PHYSICOCHEMICAL PROPERTIES AND JUVENILE PHENOLOGY OF AFRICAN WALNUT (*Plukenetia conophorum Muell Arg*) ACCESSIONS FROM SOUTHEASTERN NIGERIA

BY

UDEONYIA, OLIVER ANIEDO
PG/M.Sc/06/40837

DEPARTMENT OF CROP SCIENCE
UNIVERSITY OF NIGERIA, NSUKKA

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B. Agric (Hons), Nigeria

A DISSERTATION SUBMITTED TO THE DEPARTMENT OF CROP SCIENCE UNIVERSITY OF NIGERIA, NSUKKA, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN CROP SCIENCE (TROPICAL FRUITS AND PLANTATION CROPS)

FEBRUARY, 201

DEDICATION

This work is dedicated to Our Lord, Jesus Christ and Udeonyia family

CERTIFICATION

Udeonyia, Oliver Aniedo, a postgraduate student in the Department of Crop Science with Registration Number PG/ M.Sc/06/40837, has satisfactorily completed the requirements for research work for the degree of Master of Science in Crop Science (Tropical Fruits and Plantation Crops).

The Work embodied in this dissertation is original and has not been submitted in part or in full for any other diploma or degree of this or any other University.

_____	_____
Professor K.P. Baiyeri	Professor K.P. Baiyeri
Supervisor	Head of Department

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ABSTRACT

African walnut (*Plukenetia conophorum Muell* Arg) is a member of the family Euphorbiaceae. It is cultivated principally for the nuts which are eaten raw or served as snacks after roasting or boiling. *P. conophorum* serves many nutritional and medicinal purposes as well as good source of rural income. Despite the potentials of this plant, its existence is threatened by deforestation, urbanization and similar activities. The present study was designed in an attempt to salvage this useful plant from extinction and provide basis for its conservation. Four accessions of *P. Conophorum* were collected from Abia, Anambra, Enugu and Rivers states, southeastern Nigeria. The objectives of the research were to evaluate the effects of three manure rates on juvenile growth stage of the accessions; determine the amino acid profile of the kernels and investigate possible bio-diversity among the accessions with respect to the seed physical traits and proximate components. Four specific experiments were conducted to achieve the set objectives. Seed physical traits (edible portion, pulp weight, seed volume, seed weight, pulp (kernel) weight, seed circumference and seed coat thickness) were measured. Standard laboratory procedures were employed in determining the proximate composition (moisture content, ash, fat, crude protein, fibre and carbohydrates) and amino acids profile of raw and boiled kernels. A pot experiment was set up to evaluate the seedling emergence and growth responses to three level of pig manure applications (0, 5. and 10t/ha). Data were collected on days to seedling emergence, emergence percentage, vine length, vine base girth, number of leaves per plant, number of branches, root volume and dry matter yield and partitioning to the leaves, stem and roots. All the data were subjected to Analyses of Variance (ANOVA) following the procedures outlined for completely randomized design (CRD). Significant treatment means were separated with the aid of F-LSD at 5% probability level. All statistical analysis were done using Genstat 7.1 version 2003 software. Results indicated that all the seed physical traits showed significant ($P < 0.05$) variation across the four locations (states). Seeds from Enugu (74.08%) had the highest edible proportion followed by Abia (70.68%) and Rivers (66.65%). Anambra accession had the highest seed and pulp weight followed by Abia, Enugu and Rivers, respectively. Seed coat thickness was higher in accessions from Abia and Rivers States compared to others. The nutritional quality assessment revealed that ash, fat and moisture varied significantly ($P<0.05$) among the different accessions while carbohydrates, fibre and protein contents of the seeds did not differ across the locations. The moisture and fibre contents showed significant ($P< 0.05$) differences in the boiled and fresh seeds. Fibre content was higher in the boiled seeds, whereas the fresh seeds had higher moisture content.

The ash, carbohydrate, fat and protein contents were not influenced by processing. All the proximate contents of the seeds across locations did not differ in their response to interaction of location and processing. Location showed no significant effect on iso-leucine, leucine, phenylamine and tryptophane contents of the seeds. Seeds collected from Rivers, Enugu and Anambra States gave significantly ($P< 0.05$) higher histidine than those from Abia. Abia accession has the highest lysine content compared with those from other locations. Enugu and Rivers accessions gave significantly ($P< 0.05$) higher methionine than those from Anambra and Abia State. Enugu and Abia accessions recorded higher threonine content than Anambra and Rivers accession. The effect of processing on essential amino acids showed that the histidine, isoleucine, leucine, lysine, methionine, phenylamine, valine and threonine contents of the seeds were not influenced by processing. Except for tryptophane, all the other essential amino acids were found to be higher in the fresh seeds. The effect of location on non-essential amino acids showed that alanine, arginine, asparagine, glutamine and glutamic acid were significantly influenced by location while aspartic acid, glycine, proline, serine, trimethlisine and tyrosine did not vary among the locations. Seeds collected from Abia State gave significantly higher alanine and arginine contents than those from other locations. Asparagine content was significantly ($P < 0.05$) higher in seeds from Rivers and Abia than those from Anambra and Enugu. Glutamine acid content of seeds from Enugu and Anambra was higher than those from Abia and Rivers. The effect of processing on the non-essential amino acid components showed that glutamine, glutamic acid and trimethlisine contents of the seeds varied significantly ($P <0.05$) with processing. Glutamic acid and trimethlisine contents were higher in the fresh seeds whereas glutamine was higher in the boiled seeds. Alanine, arginine, asparagine, aspartic acid, glycine, proline, serine and tyrosine did not differ in their response to processing. Field growth performance evaluation indicated that plant height, stem girth, number of branches and vine length were all significantly ($P < 0.05$) improved with increasing manure rate. The 10 tonnes manure rate partitioned the greatest portion of the biomass to the shoot in all the accessions.

INTRODUCTION

African Walnut (*Plukenetia conophorum* Muell Arg) is a member of the family Euphorbiaceae. It has been described as a semi-wild plant found naturally in the wild (Okigbo, 1977), or may be extensively encountered in rural dwelling and in farmlands where they are protected. Walnut (*P. conophorum)* is of African origin (Nwosu, 1979); hence ñAfricanò mostly attached to its common name. It is cultivated principally for the fruits (nuts) which is edible and are eaten alone or served as snacks with kola nut when boiled. Egharevba *et al.,* 2005 also reported that the fruit is known in other African countries like Gabon, Sierra Leone, Cameroon, Madagascar and Central African Republic, where it provides income to the rural people consequently improving their economy and nutrition.

P. conophorum is a twining vine, and rarely sprawling herb, found in tropical wet and seasonally dry forest regions (Gillespie, 1993). The seeds are available in June-September when other fruits are scarce, throughout the southern states of Nigeria (Egharevba *et al*., 2005). As documented by Irvine (1990), the plant which is a perennial is also a climber requiring support of woody sticks to climb, grow and survive. The plant starts flowering between eighteen to twenty four months after planting. The importance of *P. conophorum* as an indigenous fruit climber is enormous as it is a multi-purpose crop. In most homes in southeastern Nigeria the fruits provide income to rural people, thereby improving their economy. The roots, leaves and seeds are said to have medicinal values (Johansen, 1950). The high nutrient potentials of the nut has been reported in literatures (Oke and Funsho, 1975; Ogunsua and Adebona, 1983). The plant also provides a microclimate within the forest as its branches spread on the canopy cover of forest trees (Egharevba *et al*., 2005).

The fruits are oil-bearing yielding 48-60% of light golden coloured oil which is similar to linseed oil. The oil is composed of 64% linolenic acid, 15% palmitic acid and stearic acids, 11% oleic acid and 10% linoleic acid. The oil is also known as conophor oil, and is useful in paint or varnish industry. It is edible and could be used in food preparations (Burkill, 1984). There is also a report that the cake left after extraction of the oil contains 45% protein, and has local uses for food and is obviously a good source of protein. It can be fed to livestock (Burkill, 1984).

Plukenetia conophorum is one of the non-wood forest products, which are essentially part of the forest products. According to Osemeobo and Ujo (1999), these non-wood forest products provide a safety net for most rural dwellers in many third world countries including Nigeria,

where they contribute immensely in food security. United Nations (2002) and National Planning Commission (2004) reported that these harvestable forest products accounts for 90% of rural dwellers livelihood and economic survival. However, despite the potentials of *P. conophorum*, its continuous existence among other forest products of Nigeria is threatened (UN, 2002) due to deforestation, urbanization and other similar activities.

Increases in production and availability of African walnut just like other semi-wild indigenous species, can only be possible when suitable production practices are exploited coupled with enough biochemical and biophysical knowledge. At present, there is paucity of information on the regeneration and perpetuation of this very important plant. The walnut is still in the wild. There is no evidence of efforts to fully domesticate this useful plant. The high rate of forest destruction affects their habitat. The plant could face possible extinction in the very near future. To salvage this useful plant from extinction and provide basis for its conservation, the present study was undertaken. With increased recent interest in the exploitation, conservation and domestication of less common forest products, it is important to examine the African walnut in order to ascertain its full usefulness. The objectives of this research are:

1) Evaluation of the effects of three manure rates on early growth stages of four accessions of African walnut;
2) Determination of the amino acid profile of kernels from four different accessions; and
3) Investigation of possible bio-diversity among accessions collected from different locations (states) of southeastern Nigeria, with respect to the seed physical traits and proximate components.

