.438	2.930	2.784	2.860	3.760
	20	2.363	2.842	3.789	2.661	2.865
	30	2.581	3.047	2.569	2.519	3.640
	40	2.599	3.141	2.556	2.577	3.928
Compost	0	1.806	2.087	2.082	1.944	1.683
	10	3.028	3.655	3.111	3.178	3.768
	20	3.049	3.666	3.059	3.145	3.338
	30	3.085	3.741	2.448	2.594	2.078
	40	3.215	3.898	2.367	2.704	3.228

4.7.0: Effect of growth hormones and rooting media on rooting of juvenile stem cuttings of *P. conophora*

Cuttings had rooting by the 7th week of setting as observed from the perforated rooting trays. (Plate 4.17) By the 8th week, the roots were matured enough for potting (Plate 4.18). Percentage survived rooted cuttings varied among the different types of hormone. NAA+IBA powder using sawdust as a rooting medium recorded 90% rooted cuttings. NAA powder using sand as rooting medium had 80% while IBA powder using sawdust also recorded 80%. (Fig 4.13 shows the percentage of surviving callus with IBA recording 14% in sand and18% in sawdust, NAA had12.5% and 20% in sand and sawdust respectively while IBA+NAA had 20% and 18.75%. The control had 0% because the cuttings did not root at all. Interaction between the different types of hormone showed a significant difference at $P<0.05$. The Table of means also showed a significant difference at $P= 0.05$(Table 4.30).

The highest mean number of roots (13±2) was produced by NAA + IBA hormone using saw dust. The least mean number of roots (3.±0.5) was produced by NAA hormone using saw dust. IBA recorded a mean number of (8.21±0.5) roots, while the control did not produce any root. Interaction between hormone and rooting media had a significant effect on the number of roots produced, $P<0.05$.

The longest root length, 3.11cm was obtained with IBA powder using sand as a rooting medium.

This was followed by NAA+IBA with 2.8cm and NAA alone had 2.65cm (Plate 4.19).

Interaction between hormone type and type of rooting media did not have any significant effect on root length. Although the longest tap root of 3.11cm was recorded with IBA powder, it was not significantly different from those produced by NAA and NAA + IBA powder (Table 4.32) (Fig 4.13). Immediately after the assessment rooted cuttings were potted into polythene bags filled with top soil and arranged under a winning shed for 2 weeks to stabilize, (Plate 4.20). At 14 weeks the cuttings were already established to be taken to the open nursery, (Plate 4.21).

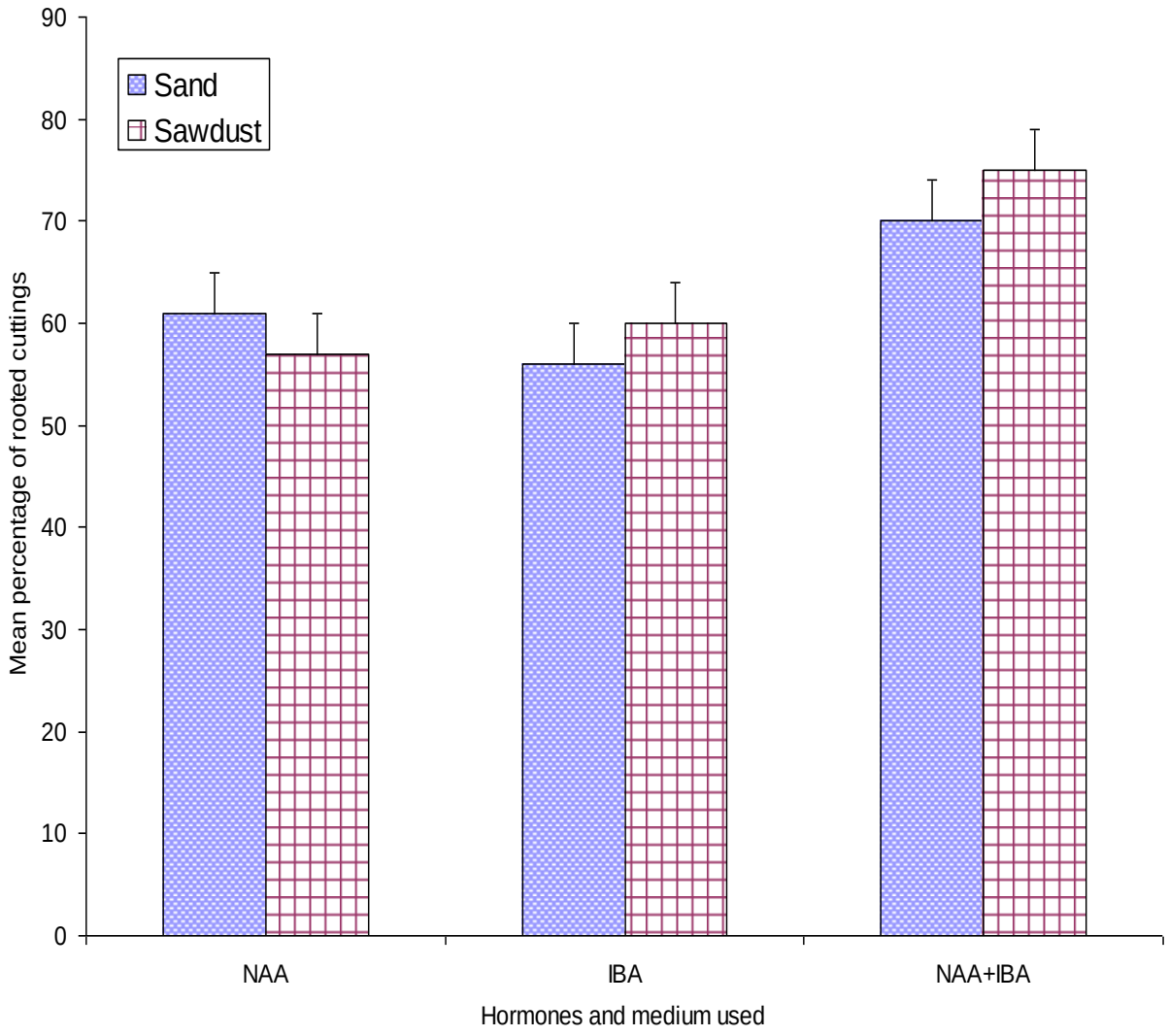


Figure 4.13: Mean percentage of rooted cuttings using NAA, IBA and NAA+IBA powder hormone in sand and saw dust.

