SOCIETY FOR UNDERUTILIZED LEGUMES (SUL)
In conjunction with Genetic Resources Centre,
International Institute of Tropical Agriculture (IITA), Ibadan.

PRESENTS

4th Annual Conference and Stakeholders Meeting
(Virtual) of the Society

THEME:
UNDERUTILIZED LEGUMES:
TOWARDS SUSTAINABLE ECOSYSTEM DEVELOPMENT AND FOOD SECURITY

KEYNOTE SPEAKER:
Prof. Nwadiugo Esiobu
Florida Atlantic University, Boca Raton FL, USA/Founder, Applied Biotech, Abuja, Nigeria.

SUB-THEMES
• Sustainable Ecosystem Development • Sustainable Food Security • Plant Health and the Changing Climate

LEAD PAPER PRESENTERS:

Prof. Lateef B. Taiwo
Institute of Agricultural Research & Training, Obafemi Awolowo University, Ibadan, Nigeria.
Topic: Cropping Practices and their Effects on Environmental Sustainability

Prof. S. G. Ado
V. C. Al-Qalam University, Katsina, Katsina State, Nigeria.
Institute of Agricultural Research Samaru, Ahmadu Bello University, Zaria.
Topic: Fighting Hunger with Underutilized Legumes: A Paradigm Shift

Dr. Lava Kumar
Head of Virology Unit
International Institute of Tropical Agriculture, Nigeria.
Topic: The Impact of Climate Change on Plant Health

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WELCOME TO SOCIETY FOR UNDERUTILIZED LEGUMES (SUL)

I welcome you to this platform and duly congratulate everyone who has opted for love and research pursuit on underutilized crop species. The many awful descriptions of this group of crop species (abandoned, almost-exiting, endangered, lesser, lost, neglected, orphaned, stigmatized, traditional, undervalued, underutilized species etc.) were so frightening that many who would have ambitiously enlisted to make a career in its research were discouraged and opted for change of research focus. I am glad to represent the interest of the many minds who have found this forte a worthwhile abode for their professional development.

We are few, we are rare, we are spread across many disciplines and we are focused with the goal: to improving awareness, research and utilization of underutilized legumes in the tropical regions of the world. Agricultural Research Institutes with mandate for these crop species are rare and agricultural platforms or centres with hosting right or program for the security of their germplasm are rarely available, because they are unfortunate crop species. How I hold in high esteem the Genetic Resources Centre (GRC), International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria for her passion in holding, freely sharing accessions of these species and supporting worthwhile research on them. Permit me to straightly say: “Working on these group of crop species would have been practically impossible; but for GRC, IITA, Ibadan, Nigeria”.

The rising research concentration on African yam bean and the quantity of requests by researchers for its genetic materials prompted Prof. Abberton Michael, the Head, GRC, IITA, Ibadan Nigeria, to organize a symposium to harness the stakeholders in African yam bean research on 18 – 19th October, 2016 for review, updates and way forward. I am sure floating a society for underutilized legume was not a prospect of the meeting; however, SUL may not have been a society today if not for the symposium. The initiative that led to the birth of SUL was taken by Dr. (Mrs.) Catherine Nnamani. Hence, the 1st National Conference & Stakeholders Forum on African Yam Bean (AYB 2017) took place 24 -26th May, 2017 at Law Auditorium, CAS Campus, Ebonyi State University, Abakaliki, Nigeria. There, the executives were constituted and the name African yam bean Conservation and Utilization Association of Nigeria (AYBCUAN) was coined.

However, for the sake of accommodating other counterpart legumes we now bear Society for Underutilized legumes (SUL), a society that is devoted to improving awareness, research and utilization of underutilized legumes in the Sub-Saharan Africa. The second National Conference came up in July, 2018 at the Covenant University, Ota, Nigeria. SUL was coined to reflect on the forgotten relevance of the traditional leguminous crops and their role in nutrition and food security. Underutilized crop species are vast, trending across all possible crop classifications. This society considers all the so called “stigmatized” crops species, so, the name: Society for Underutilized Legumes does not in any way infer the exemption of any.

There are arrays of many valuable and available natural plant/crop substitutes to food fortification; such crops are traditionally and culturally linked to our food system. Nature is peaceful enough to make our health good for a prolonged life. This is what we showcase. I choose to conclude my remark with the words of Samberg (2016): “Actors working to celebrate the traditional crops and their management strategies with the hope of preserving both the diversity and cultural values has so much to do in the face of population growth, increased access to improved seed, cultural and political pressures”. We know that we have so much to do. Coming to know that the crop of our choice is labelled and hated could be frustrating, but as at today, their awareness has improved.

We are aware of the attending problems of our crops; with dedication we are poised to bring in the needed changes in these crops to facilitate attraction, adoption and see it come back to the meal tables. Our continuous pre-to-post harvest research and information dissemination programmes will achieve this over time.

Thanks sincerely,

Daniel B. Adewale, PhD
National President, SUL
ADRESS BY THE LOCAL ORGANIZING COMMITTEE CHAIRMAN AND EDITOR-IN-CHIEF

I wish to welcome you all to the occasion of the 4th National Conference and Stakeholders meeting of the Society for Underutilized Legumes. This year’s conference is the first of its kind to be held virtually, following the outbreak of the dreaded COVID-19.

The honor and privilege of serving as Chairman of the Local Organising Committee has been bestowed upon me, and I am deeply grateful. My intellectual interest in the sciences arose from my fascination with the development of knowledge and its effect on evolution. Man's search for knowledge has led him to seek out new ways to improve his life on planet Earth, but the more he tries, the more the planet wastes. However, alongside the habitat destruction that paved the way for man's search for industrialization, hunger raged. “Go forth!” says the great book. Multiply and conquer the world; however, how could man do so on an empty stomach?

More than 75 percent of the population in many developing countries is severely malnourished, according to the FAO's 2015 global hunger forecast. According to 2015 figures, at least 10% of the population in South America and many areas of Australasia are suffering from moderate to severe food insecurity. To mention a few factors, this is focused on declining global food supplies, erratic weather conditions, illegal land use, wars and violence, and global warming.

To begin, a strategic investment in crops, especially underutilized crops, is sufficient. Many people believe that growing legumes is one way to regenerate the earth, which has been deprived of nutrients and resources as a result of over-farming. Perhaps this isn't that far-fetched. Legumes can be intercropped – thereby enhancing other crop production, organic matter enhancement, and land reclamation and remediation, as a number of legumes, such as Alfalfa, have been used in Bioremediation Strains.

The Society for Underutilized Legumes, with its National Headquarters at the Genetic Resource Centre, IITA, Ibadan, was established on May 5, 2017 and registered with the Nigerian Corporate Affairs Commission on September 8, 2018, as part of our quota to enhance environmental conservation and human and environmental sustainability.

The Society encourages and promotes the growth and development of opportunities for researchers, entrepreneurs, and organizations (universities, polytechnics, colleges, research institutes, and agencies) to disseminate research output aimed at improving the economics, use, conservation, and general development of the underutilized legume on the African continent as part of its mandate. The Journal of Underutilized Legumes was established in order to ensure proper dissemination of its research findings as well as other related issues for the benefit of the global population.

Society for Underutilized Legumes (SUL) in conjunction with Genetic Resources Centre, International Institute of Tropical Agriculture (IITA), Ibadan presents the 4th Annual (virtual) Conference and Stakeholders meeting of the Society. The event which begins today and virtually (8th December, 2020) will feature keynote speeches, plenary session and other academic engagements. Prof. Nwadiuto Esiohu, Florida Atlantic University, Boca Raton FL USA/Founder, Applied Biotech, Abuja, Nigeria is the invited keynote speaker on the theme “Underutilized legumes: Towards sustainable ecosystem development and food security”. Other lead paper presenters are Prof. Lateef B. Taiwo, Institute of Agricultural Research & Training, Obafemi Awolowo University, Ibadan, Nigeria who will be speaking on “Cropping Practices and their Effects on Environmental Sustainability”. Prof. S. G. Ado, Vice-Chancellor, Al-Qalam University, Katsina, Katsina State, Nigeria & Institute of Agricultural Research, Samaru, Ahmadu Bello University, Zaria will address “Fighting Hunger with Underutilized Legumes: A Paradigm Shift” while IITA’s Head of Germplasm Health Unit, Dr Lava Kumar will speak on “The Impact of Climate Change on Plant Health”.
The e-conference is well placed to serve its function to the least, with participants drawn from experienced research scientists and research fellows, members of academia, entrepreneurs, and stakeholders in the industry of underutilized legumes, both locally and globally.

Arrangements have been made to showcase some of the leading findings in the presentations today and tomorrow in the Journal of Underutilized Legumes, with your permission. The Journal of Underutilized Legumes is an international peer-reviewed journal that is generally seeking to be one of the world’s most rated, well-indexed, and most globally circulated journal in the nearest future. SUL is dedicated to publishing original research articles as well as reviews and short communications on issues relating to research and development and other issues geared towards the improving the economics, utilization, conservation and general development of the underutilized legumes in the African Continent and the world at large. The Journal basically covers areas of Agronomy, Crop Development, Botany, Biochemistry, Animal Nutrition, Law, Commerce, Economics, and any other Field or Discipline wherein the research/discourse is majorly based on underutilized legumes.

As this is our first virtual outing, it is hoped that all observations and criticisms on the e-conference output and other allied issues would be channeled to the Local Organising Committee to improve future outings. This will certainly also help to improve our performance and services. Finally, membership of the Society for Underutilized Legumes is open to all and sundry with great enthusiasm for underutilized legumes.

Thank you for choosing SUL.

Welcome, again.

Dr. Beckley IKHAJIAGBE, FLS, FIPMD
LOC Chairman
### Committee Chairman

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DISCLAIMER:

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CHARACTERIZATION AND MICROBIOLOGICAL EVALUATION OF PROBIOTIC ISOLATED FROM BAMBARA GROUNDNUT

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Abstract

The study was carried out to isolate, characterize, and study antimicrobial sensitivity of lactic acid bacteria (LAB) from Bambara groundnut. Dried Bambara groundnut was fermented by spontaneous method for seven days and its pH, TTA (Total Titratable acidity) and microbial load monitored for each of the fermentation days. Seven acid-producing cultures were isolated from the sample, and isolates were further classified first by phenotype. Phenotypic and biochemical characteristics led to identification of three bacterial groups. These were followed by in vitro assessment of antimicrobial activity against enteropathogenic bacteria. The most abundant type of LAB distributed in the isolates of fermented Bambara groundnut was Lactobacillus delbrueckii, followed by Lactobacillus casei in two of the isolates. Lactobacillus brevis was found in the remaining two isolates. The growth pattern at different salt concentrations revealed that the isolates were salt tolerant at 2% and 4% while at 6.5% there was no growth. At pH 4.5 and 6.0, there were also growth. The strain evaluated showed in vitro antibacterial activity against five pathogenic microorganisms namely Escherichia coli, Salmonella sp, Shigella sp, Pseudomonas sp and Staphylococcus sp using agar well diffusion method. These results suggested that various LAB were present in Bambara groundnut. The microorganisms isolated were then freeze dried using a freeze drier and then kept at a low temperature in the refrigerator so as to preserve/store the organisms for further processes. This report thereby showed that Bambara groundnut, being an underutilized legume can serve as potential candidate for probiotic nutraceuticals.

Keywords: Lactic acid bacteria, Bambara groundnut, fermentation, probiotic, isolates, nutraceuticals.

1. INTRODUCTION

Current nutritional research is aiming at health promotion, disease prevention and performance improvement. The provision of human being with required nutritional ingredients depends on both how well the host is supplied with balanced foods and what state of intestinal microecology host has. Recent studies have shown that the application of natural fermentation processes to different food sources increases their nutritional quality and decreases the anti-nutritional compounds through the action of the microbial flora responsible for the fermentation process (Farnworth, 2003). Nearly all food fermentation are the result of more than one microorganism, either working together or in sequence, but growth is generally initiated by bacteria, followed by yeasts and then moulds (FAO, 2001; Adesulu and Awojobi, 2014). Among the notable bacteria involved in improving the nutritional values, sensory properties and functional qualities of food is lactic acid bacteria (LAB). Traditional fermentation of vegetables, fruit and grains most often include a lactic acid fermentation involving many different species of LAB, that are active at different stages of the fermentation process. LAB produce several antimicrobials, including
organic acids, lactic acid, acetic acid and formic acid (Messens and De Vuyst, 2002). Additionally, among other functions, LAB improve the natural texture of products like yoghurts, ice cream and sour cream due to exopolysaccharidase production (De Vuyst and De Vin, 2001) and contribute to the aroma and flavor of fermented products. They have capacity to acidify the food, resulting in a tangy lactic acid taste and produce aromatic compounds from, for instance, amino acids upon further bio-conversion (Soro-Yao et al., 2014). With these various functions performed by LAB, they may be enhanced by implantation into various forms for specific purpose. The formulation of the dietary supplements, functional foods or herbal products into marketed medicinal products is known as “nutraceuticals”; a term which combines “nutrition” and “pharmaceuticals” (Noha, 2013). Among various nutraceuticals of major importance in diseases prevention is probiotics (Smith and Charter, 2010).

The human body, on the other hand, although created with a proper ratio of good to bad bacteria, frequently alters the ratio of bacteria as a result of today’s modern lifestyle. These lifestyle factors can affect the population of sensitive healthy bacteria. The use of antibiotics inhibits not only bad bacteria but also good bacteria, thus permitting bad bacteria, to invade the gastrointestinal tract (GIT) and multiply in high numbers that disturb the delicate balance between the good and bad bacteria. This balance also is upset by the use of oral contraceptives, steroids, exposure to radiation through x-rays and radiation therapies, excessive consumption of chlorinated water, the consumption of refined sugars and other refined foods, poor digestion, poor elimination of waste, stress, an unhealthy diet, e.t.c. (Tannock, 1995). Scientists have determined that when the delicate balance between good and bad bacteria is adversely altered, the body is overloaded with toxins and diseases.