LITERATURE REVIEW

Plukenetia conophorum Müell. (Arg), formerly known as *Tetracarpidium conophorum* (Müell. Arg.) Hutch. and Dalziel] is from the family Euphorbiaceae and commonly called the African walnut (GRIN, 2010). It is widely distributed in the southern part of Nigeria, where it is called ôUkpaô by the Igbo, óAwusaô or óAsalaô by the Yorubas and ôEporoô by the Efik and Ibibio people (Daziel, 1937). The nut is spherical in shape (Asoegwu, 1995) and has a black shell inside which is embedded a milky kernel. African walnut is a perennial climber found in the moist forest zones of sub-Saharan Africa (Oke, 1995) and sometimes found in low bush especially in Africa, America, Europe and the Asia (Hutchinson and Dalzier, 1987). It is cultivated principally for the nuts that are cooked and consumed as snacks, along with boiled corn (Oke, 1995; Edem *et al.*, 2009). Oke (1995) reported on the high nutrient potentials of the nut. Adebona *et al.,* (1988) developed a biscuit-like snack food from the nut, and highlighted on the functional significance of the oilseed.

Enujiugha (2003) noted that freshly harvested mature nut on a dry weight basis, contains 29.09% protein, 6.34% fibre, 48.9% oil, 3.09% ash and 12.58% carbohydrates. It is rich in valuable minerals like phosphorus, potassium, sodium, magnesium and zinc (Laverdine, *et al*., 2000). Its oil is edible and commands high price. Walnut oil is not used for high temperature cooking because heating normally removes the flavor and bitterness and as a result, it is used primarily as an ingredient in cold dishes such as salad dressings, where the distinct flavour is easily perceived. The oil is useful in the manufacture of paints and varnishes. The cake obtained after oil extraction can be used as source of protein for livestock or as source of nitrogen fertilizer. The nut shell could be used as cooking fuel (Asoegwu, 1995). The health benefits of walnut include lowering of cholesterol, reducing inflammation and improving arterial function (Nash and Westpfal, 1995).

The leaves are globular ovate, long and margin toothed. The bases of the leaves are broad and rounded with slender petioles up to 2 inches long. The fruits are four winged, ridged between wings and up to 3 inches in diameter. The seeds have a bitter taste and a tonic effect like Kola. They can be eaten raw, cooked, and sometimes with roasted corn. (Nuhu *et al.*, 2000; Okerulu and Ani, 2001). The plant is known in Africa especially in Nigeria for its antibacterial efficacy. Decoction of the leaves and seeds serve as beverages, relieves abdominal pains and fever. The water extracts of the roots provides soothing beverage for fever and malaria (Nuhu *et al.*, 2000; Balch and Balch, 2000).

The leaf extract of *P. conophorum* possesses antibacterial and antifungal activities (Ajaiyeoba and Fadare, 2006). The root extract of the plant also displays antibacterial activity (Ajaiyeoba, 2002). Walnut extract possesses anticancer property (Herbert *et al.*, 1998) and reduce diabetic complications (Kaneto *et al*., 1999).Walnut contains phytosterols, polyphenols , phytoestrogens (Kris-Etherton *et al*., 1999) and omega-3 fatty acid (Zambon *et al.,* 2000).The carbohydrate binding specificity of *P. conophorum* lectin shows preference for simple and unbranched oligosaccharides (Sato *et al*., 1991).

Walnut is planted under trees that can provide support for the heavy weight of the climber when fully established on the crown of the tree. The climber often takes over the crown of the tree when fully established and competes for sunlight and also affects fruiting of the host tree. Therefore, trees that do not produce high economic fruits are mostly used to serve as support for the climber. There is a crop sharing beneficial effect between Walnut plants and such trees as Kola and Cocoa. Walnut gets support from the trees while the tresses are provided with shade. Gathering and processing of the fruits is at the household level, this creates social interaction between the young and the old within the communities. In most cases, the fruits are allowed to drop after maturity and gathered by the children and women, and sometimes by the farmers themselves. The gathered fruits are allowed to rot, after which the seeds are removed and washed. In cases where the farmer was in need of money and cannot wait for the fruit to degrade, a cut is used to open the fruits and the nuts are removed.

According to a recent study by the United States of American Association for Cancer Research, mice fed the human equivalent of two ounces (56.7g) of walnuts per day developed fewer and smaller cancerous tumours. Standard testing showed that walnut consumption significantly decreased breast tumor.

The leaves, bark, and fruit of *P. conophorum* are used medicinally, and their uses include masticatory, giddiness, thrush, antihelminthic, toothache, syphilis, dysentery, and as an antidote to snakebite (Burkill, 1984). In the southern Nigeria ethnomedicine, African walnut is used as a male fertility agent and in the treatment of dysentery (Ajaiyebo and Fadare, 2006). The methanol and ethylacetate extracts of *P. conophorum* leaves have been shown to possess good antibacterial activities especially against Gram +ve organisms (Ajaiyebo and Fadare, 2006).

Ganiyu and Mofoluso (2004) reported the proximate, mineral, antinutrient composition and zinc bioavailability of some Nigerian wild seeds including *Tetracarpidium conophorum* .

Walnut is considered to be herb in traditional Chinese medicine. Walnuts are said to detoxify kidneys, strengthen the back and knees, moisten the intestines and move stool. It is believed to stop asthma and is prescribed to be taken in between bouts of asthma, but not for acute asthma. It is used by the elderly as a constipation cure (Wikipedia, 2008).

In developing countries, the high cost of animal proteins has made the search for cheap and alternative sources of proteins with desirable functional and nutritional properties highly imperative. In recent years, research attention has focused majorly on vegetable proteins as sources of low- cost proteins to supplement human diets. Several authors have demonstrated the potential application of oilseed proteins such as peanut concentrate (Wu *et al*., 2009), mustard proteins (Alireza and Bhagya, 2009) and lesser known leguminous seed fractions (Ogunwolu *et al*., 2009) for product formulation and food fortification particularly for developing countries (Wu *et al*., 2009). Due to increasing market demands on protein ingredients, underutilized oilseeds are now receiving considerable attention. Walnut is one of the neglected oil seeds in Nigeria but with great potential for increased utilization.

African walnut is equally a rich source of protein containing 20 ï 24% protein on a dry weight basis (Ogunsua and Adebona, 1983; Ige *et al.,* 1984; Ogunsua, 1987; Adebona *et al.,* 1988; Adesioye 1991; Odoemelam, 2003). After defatting, the resultant cake contains about 35 ï 40% protein (Ogunsua and Adebona, 1983; Ogunsua 1987). The amino acid composition of the nut has also been investigated by Ogunsua (1988) who reported that walnut contains essential amino acids in adequate amount for nutrition except methionine and that two unusual amino acids Ŭ-aminobutyric acid and ɔ-amino butyric acid were also present. Oyenuga *et al.* (1971) reported that Walnut nut contained low level of methionine (0.6 g/16 g N) but high cysteine (2.4 g/16 g N).

MATERIALS AND METHOD

The work involved field experiment and laboratory investigations. The early growth evaluation was conducted at the experimental research farm of the Department of Crop Science, University of Nigeria, Nsukka ($6^{0}51^{1}$E, $7^{0}29^{1}$N, 475m above sea level). Proximate analyses of samples were done at the Animal Science laboratory while Physical traits, characterization were done at Crop Science Laboratory, University of Nigeria, Nsukka. Amino acids profile was carried out at the International Institute of Tropical Agriculture (IITA), Ibadan. African walnut accessions from four southeastern states of Enugu, Anambra, Abia and Rivers were used for this study.

Land Preparation, Soil and Manure Sampling

The experimental site was cleared and the debris removed before the field experiment commenced. Soil samples were taken from six different representative locations of the site at 0-15cm depth and were bulked together into composite sample, which was air-dried. The composite air-dried soil sample was used for physicochemical analysis. The pig manure sample was analyzed. The soil analysis done included:

- Particle size
- pH
- % Organic carbon
- % Organic matter
- % Nitrogen
- % Potassium
- % Phosphorus
- % Calcium
- % Magnesium
- Cation exchange capacity (CEC)

The chemical analysis done on the pig manure included:

- pH
- % Organic carbon
- % Organic matter
- % Nitrogen
- % Potassium
- % Phosphorus

- % Calcium
- % Magnesium
- % Sodium

EXPERIMENT 1: SEED PHYSICAL TRAITS DETERMINATION

The following physical parameters were measured from the seeds of the four accessions.

1. **Percentage edible portion**

   This is the ratio of the pulp weight to the seed weight multiplied by 100 i.e. %

   $\frac{\text{Pulp weight}}{\text{Seed weight}} \times \frac{100}{1}$

2. **Mean seed volume**

   A measuring cylinder was used. A volume of water in a beaker was noted (V1), and the seeds were placed in a polythene bag, and V2 noted. Volume of the seeds was measured as V_2-V_1 (cm^3)

3. **Mean seed weight**

   The mean of 10 replicates of each accession was taken using a sensitive electronic weighing balance.

4. **Mean pulp weight**

   The mean of 10 replicates of each accession was taken using a sensitive electronic weighing balance.

5. **Seed circumference**

   The circumference of each seed was measured .Seed circumference is 2´r, where ´ (pie) =22/7, 2 is a constant and r= radius.

6. **Seed coat thickness**

   Micro meter screw gauge was used to measure the seed coat thickness.