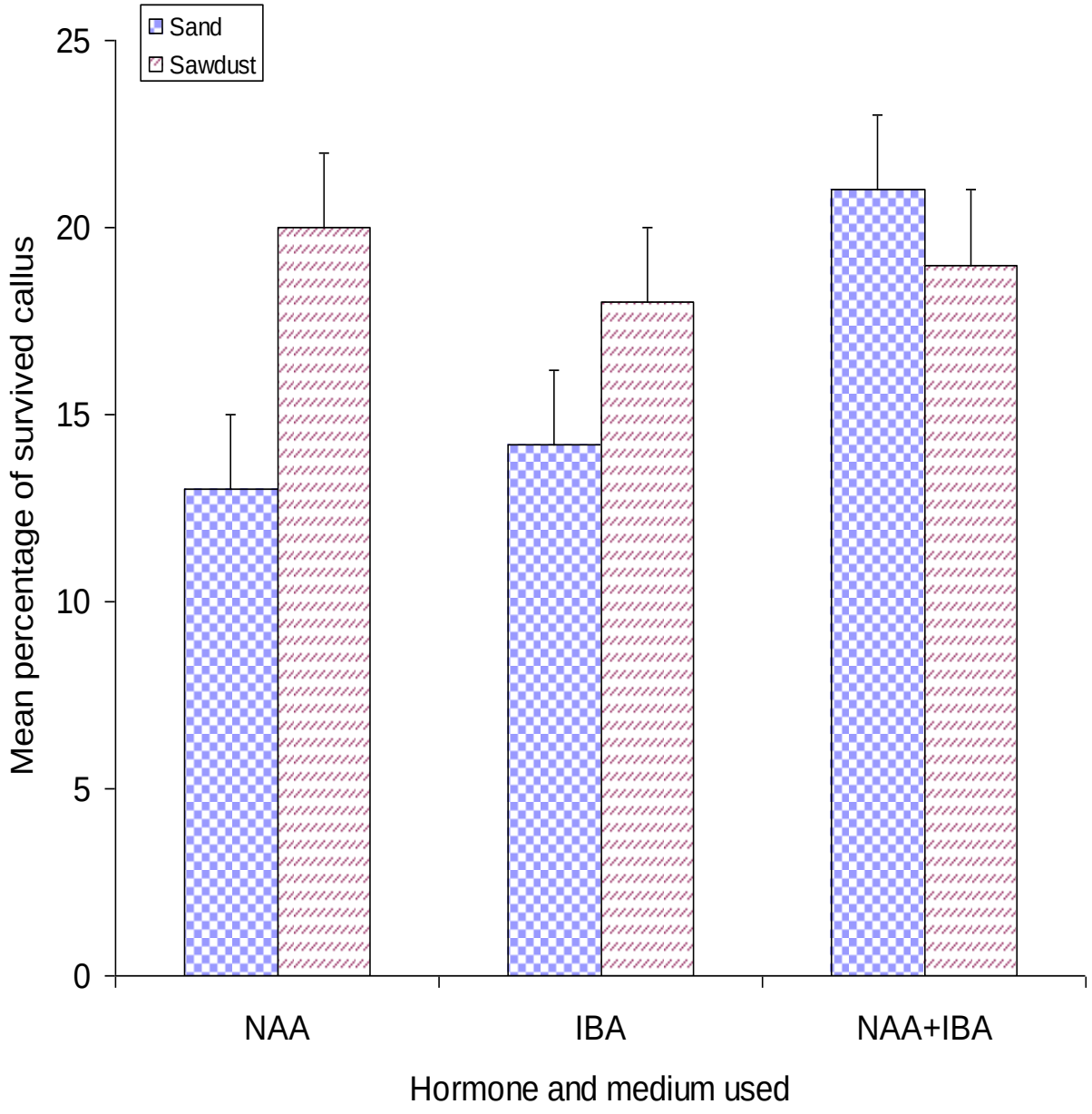


Fig.4.14: Mean percentage callus in sand and sawdust media

Table 4.32: LSD table showing percentage survival, mortality and callused cutting using powder hormones

hormones	hormones	Mean Diff.	Std. Error	Sig.
NAA	IBA	-.1667	.57478	.773
	NAA + IBA	.0000*	.57478	1.000
IBA	NAA	.1667	.57478	.773
	NAA + IBA	.1667	.57478	.773
NAA + IBA	NAA	.0000*	.57478	1.000
	IBA	-.1667	.57478	.773

Significant P < 0.05

Plate 4.10: Double node cuttings of *P. conophora* at 7 weeks showing the developing roots

Plate 4.11: Double node cuttings of *P.conophora* treated with IBA powder at 8 weeks .

Plate 4.12: 8weeks old double node rooted cuttings of *P.conophora* treated with powder IBA(A) NAA(B) IBA+NAA (C)

Plate 4.13: Potted double node rooted cutting of *P. conophora* stabilizing under the weaning shed

Plate 4.14: Established double node cuttings of *P.conophora* at 14 weeks

4.8.0 STUDY 8: Micropropagation

Embryo culture of *Plukenetia conophora*

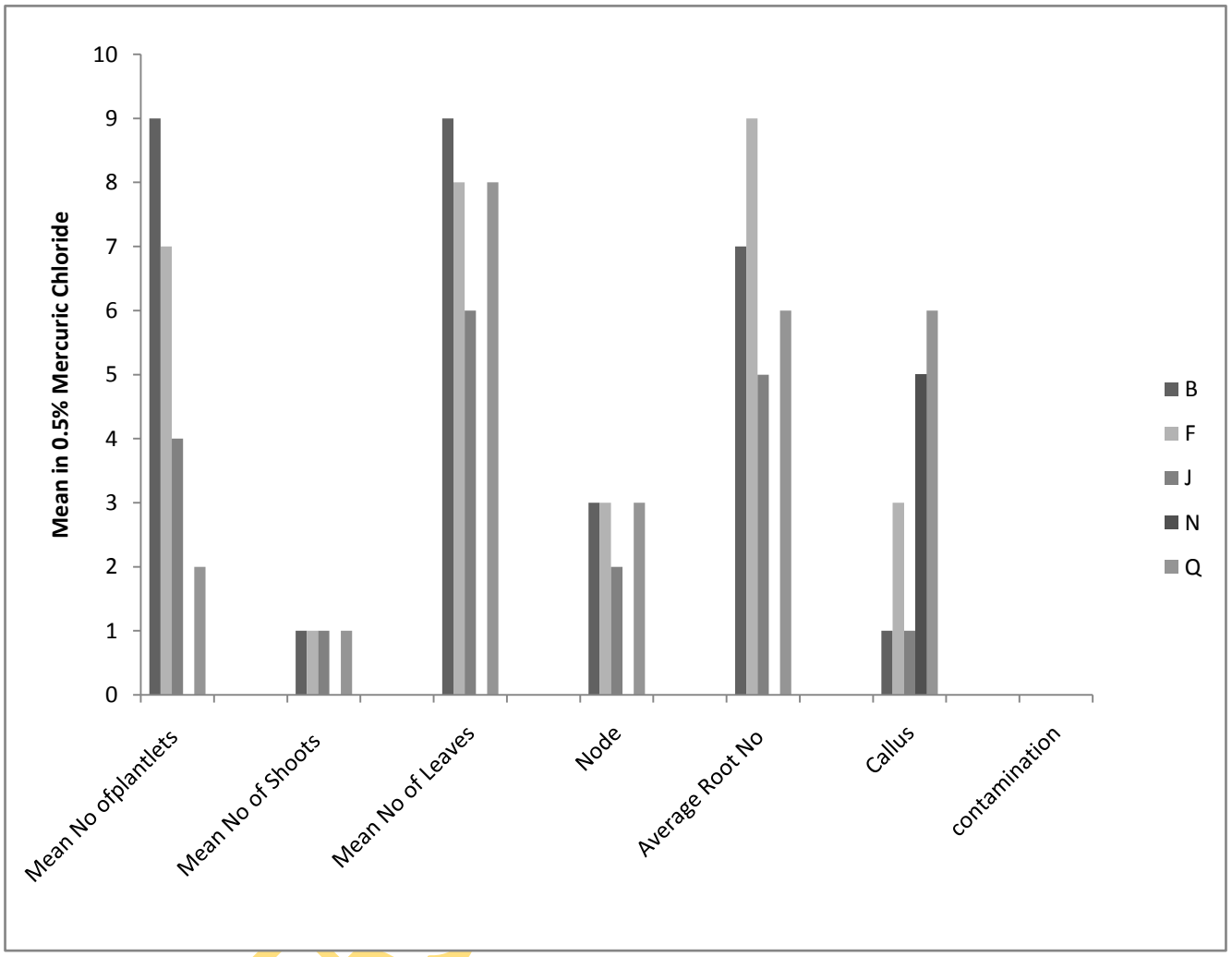
In the embryo culture the explants disinfected with 0.05% mercuric chloride performed better than those disinfected with 10% sodium hypochlorite. Shoot and root development were observed on the fifth day from explants disinfected with 0.05% mercuric chloride, while those treated with 10% sodium hypochlorite sprouted on the seventh day. The shoot and roots were obtained from the following media:

B [MS only]
F [MS + 0.1mg Kinetine + 0.3mg 24D]
J [MS + 0.3mg Kinetine + 0.1 24D]
N [MS + 0.5mg BAP only]
Q [MS + 0.1mg BAP + 0.5mg NAA]

The highest mean number of plantlets (9) was recorded in medium (B) (MS only) disinfected with 0.05% mercuric chloride (Fig.4.15). The highest mean number of leaves of (9) was also recorded in the same medium (B). Highest average number of roots (9) was recorded in medium (F) [MS + 0.1mg Kinetine + 0.3mg 24D], (Fig 4.15). The explants disinfected with 0.05 mercuric chloride did not record any contamination, whereas the explants disinfected with sodium hypochlorite though developed some shoots and roots, recorded a lot of contamination (Fig 4.16 and Plate 4.21). At 21 days the root and shoots were already well formed from the embryo, (Plates 4.22 A and 4.22 B) show the development of roots and shoots at 21 days and 30 days respectively.

Nodal culture

The explants did not develop shoots and roots in all the media used, but initial signs of growth were observed on the seventh day from medium B with the nodal stems remaining greenish, before later forming callus. However the nodal cuttings disinfected with 0.05% mercuric chloride did not show contamination but those treated with sodium hypochlorite recorded a high level of contamination.