In order to have increase access to beneficial bacteria, there is need for artificial techniques to absorb the negative effect imposed by modern life-style. Biotechnology applications in the food processing sector, therefore, target isolation, characterization, identification and manipulation of beneficial microorganisms involved in food fermentation. Isolation is done to obtain pure bacterial cultures. Pure culture is essential in the study of the morphology, physiology, biochemical characteristics, and susceptibility to antimicrobial agents of a particular bacterial strain. These life microorganisms when administered in adequate amount confer a health benefit on the host and they are termed probiotic bacteria (Kalui et al., 2010). Bacterial strains most commonly used as probiotics belong to the genera *Lactobacillus* and *Bifidobacterium*, but other organisms are also applied such as *Lactococcus* and *Enterococcus* (Enujiugha and Badejo, 2017). *Lactobacillus* species from which probiotic strains have been isolated include *L. acidophilus*, *Lactobacillus johnsonii*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus gasseri*, and *Lactobacillus reuteri*. *Bifidobacterium* strains include *Bifidobacterium bifidum*, *Bifidobacterium longum*, and *Bifidobacterium infantis* (Heller, 2001).

Probiotics can be delivered commercially either as nutritional supplements, pharmaceuticals or foods. A large number of probiotic products are available in the market in the form of milk, yoghurts, cheeses, ice creams, dairy spreads and fermented soya products. Also, special freeze-dried pharmaceutical dietary preparations are available in the form of tablets (FAO, 2002). Together with prebiotics, probiotics are often consumed as functional foods, demonstrated to be effective for the treatment or control of several diseases. Prebiotic substances, such as lactulose, lactitol, xylitol, inulin and certain non-digestive oligosaccharides, selectively stimulate the growth and activity of, for example, bifidobacteria in the colon (Ouoba et al., 2010). Other notable health benefits of probiotic products have been proposed including antimicrobial, antimutagenic, anticarcinogenic, antihypertensive properties, reduction of allergic symptoms, reduction of diarrhea and stimulation of the immune system (Scheinbach, 1998; Shah, 2000).

To achieve the benefit from their injection, the probiotics microorganisms must reach the intestine alive and in sufficient concentration, surviving the harsh conditions found during the flow through the
gastrointestinal tract (Heidebach et al., 2012). It has been reported that populations of $10^6 - 10^7$ colony forming units (CFU)/g (or CFU/ml) in the final product are established as therapeutic quantities of probiotic cultures in processed foods (Talwalkar et al., 2004; Ukeyima et al., 2010), reaching $10^8-10^9$ CFU, provided by a daily consumption of 100 g or 100 mL of food (Ying et al., 2013). At the point of consumption, the level of probiotics in the food should be $\geq 10^6$ CFU/ml. The survival of probiotic microorganisms in food products is strongly influenced by pH and post-acidification, which may occur during refrigerated storage of fermented products. Other factors such as production of hydrogen peroxide, oxygen level, temperature and food matrix also affect the microbial viability (Dave and Shah, 1997).

Probiotics have been used for centuries in food fermentation and dairy products are the main carriers of probiotic bacteria to human, as these products provide a suitable environment for probiotic bacteria that support their growth and viability (Fernanda et al., 2016). The world-wide probiotic foods are milk based and very few attempts are made for the development of probiotic foods using other fermentation substrates such as legumes. With an increase in the consumer vegetarianism in the developing countries coupled with the low purchasing power of low-income people especially in sub-Saharan Africa to consume much dairy product needed for probiotics, there is also a demand for the vegetarian probiotic products. Nondairy probiotic products have also shown a big interest among vegetarians and lactose intolerance customers. Potential sources of vegetarian probiotics are legumes due to their large distribution and important nutritive value.

Bambara groundnut (*Vigna subterranean*), an indigenous African legume known to have been domesticated in West Africa from its presumed wild ancestor (Olaleye et al., 2013). Bambara groundnut (BGN) contains about 61.3% carbohydrate, 20.7% protein and 6.0% oil (Yusuf et al., 2008) and is used as main food, snacks, relish and medicine, and has high ceremonial value (Oluwole et al., 2007). Despite its high and balanced protein content, it has little direct use because of low satiety value caused by high oil content, poor digestibility, long cooking time and persistent bitterness. Other known factors for its under-utilization is the presence of anti-nutritional factors such as tannins and trypsin inhibitors, and it has poor milling characteristics, as it does not dehull easily (Barimalaa et al., 1997). It is not part of the national or international food baskets.

As an essential step to harness the probiotics nutritional benefit of Bambara groundnut and to have increase access to beneficial probiotic bacteria locked in it due to its low satiety value, it was then subjected to fermentation process to enhance the quality parameters and functional properties of the raw material. Fermentation with well-characterized starter cultures, yeast or lactic acid bacteria (LAB), is a potential means to improve the palatability and process ability of the whole-meal flours (Salminen et al., 2005). This research therefore, targets isolation, characterization, identification and antimicrobial sensitivity of microorganisms involved in fermentation of Bambara groundnut.

2. MATERIALS AND METHODS

Dried Bambara groundnut (*Vigna subterranean*(L) Verdc) was obtained from International Institute of Tropical Agriculture, Ibadan, Nigeria. All chemicals used were of analytical grades and they include: Sodium hydroxide (NaOH), sodium chloride (NaCl), potassium dihydrogen phosphate (KH$_2$PO$_4$), hydrochloric acid (HCl), sodium citrate, trichloroacetic acid (TCA), ethanol (C$_2$H$_5$OH), iodine crystals, potassium dichromate (VII) (K$_2$Cr$_2$O$_7$), d-biotin, safrarin, distilled water, peptone water, glucose phosphate e.t.c. Media to be used are de Man, Rogosa Sharpe (MRS) broth (BIOMARK, India), nutrient agar, agar agar, muller hinton agar, bile salt agar, phenolphthalein indicator.

2.1. Bambara Groundnut Fermentation

Two hundred grams (200 g) of the sorted seeds was weighed using an analytical balance (AS 60/220.X2, India) into a sterile container and was subjected to spontaneous fermentation in which seeds were
soaked in 400 mL of distilled water in the ratio of 1:2 w/v in a clean plastic bowl with cover and allowed to ferment for seven days at 28 ± 2 °C. Fresh samples were taken from the fermented BGN for microbiological analyses. In addition, pH and total titratable acidity (TTA) of the samples were also measured. Fermentation was done in duplicate.

2.2. Determination of pH

The pH was measured using a standardized/ calibrated water test meter (Hangzhou Qi Wei, instrument Co, Ltd.). The pH meter was first calibrated using buffer solution of pH 4, pH 6 and pH 9 in order to determine the accuracy of the pH meter to be used. 10 mL of the fermented substrate was measured into a beaker and the calibrated pH meter was dipped into the measured substrate and allowed to be stabilized before the result displayed on the meter was taken and recorded. The pH was checked at 0, 1, 2, 3, 4, 5, 6 and 7th day (Prescott et al., 2008).

2.3. Temperature (°C)

The temperature of the fermentation medium was determined using a well calibrated Standard clinical thermometer. The thermometer was dipped inside the soaked Bambara groundnut and the temperature was checked at 0, 1, 2, 3, 4, 5, 6 and 7th day (Prescott et al., 2008).

2.4. Determination of Total Titratable Acidity (TTA)

Fermented substrate (10 mL) was measured into a conical flask and 90 ml of distilled water was added to it. Sodium hydroxide (0.1M) was poured into the burette to fill the burette and 2 drops of phenolphthalein indicator were carefully dropped into the substrate in the beaker and shaken. The initial volume of the alkaline in the burette was noted. The alkali was run briskly into the beaker containing the substrate and indicator which was consistently shaken until there was a sharp change of the substrate to pink. The volume of the alkali in the burette at this point was noted to enable the determination of the volume of base (titre value of alkali) used. The TTA was checked at 0, 1, 2, 3, 4, 5, 6 and 7th day and was expressed as the amount of NaOH used (ml). The Titrable acidity was then calculated using the formula in Equation 1 (Prescott et al., 2008).

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\begin{align*}
\%TTA = & \frac{\text{Average base titre (ml)} \times \text{molarity of base (mol)} \times 100}{\text{Volume of sample (ml)}}
\end{align*}
\]

2.5. Preparation of Culture Media and Diluent

The media used for both isolation and identification of microorganisms were nutrient agar (Oxoid), De Man Rogosa Sharpe (MRS) agar, bile salt agar and Muller Hinton agar. They were prepared according to manufacturer’s instruction and sterilized by autoclaving at 121 °C for 15 min at 760 psi and cooled to 45 °C before dispensing into sterile Petri dishes and left to solidify. The diluent was prepared by adding 0.85 g NaCl to 100 ml of distilled water and 9 ml each was measured into dilution bottles which were covered tightly and sterilized in an autoclave at 121 °C and 15 mmHg for 15 minutes and cooled to 45 °C.

2.6. Isolation of Lactic Acid Bacteria and Determination of Total Viable Count

Isolation of microorganisms was done by the method described by Harrigan and McCance, 1990. After sterilization of agars and diluents, the workbenches were sterilized using ethanol and cotton wool and a spirit lamp was lighted, placed on the work bench to create an aseptic environment. The sterile Petri dishes were arranged and labeled on the sterilized work bench, the cooled dilution bottles were also placed on the work bench and serial dilution was done at 3-fold dilutions (100, 10⁻¹, 10⁻² and 10⁻³). A quantity of 1ml dilution factor 10⁻² was inoculated via pour plate method on MRS agar by adding 0.3 mL of lactic acid. The Petri dishes containing the substrate and medium were incubated in an anaerobic jar which was put into an incubator at 37 °C for 48 h. Discrete colonies from each plate were sub cultured on fresh bacteriological media until pure cultures were obtained. The pure isolates obtained
were preserved in a prepared broth bottle containing nutrient agar slants and stored at 4 °C in a refrigerator before biochemical tests were further carried out.

### 2.7. Identification and Characterization of Isolates

Colonies were selected randomly and were characterized using morphological and biochemical tests such as Gram stain, Sugar fermentation, motility, catalase, e.t.c. Bacterial isolates were identified with reference to Cowan and Steel’s Manual for the Identification of Medical bacteria (Cowan, 1985) and Bergey’s manual of determinative Bacteriology (Holt, 1994). The bacterial isolates identification was based on colony morphology, cultural characteristics and biochemical tests using the methods described by Cheesebrough (2006).

### 2.8. Morphological Characterization

The morphological characteristics such as colour, shape, elevation, edge, consistency, colony surface and pigmentation of the distinct colonies were observed physically and noted with the aids of Bergeys Manual of Systematic Bacteriology (Holzapfel and Wood, 1995). The isolates subsequently screened by streaking on the appropriate sterilized agar plate till a pure colonies appears. Pure cultures of presumed lactic acid bacteria were aseptically picked and maintained on appropriate double strength sterilized media (deMann Rogosa Sharpe and extract agar slants) and broths with 10% glycerol, which were then stored in refrigerator at 4°C.

### 2.9. Biochemical Characterization

Distinct pure colonies observed to be dominant were checked for gram reactions using microscopic examination for cell morphology. Some of the key tests for identification include the following:

**Gram stain**

The method used was that described by Harrigan and McCance (1990).

**Spore stain**

The malachite green staining method was used. The staining was carried out as described by Harrigan and McCance, 1990.

**Motility test**

For this test, the hanging drop technique was employed and the technique was carried out as described by Harrigan and McCance, 1990.

**Sugar fermentation tests**

Each of the isolates was tested for its ability to ferment a given sugar with the production of acid and gas or acid only. The growth medium used was peptone water and the method used was that described by Prescott et al., 2008.

**Catalase test**

The method employed here was that described by Jagbir, 2011.

**Methyl red test**

The methyl-red test was employed to detect the production of acid during fermentation of glucose such that the pH of a culture (Prescott, 2008).

**Voges Proskauer (VP) test**

This test was carried out to detect acetoin in a bacterial broth culture. It is used to determine if an organism produces acetyl methyl cabinol from glucose fermentation. This test was carried out by the method described by Olutiola et al., (2000).
Indole test

The test was carried out using the method described by Olutiola, Famurewa and Sontag (2000). Oxidase test, starch hydrolysis test and endospore test were carried out using the method of Cheesbrough, 2006.

Coagulase test was carried with the method described by Olutiola et al., 2000.

Citrate test

Simon citrate agar of 2.4 g was dissolved in 100 mL of distilled water. About ten millilitres (10 mL) of citrate medium was dispensed into each tube and covered, then sterilized and allowed to cool in a slanted position. The tubes were inoculated by streaking the organisms once across the surface. A change from green to blue indicates utilization of the citrate.

Casein hydrolysis

Skim milk of 0.25 g was weighed and added to 25 mL of already prepared nutrient agar broth (24 g/1000 L) in a conical flask. The flask was shaken to ensure even distribution of the milk in the agar broth. 5 ml of the mixture was taken with a sterile pipette into three sterile and well labelled test tubes respectively. The test tubes were sealed with cotton wool wrapped with aluminium foil. The test tubes were then sterilized by autoclaving at 121 °C for 15 min under 15mmHg pressure. They were then cooled to about 40° C and inoculated with the isolates leaving one of the test tubes un-inoculated (serving as control). They were then incubated at 37 °C for 48 h.

Growth at Different Temperatures

This was done by a modified method of Buchanan and Gibbons (1974). Overnight isolated cultures were inoculated at 10% (v/v) in MRS broth and incubated at 15 °C, 37 °C and 45 °C for 24 hours. Total populations were determined by the streaking method, incubating the plates at 37 °C for 48 hours.

2.10. Tolerance to Bile salt

Prepared bile salt agar was poured on sterile, well-labeled Petri-dishes. The plates were gently swirled for proper distribution. The agar content of the plates was allowed to solidify. It was then inoculated with the isolates by streaking method and incubated in an anaerobic condition at 37 °C for 24-48 h.

2.11 Antibacterial Activity of Probiotic isolates

The antagonistic activity of the probiotic isolates against some pathogenic bacteria (Escherichia coli, Salmonella typhimurium, Staphylococcus sp., L. monocytogenes, Klebsiella sp., B. subtilis, P. aeruginosa, E. faecalis, and Staphylococcus aureus) was investigated using agar well diffusion. The test pathogens were seeded on a sterile molten nutrient agar. After solidification, wells were bore on seeded agar plates, and the probiotic-isolates were introduced into the wells. The plates were first incubated at 34 °C for 60 min to allow the test material to diffuse in the agar, and were then incubated at 37 °C for 18 h. After incubation, the diameters of the resulting clear zones (zones of inhibition) were measured from the center of the well in centimeters (cm) and later converted to milliliters (mL).
The flow diagram for fermentation process and eventual harvesting is shown in Figure 1.