EXPERIMENT 2: JUVENILE PHENOLOGY

Seeds from three accessions (Abia, Anambra and River states) were planted in 30kg soil in plastic buckets on 22nd October, 2011. Each bucket was separated by a distance of 0.5m x 1m. The experiment was arranged in a completely randomised design (CRD) of five replications.

The treatment consisted of three levels of pig dung as:

0 tonne per hectare

5 tonnes per hectare

10 tonnes per hectare

These were allocated at random across the three accessions forming a 3 x 3 factorial in CRD.

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Data collected included:

-Emergence studies

a) **Days to Emergence**: This is the number of days from planting to emergence.

b) Emergence percentage (E %)

$$E\ \% = \frac{\text{Number of seedlings that emerged}}{\text{Total number of seeds planted}} \times 100$$

The following parameters were measured thereafter starting from one month after the pig dung application.

- **Vine length**: this was measured in one, two and three months from the date of manure application, with a meter ruler.
- **Vine girth at 1cm above soil level:** this was measured at one, two and three months after manure application, with a micrometer screw gauge.
- **Number of leaves/ plant**: this was obtained by counting all the leaves per plant at one, two and three months from the date of manure application.
- **Number of branches/ plant**: this was obtained by counting all the branches developed by the plants at one, two and three months after manure application.
- **Dry matter analyses** following destructive sampling
    - **Dry weight of leaves, stem and root**: was determined by drying their respective fresh samples in the oven and their weights taken.
    - **Root volume**: this was obtained for each plant by water displacement measurement of the fresh root samples.

EXPERIMENT 3: PROXIMATE ANALYSES

Proximate analyses of fresh and boiled seeds of the four accessions of African walnut were carried out to determine the moisture, ash, fat, crude protein, crude fibre and carbohydrates contents. These proximate components were determined using the standard procedures of Association of Official Analytical Chemistry (AOAC, 1990). The seeds were cooked for one hour at 100 ^{0}C. The fresh and boiled seeds were ground using Thomas Miller milling machine at Crop Science Department, University of Nigeria, Nsukka.

Moisture Content Determination

The moisture content was determined using the hot air oven method (AOAC, 1990). The crucibles were washed and dried in the oven at 105 ^{0}C for 10 minutes. The crucibles were weighed again. Two grams of ground fresh and boiled seeds each were placed in crucibles and thereafter put in the oven at 105 ^{0}C until constant weight was obtained.

The difference in weight divided by the original weight expressed in percentage gave the moisture content.

$$\%\ Moisture = \frac{(Initial\ weight - final\ weight)}{initial\ weight} \times \frac{100}{1}$$

Ash Determination

The ash of an agricultural material is the inorganic residue remaining after the organic matter has been burnt off (Pearson, 1976).

Methodology:

Heat resistant crucible with cover (silica dish) was dried at 500^{0}C for 1 hour, cooled in desiccators and weighed. 2 g portion of each ground sample was transferred into the dish. The dish and the content was ignited, first gently and then at 500^{0}C (loss of chloride due to volatisation tends to occur at above 500^{0}C) for 3 hours. The crucible was removed; the lid replaced then allowed to cool before reweighing. The weight of the residue was obtained and expressed as percentage of the original sample.

$$\%\ Ash = \frac{\text{Weight of Ash}}{\text{Original weight}} \times 100$$

Fat Determination

The analysis of fat content was carried out using soxhlet extraction method (AOAC, 1990). The residues that remained after the final drying were the fat components.

Procedure:

2 g weight of fresh and boiled samples of each accession were dried in an oven for five minutes. The flask and the samples were weighed. The flasks together with the samples were taken into the oven for drying for six hours. To each ground sample, in flask, 250 ml of petroleum ether was used to extract the oil and then concentrated in oven. The oil left in the flask after the drying was weighed again.

Calculation:

Weight of oil + flask (W2)- Weight of flask (W1) = Weight of fat

$$\% \text{ Fat} = \frac{W2-W1}{2} \times 100$$

Crude Protein Determination

The crude protein content of the sample was determined by using the Kjedahl technique (Pearson, 1976). The method involved digestion of samples, distillation of digests and titration of distillate.

Digestion: One gram of each sample was put accurately in a digestion flask; 15 mls of concentrated H_2SO_4 was added in the presence of a catalyst made up of selerium powder, $NaSO_4$ and $CuSO_4$. The flask was later placed on an electrical coil heater in a fume chamber and gently boiled until clear solution was obtained. This was allowed to cool.

Distillation: Steam was passed through the Markham distillation apparatus for about 10 minutes. A mixture of 10 ml of concentrated boric acid indicator was added in a 50 ml conical flask. The flask was placed under a condenser in a way that 5 ml of the diluted digest (P. *conophorum* sample) was put in the distillation apparatus and was rinsed with water. The cup was closed with rod and 5 ml of 60% NaOH was added through the cup leaving behind a little to prevent NH2 from escaping. Steam was introduced for about 5 minutes until 20 ml of distillate was produced.

Titration: The boric acid indicator was titrated with 0.01N HCl to the end point by a change in colour of distillate from brilliant green to pinkish colour.

$$\% \text{ Crude protein} = \frac{T \times 14.01 \times 0.1 \times 6.25 \times 1000 \times 10}{1000 \times 2}$$

Crude Fibre Determination

To determine the fibre content in a sample, the protein, starch and other digestible carbohydrate and fat have to be hydrolysed out of the sample.

Two grams of sample was put into the flask. A 150 ml pre heated H_2SO_4 was added and heated to boiling for 30 minutes. A 150 ml pre heated (KOH) was also added and heated to boiling point. Also, some drops of antifoaming agent was added and boiled slowly for 30 minutes. The residue was filtered and washed three times with hot water, and washed again three times with acetone (in cold extraction unit). The washed samples were later dried at 130^0C for 1 hour, weighed and ashed at 500^0C. The resulting ashes were later weighed again.

Calculation:

$$\% \text{ Crude fibre} = \frac{W2-W3}{W1} \times 100$$

Where W1= weight of flask

W2= weight of flask + sample

W3= weight of flask + ash

Carbohydrates Determination

Carbohydrate content was obtained by difference (AOAC, 1995). The percentage values of protein, fat, ash, moisture and crude fibre was summed up and subtracted from 100%.

EXPERIMENT 4: ANALYSIS OF AMINO ACIDS PROFILE IN AFRICAN WALNUT.

The analysis of Amino acids was done using Waters 616/626 LC (HPLC) Instrument.

Amino acid analysis is a method of estimating the concentration of each individual amino acid present in a given protein or peptide samples.

The sample preparation and determination was carried out in the following stages:

1) Hydrolysis
2) Derivatisation
3) Separation of derivatised amino acids
4) Data processing/ interpretation and calculations of the final results

Step (1) Hydrolysis of samples:

A 0.5 g of the samples was put into a sterile furnaces hydrolysis tube. A 5 nmols leucine was added to the samples and then dried under a vacuum.

The tube was placed in a vial containing 10.05N HCl with a small quantity of phenol, thereby hydrolysing the protein by HCl vapours under vacuum. This stage of hydrolysis of the sample lasted for 20-23 hours at 108^0C. After this hydrolysis, the samples were dissolved in ultra-pure water (HPLC) grade, containing ethylene diamine tetra acetic acid (EDTA). The hydrolysed samples were stored in HPLC amino acid analyser bottles for further analytical operations.

Step (2) Derivatisation:

After hydrolysis, the samples were derivatised automatically with the Waters 616/626 HPLC by reacting the five amino acid, under basic situations with phenylisothiocarbamyl (PTC) amino acid derivatives. The duration for this was 45 minutes per sample, as calibrated on the instrument.

A set of standard solution of amino acids was prepared from Pierce Reference standards H (1000 umol) into auto sampler crops and they were derivatised.

These standards (0.0, 0.5, 1.0, 1.5, 2.0 umol) were used to generate a calibration file that determined the amino acids contents of the samples. After the derivatisation, a menthol

solution (1.5 N) containing the PTC-amino acids were transferred to a narrow bore Waters 616/626 HPLC system for separation.

Step (3) The HPLC Separation and Quantification:

The separation and quantification of the PTC-amino acids was done on a reverse phase (18 silica column and the PTC chromophone was automatically and digitally detected at the wavelength of 254 nm). The elution of the whole amino acids in the samples took 30 minutes. The buffer system used for the separation was 140mm sodium acetate pH 5.50 as buffer A and 80% acetonitrile as buffer B.

The program was run using a gradient of buffer A and buffer B concentration and ending with 55% buffer B concentration at the end of the gradient.

Step (4) Data interpretation and calculations:

The intensity of the chromatographic peaks was automatically and digitally identified and quantified using a Dionex chromeleon data analysis system which was attached to the Waters 616/626 HPLC system.

The calibration curve or file was prepared from the average values of the retension times (in minutes) and areas (in Au) at the amino acids in 5 standards.

Since a known amount of each amino acid in the standard loaded into the HPLC, a response factor (Au/pmol) was calculated by the software that was interphased with the HPLC. This response factor was used to calculate the amount of each of the amino acid (in pmols) in the sample and displayed on the system digitally. The amount of each amino acid in the sample was finally calculated by the software by dividing the intensity of the peak area of each (corrected for the differing molar absorptivities of various amino acids) by the internal standard (i.e. pierce) in the chromatogram and multiplying this by the total amount of internal standard added to the original sample.