B = MS only

MS = Murashige and skoog

F = MS +0.1mg kinetine + 0.3mg 24D

24D = 2,4 - dichlorophenoxyacetic acid

J = MS + 0.3mg kinetine + 0.1mg 24D

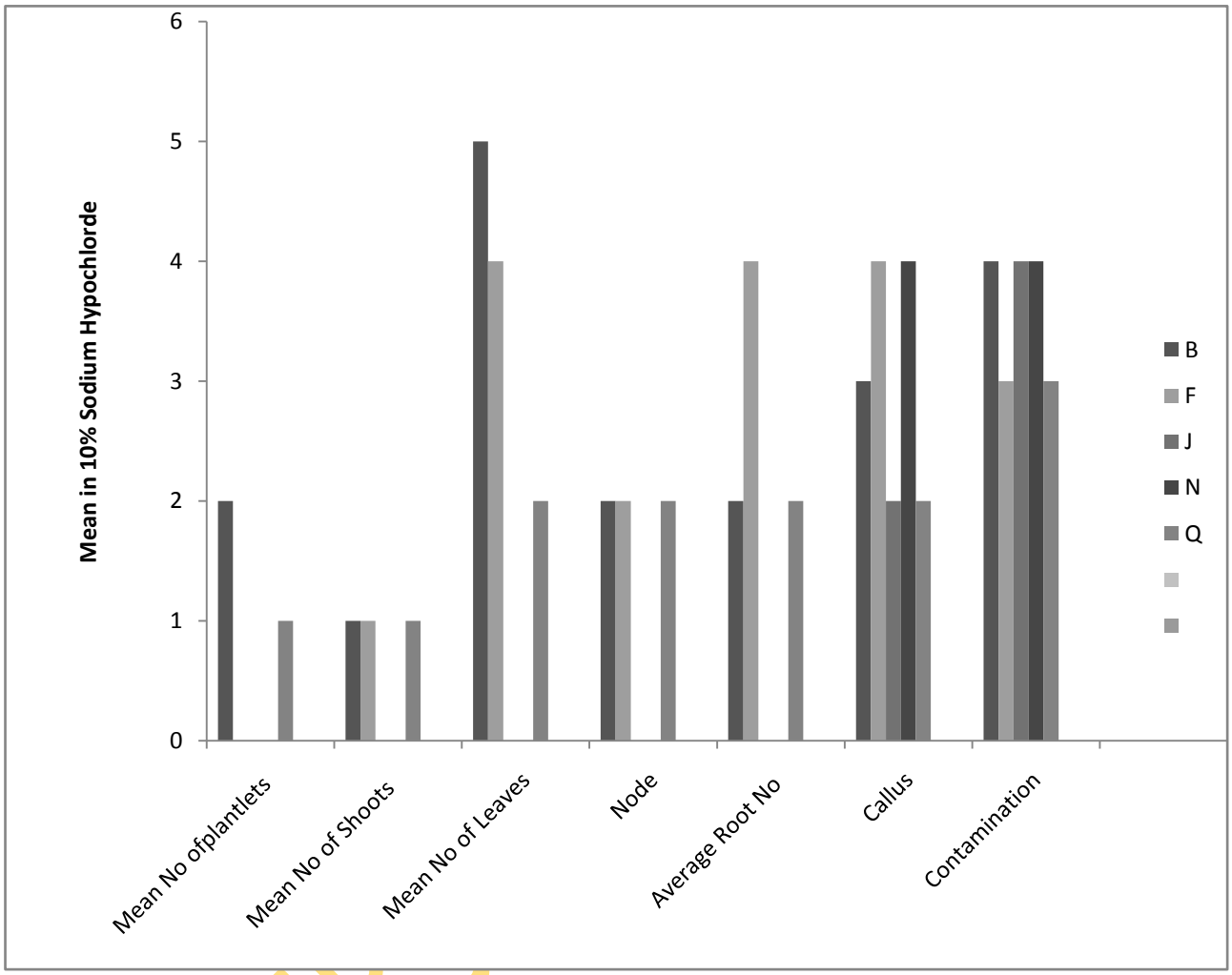
N = MS + 0.5mg BAP only

BAP = Benzylaminopurine

Q = MS + 0.1mg BAP + 0.5mg NAA

NAA = Naphthalene acetic acid

Fig. 4.15: Micro-propagation of P. *conophora* using 0.05% mercuric chloride



B = MS only

MS = Murashige and skoog

F = MS +0.1mg kinetine + 0.3mg 24D

24D = 2,4 - dichlorophenoxyacetic acid

J = MS + 0.3mg kinetine + 0.1mg 24D

N = MS + 0.5mg BAP only

BAP = Benzylaminopurine

Q = MS + 0.1mg BAP + 0.5mg NAA

NAA = Naphthalene acetic acid

Fig. 4.16: Micro-propagation of *P. conophora* using 10% Sodium hypochlorite

Plate 4.15: Micropropagation of *P. conophora* in the growth room of NACGRAB showing rooted(R), callused (CA) and contaminated (CO) explants at 24 days

Plate 4.16a: Embyro culture of *P.conophora* at 21 days showing the shoots and roots developing

Plate 4.16b Plantlets of *P. conophora* at 30 days showing well developed roots

CHAPTER FIVE

5.0 DISCUSSION

5.1 Morphological variation in seeds and seedlings of *Plukenetia conophora*

Tree improvement and breeding programmes depend on genetic diversity, which is the variation that exists within and among species. Plans for conservation are therefore often based on assessment of the eco-geographic variation of the distribution area of the population. The selection of any conservation method depends upon factors such as the variability of the species, its biology, the specified objectives of conservation, local conditions and genetic makeup of the species, (Hettasch *et al.,* 2009). Plant species with high economic importance like *Plukenetia conophora* need urgent attention to prevent them from going into extinction. This could be done by putting up organized silvicultural requirements and improvement strategies to conserve their genetic resources. Improvement involves the utilization of germplasm of plant varieties with desirable traits, which is possible when the variation in the natural populations of the species have been documented.

There was a strong inherent variability among the fruits and seeds of *P. conophora* from different sources studied. The fruits and seeds varied in their metrical characters. Fruits from Ibadan (Oyo State) had the highest size and weight. The total mean weight for Ibadan was 96.65g while Igbajo (Osun State) had the least weight of 62.38g. Ibadan source had the best performance in terms of length of liana, collar diameter, leaf area and Biomass assessment. This agrees with the findings of Gbadamosi, (2002) that variations exist in fruits of *Enantia chlorantha* from different sources. Seeds and fruits often contain energy food reserves, which are used up during germination to produce shoot and root components of the seedlings thus, a big seed is an indication of large food reserve that may manifest in strong and vigorous seedlings. This makes Ibadan a good source for establishment of an orchard. Many authors have equally observed variations among and between populations of different tree species; these include Akinyele, (2007) who investigated *Buchholzia coriacea*. Gbadamosi, (2002) studied variation in *Enantia chlorantha*. On the other hand, variations in seed and fruit size may be an indication of possible genetic or environmental variations between the sources. Ibadan had larger leaf area and flushes which is an indication of higher photosynthetic ability and growth rate. This also is in agreement with the work of Leakey *et al.,* (2003) and Atangana *et al.,* (2002) who studied the variation on morphological trait and size of different components of *Dacryoides edulis* and

Irvingia gabonensis respectively. Thus, this may suggest that before embarking on any plantation or orchard establishment, the morphology of fruit and seeds could be put into consideration so as to select suitable seed sources with desirable traits.

5.2 Effect of storage temperature and duration on viability of *Plukenetia conophora* seeds using tetrazolium test

Germplasm is the reproductive unit (genetic material) of any plant such as seed, pollen, vegetative propagules or other materials. Through germplasm collection, the genetic diversity essential to any tree improvement programme could be safe guarded, (Asaah *et al.*, 2006). In recognition of the threat to mass genetic erosion from accelerated tropical deforestation, massive germplasm collection and conservation efforts are inevitable. The physiological state and physical storage conditions of seeds greatly influence their lifespan. Since storage is an important factor in preserving viable seeds from time of collection until they are required for sowing, (Ojo, 2008), poor storage may greatly affect seed viability. Since most tropical tree species fruit seasonally, it is thus important to identify the best storage condition to maintain seed viability. Seeds are either orthodox (seeds that maintain their viability for long periods if processed (these have low moisture content) or they are recalcitrant, (seeds that lose their viability very fast, even under conditions that are normally conducive to seed longevity). *Plukenetia conophora* is a recalcitrant species. The effect of different storage temperature and duration on its viability were highly significant (<0.01). Seeds stored at refrigerator condition (7^{o}C) recorded a viability percentage of 45% between the 24^{th} and 28^{th} weeks and longest storage period of 30 weeks with 40% of seeds still viable. This is in agreement with Ojo (2008) who observed that cold storage at 5^{o}C favored the viability of *Bombax costatum*. But contrary to the findings of Eggers (2010) who stored seeds of *Trichilia dregeana*, *Trichilia emetica*, *Podocarpus henkelii* and *Syzygium cuminnii* (recalcitrant seeds) for 3 – 22 weeks at 6^{o}C, 16^{o}C and 25^{o}C (in sealed container) and inferred that storage at 6^{o}C was detrimental to the seeds when compared with storage at 16^{o}C and 25^{o}C.