**Figure 1**: Flowchart for the harvesting process of isolates from Bambara groundnut

**Bambara Groundnut** *(Vigna subterranea (L.) Verde)*

1. Cleaning and Sorting
2. Fermentation
3. Isolation
4. Characterization
5. Cell harvesting (centrifugation)
6. Freeze-drying

3. RESULTS AND DISCUSSION

3.1. Determination of pH, Total Titratable Acidity (TTA) and Microbial Load

The pH, titratable acidity (TTA) and temperature values of fermented Bambara groundnut in relation to fermentation days are shown in Figures 2 - 4. During the fermentation days, it was observed that the pH which has the initial value of 7.2 (neutral pH) decreases with the fermentation days but on the seventh day, an increase in the pH was observed. In the studies involving other legumes, Achinewhu (1987) and Ogbadu and Okagbue (1988) observed a steady increase in pH with fermentation. This difference may be due to the unique chemical composition of Bambara groundnut which is higher in starch and lower in protein than the other legumes. The fermenting microorganisms might have started fermentation by hydrolyzing available carbohydrate to acid before embarking on extensive proteolysis. Amino acids produced on proteolysis might have degraded to ammonia which may be responsible for the rise in pH. The gradual development of ammonia odor which became prominent on seventh day of fermentation was in agreement with the observed increase in pH. For this reason, the fermentation of Bambara groundnut was stopped on the 6th day.

The titratable acidity of the fermentation increases with increase in fermentation days, but on the seventh day a slight decrease was noticed. The results for both pH and titratable acidity show that acidity of the fermentation medium increases which may be related to the production of lactic acid by the fermenting microorganisms. However, the change that occur in the pH and TTA at seventh day can be linked with the microorganism succession in an environment where the lactic acid producing microorganisms begin to die of as a result of the unfavourable condition of their metabolite (lactic acid). Temperature of fermentation initially decreases from 31.5 °C of day one to 28 °C on the second day but remains constant (i.e. at 28 °C) throughout the remaining days of fermentation. The pH, TTA and temperature are shown to be correlated at different level of significance as shown in Table 1. The correlation between pH and temperature (R value of 0.882), %TTA and days of fermentation (R value of 0.948) are significant at 0.01 level. This implies that there is strong direct proportionate relationship between the pairs i.e. increase in value of one leads to increase in value of its pair. However, the correlation between % TTA and pH showed 0.05 level of significance with negative R value that implies the pair is inversely proportional.

The bacterial load of fermenting Bambara groundnut revealed that the raw sample of Bambara groundnut on the De Man Rogosa Sharpe (MRS) agar used for the isolation of lactic acid bacteria
showed no growth. After 24 hours of spontaneous fermentation, the highest bacterial count, $1.26 \times 10^5$ CFU g$^{-1}$ was observed on MRS agar which later decreased significantly ($p<0.05$) to $4.70 \times 10^4$ CFU g$^{-1}$ after 72 hours of fermentation.

Figure 2. Relationship between pH and fermentation rate

Figure 3. Relationship between %TTA and fermentation
Increase in microbial growth was observed after 48 h of fermentation which might be as a result of the metabolic activities of the organisms that produce carbon dioxide and water (Barimalaa et al., 2007). The gradual decrease observed in the lactic acid bacteria load as the fermentation progress may be due to the changes observed in the acidity of the fermenting medium. Abiola and Oyetayo (2016) reported a decrease in microbial load after 48 h of fermentation of cocoyam.

The decrease observed in bacterial load after 48 h of spontaneous fermentation may also be as a result of some bioactive substances which might have produced an inhibitory effect on other organisms involved in the fermentation. This is in line with the report of Ouoba et al. (2003, 2007), Kalui et al. (2010) and Chen (2013). Increase in microbial growth was observed after 6th day of fermentation which may be as a result of the metabolic activities of undesirable organisms.

### Table 1. Correlation between pH, % TTA and temperature

<table>
<thead>
<tr>
<th>Days</th>
<th>Temperature ºC</th>
<th>pH</th>
<th>%TTA(*10^-2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-0.556</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-0.732</td>
<td>0.882**</td>
<td></td>
</tr>
<tr>
<td>%TTA (*10^-2)</td>
<td>0.948**</td>
<td>-0.574</td>
<td>-0.829*</td>
</tr>
</tbody>
</table>

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

### 3.2. Identification of Isolates

Each of the isolates was subjected to Gram stain test and were examined under light microscope. Each of the isolates gave blue-purple colour with staining; hence they were all Gram-positive bacteria. The isolates from MRS plates were rod-like bacilli with short or rounded ends. The morphological characteristic is shown in Table 2. Spore staining and motility test were negative for all isolates on the MRS plates. This implies that none of the isolates formed spores and are non-motile. All of the isolates
on MRS plates were tested for catalase. None of them showed catalase activity. The three isolates were observed to be negative to both methyl red and Voges Proskauer indicating that they cannot produce acetoin as the end product of glucose metabolism. The three isolates were observed to be negative to coagulase test as shown in Table 3. By implication, they cannot coagulate blood and this characteristic shows that they are non-pathogenic. The yellow colouration observed during indole test indicates a negative result. This shows that the three isolates cannot produce the compound indole. Furthermore, Casein hydrolyses test for the three isolates were observed to be negative which indicates that they cannot produce the enzyme casease that breakdown casein. The result of starch hydrolysis as shown in Table 3 indicates that the three isolates were not able to hydrolyze the tested starch sample as indicated by the formation of a blue colour in the presence of iodine. The biochemical identification result for isolates on MRS plates is shown in Table 3.

Table 2. Morphological characteristics of isolated organism

<table>
<thead>
<tr>
<th>Morphological characteristics</th>
<th>Probable organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purple, short rod, irregular shape</td>
<td>Lactobacillus sub sp</td>
</tr>
<tr>
<td>Purple, short rod, regular shape</td>
<td>Lactobacillus sub sp</td>
</tr>
<tr>
<td>Purple, short rod but scanting</td>
<td>Lactobacillus sub sp</td>
</tr>
</tbody>
</table>

Table 3: Biochemical identification of isolates on MRS plates

<table>
<thead>
<tr>
<th>Biochemical Identification</th>
<th>Isolate 1</th>
<th>Isolate 2</th>
<th>Isolate 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphology</td>
<td>Bacilli</td>
<td>Bacilli</td>
<td>Bacilli</td>
</tr>
<tr>
<td>Gram stain</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Spore stain</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Motility</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Catalase</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Methyl red and Voges Proskauer</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Coagulase</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Indole</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Casein hydrolyses</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

The triple sugar iron (TSI) test revealed that isolate 1 was able to ferment all the sugars as shown to be positive in Table 4, with no production of gas and acid. Isolate 2 was not able to ferment high molecular weight lactose and sucrose but fermented low molecular weight glucose with no gas and acid production. Isolate 3 was able to ferment all the three sugars without gas and acid production. In addition to TSI test result, all the three isolate were able to ferment monitol and were all negative for xylose and citrate.

Table 4. Result of TSI and other sugar fermentation

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Lactose</th>
<th>Sucrose</th>
<th>Glucose</th>
<th>Gas</th>
<th>Acid (H2S)</th>
<th>Mannitol</th>
<th>Xylose</th>
<th>Citrate</th>
<th>Most probable Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolate 1</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td><em>Lactobacillus casei</em></td>
</tr>
<tr>
<td>Isolate 2</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td><em>Lactobacillus delbruekii</em></td>
</tr>
<tr>
<td>Isolate 3</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td><em>Lactobacillus brevis</em></td>
</tr>
</tbody>
</table>

3.3. Tolerance to bile salt

The isolates were observed to grow at different concentration (2%, 4% and 6.5%) of bile salt agar after 24-48 hours of observation. Isolate 1 and 2 showed growth at a 2% and 4% concentration but no growth
at 6.5%. Isolate 3 showed growth at 2% but no growth at 4% and 6.5% concentration. This indicates that they have tolerance to bile salt. The implication of this is that each of the isolates can survive the harsh condition of the gastrointestinal tract of both human and animal.

3.4. Growth at Different Temperatures

The isolates growth pattern at different temperatures was distinguishing within the groups as no growth was observed at 10 °C and 15 °C. However, all the isolates grew at 30 °C. This can imply that all the isolates are mesophiles. All isolated strains presented better growth at 37- 45 °C and small growth was observed at 15 °C. Based on the research carried out by Kandler and Weiss, (1989) and Cherubin, (2003), Lactobacilli grow at temperatures between 25-53 °C, where the optimal temperature generally is between 30 °C and 40 °C. According to Bergey’s Manual (Buchanan and Gibbons, 1974), the heterofermentative species grows at 45 °C and does not present any growth at 15 °C.

3.5 Antimicrobial Sensitivity

Observation reveals that the two isolates tested (1 and 2) have inhibitory capacity to destroy some pathogenic organisms over the antibiotic control (ciprofloxacin) used as shown in Figure 5. The first isolate has the highest inhibitory capacity on Salmonella sp and Staphylococcus aureus over control and isolate 2 but has no observable inhibition on Shigella sp. On the other hand, the second isolate has the widest clear zone on E. coli and Shigella sp than control and isolate indicating that it has the highest inhibitory capacity over the pathogen. However, there was no observable clear zone on Staphylococcus aureus.

![Figure 5: Antimicrobial Susceptibility.](image)

4. CONCLUSION

In this study, Lactic Acid Bacteria (LAB) was isolated, characterized, identified and stored from spontaneously fermented bambara groundnut. The results showed that isolates have probiotic properties like acid-bile tolerance and antimicrobial activity against food spoilage organisms and gastro intestinal tract (GIT) pathogens. These make them potential candidates for probiotic product, which can be beneficial to human and can then be used for nutraceuticals in the production of encapsulated products for the enhancement of immune system and proper functioning of the human GIT.
REFERENCES


FORTIFICATION OF SORGHUM FLOUR WITH *ADENOPUS BREVIFLORUS* BENT SEEDS AS INFANT FORMULA SUBSTITUTE

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Abstract

*Adenopus breviflorus* bent seeds is an underutilized protein rich seed while sorghum is one of the commonest grains used for making infant weaning food in Nigeria as palp or “Ogi”. However, this grain has been reported to be very high in carbohydrate which makes it not sufficient to give balanced diet needed by growing babies. Sorghum flour was therefore fortified with *Adenopus breviflorus* bent seeds which is a plant protein source as to prevent kwashiorkor in children. Fermented dried sorghum flour (FDSF) was fortified with roasted *Adenopus breviflorus* benth seed flour (RASF) in the ratios 90:100; 80:20; 70:30 and 60:40 respectively. The proximate and mineral compositions of the formulated mixes were determined and compared with those of some market baby food samples. Proximate analysis of sorghum shows that it has very high percentage of carbohydrate (69.8%) with protein and fat contents being (19.64%) and (4.10%) respectively. On the other hand, the proximate analysis of RASF shows that it has high percentage of fat (47.6%), high protein content (28.17%), and low carbohydrate content of (9.96%). The compounded products from fortification of sorghum with RASF shows that the carbohydrate content decreases while protein and fat content increased with increasing percentage of RASF and corresponding decrease in sorghum ratio. The organoleptic tests of the different products were also carried out. On the average, the sorghum-Adenopus mixtures were highly accepted due to the pleasant taste, flavor, colour, and odour brought about by the presence of RASF. The mineral content of these winning mixes compare favourably with those of some market samples as to meet the needs of the growing baby at much lower cost.

Key words: Fortified, Mixes, Sorghum, Mineral, *Adenopus breviflorus* benth, Protein.

INTRODUCTION

In Nigeria, one major factor for the highly prevalent kwashiorkor (a protein deficiency syndrome) is the widespread use of a low protein source marsh or pap (ogi) for weaning. Several commercial weaning mixes are being marketed nowadays but they are too expensive for the population of low socio-economic status, especially those in the rural areas. It is therefore imperative to formulate inexpensive weaning mixes of low cost and high nutritive value from locally, readily available sources to meet the nutritional demands of a growing infant. In Nigeria all mothers breast feed their babies at least for three months of life, unless there are circumstances which prevent this, (Bamiro et al 1993). Some infants sustain excellent growth over the first nine to twelve months of life despite breast milk being their main source of nutrition, (Bamiro et al, 1993). Most infants however fail to show satisfactory growth after four months of life, unless their intake is supplemented with other foods. Most of those infants formulae (milk) substitutes are expensive and not easily affordable by the average income mothers. (Bamiro et al 1993). Proteins are needed for the formation of enzymes, hormones, antibodies, haemoglobin, and antitoxins. They help to control the alkalinity of the blood and the osmotic pressure of the blood and the vessel (Uddoh, 1980). Protein malnutrition in the first two years of a child’s life may result in, permanent detrimental effect or mental development, learning ability and behaviour (Uddoh, 1980). It is
there for important to formulate inexpensive weaning mixes of low cost and high nutritive value from locally, readily available sources to meet the nutritional demands of a growing infant.

**MATERIAL AND METHOD**

**Preparation of Materials**
Sorghum seeds were bought from Oja Oba in Akure. The Sorghum seeds were handpicked to remove stones and other contaminants and fermented naturally by soaking in clean water for three days. The fermented sorghum was washed, drained and oven dried at 90 °C – 100 °C until the grains were properly dried and then milled to fine flour and kept in air tight bottle for further use and analysis. *Adenopus breviflorus benth* seeds were provided by Professor A. A. Oshodi who have done the identification and several works on the seed. The *Adenopus breviflorus benth* seeds were roasted in a clean vessel, milled into fine flour to produce RASF and stored in a labelled, clean, dried amber bottle.

**Compounding of Weaning Mixes**
Weaning mixes of sorghum and *Adenopus breviflorus benth* seeds were made by weighing 90:10, 80:20, 70:30, 60:40 grams of sorghum to *Adenopus breviflorus benth* seeds into four different clean and dried amber bottles and labelled as A1, A2, A3 and A4 respectively.

**Proximate Analysis**
Standard procedures as recommended by Association of Official Analytical Chemists were used for Sample treatment and analysis (AOAC, 1990). The Proximate analysis was carried out in triplicates and the results are in % dry weight of flour.

**Mineral Analysis and Organoleptic test**
The mineral analysis was carried out according to Oshodi, 1990. The elements determined were Sodium, Potassium, Calcium, Magnesium, Iron, Zinc and Copper.