After the pico mole by the intensity of the height of each amino acid had been ascertained by the software, the data, the digital chromatographic software extrapolate back to 5 nmoles of the internal standard (Norleucine), and displays for the total amount that was pipetted into the hydrolysis tube at the beginning of the analysis as below:

Calculation:

Mg/ml (in Extract)= Dilution factor x Peak height intensity

$$\text{Mg/ml (in sample)} = \frac{\mu\text{g/ml in extract} \times \text{sample volume}}{\text{Weight of sample}}$$

Statistical Analysis

In all the experiments, data collected were subjected to Analysis of Variance (ANOVA) following the procedures outlined for completely randomized design (CRD). Significant treatment means was separated with the aid of F-LSD at 5 % probability level. All statistical analysis was done using Genstat 7.1 version 2003 software.

Table 1: Seed Collection Centres

State	Source of seeds (locations)
Abia	Umudike-Umuahia
Anambra	Anaocha-Neni
Enugu	Amokwe-Udi
Rivers	Woji- Port Harcourt

RESULTS

The period of the experiment from October, 2011 to June, 2012 was characterized by inadequate rainfalls, low humidity and moderate temperatures (Table 2). Table 3 shows that the experimental site was characterized as sandy loam, acidic and low percentage organic matter. Also, the soil was generally low in macro and micro nutrients however, the pig manure has high pH, higher percentage organic matter, nitrogen, calcium and magnesium. The soil of the source of seeds (Table 4) are highly leached, strongly acidic, poor in plant nutrients and low to moderate cation exchange capacity (CEC). Data shown in Table 5 indicates that all the seed physical traits showed significant ($P < 0.05$) variation across the four locations (States). It was observed that seeds from Enugu had the highest percentage edible proportion followed by Abia and Rivers. With respect to seed volume, seeds from Anambra had the highest value followed by Abia, and Enugu whereas Rivers recorded the least values. Anambra State had the highest values, for seed and pulp weight followed by Abia, Enugu and Rivers, respectively. The largest seed circumference was observed in seeds from Abia State, followed sequentially by that of Anambra and Enugu States. Seeds from Rivers State had the least seed circumference. However, seed coat thickness was higher in seeds from Abia and Rivers States compared to others.

Presented in Table 6 are the proximate qualities of *P. conophorum* as influenced by location. Ash, Fat and moisture varied significantly ($P < 0.05$) across the four locations while carbohydrates, fibre and protein contents of the seeds were not significantly influenced by location effect. Moisture and ash contents were significantly ($P < 0.05$) higher in seeds from Anambra and Enugu States, followed by Rivers State. Seed fat content was significantly ($P < 0.05$) higher in seeds from Anambra and Abia States. The least fat content was obtained in seeds collected from Enugu though not statistically different from seeds from Rivers State.

In Table 7, it was observed that the ash, carbohydrates, fat and protein contents of the seeds were not significantly ($P < 0.05$) influenced by processing. However, fibre and moisture contents showed significant ($P < 0.05$) differences in the boiled and fresh seeds. Fibre content was higher in the boiled seeds, whereas the fresh seeds had higher moisture content.

The interaction effects of location (states) and processing were presented in Table 8. All the proximate qualities showed no significant ($P < 0.05$) response to interaction of the two factors (Location and processing).

Table 2: Weather Data of Oct., 2011 to June, 2012

		2011			
	Rainfall (mm)	**Temperature (^{0}C)**		**Relative humidity (%)**	
		Max	**Min**	**10am**	**4pm**
Oct	12	28.26	20.84	75.58	72.10
Nov.	2	30.33	20.77	69.3	59.47
Dec.	0.00	31.58	16.68	56.48	47.26
			2012		
January	0.00	31.65	19.77	52.23	48.74
Feb.	3	31.76	22.00	73.59	61.24
March	0.00	33.19	22.97	71.23	53.35
April	4	31.43	22.43	73.47	62.83
May	13	30.19	21.19	74.13	67.81
June	13	28.33	20.3	75.8	71.5
Average	5.22	30.75	20.77	69.09	60.48

Source: Meteorological station, Department of Crop Science, UNN

Table 3: Physicochemical properties of the experimental site and pig manure sample utilized for the study

	Substrate	
Properties	**Top soil (0-15cm)**	**Pig manure**
Clay (%)	15	-
Silt (%)	11	-
Fine sand (%)	26	-
Coarse sand (%)	48	-
Textural class	Sandy loam	
Chemicals Properties		
pH(H_2O)	4.9	7.3
pH(KCL)	4.2	7.1
Organic matter (%)	1.80	21.5
Organic carbon (%)	1.40	12.47
Nitrogen (%)	0.70	0.981
Potassium (%)	0.001143	0.72
Phosphorus (%)	0.01	0.71
Calcium (%)	0.0011	12.8
Magnesium (%)	0.00176	12.8
Sodium (%)	-	0.29
CEC	47.2	-

Table 4: Soil Characterization of the Source of Seeds

	Source of Seeds	State	Soil Characterization
1	Umudike-Umuahia	Abia	Mainly deep to very deep dark red or red soils (Ferric
2	Anoacha-Neni	Anambra	Acrisols)
3	Amokwe-Udi	Enugu	Sand to sandy clay sub-soil over lain by dark brown or red layer, poorly structured, highly leached, strongly acid and low in plant nutrients and cation exchange capacity
4	Woji-Port Harcourt	Rivers	The soils are well drained to poorly drained highly permeable reddish yellow to yellowish brown or dark grayish brown sandy loam to sandy clay loam soils developed in stratified alluvial. They are very strongly acid, have moderately amounts of organic matter and total nitrogen content, and low amounts of exchangeable bases. Soils have moderate cation exchange capacity and are classified as Orthic Acrisols.

Source: Agroecological zones of Nigeria Manual, April, 2006

Table 5: Effect of Location (States) on the seed physical traits of *Plukenetia conophorum*

Location	% edible proportion	Mean seed volume (cm^3)	Mean seed weight (g)	Mean pulp weight (g)	Seed circumference (cm)	Seed coat thickness (mm)
Abia	70.68	6.60	85.04	30.55	10.640	1.224
Anambra	64.83	7.70	112.84	37.34	9.742	1.061
Enugu	74.08	6.35	77.16	29.27	9.484	1.026
Rivers	66.65	4.65	71.87	24.59	9.038	1.179
$LSD_{0.05}$	3.09	0.85	0.52	2.28	0.21	0.14

Table 6: Effect of location on the proximate composition of *Plukenetia conophorum*

Location (states)	Ash [%]	CHO [%]	Fat [%]	Fibre [%]	Moisture [%]	Protein [%]
Abia	5.03	33.96	24.97	4.47	7.34	23.60
Anambra	5.81	32.93	25.92	5.18	8.78	21.39
Enugu	5.80	35.75	21.00	4.99	8.78	23.49
Rivers	5.07	39.10	22.33	5.18	8.03	20.84
$LSD_{0.05}$	0.65	ns	2.63	ns	0.77	ns

NS = non significance difference

Table 7: Effect of processing on the proximate composition of *Plukenetia conophorum*

Processing	Ash [%]	CHO [%]	Fat [%]	Fibre [%]	Moisture [%]	Protein [%]
Boiled	5.56	34.08	24.04	6.16	7.84	22.23
Fresh	5.20	36.55	23.02	3.75	8.62	22.43
$LSD_{0.05}$	ns	ns	ns	0.85	0.54	ns

NS = non significance difference

Table 8: Interaction effects of location (states) and processing on proximate composition of *Plukenetia conophorum*

Location (states)	Processing	Ash [%]	CHO [%]	Fat [%]	Fibre [%]	Moisture [%]	Protein [%]
Abia	Boiled	5.23	33.54	24.73	5.67	6.98	23.24
	Fresh	4.82	34.37	25.20	3.27	7.70	23.35
Anambra	Boiled	5.80	32.43	26.53	6.78	8.13	20.33
	Fresh	5.82	33.44	25.30	3.38	9.42	22.45
Enugu	Boiled	6.13	32.18	23.57	5.62	8.53	23.98
	Fresh	5.47	39.31	18.43	4.37	9.02	23.01
Rivers	Boiled	5.42	38.18	21.33	6.58	7.70	20.78
	Fresh	4.72	39.10	23.13	3.78	8.37	20.90
$LSD_{0.05}$		ns	ns	ns	ns	ns	ns

CHO = Carbohydrate
$LSD_{0.05}$ = Least significant different at 5% probability level;
ns = non significant difference

Presented in Table 9 are the essential amino acids contents of *P. conophorum* seeds collected from four states of southeastern Nigeria. Locational variation showed no significant effect on Iso-Leucine, Leucine, phenylamine and Tryptophane contents of the seeds. Seeds collected from Rivers, Enugu and Anambra states gave significantly ($P<0.05$) higher histidine value than those from Abia. Seeds collected from Abia state has the highest lysine content compared with the seeds from other locations. Lysine contents in seeds from Anambra and Enugu were significantly ($P<0.05$) higher than those from Rivers state. Seeds from Enugu and Rivers states gave significantly ($P<0.05$) higher methionine value than seeds from Anambra and Abia states. The valine content of seeds from Rivers and Enugu states were significantly ($P<0.05$) higher than that of Anambra and Abia states. Threonine content was similar for seeds collected from Enugu and Abia states which recorded higher values than those from Anambra and Rivers states.