However, Fennessy, (2002) explained that normal (orthodox) seeds could store successfully for long durations at low temperature conditions and at low moisture content, Warren, (2001) explained further that recalcitrant seeds do not store because as the storage period increases, moisture content further reduces, respiration rate declines and oil seeds will

thus lose viability. He concluded that starchy seeds tend to last longer in storage than oily seeds. In this study, cold storage of *Plukenetia conophora* at 7^{o}C favoured the viability of the species till the 7th month, which is in agreement with Piriz *et al.*, (2003) who worked on preservation of germination capacity of *Araucaria angustifolia* using refrigerated storage at 0^{o}C, 4^{o}C and 10^{o}C and concluded that germination capacities were best at 0^{o}C for 12 months. This study is also in agreement with Pasquini *et al.*, (2011) who investigated different storage conditions in recalcitrant seeds of Holm oak (*Quercus ilex L)* and concluded that low temperature and drying tolerance storage under different temperature can increase viability. Storage in ambient temperature decreased the viability of *P. conophora*; as observed in seeds stored in room temperature (28^{o}C), that lost their viability in less than 20 weeks while those buried in dry soil in plastic containers (29^{o}C) also lost their viability at 10 weeks. However extremely low temperature does not favour the viability of this recalcitrant species as seeds kept in freezer condition - 5^{o}C, caked, and lost their viability at 10 weeks.

5.3 Germination consistency and early growth parameters in seeds of different size classes of *P. conophora*.

The formation of seeds completes the process of reproduction in seed plants. Seeds have been an important development in the reproduction and spread of flowering plants. This can be seen by the success of seed plants (both gymnosperms and angiosperms) in dominating biological niches on land. Seeds are very diverse in size. Plants that produce smaller seeds may have more seeds per flower, while those with larger seeds invest more resources into those seeds and normally produce fewer seeds, (Wikipedia, 2010).

Many annuals produce smaller seeds while most perennials and woody plants have larger seeds. Within a species there are also various sizes of seeds ranging from large, medium and small size classes; however it is believed that larger seeds have more energy reserves from germination and seedling growth and produce larger, more established seedlings after germination. In this study, large seeds of *P. conophora* had the highest germination of 52% at 4th week of sowing while medium and small seeds recorded 40% and 36% respectively which is in line with the assertion by Wikipedia, (2010) that larger seeds have more energy reserves for germination. Stewart (2007) also mentioned that there is a desire for larger seeds because of a perceived increase in vigor. Early growth parameters of *P. conophora* seedlings favoured the growth of seedlings from

large seeds. At 24 weeks, the length of liana was 54.73cm, 47.25cm and 46.5cm for large, medium and small seeds respectively. This is in agreement with studies by Bofil, (1998) who investigated the effect of seed size, cotyledon reserves, and herbivory on seedling survival and growth in *Quercus rugosa and Q.laurina (Fagaceae)*.Seed size significantly affected growth of the species, The author concluded that seedlings originating from large seeds can better endure loss of cotyledon and aerial biomass and thus are better equipped to confront stress in early growth. Zekimut *et al.,* (2010) in his own study, though on an annual plant, confirmed that selection of oat genotypes with larger seed in areas displaying moisture stress will help to reduce the risk of poor stand establishment and will enable more homogenous growth. This observation was contrary to the investigations carried out by Muharrem *et al.,* (2008) who studied the interaction between seed size, germination and early growth of some Turkish cultivars of Chickpea (*Cicer arietinum* L) and recorded that small seeds germinated and grew more rapidly compared with medium and large seeds of the same cultivars.

5.4 Effect of watering regimes on growth of *Plukenetia conophora*

Water is a significant factor in dry land forest nursery and it is critical to tree growth and development in the tropics. Water is required by plants for the manufacture of carbohydrates and as a means for transportation of foods and mineral elements. Various vital physiological processes in plants such as cell division, cell elongation, leaf enlargement and chlorophyll formation depend on plant water availability, (Oyun *et al.,* 2010). In *P. conophora,* the highest values for length of liana, collar diameter, number of leaves and leaf area were observed among seedlings with daily watering. There was a high significant difference in growth of seedlings watered daily compared to other regimes. The need for watering in growth of plants was observed by Oyun *et al.,* (2010) who recorded the best growth performance by watering *Acacia senegal* twice a week. Insufficient water in plants below a critical level is usually demonstrated by changes in all structures leading to the death of the plant, (Levy and Krikum, 1993) cited by Oyun *et al.,* (2010). This assertion was in accordance with the study on *P. conophora*, whereby the seedlings that were not watered at all (control) gradually reduced in length from the 6th week through the 10th week before totally wilting, indicating that *P. conophora* is not a xerophyte. On the other hand, the seedlings that were submerged in bowl of water also wilted by the 4th week, indicating that *P. conophora* is neither a hydrophyte. The excess of water above the plant

retarded physiological processes which gradually led to the death of the plant. Plants can make virtually everything they need from water and air with a few nutrients that the roots absorb from the soil. Water is truly vital for plant growth. This agrees with Gbadamosi, (2002) who noted that vegetative traits of *Enanthia chlorantha* increased with enhanced water supply. Also the study on *P. conophora* indicated that daily watering was most suitable for the species, which confirms it a tropical rain forest liana. This observation is also in line with Akinyele, (2007) who recorded the highest values for seedling height and collar diameter in daily watering of *Buchholzia coriacea*, a tropical rainforest species. Plants grow in two ways, cell division and cell expansion. Cell division creates more cells and cell expansion is the increase in cell size. Cells grow by taking up water, if water is reduced during growth, final cell size is reduced, and this means fewer, smaller leaves, smaller fruits, shorter, thinner stems and fewer roots. Drought stress results in smaller, weaker plants. As the soil dries, the volume of water moving to the stems decrease and it becomes harder to maintain growth.

5.5 Influence of height of decapitation on the coppicing potential of juvenile *P. conophora*.

The apex of the stem of a typical plant has an actively growing apical bud. It produces additional nodes and internodes to add to the length of the shoot. In some plants the lateral bud located in the axil of each leaf does not grow to form branches as a result of the control the active meristematic tissues at the shoot apex exert on them, this condition is known as apical dominance. It is caused by the apical bud producing IAA (auxin) in abundance. When the apical bud is removed, the source of IAA is removed, thus giving rise to the growth of lateral buds, (Wikipedia, 2010). To form an ortet base for production of ramets, there is need to have several juvenile branches developing from the plant, hence the need for decapitation of the plant so as to remove the dominant hormone (IAA) to allow shoot (branch) development, (Fletcher, 2002). The study on decapitation of *P. conophora*, had the best shoot development recorded in seedlings decapitated at 20cm. Collar diameter, number of coppices and number of leaves were significantly affected by height of decapitation. This is in line with the findings of several authors that the number of coppice shoots produced depends on the height of severance of the plant, (Magingo and Dick, 2001; Gbadamosi, 2002 and Akinyele, 2007). With a species like *P. conophora* in which the seeds form the bulk of consumption by man and rodents, the ability of its seedlings to produce flushes of juvenile coppice shoots is crucial to production of active ortets

for vegetative propagation. Higher heights contain more buds on the stem of a plant and this gives rise to more shoot production. Photosynthetic reinvigoration of leaves following shoot decapitation and accelerated growth of coppice shoot of *Populous deltoidses Bartr* and *Populus maxmowic zii*. Nigra were studied by Timothy *et al.*, (2006) to determine changes in photosynthesis and water relations of leaves left for re-growth. Result showed a manifold increase in net photosynthesis indicating a rapid substantial reinvigoration. This principle could also be applicable to *P. conophora.*