**Organoleptic Test**
The organoleptic test of the compounded weaning mixes was carried out to determine the acceptability of the samples. The samples were given to some people to rate the following parameters: taste, colour, odour, texture, flavour and general acceptability. The remarks given were graded as follows:
- 5 = Excellent
- 4 = Very good
- 3 = Good
- 2 = Fair
- 1 = Poor

The result is the average of the different results of members of the panel.

**RESULT AND DISCUSSION**

The result of the proximate analysis of sorghum and *Adenopus breviflorus benth* seeds in Table 1 shows that sorghum has high carbohydrate content (69.88%) and low fat and protein contents of 4.10% and 13.60% respectively. On the other hand, *Adenopus breviflorus benth* seeds has higher fat (47.65%), protein content (28.17%) and lower carbohydrate value (9.96%). This shows that sorghum when given to infants as ogi (infant weaning palp) cannot meet the daily required nutritive value needed for growing babies. RASF has higher fat and protein contents of 47.60% and 28.17% respectively than FDSF (Table 2).

Table 4 shows manufacturers’ specifications for the nutritive values of some market samples of baby weaning food samples. The carbohydrate contents of these market samples ranged from 55.40% to 66.40%, the protein content ranged from 11.90% to 17.00%. The fat content ranged from 9.00% to
27.70 %, moisture content ranged from 2.00% to 4.00 % and ash content ranged from 2.30 % to 3.80 %. Comparing these values with the proximate analysis of the fortified sorghum with RASF (Samples A), the carbohydrate content ranged from 50.90 % to 67.90 %, protein content ranged from 17.60 % to 21.70 % and fat ranged from 10.00% to 21.00 %, moisture content ranged from 2.03 % to 8.08 %; showing that RASF mixes with FDSF gives good qualities in terms of the protein and fat contents meet the required nutrients for baby weaning food. However, the results of the data for samples A1 to A4, shows that there is gradual decrease in carbohydrate contents, while there is approximately increase in protein and fat contents of the samples, Table 2.

The sorghum- *Adenopus breviflorous benth* mixes also show similar trend as seen in Table 2. Carbohydrate content ranged from 60 % to 72.67 %, protein (9.00 to 16.50) %, fat (6.00 to 9.03) %. This will help to meet the daily protein requirement of 2.2g/100 Kcal for 3 months old infants and 1.6g/100 Kcal for infants over 3 months (Niels, 1994)

Considering the mineral content in Table 3, sodium ranged from 95 mg/ 100g to 113 mg/ 100 g, calcium (263 to 361) mg/ 100 g, showing that they have high mineral content needed for the formulation of strong bones and other functional growth required by babies. These also conform with the manufacturers’ specifications in Table ---. Sodium (85.00 – 245.00) mg/100g, calcium (390 - 600) mg/100g and potassium (570 - 740) mg/100g.

Table 1: Proximate Composition of Fermented, Dry Sorghum flour (FDSF) and Roasted *Adenopus breviflorous benth* Seeds Flour (RASF) (%)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sorghum flour</th>
<th><em>Adenopus breviflorous benth</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>13.64</td>
<td>28.17</td>
</tr>
<tr>
<td>Fat</td>
<td>4.10</td>
<td>47.65</td>
</tr>
<tr>
<td>Fibre</td>
<td>1.30</td>
<td>4.90</td>
</tr>
<tr>
<td>Moisture</td>
<td>1.30</td>
<td>4.43</td>
</tr>
<tr>
<td>Ash</td>
<td>1.45</td>
<td>4.15</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>69.88</td>
<td>9.96</td>
</tr>
</tbody>
</table>

Table 2: Proximate composition of Compounded Sorghum flour fortified with Roasted *Adenopus breviflorous benth* seed flour (RASF)

<table>
<thead>
<tr>
<th>Parameter (%)</th>
<th>Ratio of Sorghum to RASF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>15.75</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>67.95</td>
</tr>
<tr>
<td>Fat</td>
<td>10.30</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>2.15</td>
</tr>
<tr>
<td>Ash</td>
<td>1.84</td>
</tr>
<tr>
<td>Moisture</td>
<td>2.03</td>
</tr>
</tbody>
</table>
Table 3: Mineral content of Fortified Sorghum Mixes (ppm)

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Na</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>Cu</th>
<th>Zn</th>
<th>Fe</th>
<th>Mn</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>95</td>
<td>400</td>
<td>280</td>
<td>34</td>
<td>0.53</td>
<td>4.60</td>
<td>16</td>
<td>0.90</td>
</tr>
<tr>
<td>A2</td>
<td>101</td>
<td>410</td>
<td>289</td>
<td>31</td>
<td>0.23</td>
<td>4.30</td>
<td>16</td>
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<td>460</td>
<td>304</td>
<td>29</td>
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<td>3.90</td>
<td>15</td>
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<td>463</td>
<td>361</td>
<td>28</td>
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<td>4.10</td>
<td>17</td>
<td>1.10</td>
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Table 4: Proximate Composition (%) and Mineral Content (ppm) of Baby Weaning Market Samples

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Trade Names of Weaning Food Sample</th>
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<tr>
<td></td>
<td>Nutrend</td>
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<td>Protein</td>
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<td>Ash</td>
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<td>Moisture</td>
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<td>Fat</td>
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<td>Carbohydrate</td>
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<td>Na</td>
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<td>K</td>
<td>570</td>
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<td>Ca</td>
<td>390</td>
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<td>7.00</td>
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<td>Mn</td>
<td>ND</td>
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References


COMPARATIVE NUTRITIONAL PROFILING AND MINERAL PROFILING OF FLOURS FROM RAW AND NATURALLY FERMENTED SWAMP PEA SEED GROWN IN NSPRI ONIREKE IBADAN

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Abstract

This study was conducted to profile and evaluate the nutritive value of Raw and Fermented swamp pea flour from its seed grown and harvested at Nigerian Stored Products Research Institute (NSPRI) Ibadan, Oyo State, Nigeria. Dried seeds of swamp pea harvested were divided into two portions, one portion was subjected to natural fermentation and the other portion was not fermented (i.e., raw). Both the raw and fermented seed were dried using NSPRI multi-crop dryer and grinded into fine flour. Flours from Raw and naturally fermented seeds of swamp pea were analyzed for their nutritional composition. Proximate composition for the raw flour sample gave the following results: 6.00 ± 0.01 % moisture, 33.58 ± 0.00 % protein, 11.48 ± 0.01 % Crude fat and 23.30 ± 0.02 % carbohydrates. The moisture content of the fermented flour of swamp pea was higher than that of the raw flour. The crude protein value of fermented flour was observed to be higher than the value for the raw flour. Protein content of the fermented flour was observed to have considerably increased and statistically (p<0.05) different. Comparatively, the order of mineral elements present in the raw flour in (mg/100g) was K >Ca> Na> P> Zn > Fe> Cu>Pb>Ni > Cd, while the order in fermented sample was K>Ca> Na> Fe> P> Zn> Cu>Pb> Ni and Cd which was not detected. Both the raw and fermented swamp pea seed samples were viably dense with mineral matter, particularly potassium, Calcium, Sodium, Phosphorus, Zinc, Iron and Copper which were found to be in abundance in seed. Hence, with these observed appreciable nutritional and mineral profile from the two treatments, this revealed that both raw and fermented swamp pea seed sample would contribute immensely to good nutritional status in biological systems, if proper attention is geared towards its further utilization.

Key word: Swamp pea seed, mineral, nutritive value, proximate

1. INTRODUCTION

Swamp pea is a large, very tall annual legume scientifically known as Sesbania sesban and known as Zarmakee by the Hausas in the Northern part of Nigeria (Ishola et al., 2018). It grows and thrives in warm weather locations. Swamp pea seeds sown were propagated by seed and scarified by dipping seeds in water heated to below boiling for 30 seconds to improve germination. The seeds were sown in April 2019 and harvested in June 2019. The stalks of this plant reach a height of 8 to 12 feet tall with long slender seed pods that shattered when mature within two months and four weeks. Although, location of a plant, could also affect the nutritional status of plants, because soil nutrients status and management has
potentials to affects nutrients concentration in plant parts consumed as food and feed (Kihara et al., 2020). Swamp pea plants grows well in temperate region and under eight (8) to twelve (12) weeks after germination, the plants are ready to plow. Swamp pea is valuable as a green manure crop because of its tremendous growth (Evans and Rotor, 1987; Evans, 2001). Swamp pea is particularly suited for planting in orchards because it protects the young trees during hot weather, and due to its nitrogen fixing potentials, enriches the soil at the same time. The plant grows in the wide and the seed are often wasted due to less importance attached to it. Considering the economic value of this seed in India where about eight tins cost as much as 1000 Indian Rupee which is approximately equal to 5,200 Naira equivalent of Nigeria currency (www.amazon.in/RK-seeds). Economically, swamp pea seed utilization in Nigeria is next to useless because the seeds are allowed to waste away and need to be properly utilized. Presently, the seed is out of stocks on major plant seed sales shops in India and Nigeria could grab this opportunity to become major supplier of this seed to the outer world.

In developing countries like Nigeria, legumes are probably the second most important source of food next to cereal grains and are consumed worldwide as a major source of protein (Onwurafor et al., 2014; Ishola et al., 2018). Thus, legumes are good source of protein in the population where the staple food largely consumed is rich in carbohydrate and less of protein (Onwurafor et al., 2014). This leguminous seed, Swamp pea seed, a cream colored seed with a smooth seed coat at maturity is one of the non-popular underutilized legumes in Nigeria. Up till now swamp pea seeds flour is not yet utilized adequately in this part of the world. Due to the hard nature of the seed coat, there is need for safe processing method to make the seed nutrients bio-accessible and more utilizable. Therefore, profiling the nutritional potentials of this seed by evaluating the nutritional quality of its flour and creating more awareness for it utilization would be a step in the right direction. Nutritional evaluation of a plant seed reveals its functionality (i.e., nutritional profiles) that as well determines its possible utilizations. Therefore, this study aimed at comparatively profiling the nutritional and mineral composition of raw and fermented swamp pea seed locally grown at Nigerian Stored Products Research Institute, Ibadan Zonal Office,

2. Materials and Methods
2.1. Source of plant materials and authentication
The swamp pea seeds were grown and collected at the premises of Nigerian Stored Products Research Institute, Ibadan Zonal Office (7°23’36”N 3°52’36”E) in Ibadan North West L.G.A of Oyo State and identified at the Department of Forest Conservation Protection of Forest Research Institute of Nigeria, Ibadan Nigeria.

2.2. Preparation of dry milled flour from Raw Swamp pea seeds ((Sesbania sesban))
Dried Swamp pea seeds were washed by mixing the sample with distilled water at a ratio of 1:2 w/v, drained and dried using NSPRI Multi-crop dryer. The dried grains were milled, sieved, packaged and stored in air tight container until further use for experiments.

2.3 Preparation of dry milled flour from fermented Swamp pea seeds:
Natural fermentation was carried out by mixing the sample with distilled water at a ratio of 1:2 w/v. The sample was withdrawn at period of 72 h (i.e., steeped into water for three days). After the fermentation period, each flour sample was transferred to aluminum dish and dried using NSPRI Multi-crop dryer) for 1hr. The fermented dried samples were finely ground, sieved and stored in polyethylene bags at 4°C for subsequent analysis. (Ishola et al., 2018)
2.4. **Proximate Determination**

The moisture, ash, crude fibre, crude fat and crude protein present in Swamp Pea seeds were determined based on the method described by Association of official Analytical Chemist AOAC (2010).

### 2.4.1 Determination of moisture content of samples

The moisture content was determined according to AOAC (2010). About 5g each of the processed flour samples were weighed into clean empty Petri dish ($W_1$) of known weight. The weight of the Petri dish and the sample was taken and recorded as ($W_2$). The petri dish was placed in a preset oven at 105° C for 3h in order to reduce the moisture. The samples were removed and kept inside desiccators for 30 minutes so that it can cool. After cooling the dish was brought out and weighed on weighing and weighed on a weighing balance. The weight was recorded as $W_3$.

#### Calculation

The moisture content of the sample is calculated using the following equation

$$Moisture(\%) = \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1} \quad (1)$$

Where:

- $W_1$ is weight of empty petri dish
- $W_2$ is the weight of petri dish and sample before drying
- $W_3$ is the weight of petri dish and sample after drying

### 2.4.2 Determination of ash content

The ash contents of the flour samples was determined as described by AOAC (2010) method. About two grams (2g) of the flour sample was weighed ($W_1$) into clean, dried pre-weighed crucible and the weight was recorded as ($W_2$). The crucible with the flour was placed on a muffle furnace and the temperature was increased to 550°C for 3 h in order to allow the sample to ash. After ashing the crucible was continued until a white ash was obtained. The crucible with the was cooled in a desiccator and then weighed ($W_3$).

$$\text{Ash} = \frac{\text{weight of ash}}{\text{weight of sample}} \times \frac{100}{1} \quad (2)$$

$$\text{Ash} = \frac{W_2 - W_3}{W_1} \times \frac{100}{1}$$

Where:

- $W_1$ = the weight of cleaned, dried, ignited and cooled crucible
- $W_2$ = the weight of the sample and crucible before ashing
- $W_3$ = the weight of crucible and sample after cooling in an airtight desiccator

### 2.4.3 Determination of crude fat content

The fat content was determined according to AOAC (2010) method. About five grams (5 g) each of the flour samples were weighed into a pre-weighed filter paper, weighed dried in an oven and tied with thread. The filter paper contain the flour sample was placed in the receiver of the soxhlet apparatus. Hexane of boiling point range 60-68 °C was used as solvent for the extraction; also a 500 ml round bottom flask was filled to ¾ with the solvent. The flask was fitted to the soxhlet apparatus with a reflux condenser and placed in an electro mantle heater. Extraction took place as the solvent refluxed several time and continue for 4 h until the condenser was detached. The filter paper containing the defatted
sample was removed and dried to a constant weight in an oven at 50°C. The difference in weight before extraction and after extraction was recorded in order to obtain the value of the fat extracted.

\[
\% \text{Fat content} = \frac{\text{Weight of fat extracted}}{\text{Weight of sample}} \times 100 \quad (3)
\]

### 2.4.4 Crude fibre content determination

The fibre was determined by AOAC (2010) method. Ten grams (10 g) of the flour samples was weighed and transferred into a 500 ml conical flask. About 200 ml of 1.25H_2SO_4 was added and the sample was boiled for 30 mins. After boiling, the mixture was poured into white cloth under gentle suction using a butcher funnel and rinsed well with hot distilled water. The material was transferred into a conical flask containing 200 ml of 1.25 NaOH and boiled for another 30 mins while shaking gently to avoid spillage. The sample solution was filtered and washed with ethanol and repeatedly with ether to remove any remaining fat. The residue was transferred into a clean dried crucible, oven dried and cool in the desiccators and re-weighed (W_2). The crucible was placed in the muffle furnace (model 48000 J.R, furnaces and oven PVT Ltd.) at 450°C for 2h, cooled in the desiccator and re-weighed (W_3).

\[
\% \text{Crude Fibre} = \frac{\text{weight of the digested sample} - \text{weight of the ashed}}{\text{Weight of sample}} \times \frac{100}{1} \quad (4)
\]

Where: 
W_1 = Weight of the sample
W_2 = weight of digested sample + crucible after ove drying
W_3 = Weight of sample + crucible after ashing

### 2.4.5 Crude protein content determination

The crude protein was determined using the method of AOAC (2010) method. The method is in three stages

(i) Digestion stage

This stage involves digestion of the samples. Five grams (5g) of the flour sample was weighed (W_1) into 50 ml micro- Kjeldal flask and digested with 10 ml concentrated, H_2SO_4 using a digestion catalyst mixture (0.5 g Selenium). The flask was heated on an electro-thermal heater until a clear solution was obtained. The digest was allowed to cool after which the digest was diluted into a 100 ml distilled water standard flask. The sample was transferred to the kjeldahl distillation unit.