Effect of processing on the essential amino acids of *P. conophorum* seeds is presented in Table 10. It was observed that the Histidine, Isoleucine, leucine, lysine, methionine, phenylamine, valine and Threonine contents of the seeds were not influenced by processing. Except for tryptophane, all the other essential amino acids were found to be numerically higher in the fresh seeds. Tryptophane content was significantly ($P < 0.05$) higher in the boiled seeds.

Interaction effects of location and processing on the essential amino acid components is shown in Table 11. Histidine and lysine varied significantly ($P < 0.05$) across locations and processing treatment while the others were not significantly influenced by the interaction effects. In Anambra and Rivers States, Rivers histidine was found to be higher in the boiled seeds, whereas fresh seeds in Enugu had higher histidine content. Histidine content of seeds from Abia was not influenced by processing. Except for seeds from Anambra state, lysine was higher in the fresh seeds in every other state. Lysine content was found to be exceptionally high (2.91) in fresh seeds from Abia state. A similar high value (2.34) was recorded in boiled seeds from Anambra state. Lysine was particularly poor in seeds from Rivers state. A majority of the essential amino acids were found to be higher in the fresh seeds across the locations.

Table 12 shows that alanine, arginine, asparagine, glutamine and glutamic acid were statistically ($P < 0.05$) influenced by location while aspartic acid, glycine, proline, serine, trimethlisine and tyrosine did not vary significantly among the different locations. Seeds

collected from Abia State gave significantly higher alanine value than seeds collected from the other locations. The alanine contents of seeds from Enugu and Rivers States were similar but statistically different from seeds collected from Anambra States. Seeds collected from Abia gave significantly ($P < 0.05$) the highest arginine value followed by Anambra. Arginine content was however similar with seeds from Anambra, Enugu and Rivers. Asparagine content was significantly higher in seeds from Rivers than those from Anambra and Enugu locations but similar with seeds from Abia. Seeds from Abia state gave significantly higher glutamine value than the seeds from the other locations.

Glutamic acid content of seeds from Enugu location was significantly ($P < 0.05$) higher than the seeds from Abia and Rivers locations but similar with those from Anambra.

Table 9: Effects of location (states) on the essential amino acids (ppm) composition of Plukenetia conophorum

Location (States)	Histidine	Iso-Leucine	Leucine	Lysine	Methionine	Phenylalanine	Tryptophan	Valine	Threonine
Abia	0.186	1.216	2.35	2.20	0.113	0.86	1.22	0.436	1.02
Anambra	0.573	1.342	1.68	1.52	0.319	0.68	1.72	0.583	0.86
Enugu	0.831	0.794	1.89	1.04	0.927	0.84	0.88	1.278	1.73
Rivers	1.513	0.789	1.83	0.16	0.590	0.47	1.14	1.546	0.66
$LSD_{0.05}$	1.094	ns	ns	0.68	0.47	ns	ns	0.54	0.71

Ns = non-significant difference

-Table 10: Effects of processing on the essential amino acid (ppm) composition of *Plukenetia conophorum*

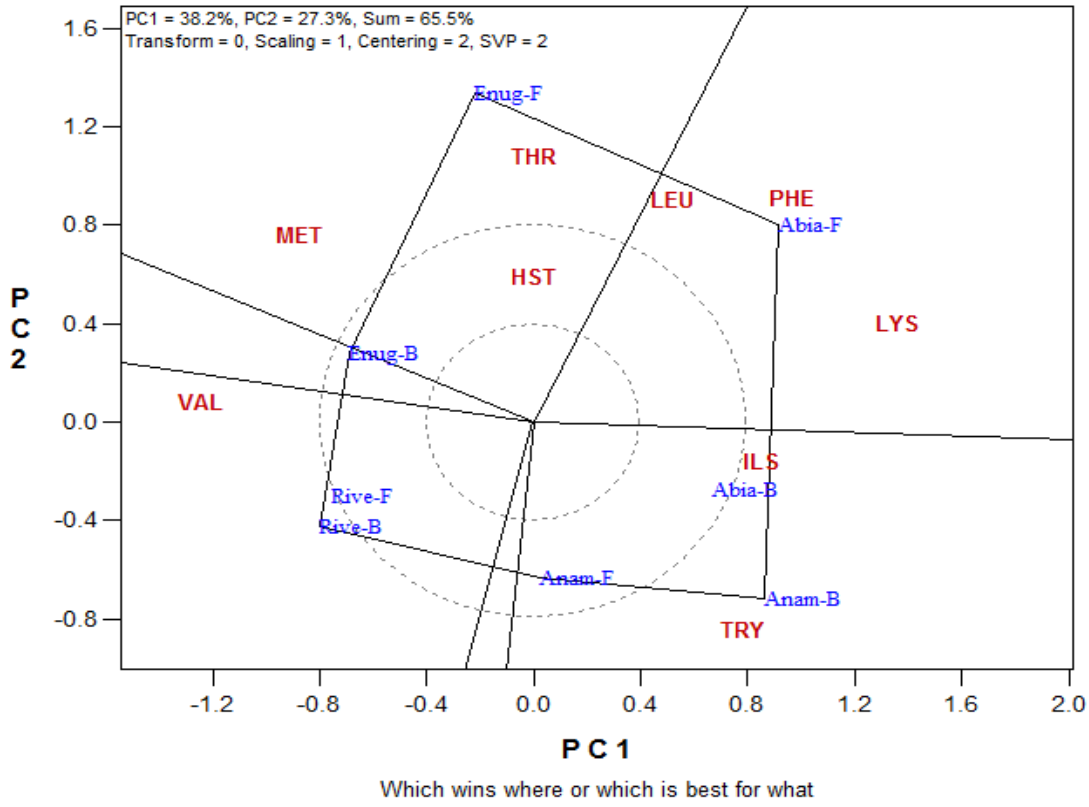
Processing	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Tryptophan	Valine	Threonine
Boiled	0.587	0.895	1.56	1.16	0.381	0.71	1.51	0.931	1.06
Fresh	0.776	1.176	2.32	1.30	0.593	0.72	0.97	0.990	1.07
$LSD_{0.05}$	ns	ns	ns	ns	ns	ns	0.45	ns	ns

Ns = non-significant difference

Table 11: Interaction effects of location (states) and processing on the essential amino acid (ppm) composition of *Plukenetia conophorum*

Location (States)	Processing	Histidine	Iso-Leucine	Leucine	Lysine	Methionine	Phenylalanine	Tryptophan	Valine	Threonine
Abia	Boiled	0.71	1.020	1.73	1.94	0.009	0.83	1.47	0.328	0.82
	Fresh	0.711	1.412	2.97	2.91	0.217	0.90	0.96	0.545	1.22
Anambra	Boiled	0.448	1.026	1.51	2.34	0.182	0.82	2.31	0.264	0.89
	Fresh	0.206	1.658	1.85	0.71	0.455	0.54	1.14	0.901	0.84
Enugu	Boiled	0.390	0.713	1.47	0.73	0.719	0.73	0.90	1.574	1.70
	Fresh	0.755	0.874	2.32	1.36	1.134	0.96	0.85	0.982	1.76
Rivers	Boiled	0.793	0.819	1.52	0.09	0.614	0.47	1.34	1.558	0.84
	Fresh	0.675	0.758	2.14	0.22	0.565	0.47	0.95	1.533	0.47
$LSD_{0.05}$		0.44	ns	ns	0.96	ns	ns	ns	ns	ns

Ns = non-significant difference



Hst = Histidine
Ils = Iso- Leucine
Leu = Luecine
Lys = Lysine
Met = Methionine
Phe = Phenylalanine
Try = Tryptophan
Val = Valine
Thr = Threonine

Fig. 1: GGE- Biplot analysis on the interaction effects of location (states) and processing on the essential amino acid composition of *P. conophorum*

Table 12: Effects of location (states) on the non-essential amino acids (ppm) composition of *Plukenetia conophorum*

Location (states)	Alanine	Arginine	Asparagine	Aspartic acid	Glutamine	Glutamic Acid	Glycine	Proline	Serine	Trimethylisine	Tyrosine
Abia	2.28	0.923	1.90	0.091	2.33	0.49	2.07	1.15	1.33	0.156	0.80
Anambra	0.88	0.334	1.17	0.340	0.88	1.44	1.42	0.99	1.27	0.113	0.60
Enugu	1.27	0.114	0.35	0.234	1.77	2.01	1.28	0.53	1.29	0.286	1.08
Rivers	1.24	0.042	2.20	0.365	0.38	0.16	1.08	0.55	1.10	0.237	1.22
$LSD_{0.05}$	0.10	0.53	0.73	ns	0.43	0.64	ns	ns	ns	ns	ns

Ns = non-significant difference

Table 13 showed the effect of processing on the non-essential amino acid components of *P. conophorum* seeds. Glutamine, glutamic acid and Trimethlisine contents of the seeds varied significantly ($P < 0.05$) with cooking processing. Glutamic acid and trimethlisine contents were higher in the fresh seeds whereas glutamine was higher in the boiled seeds. Alanine, arginine, asparagine, aspartic acid, glycine, proline, serine and tyrosine showed no significant ($P < 0.05$) response to processing.

Data in Table 14 show the interaction effects of location (states) and processing on the non-essential amino acid components. Asparagine, glutamine and glutamic acid contents varied significantly ($P < 0.05$) among the seeds collected from all the locations while the others were not significantly influenced by the interaction effects.