5.6 Organic fertilizer and early growth of *P. conophora*

There is need to augment soil fertility for growth of most tree species. Although forest soils are known to be rich in organic matter, decomposition is hindered by some conditions in the forest soil such as unavailability of water and soil micro organisms at certain times thus preventing these soils from supplying the necessary nutrient required by the tree on demand (Vanlauwe *et al.,* 2001). With the application of organic manure to the forest floor mobilization of soil nitrogen and phosphorus by soil micro organism is increased and there is a buildup of organic carbon if organic fertilizer is applied to the plants regularly, which provides a capital of nutrients that are slowly released. At the same time, the soil's buffering capacity for water, cation and acidity is increased, (Bolland *et al.,* 2001). Various plant species differ in their preference for fertilizer type and dosage. The study on fertilizer requirement of *P. conophora* indicated that fertilizer source and dosage were highly significant on the leaf area (<0.001PV) which are mainly the photosynthesis apparatus of plant for food production and subsequently growth. The highest values for growth were recorded in seedlings treated with compost; which could be due to the presence of high organic carbon (38.3g/kgdw), Potassium (29.2g/kgdw) and calcium (21.5g/kgdw) present in these sources more than in other sources (Table 4.25). This observation is in agreement with Fasina *et al.,* (2002) who inferred that application of different levels of poultry manure on *Telfairie occidentalis* (ugu) increased highly the vegetative growth of the plant. Fertilizer investigations on *P. conophora* were also in agreement with Fagbayide and Adekunle, (2002) on influence of poultry manure on early growth of passion fruit (*Passiflora edulis Var Flavicarpa*). They found a significant difference in growth over control on seedlings applied with different rates of poultry manure. *P. conophora* treated with poultry manure was second highest in the production of leaf area (89.93cm^2). This could be attributed to the high

Nitrogen content, 16.1g/kgdw for compost and 17.3g/kgdw for poultry manure. Nitrogen is an important constituent of chlorophyll, amino acid and nitric acid (Sani *et al.,* (2000).

5.7 Vegetative propagation of *P. conophora*

The use of vegetative propagation makes it possible to capture and transfer to the new tree all the genetic potential (additive and non- additive variation) from the ortet for certain characteristics. With low narrow-sense heritability, it has been demonstrated that genetic gains can be doubled by using vegetative propagation, (Hettasch *et al.,* 2009). Another valuable advantage is that in some situations, vegetative propagation can contribute to accelerating the realization of results from tree improvement activities, since the desired genetic qualities of selected trees can be utilized rapidly without having to wait for seed production, before producing propagules for operational planting. This is the case of *P. conophora*, a seasonal fruiting liana with recalcitrant seeds. The possibility of rooting double- node- stem cuttings of *P. conophora* is crucial to its multiplication and germplasm conservation strategy. Most plant species require hormone at varied concentrations to enhance the rooting potentials of their nodal cuttings. In this study, juvenile double node cuttings of *P. conophora* were treated with varied concentrations of indole 3- butyric acid (IBA), Naphthalene acetic acid (NAA) and a combination of IBA+ NAA at 100ppm, 150ppm and 200ppm according to the standard used in Forestry Research Institute of Nigeria Ibadan (FRIN). Two leaves halved in size were left on the cuttings for photosynthesis and to produce rhizocaline, an unidentified rooting co-factor which enhances rooting (Herman *et al.,* 1991). These treatments produced a negative result contrary to studies by Gbadamosi, (2002) and Akinyele, (2007) who worked on vegetative propagation of *Enantia chlorantha* and *Buchholzia coriacea* (Engler) respectively using juvenile stem cuttings.

However, cuttings of *P. conophora* produced successful results using powdery form of IBA, NAA and IBA+ NAA at a rate of 8g kg-1 applied to the base of the cutting, a standard used by the Centre for Scientific Research (CSIR) South Africa. These findings were in agreement with Hettasch *et. al.,* (2009) who applied IBA hormone in powdery form at 8g kg-1 on *Pinus elliottii* and *Pinus caribaea* cuttings, and recorded 90% rooting with an increase in quantity and quality of roots produced. Using sand or sawdust as a rooting medium did not show any significant difference. However form and type of hormone had a significant difference from the control (no hormone) indicating that *P. conophora* is not an "easy rooter". The possibility of rooting this

species may solve the problem of non – availability of seeds at the off season periods and the effect of consumption by man and rodents. Cuttings have been widely used in clonal propagation of forest trees, where large number of planting stocks are required and recently in local fruit trees like *Irvingia gabonensis, Ricinodendron heudelotii* and *Dacryoides edulis*. Also in medicinal plants like *Prunus africana* and *Pausinystallia johimbe* as recorded by various authors including Mialoundama *et al.,* (2002), Tchoundjeu *et al.,* (2002) and Ngo Mpeck *et al.,* (2003).

5.8 Micro propagation of *P. conophora*

Micro propagation facilitates rapid multiplication and conservation of plant genetic resources, (Hettasch *et al.,* 2009). However tree species have different biochemical nature and variations in their physiological and growth responses to different media. The single node cuttings treated with sodium hypochlorite recorded a high load of contamination in most of the media tested in the study. But for those treated with 0.05% mercuric chloride, there was no contamination, but neither shoots nor roots developed in all the media used except two that formed callus. This was contrary to the reports of Gbadamosi, (2002) who observed shoot and root growth in culture of single node cuttings of *Enantia chlorantha*; and Akinyele, (2007) who also recorded shoot and root initiation in in-vitro culture of single node cuttings of *Buchholzia coriacea*. The poor root and shoot initiation in *P. conophora* could be due to heavy load of extractives in the tissues, which is supported by the findings of Rao, (1988) who stressed that most shoots of tropical trees may not root at all as a result of their tissues having heavy deposits of tannin and phenolic compounds. Micro propagation of woody plants and propagation by tissue culture is indeed applicable to the "difficult to propagate" species, and equally as important. It may offer economic advantages for some species which are considered relatively "easy to propagate".

There are a variety of techniques which can be used to get plant development in-vitro (That is by techniques such as tissue culture). These techniques are dependent upon the species in question. Single cells of leaf tissue can regenerate whole plants as can shoot tips, lateral buds or stem section. Shoot tip culture is the method in widest use for mass propagation of woody species, (Obembe, 2000). An actively growing shoot tip is surface sterilized and placed on a defined culture medium under sterile conditions. This culture medium contains inorganic and organic salts (macronutrients micronutrients and vitamins) as well as an energy source (Sucrose or table sugar) growth regulators and agar to gel the medium. The rapid proliferation of shoots results in

masses of shoots being produced from a single shoot tip. Up to a hundred shoots may be produced in as little as eight to twelve weeks from a single tip. The number of shoots produced and the rapidity of shoot proliferation vary between species. Rathore *et al.,* (2005) applied micro propagation protocols to cloning of *Balanites aegyptiaca* , the nodal explants produced multiple shoots in-vitro by activation of axillary meristems on MS medium +0.45um BAP.

However the embryo culture of *P. conophora* was successful. Explants disinfected with 0.05 percent mercuric chloride produced shoots and roots from the fifth day, while those disinfected with 10% sodium hypo chloride produced shoot and root on the seventh day from five different media concentrations. This is in agreement with the findings of Fan and Grossnickle, (1999) who successfully raised *Picea spp* from embryo culture. With the success obtained in this study, capacity for shoot and root morphogenesis of *P. conophora* through embryo culture could be established. Cloned plants could be generated for conservation purpose.

CHAPTER SIX

6.0. Summary and Conclusion

6.1. Summary of results

Investigations on the improvement and conservation of *Plukenetia conophora* (MULL ARG) was carried out using seeds from Ibadan (Oyo State), Ijebu Ode (Ogun State), Akure (Ondo State) and Igbajo (Osun State).

Phenological observations indicated that *P. conophora* undergoes active vegetative growth from September to early November, flowers between November and December while fruits set in from February through April.

Morphological variations were observed in fruits and seeds from the four sources. Ibadan had the highest mean fruit weight of 96.64g; Ijebu Ode had 81.46g while Akure and Igbajo had 65.34g and 62.38g respectively. With respect to mean length of liana for 24 weeks, Ibadan had the highest value of 224.8cm; Ijebu Ode recorded 199.5cm, Igbajo and Akure had 188.3cm and 182.4cm respectively. Seed sources had significant effect on mean collar diameter at $P<0.05$. Ibadan recorded the highest with 1.82cm while Igbajo had the least 1.28cm. It was observed that collar diameter of *P. conophora* reduces with increase in age in the early growth.