(ii) Distillation

It involves the steam distillation of the digest which 10 ml of 40% NaOH solution was added to release the ammonia. Three drops of mixed indicator was added to the receiving flask containing 10 ml of 2% boric acid solution to give a pink colour solution. The sample was distilled until about 50 ml of the distillate was collected in the receiving flask. A colour change from red wine to green was observed, indicating the presence of ammonia

Equation: Sample + Conc. H_2SO_4 → (NH_4)_2SO_4

\[(NH_4)_2SO_4 + 2NaOH → 2NH_3 + Na_2SO_4 + 2H_2O\]

The collected ammonia forms complex with the boric acid as

\[\text{NH}_4^+ + \text{H}_2\text{SO}_4 \rightarrow 2\text{NH}_4^+ + \text{B}_3\]

(iii) Titration Stage

It involves the titration of resulting solution in the conical flask against 0.1M HCl solution until a colour change from green to red wine is obtained including the end point.

\[\text{NH}_4^+ + \text{HCl} + \text{H}_2\text{O} \rightarrow \text{NH}_4\text{Cl} + 2\text{H}_2\text{O}\]

Percent nitrogen was calculated as:
% Nitrogen = \frac{\text{Titre value} \times \text{molarity of acid used} \times 0.014 \times 100}{\text{Weight of the sample}} \quad (5)

Where 14 is nitrogen molecular weight and 6.25 is the dilution factor.

% Crude protein = \% \times 6.25 \text{ (conversion factor)}

\subsection*{2.4.6 Carbohydrate content determination by difference}

The carbohydrate content of the flour samples were determined by difference AOAC (2010) method. The carbohydrate content was given by the expression below.

\% \text{Carbohydrate} = 100(\% \text{ash} + \% \text{crude protein} + \% \text{fat} + \% \text{crude fibre} + \text{moisture}) \quad (6)

\subsection*{2.5 Mineral Determination}

Five grams (5g) of the flour samples was digested using 15ml of HCl and 5ml of Nitric acid (3:1). Mineral compositions of the digested samples (seeds and pods) were determined using Shimadzu AAS 6800 Atomic absorption spectrophotometer according to the method of Hernandez et al. (Hernandez et al., 2004).

\subsection*{2.6 Energy Value Determination}

The gross energy (MJ/kg) of the optimized and control flours were determined by using Gallenkamp Adiabatic bomb calorimeter (Model CBB-330-01041; UK). 1 g moisture-free sample of optimized flour samples were taken respectively. The sample were combusted using the Adiabatic CC01/M3 Microprocessor Bomb Calorimeter (Toshniwal Technologies Pvt. Ltd., India). Pure and dry benzoic acid (Merck, Germany) was run as standard. Energy content of flour was calculated as follows: \( W = \frac{(H \times M) + E_1 + E_2}{T} \), where \( W \) = Energy equivalent of calorimeter in calories per degree centigrade, \( H \) = Heat of combustion of standard benzoic acid in calories per g, \( M \) = Mass of standard benzoic acid sample in g, \( T \) = Corrected temperature rise in degrees centigrade, \( E_1 \) = Correction for heat of combustion of threads in calories, and \( E_2 \) = Correction for heat of combustion of firing wire in calories. Each sample was run in triplicate, taking the mean of all runs as final energy content. (McGill et al., 2004)

\subsection*{2.7 Statistical analysis}

Data were analysis using Independent-Sample T-test and results were presented as means (±SD). Values of \( p < 0.05 \) were considered as statistically significant as described by Yalta and Talha (2008).

\section*{3.0 RESULTS AND DISCUSSION}

The result of the proximate composition of the flour from the raw (RSPS) and fermented Swamp pea seeds (FSPS) are presented in Table 1.

The moisture content of the raw and fermented flour sample were 6.00 and 8.15 g/100g respectively which were observed to be significantly difference at \( p < 0.05 \). Fermentation process was observed to increase the moisture content of the fermented sample. The value of the Crude protein obtained for both raw and fermented flours sample also were observed to be 33.58 and 35.67g/100g respectively. The protein value of fermented flour was observed to be higher than the value for the raw flour which could be implicatively to be due to increase in microbial mass during fermentation causing extensive hydrolysis of the protein molecules to amino acids and other simple or lower molecular weight peptides (Ishola et al., 2018). It may also be attributed to the degraded structural proteins that are integral part of the microbial cells (Tortoral et al., 2002). The increase in crude protein in fermented Swamp pea seed flour (SPSF) is also an indication that fermentation process has improved the protein content of the SPSF and therefore a possible alternative source of high quality plant protein for animal and man that cannot afford animal protein in their diets. The ash content values were also of interest both in the raw...
and fermented state. The ash content value of 6.96 obtained for FSPSF was fairly higher than 5.20g/100g value obtained for the RSPSF. The increase in ash content may be due to poor leaching of soluble minerals into the processing water during the fermentation period or the fermenting microorganisms might not be able to use it for metabolic activities (Tortora et al., 2002; Ishola et al., 2018).

The crude fat values for the flour samples of SPSF were also determined. The crude fat value obtained for RSPSF is 11.48g/100g, while that obtained for FSPSF is 10.55g/100g. The values which was observed to decrease as a result of fermentation process were significantly difference at p<0.05 and agreed with the 2018 earlier report of Ishola et al. on the influence of fermentation processes on mineral composition of Zamarkee seed. Decrease in crude fat value of FSPSF signals a poor or in extensive breakdown of large molecules of fat into simple fatty acids (Sanni and Ogbonna, 1991; Ishola et al., 2018). This observation could also be due to the utilization of oxidized lipids to generate energy for the growth and cellular activities of the microorganism (Achi and Okereka, 1999).

The crude fibre values for both flours were 20.44 and 18.24 g/100g respectively. RSPSF had the highest fibre content than FSPSF crude fibre content. The decreased levels of fibre content in fermented Swamp pea seed flour agreed with the result of (Eka, 1980; Oboh, 2006; Butt and Batool, 2010) that fermented foods such as legumes has lower fibre content. This observation could be due to the utilization of oxidized lipids to generate energy for the growth and the cellular activities (Sanni and Ogbonna, 1991).

The carbohydrate value determined by difference for RSPSF and FSPSF were 23.30 and 23.03 g/100g respectively. A slight decrease in the carbohydrate content of the fermented flour sample could be attributed to the conversion of oligosaccharides to simple sugars or the utilization of the carbohydrate nutrient in the legume as source of energy by the fermenting microorganisms for growth, metabolism and as carbon source in order to synthesize cell biomass (Madigan et al., 2002; Oladunmoye, 2007). The energy value in Kcal was also determined for the flour samples of swamp pea seed and the results obtained were presented in Table 1. The energy value of the fermented flour (FSPSF) of 27.42Kcal was observed to be higher than that of the raw flour (RSPSF) of 25.15Kcal which were significantly (p<0.05) difference. Fermentation process was observed to increase the energy value of the flour.

The Protein content, Ash content, moisture contents and energy value of the flours were observed to increase with fermentation process and are statistically p<0.05 difference, whereas crude fibre, crude fat, and carbohydrate were not favored by the fermentation process as values were observed to decrease with the process as earlier reported in 2018 by Ishola et al. The result have shown that swamp pea seeds in the raw and fermented forms have enough nutrients to satisfy protein-energy requirements of populations in the developing countries that rely much on starchy staples.
Table 1: Proximate composition and energy value of flours from raw and fermented swamp pea seed

<table>
<thead>
<tr>
<th>Sample</th>
<th>Proximate composition (g/ 100g)</th>
<th>Energy Value (Kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Moisture</td>
<td>Crude protein</td>
</tr>
<tr>
<td>RSPSF</td>
<td>6.00 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.58 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>FSPSF</td>
<td>8.15 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.67 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Results are mean values of triplicate determination ± standard deviation. Mean value within the same column having the same letter are not significantly different at P<0.05.

Where,

RSPSF - Raw Swamp Pea Seed Flour.

FSPSF - Fermented Swamp Pea Seed Flour.
The mineral composition (mg/100g) of flours from the raw and fermented Swamp pea seed are presented in Table 2. The Calcium (Ca) content of the flours from RSPS and FSPS were 125.14 mg/100g and 168.35 mg/100g respectively (Table 2). The fermented flour sample, FSPS had the highest calcium content of 168.35 mg/100g, which is significantly different (p < 0.05) from the calcium content value of the raw sample. The Ca content of the fermented sample was observed to increase significantly. Ca principal physiological function in humans apart from its role in maintaining the skeleton is as an essential intracellular messenger in cells and tissue throughout the body.

The phosphorus contents of the flours for both RSPSF and FSPSF were 17.44 and 11.76 mg/100g respectively. Fermentation process was observed to significantly decrease the phosphorus value in FSPSF. The iron (Fe) content, Sodium (Na), Potassium content (K) and Zinc Content (Zn) for both the raw and fermented sample were (15.96 and 12.10 mg/100g), (35.58 and 23.80 mg/100g), (635.76 and 503.82 mg/100g), and (14.38 and 11.25 mg/100g) respectively (Table 2). More also, the Copper (Cu) content, Cadmium (Cd) content, Lead (Pb) content, Manganese (Mn) content and Nickel (Ni) content for both raw and fermented flour of swamp pea seed were (4.33 and 2.46 mg/100g), (0.00 and 0.00 mg/100g), (0.08 and 0.00 mg/100g), (17.44 and 11.76 mg/100g), and (0.08 and 0.00 mg/100g), respectively (Table 2).

A comparative increase in calcium content of FSPSF was observed through the fermentation process. Although, the value of potassium content in the fermented sample was otherwise (i.e., lesser compared to its value in the raw sample). But, based on the general observations of the results, the level of calcium and potassium were significantly higher in both flour produced from the Swamp pea seed, and this indicate one of the possible potentials of the flours to be active against elevated blood pressure, especially for hypertensive patients (Adepoju, 2007; Adepoju and Adeniji, 2008; Ishola et al., 2018). Also, of note is the trace of heavy metals observed in the raw flour sample (i.e., could be due to level of heavy metals contamination in the soil where the seed was harvested from) which through fermentation process was not detected in the fermented sample (FSPSF) (Roth and Townsend, 2003; Oboh, 2006; Oladunmoye, 2007). Considering the observed results of the mineral elements evaluated from the flour samples, both FSPSF and RSPSF could be formulated into instant flours for convalescence and in the formulation of baby foods as these categories of humans require high levels of minerals for growth and repair.
Table 2: Mineral Composition of the flours from Raw and Fermented Swamp pea seed

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mineral Composition (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ca</td>
</tr>
<tr>
<td>RSPSF</td>
<td>125.14±0.02^a</td>
</tr>
<tr>
<td>FSPSF</td>
<td>168.35±0.17^c</td>
</tr>
</tbody>
</table>

Results are mean values of triplicate determination ± standard deviation. Mean value within the same column having the same letter are not significantly different at P<0.05.

Where, \(168.35±0.17^c\ 125.14±0.02^a\)

RSPSF - Raw Swamp Pea Seed Flour
FSPSF - Fermented Swamp Pea Seed Flour
4.0 Conclusion

The evaluation of the two swamp pea seed flours in terms of the mineral elements, energy values and the proximate composition results of both the raw and fermented sample revealed good sources of healthy foods. The investigation further revealed that the fermented sample flour had a better nutritional profile in terms of protein and ash content which is implicative as a possible alternative source of natural protein and micronutrients, if properly employed in food formulations. Also, natural Fermentation, a biological food process was observed to have a considerable reduction effect on the value of all the mineral elements (Fe, Zn, Cu, Pb, Ni, Cd, P, Na, and K) understudied, except for calcium element level which increased considerably through the process. Furthermore, the investigation also revealed that both the raw and fermented swamp pea seed samples were dense with micronutrients, particularly potassium, Calcium, Sodium, Phosphorus, Zinc, Iron and Copper which were found to be in abundance in the seed. Further to this, Calorie- protein value of both the raw and fermented flour signals a possible staple in confronting the menace of calorie-protein deficiency. Therefore, with these observed appreciable nutritional and mineral profile from the two treatments, this revealed that both raw and fermented swamp pea seed sample would contribute immensely to good nutritional status in biological systems. Hence, a possibility of becoming one of the reliable future supplements to improve the nutritional value of food for both human and animal diet in the nearest future.

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R&D.


THE MORPHOLOGICAL GROWTH RESPONSES OF AFRICAN YAM BEAN (SPHENOSTYLIS STENOCARPA) IN A CADMIUM POLLUTED SOIL.

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Abstract
This study investigated the morphological growth responses of African yam bean (AYB) (sphenostylis stenocarpa) in a cadmium polluted soil. Soil was polluted with cadmium (as CdCl2) on the basis of the statutory ecological screening values (ESV); 0, 2.5 and 5 times the ESV. The morphology of polluted by cadmium was assessed using five AYB accessions namely; TSs-91, -92, -93, -94, and -95 respectively. The parameters studied were the shoot height, number of leaves, leaflet area folded, curled and forage leaves by insects. Results showed that Cadmium pollution resulted to significant reduction in all plant parameters studied during the 20 weeks period of observation. The shoot height of TSs-92 had a reduced difference of 8.9 cm compared to the control. There was no significant difference in leaflet area between the TSs-94 and the control. The highest number of leaf curling and folding were more observed in the Cd-5ESV treatments. In conclusion, TSs-92 has the potential to perform in Cd stressed soil and further research be carried out to check it response at molecular level.