Asparagine content of the seeds was highest in seeds from Abia State. In Abia State, asparagine was higher in the fresh seeds whereas boiled seeds had higher asparagine content in other states. Glutamine was highest in seeds from Abia. The glutamine content, just like the glutamic acid content, was consistently higher in the boiled seeds across the locations. In Abia State, glutamine and glutamic acid contents were not influenced by processing. A majority of the non-essential amino acids was found to be higher in boiled seeds.

Table 13: Effects of processing on the non-essential amino acid (ppm) composition of *Plukenetia conophorum*

Processing	Alanine	Arginine	Asparagine	Aspartic acid	Glutamine	Glutamic acid	Glycine	Proline	Serine	Trimethylisine	Tyrosine
Boiled	1.70	0.332	1.06	0.289	2.06	0.30	1.68	0.74	1.27	0.10	0.98
Fresh	1.14	0.375	0.76	0.226	1.34	1.02	1.25	0.87	0.87	0.296	0.87
$LSD_{0.05}$	ns	ns	ns	ns	0.69	0.46	ns	ns	ns	0.19	ns

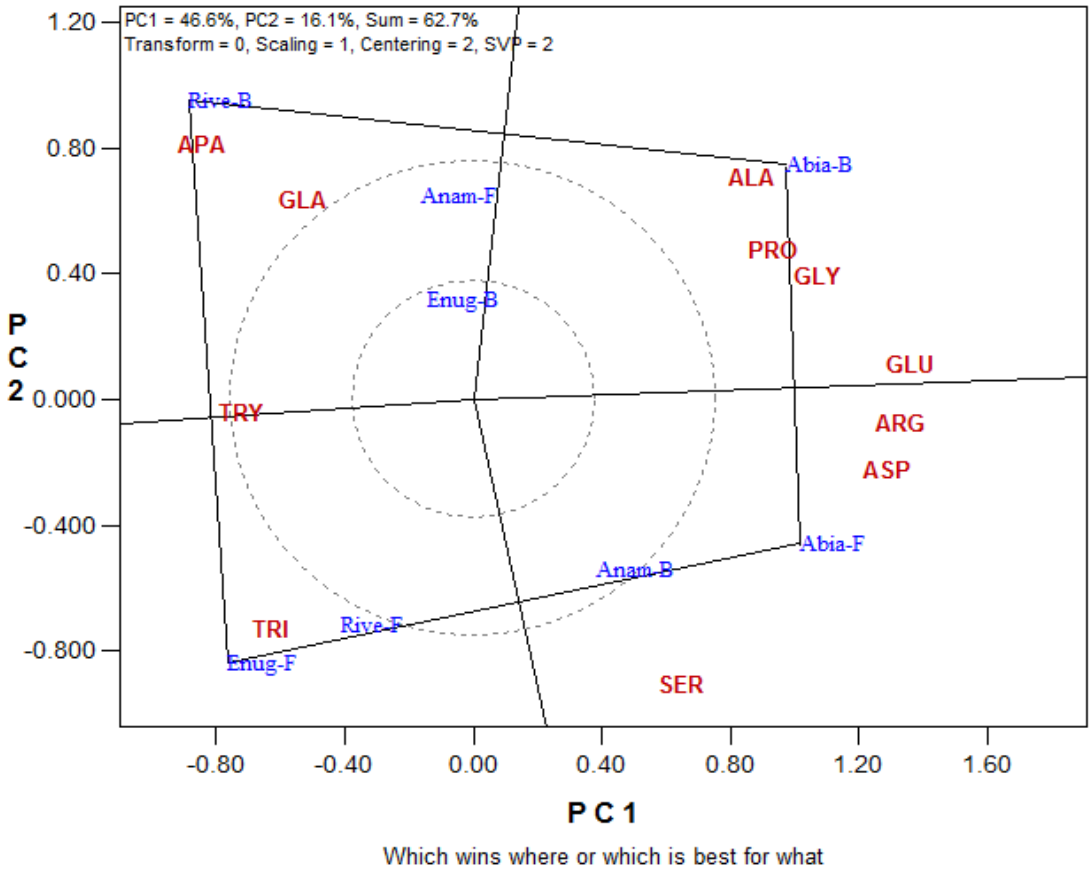
27

Ns = non-significant difference

Table 14: Interaction effects of location (states) and processing on the non-essential amino acid(ppm) composition of Plukenetia conophorums

Location (States)	Processing	Alanine	Arginine	Asparagine	Aspartic acid	Glutamic	Glutamic acid	Glycine	Proline	Serine	Trimethylisine	tyrosine
Abia	Boiled	2.47	0.683	1.42	0.102	5.42	0.37	2.75	1.22	1.06	0.103	0.77
	Fresh	2.08	1.164	2.38	0.080	5.42	0.37	1.39	1.08	1.60	0.2	0.82
Anambra	Boiled	0.91	0.432	1.99	0.236	3.87	0.43	1.47	0.70	1.73	0.061	0.79
	Fresh	0.84	0.237	0.36	0.444	1.52	0.18	1.36	1.27	0.81	0.164	0.42
Enugu	Boiled	1.64	0.161	0.57	0.274	1.20	0.81	1.78	0.43	1.39	0.054	0.73
	Fresh	0.90	0.067	0.14	0.194	0.80	0.50	0.78	0.64	1.19	0.519	1.48
Rivers	Boiled	1.75	0.053	0.25	0.546	1.29	1.25	0.72	0.59	0.98	0.183	1.64
	Fresh	0.72	0.034	0.15	0.184	0.48	0.17	1.45	0.50	1.32	0.291	0.79
$LSD_{0.05}$		ns	ns	1.03	ns	0.98	0.91	ns	ns	ns	ns	ns

Ns = non-significant difference



Ala = Alanine
Arg = Arginine
Asp = Asparagine
Apa = Aspartic acid
Glu = Glutamic
Gla = Glutamic
Gly = Glutamic acid
Prof = Proline
Ser = Serine
Tri = Trimethylisine
Try = Tyrosine

Fig 2: GGE ï Biplot analysis on the interaction effects of location (states) and processing on the non-essential amino acid composition of *P. conophorum*

Table 15 showed a non significant location effect on seedling emergence traits. The Rivers accession however performed better than the other two accessions. It had the earliest emergence (24.81) and the highest emergence percentage.

There were non- significant variation on the seedling growth parameters studied at first, second and third months after manure application (Table 16). There was a general increase in values of parameters measured with time. The performance of Abia accession was the best at the third month after manure application.

Manure significantly ($P<0.05$) influenced all the seedling growth parameters studied except the vine length at one and two months after mature application (Table 17). The plant girth, number of branches, number of leaves and vine length of the seedlings were improved by increasing the manure rate from 5 to 10 tonnes per hectare. The entire plant stature, the plant girth, number of branches and leaves and vine length were all significantly ($P < 0.05$) improved with an increase in manure rate over time. These improvements observed in seedling growth parameters with increase in manure rate means that better plant nutrition could lead to increased productivity of this plant.

Table 18 showed a significant ($P < 0.05$) location-by-manure interaction effect in most of the seedling growth parameters studied at the first month after manure application. The 10 tonnes application rate produced the best results in most cases. This was evident in the high values recorded for the plant girth, number of branches, number of leaves and vine length. However, O tonne and 5 tonnes application rates were superior to 10 tonnes in the number of branches, number of leaves and vine length in Anambra accession.

Data presented on Table 19 is the interaction effect of location and manure rate on the seedling growth parameters of *P. conophorum* studied at 2 months after manure application. There were non-significant interaction effect in most of the studied parameters. Plant growth parameters generally increased with incremental doses of manure in the three accessions. There was a significant ($P < 0.05$) interaction effect on the number of leaves per plant. In Abia and Rivers accessions, the 10 tonnes per hectare manure application rate produced the greatest number of leaves, followed by the 5 tonnes rate, whereas number of leaves did not vary much in Anambra accession.

Table 20 shows the interaction effect of location and manure rate on the seedling growth parameters of *P. conophorum* studied at 3 months after manure application. Almost the same trend was maintained as reported in Table 21. There were non-significant interaction effect in

most of the studied parameters. Plant growth generally increased with incremental doses of manure in the three accessions. There was a significant ($P < 0.05$) interaction effect on the number of leaves per plant. In all the accessions, the 10 tonnes per hectare manure application rate produced the greatest number of leaves. The seedlings growth in 5 tonnes and O tonnes of manure rates were inferior in most traits considered.