Total biomass at the end of the experimental period of 24 weeks (6 months) showed that Ibadan had the highest biomass accumulation of 45.77g, followed by Igbajo with 48.61g while Ijebu Ode and Akure had 37.64g and 33.55g respectively. Ibadan source had the best performance in early growth and Akure had the least.

There was a significant difference between viability of fresh seeds and those stored in different media and different temperature levels viz: Refrigerator (7°C), Freezer (-5°C), Plastic containers filled with dry soil (29°C) and room temperature (28°C). The best storage medium was Refrigerator (7°C) which retained 40% viability at the 30th week of storage and the poorest was storing at 29°C in dry soil, which retained only 15% viability at the 10th week of storage.

Seeds of *P. conophora* differ in weight and size. Large seeds had a mean weight of 13.20±0.02g; medium seeds recorded a mean weight of 10.36± 0.02g while small seeds had 7.25± 0.02g.

The small size seeds had the highest mean number of leaves (11.6), while the medium and large seeds recorded 10.92 and 10.73 respectively. This was as a result of long internodes observed in

seedlings from large seed class. However, there was no significant difference in seedling biomass accumulation (leave, stem and root dry weights)

Watering regimes had significant effect on early growth of *P. conophora.* Seedlings watered daily had the highest mean liana length of 140.8cm, diameter growth of 0.79± 0.02cm, leaf area, (95cm^2),and total dry weight (TDW) of 26.84g. This was followed by four days (4 days) watering with 126cm length, 0.72± 0.02 diameter and TDW of 19.42g while seven days watering interval recorded 42.1cm length, 0.60± 0.02m diameter and 14.12g TDW. The seedlings submerged in water and the control (not watered at all) could not survive the conditions, they wilted after the 8th week.

Height of decapitation had significant effect on coppicing of the species. The longest coppice of 202cm was obtained from seedlings decapitated at 20cm above collar. The highest coppice for seedlings decapitated at 15cm was 36cm while the maximum length among seedlings decapitated at 10cm was 9.9cm.Diameter of sprout was affected significantly by height of decapitation. Seedlings decapitated at 20cm, 15cm and 10cm above the collar had the following mean collar diameter 0.77± 0.02cm, 0.16± 0.02cm, and 0.05± 0.02cm respectively. Also, number of coppices and leaves were significantly affected by decapitation. Seedling decapitated at 20cm recorded six coppices shoots, 15cm decapitation, produced five, while 10cm decapitation had only three. The control (not decapitated) had only one (1) coppice shoot. Number of leaves for each height of decapitation differed significantly. The highest mean number of leaves (12) was recorded in seedlings decapitated at 20cm height, while those for 15cm and 10cm heights had 9.75 and 5 respectively.

The highest mean length of liana, 242.5cm was recorded among seedlings treated with compost at 40g/2kg soil, while the least length, 164cm was obtained in the control (No fertilizer). Collar diameter growth differed significantly under different fertilizer sources. The highest was 0.98cm obtained from compost manure at 30g/2kg soil. Poultry waste had 0.84cm from seedling treated with 20g/2kg soil, while seedlings treated with cowdung manure had 0.80cm from 40g/2kg soil. The control had the least collar diameter of 0.63cm.

Vegetative propagation of *P. conophora* using stem cutting was successful, percentage survived and rooted plants varied significantly among the type and state of hormone and rooting media at $P<0.05$. The highest survival percentage 100% (80% rooted and 20% callused cuttings) was

obtained using NAA + IBA in powder form at a rate of 8g/kg^{-1} applied to the base of cuttings using sawdust as a rooting medium. The highest mean number of roots 14 per cutting was produced by NAA+IBA using sawdust. Cuttings treated with IBA alone using river sand recorded 8 roots,while the longest mean taproot length of 3 was also recorded in IBA using sand. The least mean number of roots was 3 which was obtained from NAA using sawdust. The control (no hormone) did not root at all, indicating that *P. conophora* is not an"Easy rooter".

Micro propagation of *Plukenetia conophora* by means of tissue culture using embryo and nodal cuttings as explants was investigated. This was to provide baseline information on requirements for the species tissue survival in-vitro. The best sterilization procedure was the stepwise treatment with 70% ethanol for 20 minutes and 0.05% mercuric chloride for 10 minutes. Shoot and root development were observed on the fifth day from explants disinfected with 0.05% mercuric chloride while those treated with 10% sodium hypochlorite started sprouting on the seventh day. In the embryo culture roots and shoots were obtained from the following five media: B (MS only), F (MS + 0.1mg kinetine + 0.3mg 24D), J (MS + 0.3mg kinetine + 0.1mg 24D) N (MS + 0.5mg BAP only). Q (MS + 0.1mg BAP + 0.5mg NAA).The highest mean number of plantlets (9) was recorded in medium B (MS only). The highest number of leaves (9) was recorded in the same medium (B). The highest average number of root (9) was recorded in medium (F) The nodal culture explants did not develop shoots and roots in all the media used, but had callus formation. The findings of this investigation have provided baseline information upon which future in-vitro studies on *Plukenetia conophora* can be based. Despite the inherent accumulation of phenolics which constitute the problem facing micro propagation of oily seeds like *P. conophora*, as suggested by Obembe (2000), appropriate explants and the suitable package of medium requirement for their survival and root induction has been successfully established.

6.2 Conclusion

This study comprises an aspect of improvement and conservation of *P. conophora*. There were variations in fruits and early growth of seedlings from different provenances which formed a vital aspect of selection and improvement strategies of plant species. Thus seeds from Ibadan that proved best could be used for plantation establishment. Seeds of *P. conophora* are recalcitrant, but the study has shown that with storing at 7°C in a refrigerator, seeds could be viable for 30 weeks and held in this storage until the sowing period. Large seeds gave the highest germination percentage and can therefore be used to produce vigorous and homogeneous seedlings.

The availability of water is critical to plant growth as vital physiological processes depend on it. Thus, the study on effect of watering regimes on growth of *P. conophora* shows that adequate water is required for its development. The plant is neither a hydrophyte nor a xerophyte, it is therefore a mesophyte. Hence it cannot perform well in waterlogged and desert environments. The study on organic fertilizer shows that compost which is readily available and affordable could be used to increase growth of the species.

On macro-propagation studies, it could be concluded that for a good ortet base, decapitating the liana at 20cm height above collar is ideal, as this will produce more juvenile cuttings for vegetative propagation. The species could therefore be conserved and rapidly multiplied using macro vegetative propagation to avert the problems of non availability of seed and extinction threat. This study has provided baseline information for further studies on in-vitro and maro propagation of *P. conophora.*

6.3 Recommendation

From this study, it could be suggested that;

1. *Plukenetia conophora* from Ibadan could be used for plantation establishment.
2. Storing the seeds of *Plukenetia conophora* in a refrigerator at 7^{o}C could sustain the viability for 30 weeks until the sowing period.
3. Planting out of *Plukenetia conophora* seedlings on the field should be done at the peak of the raining season (May to August) to enable it establish before the dry season sets in by October.

4. Organic fertilizer which is cheaper and affordable than chemical fertilizer could be used to enhance the growth of the seedlings on the field.
5. To have a good ortet base for juvenile cuttings, seedlings should be decapitated at 20cm above collar
6. Vegetative propagatiom by double node cuttings and the use of embryo in-vitro culture could serve as an alternative planting stock to propagation by seeds since the seeds of *P conophora* recalcitrant and seasonal

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APPENDICES

Appendix 1: Analysis of Variance for Effect of Fruit Source on Fruit Weight of *Plukenetia conophora*

Source	Degree of freedom	Sum of square	Mean square	F	P-value
Sources	3	30171.084	10057.028	24.020	.000*
Fruit category	3	61352.056	20450.685	170.863	.000*
Sources x Fruits	9	9822.675	1091.408	9.118	.000*
Error	144	17236.481	119.698		
Total	160	1053938.733			
Corrected Total	159	118582.296			

***Significant at P< 0.05**

Appendix 2: Effect of number of seeds on Fruit Weight of *Plukenetia conophora*

No of seeds Per fruit	N	Subset 1	2	3	4
Fruits with 4 seeds	40				104.0608[a]
Fruits with 3 seeds	40			83.9378[b]	
Fruits with 2 seeds	40		66.0138[c]		
Fruit with 1 seed	40	51.8235[d]			
Sig		1.000	1.000	1.000	1.000

LSD *Significant at P< 0.05

Appendix 3: Effect of Source on Fruit Weight of *Plukenetia conophora*

		Subset		
Fruits sources	N	1	2	3
Ibadan (Oyo)	40			96.6459[a*]
Ijebu-Ode (Ogun)	40		81.4649[b*]	
Igbajo (Osun)	40	62.3845[c]		
Akure (Ondo)	40	65.3407[c]		
		229	1.000	1.000

LSD *Significant at P< 0.05

Means with the same letters are not significantly difference.