Keywords: Cadmium, pollution, legumes, Sphenostylis stenocarpa, plant

1.0 INTRODUCTION

Plants depend on a blend of nutrients in the soil and air, including solar energy, CO2, and H2O for photosynthetic processes (Ikhajiagbe, 2016). These nutrients are referred to as essential elements and play a pivotal role in the structure of enzymes and proteins. They are required by plants in tiny amounts for their growth, metabolism and development. However, excess concentration of both essential and non-essential metals can lead to reduction and inhibition of plant growth (Munzuroglu and Zengin, 2006). Soil as a dynamic component of terrestrial ecosystem is essential for the growth of plants but this is not without variations in soil characteristics such as heavy metal pollution (Ikhajiagbe et al., 2018). Heavy metals belong to the group of non-biodegradables, persistent inorganic chemical constitutes with the atomic mass over 20 and the density higher than 5g/cm³. Their occurrence in many fertilizers and some pesticides is alarming. Cadmium is predominantly found in phosphatic fertilizers due to the presence of cadmium as an impurity in phosphate rocks. A lot of attention is given to cadmium in plant nutrition and soil science because of its toxic outcome which varies from growth reduction, wilting, chlorosis and cell death (Gallego et al., 2012; Van-Assehe and Clijsters, 1990).

Cadmium, a highly toxic metal pollutant of soils inhibits root and shoot growth, yield production, nutrient uptake and homeostasis, and is frequently accumulated by agriculturally important crops including legumes with a significant potential to impair animal and human health. The effect of Cd on the inhibition of seed germinant, growth and yield suppression
have been reported in cowpea and AYB, and are concentration dependent (Ohanmu et al., 2017). From the point of view of agricultural productivity and food security, there is an agreement to increase food productivity by the cultivation of underutilized crops in marginal soils that have the ability to withstand heavy metal contamination while restricting them from their productive parts. African yam bean which belongs to the family of Fabaceae is a vigorously climbing herbaceous vine whose height can reach 1.5–3 m or more. The main vine/stem produces many branches which also twine strongly on available stakes. Its utilization has links with sociocultural values in the cultures of some ethnic groups within and outside the area. In 2009, the Genetic Resources Information Network (GRIN) affirmed that center of diversity to spread from the west through to the east and southern parts of Africa and these areas are suspected to host the genetic resources of AYB. It has remarkably low susceptibility to most field and storage leguminous pests (Omitogun et al., 1999, Ohanmu and Ikhajiagbe, 2018). This plant was selected due to its widespread, culinary and medicinal values it possesses especially in the rural parts of Edo state. The aim of this study was to determine the morphological growth response of Sphenostylis stenocarpa in a cadmium contaminated soil.

2.0 MATERIALS AND METHODS

The experiment was carried out in the Botanical garden of Plant Biology and Biotechnology, University of Benin, Benin city. Five accessions of AYB were procured from the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria as shown below.

<table>
<thead>
<tr>
<th>Accession name</th>
<th>ID</th>
<th>Accession number</th>
<th>Country of origin</th>
<th>Cultivar name</th>
</tr>
</thead>
<tbody>
<tr>
<td>African yam bean (Sphenostylis stenocarpa)</td>
<td>TSs-91</td>
<td>95993</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>TSs-92</td>
<td>95994</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
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<td>TSs-93</td>
<td>95995</td>
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<td>NA</td>
</tr>
<tr>
<td></td>
<td>TSs-94</td>
<td>95996</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>TSs-95</td>
<td>95997</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Soils were collected from three different points at 0 – 30 cm depths using a soil auger at the botanical garden, where the experiment was carried out in the Department of Plant Biology and Biotechnology, University of Benin, Benin City. The soils were polluted with cadmium in the form of cadmium chloride (CdCl₂) using 0 Cd-ESV, 2.5Cd-ESV, 5 Cd-ESV (Ecological screening value).

2.1 Experimental procedure

The experiment consists of three (3) treatments, seeds of 5 accessions replicated three times. There were 3 blocks comprising 5 treatments per block, one (1) plant per polythene bag making a total of forty-five (45) plants arranged in a randomized block design (RBD). The experimental setup was three (3) months. The morphological parameters were collected on a weekly and bi-weekly basis such as shoot height, determined by measuring the height of the plant from the apex to the ground level using a meter rule (cm), number of leaves (by manual counting), leaflet area was determined by multiplying the length and breadth of the leaf, while number of folded, curled and forage leaves by insects by manual counting of leaf folding (leaves that folds inwards from the leaf side), leaf curling (leaves that curls inwards...
from the leaf tip) and leaf foraging was physical observation of any sign of injuries on the leaf by insects. This was done at the end of the experiment.

### 3.0 RESULT AND DISCUSSION

The progression in plant height of African yam bean accession (TSs-91, TSs-92, TSs-93, TSs-94 and TSs-95) to cadmium pollution is presented in Fig 1. Cadmium pollution resulted in reduction in plant height 6 weeks after sowing with TSs-91 exposed to Cd-5ESV having a suppressed height of 21.55cm as compared to an increased height of 32.25cm in control. This effect could be as a result of cadmium toxicity which possibly hindered nutrient availability. Plant nutrient is based not only on the presence of mineral nutrients in soils but on their availability (Scwab and Banks, 1999). However, the various plant accessions responded differently to the effect of cadmium with increase in concentration. At 12 weeks after sowing, the height difference between TSs-92 and TSs-95 in the Cd-5ESV soil were 8.9 cm and 29.18 cm when compared with their respective controls. Hence, TSs-92 is a better accession in term of plant height to cadmium pollution.

Cadmium pollution resulted to a decrease in the number of leaves with increase in the metal concentration in all studied accessions (Fig. 2). The effect of cadmium toxicity had no effect on the number of leaves until 4 weeks after sowing, the metal resulted to a 22.67 and 17.67 mean number of leaves in TSs-94 sown in Cd-2.5ESV and Cd-5ESV as compared to 25.18 leaves in control. Although there was a general reduction in the number of leaves 10 – 12 WAS in all treatments, cadmium pollution further enhanced the reduction of TSs accessions with a difference of 42 number of leaves in TSs-91 between the Cd-5ESV treatment and control.

Figure 3 shows the effect of cadmium pollution on leaflet area of African yam bean accessions. Leaflet area increased progressively in TSs accessions irrespective of treatments or accessions. However, cadmium pollution resulted in suppression of leaflet area with the highest reduction occurring with increase in metal concentration. For example, at 3 weeks after sowing, cadmium toxicity resulted to suppression in leaflet area (TSs-91) from 14.17 cm² in the control to 10.46cm² in Cd-2.5ESV and 7.76cm² in Cd-5ESV. At 12 weeks after sowing, cadmium pollution resulted to the highest leaflet area difference of 29.98cm² in TSs-95 between the control and Cd-5ESV. However, AYB accessions responded differently to cadmium toxicity with TSs-93 having more resistance to the metal toxicity than the other studied accessions.
Fig 1: Shoot height of African yam bean accessions in different concentrations of Cadmium.
Fig 2: leaf of African yam bean accessions in different concentrations of Cadmium.
3.1 Other observable morphology of Africa yam bean during the plant’s growth cycle

The effects of cadmium pollution on leaf folding, curling and foraging by insects during the plant’s lifetime has been reported (Table 2). Cadmium pollution resulted in an increased folded leaf in the older plants than the younger plant with increase in metal concentration. Take for instance, TSs-91 in the Cd-5ESV had an increased folded leaf of 8.41, 36.08 and 30.48 in the young plant (YP), intermediate (IP) and old plant (OP) respectively as compared to 0, 1.31 and 0 in the control (YP, IP and OP). This is in line with the work carried out by Ohanmu and Ikhajiagbe (2018) that cadmium may have been transported to older leaves as a survival strategy in order to overcome the stress exerted by the metal. It was observed from
the data that the YP had no presence of insect foraging when compared to the IP and OP irrespective of treatments. The highest number of leaf foraging was observed in the IP with TSs-93 having the highest recorded foraged mean leaf number of 19.15

Table 2: Other observable morphology of Africa yam bean during the plant’s growth cycle

<table>
<thead>
<tr>
<th>Plant Accession</th>
<th>Cd. Conc. (ESV)</th>
<th>Folded leaves</th>
<th>Curled leaves</th>
<th>Leaves with sign of foraging by insects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>YP</td>
<td>IP</td>
<td>OP</td>
</tr>
<tr>
<td>TSs-91</td>
<td>0</td>
<td>0</td>
<td>1.31</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>4.00</td>
<td>14.13</td>
<td>11.97</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>8.41</td>
<td>36.08</td>
<td>30.48</td>
</tr>
<tr>
<td>TSs-92</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.77</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>5.88</td>
<td>12.25</td>
<td>23.65</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>10.75</td>
<td>44.56</td>
<td>46.51</td>
</tr>
<tr>
<td>TSs-93</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.74</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>5.04</td>
<td>10.08</td>
<td>13.20</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>6.37</td>
<td>33.59</td>
<td>26.92</td>
</tr>
<tr>
<td>TSs-94</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4.13</td>
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<td></td>
<td>2.5</td>
<td>13.30</td>
<td>13.43</td>
<td>35.90</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>14.83</td>
<td>25.92</td>
<td>46.18</td>
</tr>
<tr>
<td>TSs-95</td>
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<td>0</td>
<td>6.09</td>
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<td></td>
<td>2.5</td>
<td>10.63</td>
<td>13.24</td>
<td>25.71</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>15.62</td>
<td>22.88</td>
<td>40.64</td>
</tr>
</tbody>
</table>

**CONCLUSION AND RECOMMENDATION**

The present study determined the morphological growth responses of African yam bean in a cadmium polluted soil. There was difference in the response of various accessions to cadmium treatments. It was recorded that TSs-92 produced a better morphological characteristic irrespective of the metal concentration and is recommended for further investigation to determine its resistance to cadmium polluted soil for food security.

**REFERENCE**


MORPHOLOGICAL CHARACTERIZATION OF SELECTED ACCESSIONS OF VIGNA SUBTERRANEAE (BAMBARA GROUNDNUT)

Motunrayo Funmilola Adeboye, Micheal Abberton, Olaniyi Oyatomi, Odunayo Joseph Olawuyi

Department of Botany, University of Ibadan, International Institute of Tropical Agriculture

ABSTRACT

The morphological characterization of accessions of Bambara groundnut was carried out to assess genetic variability and association among Bambara groundnut accessions based on agro-morphological characters. All the accessions originated from Nigeria and they include; TVSu-83, TVSu-119, TVSu-129, TVSu-179, TVSu-261, TVSu-263, TVSu-275, TVSu-277, TVSu-280, TVSu-331, TVSu-334, TVSu-346, TVSu-349, TVSu-356, TVSu-357, TVSu-361, TVSu-363, TVSu-365, TVSu-367 and TVSu-2090. The accessions were selected from the database of GRC Bambara groundnut accessions from accessions that have not been completely characterized. The accessions were significantly higher at P<0.01 for plant height, plant spread, terminal leaflet width, petiole length, pod width, shell thickness and 100-seed weight, while terminal leaflet length and number of terminal leaves and seed length were significant at P<0.05. The highest mean number of terminal leaves of 53.03 was recorded for TVSu-275. This indicate that variations exist among the accessions. All the accessions were found to have bunchy growth habit. Accession TVSu-356 had the highest yield of 13.22g per plant. TVSu-346 is significantly higher for pod length, but different from other accessions. The pod width, seed length and seed width in TVSu-367 are significantly higher and different from other accessions. TVSu-277 had the highest plant height (29.67cm), while TVSu-334 had the highest plant spread (54.93cm) and peduncle length (6.63mm). TVSu-365 is significantly higher for terminal leaflet length, while terminal leaf width was higher in TVSu-263. Plant height is significant and correlated to terminal leaflet width (r=0.52), petiole length (r=0.93) and plant spread (r=0.58), while petiole length is associated with plant spread (r=0.54) at p<0.01. Seed weight is negatively related with plant height (r= - 0.52; p<0.05). The results of this work show the importance of the crop as revealed by agro-morphological descriptors in the study, and it serves to form a basis for selection in agricultural improvement programs and nutrition studies.

Keywords: Bambara groundnut, characterisation, accessions, association

1.0 INTRODUCTION

Bambara groundnut (Vigna subterranea [L.] Verdc.) is an African indigenous legume that has been ranked as the third most important grain legume, after groundnut (Arachis hypogaea L.) and cowpea (Vigna unguiculata) in semi-arid Africa (Rachie and Silvester, 1977). Bambara groundnut, have an important socio-economic role in Tropical Africa, where they are part of tradition in culinary habits (Massawe et al., 2005, Brink et al., 2006). It originated in West Africa and has considerable genetic diversity (Mohammed et al., 2016). It is an annual, herbaceous, and intermediate plant with creeping stems at ground level (Bamshaiye et al., 2011). Bambara groundnut is known as a well adapted crop to extreme conditions such as
drought (Ouedraogo et al., 2008). The crop is widely distributed and grown throughout Africa where small scale farmers currently grow unimproved and heterogenous landraces (Mohammed et al., 2016). The centre of origin of Bambara groundnut is North-Eastern Nigeria and Northern Cameroon, in West Africa. The species is also sparsely grown in some Asian countries such as India, Malaysia, Philippines and Thailand (FAO, 2019).

Bambara groundnut possesses various economic importance such as nitrogen-fixing ability (Yakubu et al., 2010), drought tolerance (Bamshaiye et al., 2011), and photoperiod control (Kendabie et al., 2012). The leguminous plant has compound leaves of 3 leaflets and stems grow downwards into the soil taking the developing seeds with them. The pods are produced below the ground enclosing the seeds. Each pod contains one or two seeds that are round, smooth and hard when dried. It has high carbohydrate content (65%), relatively high protein content (18%), as well as fatty content (6.5%), making the nut a complete food (Mahazib et al., 2013).