Table 15: Effect of location (states) on seedling emergence of *P. conophorum*

Location	Days to emergence	Emergence percentage (%)
Abia	25.78	25.0
Anambra	30.05	30.8
Rivers	24.81	33.3
LSD 0.05	ns	ns

NS = non significance difference

Table 16: Effect of location (states) on the Seedling Growth Parameters of *P. conophorum* over three (3) months after manure application

Location (States)	Plant Girth (mm)	No of Branches	No of Leaves	Vine length(cm)
1 month after manure application				
Abia	6.31	2.61	41.2	118.8
Anambra	6.61	3.13	42.5	108.2
Rivers	6.72	2.14	36.8	101.6
$LSD_{0.05}$	ns	ns	ns	ns
2 months after manure application				
Abia	8.44	4.11	57.6	148
Anambra	8.34	3.96	49.2	134
Rivers	8.96	4.67	56.2	140
$LSD_{0.05}$	ns	ns	ns	ns
3 months after manure application				
Abia	9.56	5.50	59.7	160.5
Anambra	8.01	4.91	44.9	149
Rivers	9.06	4.61	55.9	118.9
$LSD_{0.05}$	ns	ns	ns	ns

NS = non significance difference

Table 17: Effect of manure rates on the seedling growth parameters of *P. conophorum* over three (3) months after manure application

Manure (t/ha)	Plant Girth(mm)	No of Branches	No of Leaves	Vine length (cm)
1 month after manure application				
0	5.81	1.80	32.5	100.9
5	6.06	2.55	35.2	110.8
10	7.76	3.53	52.7	117.0
$LSD_{0.05}$	1.05	0.78	12.06	ns
2 months after manure application				
0	7.88	3.32	35.8	121
5	8.17	3.84	45.8	139
10	9.68	5.58	81.4	161
$LSD_{0.05}$	1.12	1.21	19.88	ns
3 months after manure application				
0	7.92	4.22	38.0	111.5
5	8.73	4.61	43.2	119.1
10	9.97	6.19	97.3	197.8
$LSD_{0.05}$	1.28	1.03	16.32	46.11

NS = non significance difference

Table 18: Interaction effect of location (States) and manure rate on the seedling growth parameters of *P. Conophorum* at 1 month after manure application

Location (states)	Manure (t/ha)	Plant Girth(mm)	No of Branches	No of Leaves	Vine length (cm)
Abia	0	5.25	1.50	32.0	107.0
	5	6.50	2.00	29.5	80.5
	10	7.17	4.33	62.0	169.0
Anambra	0	6.30	2.40	41.6	115.2
	5	5.52	3.99	52.8	137.4
	10	8.00	3.00	33.0	71.9
Rivers	0	5.88	1.50	23.7	80.5
	5	6.17	1.67	23.3	114.3
	10	8.12	3.25	63.2	110.0
$LSD_{0.05}$		ns	1.36	21.79	61.28

NS = non significance difference

Table 19: Interaction effect of location (States) and manure rate on the seedling growth parameters of *P. conophorum* at 2 months after manure application

Location (States)	Manure (t/ha)	Plant Girth (mm)	No of Branches	No of Leaves	Vine length (cm)
Abia	0	6.65	3.32	26.0	113.0
	5	9.00	3.84	56.0	130.0
	10	9.67	5.58	90.7	201.0
Anambra	0	8.50	3.20	50.4	160.0
	5	7.52	4.02	48.3	141.0
	10	9.00	4.67	49.0	99.0
Rivers	0	8.50	3.75	31.0	91.0
	5	8.00	3.50	33.0	145.0
	10	10.38	6.75	104.5	184.0
$LSD_{0.05}$		ns	ns	34.45	ns

NS = non significance difference

Table 20: Interaction effects of location (States) and manure rate on the seedling growth parameters of *P. conophorum* at 3 months after manure application

Location (States)	Manure (t/ha)	Plant Girth(mm)	No of Branches	No of Leaves	Vine length (cm)
Abia	0	7.50	5.00	32.0	106.1
	5	10.50	4.50	60.0	152.0
	10	10.67	7.00	87.0	223.3
Anambra	0	8.00	4.40	47.4	155.8
	5	7.02	4.99	35.2	138.6
	10	9.00	5.33	52.0	152.7
Rivers	0	8.25	3.25	34.5	72.5
	5	8.67	4.33	34.3	66.7
	10	10.25	6.25	99.0	217.5
$LSD_{0.05}$		ns	ns	28.26	ns

NS = non significance difference

Results presented on Table 21 presented indicate that the leaf dry weight and root volume were significantly ($P < 0.05$) influenced by effect of location. The Abia accession recorded the best results in all the seedlings dry matter yield across the locations followed by Rivers accessions.

Location has a non-significant effect on most of the dry matter partition studied (Table 22). Significant ($P < 0.05$) variability exists only in the dry matter partitioned to the leaf portion. Abia accession partitioned ($P < 0.05$) higher dry matter yield to the leaf portion than the other two accessions. The Abia accession also produced the highest dry matter yield

Table 23 shows a non-significant effect of manure on most of the dry matter yield estimated meaning that manure had similar effects on all the accessions. For example the 10 tonnes manure application rate partitioned the greatest portion of the biomass in all accessions except the root portion. At this manure rate, the plants needed little efforts to absorb nutrients hence its inferior in root yield. The leaf and vine dry matter yields were significantly ($P < 0.05$) influenced as the rate of manure is increased. The 10 tonnes manure rate produced the highest dry matter yield and partitioned a greater dry matter yield to the leaf and shoot portions. Root and vine components were higher in plants grown with O and 5 tonnes rates.

In Abia and Rivers accessions, the dry matter yield was highest in plants that received 10 tonnes of pig manure, but O and 5 tonnes applications rates produced higher dry matter yield in the Anambra Accession. Dry matter partitioning pattern did not vary significantly with manure application in the Rivers and Abia accessions. In the Anambra accession plants that received manure partitioned greater proportion of the accumulated dry matter to the leaf and vine components.

The interaction effect of location-by-manure on the dry matter partitioning significantly ($P < 0.05$) influenced the percentage dry matter partitioned to the leaf and vine portions and the total dry matter yield. The percentage dry matter partitioned to the shoot and root portions were not influenced by this interaction.

Table 21: Effects of location (states) on the seedling dry matter yield of *P. conophorum* at three (3) months after manure application

Location (States)	LDW(g)	VDW(g)	RDW(g)	Rt Vol (ml)
Abia	8.85	12.79	8.84	62.2
Anambra	5.28	9.17	6.64	35.4
Rivers	6.39	10.66	7.45	55.6
$LSD_{0.05}$	2.19	ns	ns	14.23

LDW = Leaf dry weight, VDW = Vine dry weight, RDW = Root dry weight, Rt Vol = Root volume, NS = non significance difference

Table 22: Effect of locations (states) on the dry matter partitioning of *P. conophorum*

Location (states)	LvDM%	Vine DM%	RtDM%	TDMY (g)
Abia	29.55	42.3	28.1	30.5
Anambra	25.75	42.1	32.1	21.1
Rivers	25.38	39.5	35.1	24.5
$LSD_{0.05}$	2.74	ns	ns	ns

NS = non significance difference
LvDM% = %Dry matter partitioned to the leaf portion
VineDM% = % Dry matter partitioned to the vine portion
RtDM% = %Dry matter partitioned to the root portion
TDMY(g) = Total dry matter yield

Table 23: Effect of manure rate on the dry matter partitioning of *P. conophorum*

Manure rate (tonnes/ha	LvDM%	Vine DM%	RtDM%	TDMY(g)
O	26.32	40.8	32.9	17.1
5	24.36	43.5	32.2	21.3
10	29.99	39.7	30.3	37.7
$LSD_{0.05}$	2.74	8.92	ns	ns

NS = non significance difference
LvDM% = Dry matter partitioned to the leaf portion
VineDM% = % Dry matter partitioned to the vine portion
RtDM% = %Dry matter partitioned to the root portion
TDMY(g) = Total dry matter yield

Table 24: Interaction effects of location (states) and manure rate on the dry matter partitioning of *P. conophorum*

Location (states)	Manure(t/ha)	LvDM%	Vine DM%	RtDM%	TDMY (g)
Abia	0	28.80	46.9	24.3	15.3
	5	31.61	40.7	27.7	25.5
	10	28.22	39.4	30.1	50.7
Anambra	0	23.96	39.6	36.4	23.6
	5	19.51	50.6	30.0	24.3
	10	33.78	36.1	30.1	15.5
Rivers	0	26.20	35.7	38.1	12.5
	5	21.95	39.2	38.9	14.2
	10	27.97	43.6	28.4	46.8
$LSD_{0.05}$		4.74	9.86	ns	15.45

NS = non significance difference
LvDM% = Dry matter partitioned to the leaf portion
VineDM% = % Dry matter partitioned to the vine portion
RtDM% = %Dry matter partitioned to the root portion
TDMY(g) = Total dry matter yield

DISCUSSION

Seed Physical Characters

The observed diversity in African walnut seed characters in this study could be ascribed in part to differences in the physical environment from where the accessions were collected. While Abia, Anambra and Enugu States are in the humid low land zone in the semi-deciduous forest and derived savanna vegetation, Rivers State is in the humid/Niger Delta zone which is in the forest water swamp and evergreen forest zones (Ojanuga, 2006).

Different locations may have differences in their climatic and edaphic factors. Varying weather and soil conditions may result to varying nutrients concentrations in the different parts of the plants including the seeds (Ugwuoke *et al*., 2001). Labanauskas *et al*., (1981) reported that water stress during flowering and pod-filling in cowpea reduced seed yield by 44 and 29 percent, respectively: and water stress during both flowering and pod-filling stages reduced seed yield by 67 percent. Since the locations from where the accessions were collected differed, it is possible that they may have experienced variable moisture deficit at various stages of the plant growth and as such may have resulted to the differences in the seed weight. Meanwhile, the seed and pulp weight, and seed volume were statistically similar.

Proximate Composition of the Kernel Pulp

The higher amount of carbohydrate found in the Rivers seeds may be attributed to more adequate rains (moisture) which is necessary for optimum photosynthesis (Roberts, 1976). According to Pearson (1976), plant food that provides more than 12% of its calorific value from protein is considered good source of protein. Therefore, *P. conophorum* seeds containing 20-23% protein meet this requirement. This result is similar to the findings of Ogunsua and Adebona (1983), Ige *et al*. (1984), Ogunsua (1987), Adebona *et al*., (1988), Adesioye (1991) and Odoemenam (2003)., A similar oilseed-African melon (*Cucumeropsis mannii* Naudin) collected from Cameroon shows that *C. mannii* has crude protein content of 31.4% and all the essential amino acids (Samuel, et al., 2011).