Appendix 4: Effect of Source on Early Growth of *Plukenetia conophora*

Source of variation		Degree of freedom	Sum of square	Mean square	F	P-level
	Sources	3	0.44981	0.14994	3.09	0.037*
COLLAR	Error	44	2.13514	0.04853		
DIAMTER	Total	47	2.58495			
(CM)						
LENGTH	Source Error	3	2026	675	0.28	0.842
OF LIANA	Total	44	107583	2445		
		47	109608			
	Source Error	3	33.09	11.03	0.99	0.405
NO OF	Total	44	488.37	11.10		
LEAVES		47	521.46			

***Significant at P< 0.05**

Appendix 5: Mean biomass assessment and leaf area of seedlings of *P. conophora* as affected by seed source at 4, 20 and 24 weeks after germination

Source	Leaf dry weight (g)	Stem dry weight (g)	Root dry weight (g)	Total biomass (g)	Leaf area (cm^2)
4 weeks					
Ibadan	0.75	0.50	6.59	7.81	88.31
Ijebu-Ode	0.70	0.49	6.23	7.46	85.43
Akure	0.69	0.50	5.60	6.78	83.24
Igbajo	0.71	0.50	6.06	7.27	86.53
20 weeks					
Ibadan	3.93	6.29	29.75	39.97	90.11
Ijebu-Ode	3.32	5.10	28.24	36.66	89.27
Akure	3.13	5.97	24.88	33.98	87.15
Igbajo	3.88	6.17	27.19	37.24	88.0
24 weeks					
Ibadan	3.17	6.21	36.39	45.77	90.01
Ijebu-Ode	2.12	5.16	30.36	37.64	86.51
Akure	2.09	5.11	26.35	33.55	85.04
Igbajo	3.11	6.14	29.36	38.61	86.21

Appendix 6: Analysis of Variance for Effect of Seed Source on Biomass and Leaf Area

Source of variation		Degree of freedom	Sum of square	Mean square	F	P-level
Leaf.Dry Weight	Source	3	0.579	0.193	0.14	0.933 Ns
	Error	20	25.983	1.349		
	Total	23	27.562			
Stem.Dry Weight	Source Error	3	1.069	0.356	0.06	0.980 Ns
	Total	20	116.906	5.845		
		23	117.975			
Root Dry Weight	Source Error	3	60.1	20.0	0.16	0.922 Ns
	Total	20	2517.0	125.8		
		23	2577.1			
Leaf Area	Source Error	3	87.69	29.23	2.09	0.133 Ns
	Total	20	279.51	13.98		
		23	367.20			

Ns = Not sig.

Appendix 7: Analysis of Variance for Effect of Storage Temperature on Number of seeds that Changed to Red Colour

Source of variance	Degree of freedom	Sum of square	Mean square	F	F.pr
Temp	3	1407.30	469.10	14.71	<0.001*
Residual	60	1909.31	31.82		
Total	63	3316.61			

***Significant at P< 0.001**

Appendix 8: Analysis of Variance for the Effect of Storage Temperature on Seed Viability Of *P.conophora*

Source of variance	Degree of freedom	Sum of square	Mean square	F	F.pr
Temp	3	35406.4	11802.1	14.62	<.001*
Residual	59	47619.4	807.1		
Total	62	82915.1			

***Significant at P< 0.001**

Appendix 9: Table of means for the Effect of Storage Temperature On viability of *Plukenetia conophora*

Treatment (Temp)	Parameter Changed red	Measured Unchanged	% Viable
5^0C	4.19	10.81	20.9
7^0C	15.00	6.25	75.0
28^0C	4.94	8.07	24.0
29^0C	3.56	12.69	17.8
LSD	3.989	NS	20.10

Appendix 10: Early Growth Performance in Seeds of Different size Classes of *Plukenetia conophora*

WK 2			WK 4			WK 6			WK 8			WK 10			WK 12			WK 14			WK 16			WK 18			WK 20			WK 22			WK 24		
Length	Stem dia	Leaf count	Length	Stem dia	Leaf count	Length	Stem dia	Leaf count	Length	Stem dia	Leaf count	Length	Stem dia	Leaf count	Length	Stem dia	Leaf count	Length	Stem dia	Leaf count	Length	Stem dia	Leaf count	Length	Stem dia	Leaf count	Length	Stem dia	Leaf count	length	Stem dia	Leaf count	Length	Stem dia	Leaf count
																		LARGE																	
19.45	1.05	3.92	50.33	1.01	7.00	85.78	1.14	9.90	97.04	1.22	10.75	97.42	1.23	10.45	86.78	1.21	11.95	77.58	1.23	13.6	64.51	0.64	9.58	59.57	1.11	8.05	54.78	1.13	8.0	53.59	1.01	10.00	54.73	1.08	10.73
															MEDIUM																				
18.8	0.9	4.2	45.5	0.97	7.4	62.9	0.95	9.53	77.54	1.02	8.61	82.27	1.04	9.83	84.59	1.06	10.56	74.64	1.07	11.24	59.21	0.94	9.33	52.19	0.78	7.56	46.25	0.52	7.13	44.29	0.99	8.77	47.25	0.71	10.92
																		SMALL																	
17.93	0.76	4.2	33.5	0.77	8.0	69.0	0.91	10	87.0	17.0	10.6	94.8	5.9	17.0	95.6	1.10	11.2	76.1	0.92	12.9	64.8	0.71	11.9	50.8	0.76	9.8	49.2	0.43	8.9	51.3	0.46	9.9	46.5	0.55	11.6

Appendix 11: Effect of seed size on biomass of *Plukenetia conophora*

4^{th} Week	Leaf dry weight (g)	Stem dry weight (g)	Root dry weight (g)	Total dry weight (g)	Leaf area (cm^2)
Large	0.70	0.42	7.12	8.24	82.57
Medium	0.64	0.41	6.85	7.90	80.22
Small	0.69	0.40	6.60	7.69	79.59
24^{th} Week					
Large	0.72	0.52	31.66	32.90	89.64
Medium	0.69	0.51	28.60	29.80	86.52
Small	0.69	0.51	27.90	29.10	86.41

Appendix 12: Analysis of Variance for the Effect of watering regime on the Early Growth of *Plukenetia conophora*

Source of variation		Degree of freedom	Sum of square	Mean square	F	P-level
Variable Length of liana	Treatment	4	55159.9	13790.0	25.15	<0.001**
	Residual	42	23024.5	548.2		
	Total	46	62040.6			
Collar diameter	Treatment	4	0.51641	0.12910	8.94	<0.001**
	Error	41	0.59229	0.01445		
	Total	45	0.88480			
No of leaves	Treatment	4	293.501	73.375	10.35	<0.001**
	Error	41	290.738			
	Total	45	531.124	7.091		
Total Biomass	Treatment	3	47.7	15.9	0.08	0.969ns
	Error	20	3857.7	192.9		
	Total	23	3905.4			

***Significant at P< 0.001**

Appendix 13: Analysis of Variance for Influence of Height of Decapitation on Mean Diameter of Coppice

Source of variation	Degree of freedom	Sum of square	Mean square	F	F.pr
Height	2	0.27477	0.13738	7.32	0.003*
Residual	27	0.50645	0.01876		
Total	29	0.78121			

***Significant at P< 0.01**

Appendix 14: Analysis of Variance for Effect of Height of Decapitation on Number of Coppices

Source of variation	Degree of freedom	Sum of square	Mean square	F	F.pr
Height	2	19.467	9.733	6.04	0.007*
Residual	27	43.500	1.611		
Total	29	62.967			

***Significant at P< 0.01**

Appendix 15: Analysis of Variance for the Effect of Organic Fertilizer Source and Dosage on length of Liana

Source of variation	Degree of freedom	Sum of square	Mean square	F	F.pr
Fertilizer source	3	16031	5344	1.30	0.277 Ns
Rate	3	236	79	0.02	1.996 Ns
Fert. Source. Rate	9	1165	129	0.03	1.000 Ns
Error	176	725110	4120		
Total	191	742542			