Bambara groundnut seed is highly nutritious and chemical analyses showed that it contains 32.72% of total essential amino acids and 66.10% of total non-essential amino acids (Amarteifio et al., 2006). Lysine is the major essential amino acid and represents 10.3% of the total essential amino acid. Bambara groundnut is also a good source of leucine and contains a reasonable amount of phenylalanine, histidine and valine (Ouedraogo et al., 2007). The fatty acid content is predominantly palmitic, linoleic and linolenic acids (Minka and Bruneteau, 2000). Bambara groundnut is traditionally eaten as a boiled bean, or added to stews, or it can be made into a sweetened pudding (Mohammed et al., 2016). The flour has strong water and oil binding qualities, and it is therefore widely used to make indigenous bread, or to make milk similar to soya milk (Okpuzor et al., 2010). The young fresh seeds may be boiled and eaten as a snack in a manner similar to boiled peanut, and can be made into pudding (or steamed-paste) which is called Moi–Moi or Okpa (bean porridge) in some parts of Nigeria (Okpuzor et al., 2010). Bambara groundnut is an important source of affordable protein in the diets especially in regions where animal protein is comparatively expensive. The crop is richer in essential amino acids than other legumes and has a higher protein score (80%) than soya bean (74%) and cowpea (64%), meaning that Bambara groundnut has relatively more protein available for human metabolism than the other common legumes in Africa (Mubaiwa et al., 2018).

The sustainable conservation and utilization of these plants’ genetic diversity and the characterization of neglected and under-utilized species (NUS) are important food sources addressing food insecurity (Atoyebi et al., 2017). Research into NUS is especially important and necessary due to the need to secure the basis of food production and thus help to provide a balanced nutritional diet for the rural population of many developing countries, like Nigeria, Burkina-Faso, Mali and other African countries (Atoyebi et al., 2017).

Neglected and under-utilized species (NUS) such as Bambara groundnut have traits and properties that are important to provide varied and nutritionally balanced diets. However, this crop, although prominent in rural communities where small scale farmers grow unimproved and heterogenous landraces, has been largely neglected by researchers. Unlike soybean (Glycine max) which has received considerable scientific and financial supports since its introduction, Bambara groundnut has received limited support from governmental or international agencies and has largely been ignored by the research community (Oyeyinka et al., 2015).
Studies of agro-morphological parameters has been an important approach in exploiting the genetic diversity of crops. This especially makes under-utilised crops more attractive to farmers through identifying appropriate morphotypes and farming practices that can help to determine the choice of germplasm to be used in specific climatic condition studies or in nutrition related research. The creation of options for farmers in the agricultural production system is essential, as it drives the ability of society to address diverse livelihood problems, through the farming system and genetic diversity created; thus enhancing the popularity of such crops for consumption (Padulosi et al., 2011).

2.0. MATERIALS AND METHODS

2.1. Germplasm Collection
In this study, a total of twenty (20) accessions of Bambara groundnut were obtained from Seed Bank, Genetic Resources Center of International Institute of Tropical Agriculture (IITA). The accessions were randomly selected from the database of uncharacterized Bambara groundnut accessions of Nigerian origin.

2.2. Experimental Site and Design
Morphological characterization was carried out on one of the experimental fields of the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo state, Southwestern Nigeria (latitude 7° 30 ′ N and longitude 3° 54 ′E). The experiment was laid in three replicates using a Randomized Complete Block Design (RCBD). Each plot within a replication consisted of ten (10) accessions. The position of each accession within the plot was randomized. Each accession within the plot was grown in a one row of 3 m length. A spacing of 75 cm between rows and 25cm between plants in a row was used. Adequate agronomic practices recommended were followed during the crop-growing period. Irrigation was used to complement little rainfall that stopped midway of the plant cycle.

2.3. Method of Data Collection
Data were collected based on Bambara groundnut descriptor provided by IPGRI for both selected pre and post harvesting observations, which were recorded from three to five (3-5) randomly selected plants from each genotype out of ten (10) plant stands in each replication for all selected characters studied in this research.

The following morphological parameters were measured: growth habit, peduncle length, petiole length, number of terminal leaflet leaves, terminal leaflet length, terminal leaflet width, terminal leaflet shape, plant spread, plant height, dry pod colour, seed shape, seed length, seed weight, seed width, pod length, pod width, pod shape, shell thickness, 100-seed weight and yield per plant.

3.0. RESULTS AND DISCUSSION

3.1. Data Analysis
Data obtained were subjected to one way analysis of variance (ANOVA) using Statistical Analysis Software (SAS) version 9.4. Means (averages) were separated using Duncan Multiple Range Test (DMRT) at 95% probability level. Dendrogram was constructed to show relatedness among the accessions collected. Correlation matrix was used to highlight the various relationships that exist among the studied parameters.
3.2. Mean Square Variance of Quantitative Growth Characters in Twenty Accessions of Bambara Groundnut

The result of the mean square variance of the quantitative growth characters of twenty accessions of Bambara groundnut is shown in Table 4.1. The accessions were found to be highly significant at $P<0.01$ for plant height, plant spread, terminal leaflet width and petiole length while terminal leaflet length and number of terminal leaves were significant at $P<0.05$.

Table 3.2

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df(n-1)</th>
<th>PH</th>
<th>PS</th>
<th>TLL</th>
<th>TLW</th>
<th>NTL</th>
<th>PetL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>21</td>
<td>15.39</td>
<td>75.6</td>
<td>204.29</td>
<td>54.12</td>
<td>135.92</td>
<td>1359.62</td>
</tr>
<tr>
<td>Rep</td>
<td>2</td>
<td>9.94*</td>
<td>185.62</td>
<td>78.35**</td>
<td>59.97*</td>
<td>30.24*ns</td>
<td>615.05**ns</td>
</tr>
<tr>
<td>Accession</td>
<td>19</td>
<td>15.97**</td>
<td>64.02**</td>
<td>217.54*</td>
<td>53.51**</td>
<td>147.04*</td>
<td>1438**</td>
</tr>
<tr>
<td>Error</td>
<td>38</td>
<td>4.23</td>
<td>23.99</td>
<td>113.21</td>
<td>8.81</td>
<td>74.1</td>
<td>503.79</td>
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<tr>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>

Key: PH= Plant Height, PS= Plant Spread, TLL= Terminal Leaflet Length, TLW= Terminal Leaflet Width, NTL= Number of Terminal Leaves, PetL= Petiole Length

3.3 Mean Square Variance of Quantitative Yield Characters in Twenty Accessions of Bambara Goundnut

The result of the mean square variance of the quantitative yield characters of twenty accessions of Bambara groundnut is shown in Table 3.3. The accessions were found to be not significant at $P>0.05$ for peduncle length, yield per plant, pod length, seed width and seed weight while they were found to be highly significant at $P<0.01$ for pod width, shell thickness and 100-seed weight. Seed length was found to be significant at $P<0.05$.

Table 3.3

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df(n-1)</th>
<th>PdclL</th>
<th>Y/P</th>
<th>PdL</th>
<th>PdW</th>
<th>SdL</th>
<th>SdW</th>
<th>ShThk</th>
<th>SdWg</th>
<th>HdSdWg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>21</td>
<td>0.8</td>
<td>23.83</td>
<td>7.57</td>
<td>3.68</td>
<td>2.49</td>
<td>1.12</td>
<td>0.13</td>
<td>1469.85</td>
<td>801.9</td>
</tr>
<tr>
<td>Rep</td>
<td>2</td>
<td>1.57**</td>
<td>17.59**</td>
<td>1.93**</td>
<td>3.24**</td>
<td>3.80*</td>
<td>1.22**</td>
<td>0.08**</td>
<td>669.15**</td>
<td>0</td>
</tr>
<tr>
<td>Accession</td>
<td>19</td>
<td>0.72**</td>
<td>24.49**</td>
<td>8.16**</td>
<td>3.73**</td>
<td>2.35*</td>
<td>1.11**</td>
<td>0.14**</td>
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<td>886.31**</td>
</tr>
<tr>
<td>Error</td>
<td>38</td>
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<td>15.87</td>
<td>4.62</td>
<td>1.01</td>
<td>0.86</td>
<td>0.43</td>
<td>0.04</td>
<td>943.44</td>
<td>0</td>
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<tr>
<td>Corrected total</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Key: PdclL= Peduncle Length, Y/P= Yield per Plant, PdL= Pod Length, PdW= Pod Width, SdL= Seed Length, SdW= Seed Width, ShThk= Shell Thickness, SdWg= Seed Weight, HdSdWg= 100-Seed Weight

3.4 Qualitative Growth and Yield characters of Twenty Bambara Groundnut Accessions
The result of the qualitative growth and yield characters of twenty Bambara groundnut is shown in Table 3.4.

3.4.1 Terminal Leaflet Shape

3.4.2 Pod Shape

3.4.3 Dry Pod Colour
TVSu-331, TVSu-357 and TVSu-367 have brown pod colour while all the other accessions have yellowish brown pods.

3.4.4 Growth Habit
All of the twenty accessions are bunchy.

3.4.5 Seed Shape
Accessions TVSu-119 and TVSu-363 have round seeds while all of the remaining accessions have oval-shaped seeds.
Table 3.4: Seed quality of the selected accessions

<table>
<thead>
<tr>
<th>Accn</th>
<th>Terminal Leaflet Shape</th>
<th>Pod shape</th>
<th>Dry Pod Colour</th>
<th>Growth Habit</th>
<th>Seed Shape</th>
</tr>
</thead>
<tbody>
<tr>
<td>TVSu-83</td>
<td>Oval</td>
<td>ending in a point with a nook on the other side</td>
<td>Yellowish brown</td>
<td>Bunchy</td>
<td>Oval</td>
</tr>
<tr>
<td>TVSu-119</td>
<td>Lanceolate</td>
<td>ending in a point round on the other side</td>
<td>Yellowish brown</td>
<td>Bunchy</td>
<td>Round</td>
</tr>
<tr>
<td>TVSu-129</td>
<td>Lanceolate</td>
<td>ending in a point with a nook on the other side</td>
<td>Yellowish brown</td>
<td>Bunchy</td>
<td>Oval</td>
</tr>
<tr>
<td>TVSu-179</td>
<td>Oval</td>
<td>ending in a point round on the other side</td>
<td>Yellowish brown</td>
<td>Bunchy</td>
<td>Oval</td>
</tr>
<tr>
<td>TVSu-261</td>
<td>Lanceolate</td>
<td>ending in a point with a nook on the other side</td>
<td>Yellowish brown</td>
<td>Bunchy</td>
<td>Oval</td>
</tr>
<tr>
<td>TVSu-263</td>
<td>Oval</td>
<td>ending in a point round on the other side</td>
<td>Yellowish brown</td>
<td>Bunchy</td>
<td>Oval</td>
</tr>
<tr>
<td>TVSu-275</td>
<td>Lanceolate</td>
<td>ending in a point round on the other side</td>
<td>Yellowish brown</td>
<td>Bunchy</td>
<td>Oval</td>
</tr>
<tr>
<td>TVSu-277</td>
<td>Lanceolate</td>
<td>ending in a point round on the other side</td>
<td>Yellowish brown</td>
<td>Bunchy</td>
<td>Oval</td>
</tr>
<tr>
<td>TVSu-280</td>
<td>Oval</td>
<td>ending in a point with a nook on the other side</td>
<td>Yellowish brown</td>
<td>Bunchy</td>
<td>Oval</td>
</tr>
<tr>
<td>TVSu-331</td>
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<td>ending in a point with a nook on the other side</td>
<td>Brown</td>
<td>Bunchy</td>
<td>Oval</td>
</tr>
<tr>
<td>TVSu-334</td>
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<td>ending in a point with a nook on the other side</td>
<td>Yellowish brown</td>
<td>Bunchy</td>
<td>Oval</td>
</tr>
<tr>
<td>TVSu-346</td>
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<td>ending in a point round on the other side</td>
<td>Yellowish brown</td>
<td>Bunchy</td>
<td>Oval</td>
</tr>
<tr>
<td>TVSu-349</td>
<td>Lanceolate</td>
<td>ending in a point with a nook on the other side</td>
<td>Yellowish brown</td>
<td>Bunchy</td>
<td>Oval</td>
</tr>
<tr>
<td>TVSu-356</td>
<td>Lanceolate</td>
<td>ending in a point round on the other side</td>
<td>Yellowish brown</td>
<td>Bunchy</td>
<td>Oval</td>
</tr>
<tr>
<td>TVSu-357</td>
<td>Oval</td>
<td>ending in a point with a nook on the other side</td>
<td>Brown</td>
<td>Bunchy</td>
<td>Oval</td>
</tr>
<tr>
<td>TVSu-361</td>
<td>Lanceolate</td>
<td>ending in a point round on the other side</td>
<td>Yellowish brown</td>
<td>Bunchy</td>
<td>Oval</td>
</tr>
<tr>
<td>TVSu-363</td>
<td>Oval</td>
<td>ending in a point with a nook on the other side</td>
<td>Yellowish brown</td>
<td>Bunchy</td>
<td>Round</td>
</tr>
<tr>
<td>TVSu-365</td>
<td>Oval</td>
<td>ending in a point with a nook on the other side</td>
<td>Yellowish brown</td>
<td>Bunchy</td>
<td>Oval</td>
</tr>
<tr>
<td>TVSu-367</td>
<td>Oval</td>
<td>ending in a point with a nook on the other side</td>
<td>Brown</td>
<td>Bunchy</td>
<td>Oval</td>
</tr>
<tr>
<td>TVSu-2090</td>
<td>Lanceolate</td>
<td>ending in a point with a nook on the other side</td>
<td>Yellowish brown</td>
<td>Bunchy</td>
<td>Oval</td>
</tr>
</tbody>
</table>

3.5. Quantitative Growth Characteristics in Twenty Bambara Groundnut Accessions

The results of the quantitative growth characteristics in twenty accessions of Bambara groundnut are shown in Table 3.5.

3.5.1. Plant Height

Plant height was found to be highly significant for $P<0.05$ at 0.0002. TVSu-277 was found to be the most significant accession for plant height. Accessions TVSu-261, TVSu-275, TVSu-280, TVSu-357, TVSu-361 and TVSu-363 were found to be the most similar for this character. TVSu-277 was recorded to have the highest value for plant height at 29.67cm, followed by TVSu-331 at 27.90cm and TVSu-119 at 25.33cm. TVSu-2090 at 20.50cm and TVSu-365 at 20.67cm recorded the least values.