The higher protein values in Abia, Enugu and Anambra seeds agrees with Maranz and Wiesman (2004) who reported a pattern of increasing fruit protein content with increased dryness. Since soil nitrate is highly mobile and easily leached (Lombin, 1986), it is likely that

the wetter zone (Rivers State) afforded greater opportunities for leaching losses hence the disparity in observed seed protein content.

The crude fibre content of 4.47-5.18% is low when compared to *lpomea batatas* (7.20%), *Talinum triangulare* (6.20%), *Corchorus olitorus* (7.0%), and *Vernonia amagydalina* (6.5%) (Akindahunsi and Salawu, 2005; Antia *et al*., 2006). Meanwhile, Enujiugha and Ayedele ï Oni (2003) noted that the fibre content is within the range for most oilseeds. Adequate intake of dietary fibre can lower the serium cholesterol level, risk of coronary heart disease, hypertension, colon and breast cancer (Ishida *et al*., 2000).

The percentage of ash in a food material is an indication of its inorganic content (ENCARTA, 2005). The low ash content of *P. conophorum* (5.2-5.6%) is reflective of the low mineral content of the oilseed, although it has higher ash content than *Pentaclethra macrophylla* (Benth) with an ash content of 2.1 -2.8% (Enujiugha and Agbede, 2000). However, compared with some other legumes and oilseeds, conophor nut may not be an adequate source of essential minerals.

The moisture content (dry weight) of the seeds across the locations did not vary comparatively. The low moisture content of the seed shows that it could be stored for sometimes without deteriorating (Edem *et al*., 2009). The fat content of the seeds varied from 21-25.9%. The results revealed that *P. conophorum* is an oilseed that could be used in commercial vegetable production. Maranz and Wiesman (2004) reported fat content of 20-50% in shear butter. Comparing the fat content of *P. conophorum* observed in the present study with some other crops, it does not seem to indicate an unimpressive performance.

Enujiugha and Ayodele Oni (2003) reported some significant concentrations of phytates and tannins in raw conophor nut. Tannins usually form insoluble complexes with proteins, thereby interfering with the bioavailability. Phytates on the other hand chelate certain mineral elements, especially Ca, Mg, Fe and Zn, rendering them metabolically unavailable (Forbes and Erdman, 1983). Some of these anti-nutritional factors are known to leach into processing water during hydrothermal treatment. Baiyeri *et al.* (2011) reported that most of the mineral and proximate qualities of plantain were significantly influenced by the cooking method employed. Ash, fat, protein, dry mater content, iron and potassium contents were found highest in the roasted fruits, whereas, pro-vitamin A was lowest in fruits boiled directly in water. This observation could be as a result of leaching or volatilization losses through the cooking medium (water). Significant losses in various minerals including

Ca, Mg, P, Fe, Na and Cl were observed in the roots and fresh leaves of cassava as a result of boiling (Ebuehi, 2005). These reported cases of the effects of processing could probably explain variable nutrient concentration in the fresh and cooked seeds of *P. conophorum*.

Amino Acid Profile of *Conophorum* Seeds.

In this study, the twenty amino acids commonly found as components of proteins (McDonald *et al*., 1995), were determined. The results indicated that *P. conophorum* contains all the essential amino acids in adequate levels when compared with the recommended amino acid requirement for an adult (FAO/WHO, 2007). Oyenuga *et al*., (1971) reported high levels of cysteine (4.90%) and tryptophane (4.45%), and abundance of glutamic and aspartic acids and arginine in *conophor* nut. Similarly, Ogunsua (1988) reported that a conophor nut contains all the essential amino acids in adequate levels for nutrition except methionine. Also, similar results were obtained in African melon oil seed (*Cucumeropsis mannii* Naudin) by Samuel *et al*., (2011).

Destruction of essential amino acids varies between amino acids as well as between different cooking method. Levels of the essential amino acid lysine dropped significantly in sweet potatoes that were canned or dehydrated and not as severally in sweet potatoes that were baked (Hill Cindy, 1982). Cooking decreases lysine, histidine and arginine, the effect being highly significant. Tryptophan decreased, the effect being significant. In addition, protein solubility decreased with increased cooking time (Abdel-Hamid Youssef Abdel-Rahman, 1983). Also, the effect of domestic cooking on the content of amino acids and antinutrients of Phaseolus angularis, Phaseolus calcaratus and Dolichos lablab showed that heat treatment (30 min.) caused significant declines in apparent recovery of most of the essential amino acids except leucine and phenylalanine for P. calcaratus (Chi-Fai et al., 1997). Adeyeye (2000) reported that cooking raw groundnut enhanced the amino acid levels of Asparagine, Serine, Glutamine, Proline, Arginine, Alanine, Valine, Leucine and Phenylamine. He also stated that the following essential amino acids were reduced by both cooking and roasting; Lysine (15.9-27.6%), Histidine (4.23-16.5%), Threonine (40.1-60.6%), Methionine (38.0-63.4%) and Iso-leucine (13.3-31.8%).

It has been documented that most diets in developing countries are deficient in protein, especially, essential amino acids (Ladeji *et al*., 1995). Several studies have documented that children and adults consume less than 10% protein per day which is lower than the recommended daily requirement of 16% protein (Jansen and Howe, 1964; Byrd-

Bredbenner *et al.*, 2007). Results from the present study confirmed that protein in *Plukenetia conophorum* is of high quality because it has all the essential amino acids; indicating that this oilseed has the potential of meeting the protein needs of children and adults in developing countries.

Juvenile growth Phenology

There was apparent non-variability in days to onset of seedling emergence as influenced by location. The long days to seedling emergence observed in the seeds across the locations could be attributed to the physiology of the seeds. Loss of weight during storage and handling may have influenced dormancy of the seeds. Faiz *et al*., (1984) reported a loss of weight in witchweed seeds during storage. This may have led to poor food reserve which inhibited the growth and development of the seed embryo retarding germination. Moreover, the seeds were grown during the dry periods of October through April to June. Inadequate water supply during the growth period may have contributed to the poor seedlings emergence. The reduction in the seed viability might probably be associated with loss of moisture during storage.

Pig manure significantly ($P<0.05$) influenced the general performance and productivity of the crop. The findings of the study clearly showed gradual increases in growth parameters with incremental manure doses. There were increases in plant height, stem girth, number of branches and leaves with manure application. Interestingly, plants that received 10t/ha of pig manure performed better than the other levels (0 and 5 tonnes). This is in line with the work of Giwa and Ojeniyi (2004), who reported that higher rates of pig manure proved effective in ensuring better performance in tomato. Onweremadu *et al*. (2003) and Mbagwu *et al*. (1994) recorded increase in soil productivity as a result of pig manure application. Similar growth responses were observed in several other species including plantains (Obiefuna, 1990; Baiyeri and Tenkouano, 2007) and passion fruit (Ani and Baiyeri, 2008). Animal manure is a valuable source of crop nutrients and organic matter which can improve soil biophysical conditions thereby making the soil more productive and sustainable for food production (Baiyeri and Tenkouano, 2007).

The effect of location did not influence the plant girth, number of branches, number of leaves and vine length over the three months growth period after manure application. The slight variability indicates that the accessions were similar in behaviour. This slight variability is probably not genetic, but more of environmental effect.

There were significant ($P < 0.05$) location by manure interaction effect in most of the growth parameters studied in the first month after manure application. The 10 tonnes manure application rate produced the best results. At two and three months after manure application, there were non-significant interaction effect in most of the growth parameters measured. Plant growth generally increased with incremental doses of manure. In all the accessions, the 10 tonnes per hectare manure application rate produced the best plant growth. The 10 tonnes manure rate produced the highest dry matter yield and partitioned a greater proportion to the leaf and shoot portions. Root and vine components were higher in plants grown with O and 5 tonnes rates.

CONCLUSION AND RECOMMENDATION

The observed diversity in the accessions physical characters were probably not genetic but would be ascribed to differences in their physical environment which are climatic and edaphic factors. The higher amount of carbohydrate in the wetter zone (Rivers) seeds may be attributed to more adequate rains (moisture) which is necessary for photosynthesis. The protein content was higher in Abia, Enugu and Anambra accessions than Rivers. The fat content of Anambra and Abia accessions was comparatively higher than others which led to their higher energy content. Ash content was similar across the accessions. Anambra and Rivers accessions had similar and higher fibre values than the other two accessions. Results from this study confirmed that *P. Conophorum* contains all the essential and non-essential amino acids in adequate levels, indicating that the oil seed has the potential of meeting the protein needs of children and adults. The findings of the study clearly showed that the entire plant stature; the plant height, stem girth, number of branches, leaves and vine length were all significantly ($P < 0.05$) improved with an increase in manure rate. The 10 tonnes per hectare of pig manure performed better in the growth parameters than other levels (O and 5 tonnes).

The application of 10 tonnes of pig manure is recommended for African walnut growers particularly on the highly weathered oxisols of the humid tropics, not only for improved harvest but also for soil fertility maintenance and yield sustainability. Higher manure rates could be further evaluated on the yield and growth behaviour of African walnut.

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