Ns = Not significant

Appendix 16: Analysis of Variance for the Effect of Organic Manure on Collar Diameter

Source of variation	Degree of freedom	Sum of square	Mean square	F	F.pr
Fertilizer source	3	0.35053	0.11684	3.80	0.011*
Rate	3	0.00022	0.00007	0.00	1.000ns
Fert. Source. Rate	9	0.00921	0.00102	0.03	1.000ns
Error	176	5.41573	0.03077		
Total	191	5.77569			

***Significant at P< 0.05**

Appendix 17: Analysis of Variance for the Effect of Organic Manure on number of leaf

Source of variation	Degree of freedom	Sum of square	Mean square	F	F.pr
Fertilizer source	3	487.1	162.4	0.20	0.894 Ns
Rate	3	181.2	60.4	0.08	0.973 Ns
Fert. Source. Rate	9	148.4	16.5	0.02	1.000 Ns
Error	176	141001.7	801.1		
Total	191	141818.4			

Ns = Not significant

Appendix 18: Analysis of Variance for the Effect of Organic Fertilizer on Leaf Dry Weight

Source of variation	Degree of freedom	Sum of square	Mean square	F	F.pr
Fertilizer source	3	51452	17.151	2.15	0.101 Ns
Rate	3	3.589	1.196	0.15	0.929 Ns
Fert. Source. Rate	9	1.861	0.207	0.03	1.00 Ns
Error	80	638.539	7.982		
Total	95	695.440			

Ns = Not significant

Appendix 19: Analysis of Variance for the Effect of Organic Fertilizer on Stem Dry Weight of *P. conophora*

Source of variation	Degree of freedom	Sum of square	Mean square	F	F.pr
Fertilizer source	3	157.07	52.36	2.55	0.061 Ns
Rate	3	11.84	3.95	0.19	0.901 Ns
Fert. Source. Rate	9	18.87	2.10	0.10	1.000 Ns
Error	80	1639.30	20.49		
Total	95	1827.08			

Ns = Not significant

Appendix 20: Analysis of Variance for the Effect of Organic Fertilizer on Root Dry Weight

Source of variation	Degree of freedom	Sum of square	Mean square	F	F.pr
Fertilizer source	3	561.8	187.3	0.67	0.572 Ns
Rate	3	542.7	180.9	0.65	0.586 Ns
Fert. Source. Rate	9	870.2	96.7	0.35	0.956 Ns
Error	80	22310.8	278.9		
Total	95	24285.5			

Ns = Not significant

Appendix 21: Analysis of Variance for the Effect of Organic Fertilizer on Total Dry Weight

Source.of variation	Degree of freedom	Sum of square	Mean square	F	F.pr
Fertilizer source	3	3271.8	1090.6	2.50	0.066 Ns
Rate	3	165.8	55.3	0.13	0.944 Ns
Fert. Source. Rate	9	84.5	9.4	0.02	1.000 Ns
Error	80	34956.9	437.0		
Total	95	38479.0			

Ns =Not significant

Appendix 22: Analysis of Variance for the Effect of Organic Fertilizer on Leaf Area

Source of variation	Degree of freedom	Sum of square	Mean square	F	F.pr
Fertilizer source	3	2470.36	823.45	9.51	<.001**
Rate	3	3259.62	1086.54	12.55	<.001**
Fert. Source. Rate	9	219.38	24.38	0.28	0.978
Error	80	6926.73	86.58		
Total	95	12876.09			

***Significant at P<. 001**

Appendix 23: Analysis of Variance for Effect of Hormone and Rooting Media on Number of Root per cutting

Source	Degree of freedom	Sum of square	Mean square	F	p-value
Hormone	2	5759.271	3379.636	191.904	000**
Media	1	8.000	8.000	.454	.501 Ns
Hormone + media	2	38.560	19.280	1.095	.336 Ns
Error	444	7819.333	17.611		
Total	450	44654.000			

***significant at P<0.05**

Ns = Not significant

Appendix 24: Table of means and LSD for number of root per cutting

		Subject		
Powder hormone	N	1	2	3
NAA	150	3.4267[c]*	8.1606[b]*	
IBA	150			12.9200[a]*
NAA + IBA	150			
Sig		1.000	1.000	1.000

LSD* Significant at P<0.05

NOTE: Means with the same letters under each column are not significantly different from each other.

Appendix 25: Analysis of Variance for the effect of hormone and rooting media on Length of taproot of *P. conophora*

Source	Degree of freedom	Sum of square	Mean square	F	p-value
Hormone	2	19.543	9.771	2.425	0.090 Ns
Media	1	12.235	12.235	3.037	0. 082 Ns
Hormone +media	2	1.873	.937	.232	.793 Ns
Error	444	1788.911	4.029		
Total	450	5254.900			
Corrected Total	449	1822.563			

Ns = Not significant

Appendix 26: Embryo Culture of *Plukenetia conophora* (Walnut) Mean of 5 Replicates

0.05% Mercuric Chloride

Media	Mean No of plantlets	Mean No of Shoot per Plantlet	Mean No of Leaf	Node	Average No of Root	Callus	Contamination
B	9	1	9	3	7	1	-
F	7	1	8	3	9	3	-
J	4	1	6	2	5	1	-
N	-	-	-	-	-	5	-
Q	2	1	8	3	6	6	-
10% Sodium Hypochlorite							
B	2	1	5	2	2	3	4
F	-	1	4	2	4	4	3
J	-	-	-	-	-	2	4
N	-	-	-	-		4	4
Q	1	1	2	2	2	2	3

Key

B = MS Only

F = MS + 0.1mg kinetine + 0.3mg 24D

J = MS + 0.3mg kinetine + 0.1mg 24D

N = MS + 0.5mg BAP only

Q = MS + 0.1mg BAP + 0.5mg NAA

MS – Murashige and Skoog

24D – 2, 4- dichlorophenoxyacetic acid

BAP – Benzeylaminopurine

NAA – Naphthalene Acetic Acid

Appendix 27: Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1811.256[a]	17	106.544	21.500	.000
Intercept	4066.944	1	4066.944	820.684	.000
setnos	.000	0			
surviving	.000	0			
Code	1092.711	4	273.178	55.126	.000
setnos * surviving	.000	0			
setnos * code	.000	0			
surviving * code	.000	0			
setnos *surviving * code	.000	0			
Error	356.800	72	4.956		
Total	6235.000	90			
Corrected Total	2168.056	89			

a. R Squared = .835 (Adjusted R Squared = .797)

Appendix 28: Percentage survival and mortality of cuttings

	% survival & mortality	% survival & mortality	Mean Diff.	Std. Error	Sig.
LSD	S NO SAND	S NO SAWDUST	3.8667*	.81286	.000
		S C SAND	-.2000	.81286	.806
		S C SAWDUST	3.7333*	.81286	.000
		M SAND	-1.9333*	.81286	.020
		M SAWDUST	5.8000*	.81286	.000
	S NO SAWDUST	S NO SAND	-3.8667*	.81286	.000
		S C SAND	-4.0667*	.81286	.000
		S C SAWDUST	-.1333	.81286	.870
		M SAND	-5.8000*	.81286	.000
		M SAWDUST	1.9333*	.81286	.020
	S C SAND	S NO SAND	.2000	.81286	.806
		S NO SAWDUST	4.0667*	.81286	.000
		S C SAWDUST	3.9333*	.81286	.000
		M SAND	-1.7333*	.81286	.036
		M SAWDUST	6.0000*	.81286	.000
	S C SAWDUST	S NO SAND	-3.7333*	.81286	.000
		S NO SAWDUST	.1333	.81286	.870
		S C SAND	-3.9333*	.81286	.000
		M SAND	-5.6667*	.81286	.000
		M SAWDUST	2.0667*	.81286	.013
	M SAND	S NO SAND	1.9333*	.81286	.020
		S NO SAWDUST	5.8000*	.81286	.000
		S C SAND	1.7333*	.81286	.036
		S C SAWDUST	5.6667*	.81286	.000
		M SAWDUST	7.7333*	.81286	.000
	M SAWDUST	S NO SAND	-5.8000*	.81286	.000
		S NO SAWDUST	-1.9333*	.81286	.020
		S C SAND	-6.0000*	.81286	.000
		S C SAWDUST	-2.0667*	.81286	.013
		M SAND	-7.7333*	.81286	.000

*. The mean difference is significant at the .05 level.