3.5.2. Plant Spread

Plant spread was found to be significant for $P<0.05$ at 0.04. TVSu-334 was found to be the most significant for plant spread. TVSu-119, TVSu-129, TVSu-179 and TVSu-83 were found to be the most similar accessions for this character. TVSu-334 at 54.93cm and TVSu-280 at 54.33cm were observed to have the highest values for plant spread, while TVSu-349 at 40.67cm, TVSu-357 at 40.73cm and TVSu-361 at 40.40cm recorded the least values.
3.5.3 Terminal Leaflet Length

Terminal leaflet length was found to be significant for $P<0.05$ at 0.04. TVSu-365 was found to be the most significant for this character. TVSu-129, TVSu-2090, TVSu-280, TVSu-346, TVSu-356, TVSu-357 and TVSu-83 were found to be the most similar for this character. TVSu-365 at 78.33mm was observed to have the highest terminal leaflet length, followed by TVSu-331 at 72.33mm and TVSu-277 at 71.33mm. The least values recorded were that of TVSu-275 at 40.67mm and TVSu-261 at 49.00mm.

3.5.4 Terminal Leaflet Width

Terminal leaflet width was found to be highly significant for $P<0.001$ at 0.0001. TVSu-129 and TVSu-263 were found to be the most significant for this character. TVSu-280, TVSu-346, TVSu-349, TVSu-356, TVSu-363, TVSu-367 and TVSu-83 were the most similar accessions for this character. TVSu-129 at 34.67mm was observed to have the highest value of terminal leaflet width, followed closely by TVSu-263 at 34.53mm and TVSu-277 at 32.67mm. The least values observed were those of TVSu-275 at 21.33mm, and then TVSu-119 at 22.00mm and TVSu-365 at 22.00mm having equal values.

3.5.5 Number of Terminal Leaves

Number of terminal leaves was found to be significant for $P<0.05$ at 0.03. TVSu-275 was found to be the most significant accession for this character. TVSu-346, TVSu-356, TVSu-361 and TVSu-367 were found to be the most similar accessions for this character. The accessions with the highest number of terminal leaves are TVSu-275 at 53.03, TVSu-119 at 51.84, and TVSu-334 at 50.17, while TVSu-261 at 27.88 and TVSu-363 at 33.88 were recorded to have the least values.

3.6. Quantitative Yield Characters in Twenty Bambara Groundnut Accessions

The results of the quantitative yield characters of twenty Bambara groundnut accessions are shown in Table 3.6.

3.6.1 Peduncle Length

Peduncle length was found to be not significant for $P<0.01$ at 0.76. TVSu-334 was found to be the most significant accession for this character. TVSu-119, TVSu-129, TVSu-179, TVSu-2090, TVSu-261, TVSu-263, TVSu-275, TVSu-277, TVSu-331, TVSu-349, TVSu-357, TVSu-361, TVSu-363, TVSu-365, TVSu-367 and TVSu-83 were all found to be similar for this character. TVSu-334 at 6.63mm had the highest value for peduncle length, followed by TVSu-356 at 6.30mm and TVSu-365 at 6.03mm. TVSu-346 at 4.50mm and TVSu-280 at 4.87mm recorded the least values for this parameter.
3.6.2 Petiole Length

Petiole length was found to be highly significant for P<0.05 at 0.003. The most significant accession for this character was found to be TVSu-277. Accessions TVSu-129, TVSu-261, TVSu-263, TVSu-275 and TVSu-367 were found to be the most similar. TVSu-277 at 205.33mm was observed to have the highest values for petiole length, followed by TVSu-331 at 186.67mm and TVSu-346 at 183.33 while TVSu-365 at 108.33mm had the lowest value.

3.6.3 Yield/Plant

Yield per plant was found to not be significant for this character for P<0.01 at 0.12. TVSu-261 was found to be the most significant accession. TVSu-280, TVSu-349, TVSu-356, TVSu-357, TVSu-363 and TVSu-367 were found to be very similar. Accessions TVSu-129, TVSu-179, TVSu-331, TVSu-334, TVSu-365 and TVSu-83 were also found to be similar. TVSu-356 at 13.22g, TVSu-357 at 12.87g and TVSu-349 at 12.75g had the highest values for yield/plant while TVSu-119 at 3.25g and TVSu-277 at 5.53g had the least values.

3.6.4 Pod Length

Table 3.5: Leaf quality

<table>
<thead>
<tr>
<th>Accn</th>
<th>PH (cm) ± SD</th>
<th>PS(cm) ± SD</th>
<th>TLL (mm) ± SD</th>
<th>TLW (mm) ± SD</th>
<th>NTL ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>TVSu-119</td>
<td>25.33 ± 0.58**</td>
<td>51.33 ± 2.52**</td>
<td>51.33 ± 8.08**</td>
<td>22 ± 2*</td>
<td>51.84 ± 16.04**</td>
</tr>
<tr>
<td>TVSu-129</td>
<td>24.67 ± 2.08**</td>
<td>51 ± 7.55**</td>
<td>63.67 ± 5.51**</td>
<td>34.67 ± 4.16*</td>
<td>39.63 ± 4.21**</td>
</tr>
<tr>
<td>TVSu-179</td>
<td>24.8 ± 1.04**</td>
<td>51.33 ± 2.89**</td>
<td>68.33 ± 2.89**</td>
<td>32.33 ± 4.62**</td>
<td>49.3 ± 12.48**</td>
</tr>
<tr>
<td>TVSu-2090</td>
<td>20.5 ± 2.18</td>
<td>43.73 ± 5.83**</td>
<td>61.67 ± 7.64**</td>
<td>27 ± 4de</td>
<td>36.3 ± 10.8**</td>
</tr>
<tr>
<td>TVSu-261</td>
<td>23 ± 1*</td>
<td>47 ± 7.21**</td>
<td>49 ± 7.94**</td>
<td>27 ± 2f</td>
<td>27.88 ± 6.43f</td>
</tr>
<tr>
<td>TVSu-263</td>
<td>24.33 ± 1.15**</td>
<td>43.83 ± 7.57**</td>
<td>56 ± 3.46**</td>
<td>34.53 ± 0.5a</td>
<td>49.35 ± 6.38**</td>
</tr>
<tr>
<td>TVSu-275</td>
<td>22.17 ± 1.04**</td>
<td>47.33 ± 9.02**</td>
<td>40.67 ± 4.04</td>
<td>21.33 ± 1.53f</td>
<td>53.03 ± 4.04f</td>
</tr>
<tr>
<td>TVSu-277</td>
<td>29.67 ± 3.21</td>
<td>49.5 ± 5.07**</td>
<td>71.33 ± 2.31**</td>
<td>32.67 ± 2.08**</td>
<td>35.88 ± 8**</td>
</tr>
<tr>
<td>TVSu-280</td>
<td>23 ± 1.73**</td>
<td>54.33 ± 0.58**</td>
<td>61.33 ± 4.62**</td>
<td>30.33 ± 2.89**</td>
<td>37.97 ± 5.93**</td>
</tr>
<tr>
<td>TVSu-331</td>
<td>27.9 ± 1.56</td>
<td>53.37 ± 5.76**</td>
<td>72.33 ± 2.31**</td>
<td>33.33 ± 5.77**</td>
<td>36.68 ± 3.72**</td>
</tr>
<tr>
<td>TVSu-334</td>
<td>24.67 ± 0.58**</td>
<td>54.93 ± 6.96**</td>
<td>67.67 ± 5.77**</td>
<td>29 ± 1.73**</td>
<td>50.17 ± 3.9**</td>
</tr>
<tr>
<td>TVSu-346</td>
<td>26.5 ± 2.08**</td>
<td>48.07 ± 3.35**</td>
<td>61.67 ± 8.08**</td>
<td>31.67 ± 2.89**</td>
<td>42.43 ± 3.16**</td>
</tr>
<tr>
<td>TVSu-349</td>
<td>21.67 ± 3.06**</td>
<td>40.67 ± 4.51**</td>
<td>59.33 ± 4.04**</td>
<td>30 ± 2**</td>
<td>37.68 ± 10.5**</td>
</tr>
<tr>
<td>TVSu-356</td>
<td>23.5 ± 2.78**</td>
<td>46.67 ± 5.79**</td>
<td>63 ± 3.61**</td>
<td>31.33 ± 1.53**</td>
<td>46.1 ± 9.69**</td>
</tr>
<tr>
<td>TVSu-357</td>
<td>22.63 ± 4.05**</td>
<td>40.73 ± 5.22**</td>
<td>61 ± 10.54**</td>
<td>28 ± 5.29**</td>
<td>35.93 ± 4.48**</td>
</tr>
<tr>
<td>TVSu-361</td>
<td>22 ± 1.73**</td>
<td>40.4 ± 5.13**</td>
<td>55 ± 8.89**</td>
<td>22.33 ± 3.21**</td>
<td>47.03 ± 4.48**</td>
</tr>
<tr>
<td>TVSu-363</td>
<td>22.1 ± 0.85**</td>
<td>45.67 ± 6.35**</td>
<td>60 ± 8.66**</td>
<td>30.33 ± 0.58**</td>
<td>33.88 ± 8.91**</td>
</tr>
<tr>
<td>TVSu-365</td>
<td>20.67 ± 2.08**</td>
<td>42.03 ± 4.57**</td>
<td>78.33 ± 38.89*</td>
<td>22 ± 2f</td>
<td>35.2 ± 10.49**</td>
</tr>
<tr>
<td>TVSu-367</td>
<td>24.33 ± 3.51**</td>
<td>50.07 ± 7.54**</td>
<td>57 ± 6.08**</td>
<td>30.67 ± 3.21**</td>
<td>45.37 ± 10.1**</td>
</tr>
<tr>
<td>TVSu-83</td>
<td>23.17 ± 1.44**</td>
<td>51 ± 1**</td>
<td>61.33 ± 1.15**</td>
<td>30.67 ± 0.58**</td>
<td>42.1 ± 10.86**</td>
</tr>
<tr>
<td>CV</td>
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<td>10.27</td>
<td>17.44</td>
<td>10.21</td>
<td>20.64</td>
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<tr>
<td>F value</td>
<td>0.0002**</td>
<td>0.005**</td>
<td>0.04**</td>
<td>&lt;.0001**</td>
<td>0.03*</td>
</tr>
</tbody>
</table>

KEY: PH= Plant Height, PS= Plant Spread, TLL= Terminal Leaflet Length, TLW= Terminal Leaflet Width, NTL= Number of Terminal Leaves, SD= Standard Deviation

Means with the same letters are not significantly different at P≤0.05
Pod length was found to be not significant for P<0.05 at 0.06. TVSu-346 was found to be the most significant for this character. TVSu-179, TVSu-275, TVSu-277, TVSu-331 and TVSu-363 were found to be the most similar accessions. TVSu-346 at 21.48mm recorded the highest value for pod length, followed by TVSu-334 at 20.97mm and TVSu-349 at 20.47mm while TVSu-261 at 15.85mm and TVSu-2090 at 15.94mm were observed to have the least values.

3.6.5 Pod Width

Pod width was found to be highly significant for P<0.01 at 0.0003. The most significant accession for this character is TVSu-367. TVSu-331, TVSu-334 and TVSu-346 are similar. TVSu-119, TVSu-356 and TVSu-363 are also similar accessions for this character. TVSu-367 at 15.60mm was observed to have the highest value for pod width, followed by TVSu-83 at 15.06mm. The least values recorded were from accessions TVSu-365 at 11.37mm and TVSu-2090 at 11.94mm.

3.7. Quantitative Seed Characters of Twenty Bambara Groundnut Accessions

The results of the quantitative seed characters of twenty accessions of Bambara groundnut are shown in Table 3.7.

3.7.1. Seed Length

Seed length was found to be highly significant for P<0.01 at 0.004. TVSu-367 was found to be the most significant accession for this character. Accessions TVSu-119, TVSu-2090, TVSu-261, TVSu-280, TVSu-349, TVSu-356, TVSu-357 and TVSu-361 were the most similar for this character. TVSu-367 at 13.15mm was observed to have the highest value for seed length, followed by TVSu-334 at 12.71mm and TVSu-83 at 12.48mm while the least values observed were those of TVSu-263 at 9.23mm and TVSu-277 at 10.06mm.

3.7.2. Seed Width

Seed length was found to be highly significant for P<0.01 at 0.006. The accession that is most significant for this character is TVSu-367. TVSu-119, TVSu-129, TVSu-179, TVSu-261, TVSu-275, TVSu-361 and TVSu-363 were found to be the most similar accessions. The accession recorded to have the highest seed width was TVSu-367 at 10.47mm, followed by TVSu-357 at 10.35mm. Those with the least values are TVSu-277 at 8.36mm and TVSu-263 at 8.43mm.
Seed weight was found to be not significant for P ≥ 0.05. The most similar accessions are TVSu-2090, TVSu-275, TVSu-280, TVSu-349, TVSu-356, TVSu-357, TVSu-363 and TVSu-367. TVSu-261 at 112.30g, TVSu-356 at 94.77g were observed to have the least values.

3.7.3. Shell Thickness

Shell thickness was found to be highly significant for P < 0.01 at 0.0003. The accessions that are most similar are TVSu-129, TVSu-2090 and TVSu-361 and TVSu-349, TVSu-367 and TVSu-83. TVSu-119, TVSu-346 at 1.13mm and TVSu-334 at 1.12mm were observed to have the highest values for shell thickness while TVSu-365 at 0.53mm and TVSu-280 at 0.53mm were observed to have the least values.

3.7.4. Seed Weight

Seed weight was found to be not significant for P < 0.05 at 0.09. The most similar accessions are TVSu-2090, TVSu-275, TVSu-280, TVSu-349, TVSu-356, TVSu-357, TVSu-363 and TVSu-367. TVSu-261 at 112.30g, TVSu-356 at 101.17g, and TVSu-356 at 94.77g were
observed to have the highest values for seed weight while TVSu-119 at 23.83g had the least value.

3.7.5. 100-Seed Weight

100-seed weight was found to be highly significant for P<0.01 at <.0001. All of the accessions vary greatly from one another. TVSu-83 was found to be the most significant for this character. TVSu-83 at 115.62g and TVSu-349 at 100.02g were observed to have the highest values of 100-seed weight while TVSu-365 at 46.62g and TVSu-263 at 50.82g recorded the least values.

3.8. Dendrogram Showing Relationship Among Twenty Bambara Groundnut Accessions

The dendrogram (Figure 1) shows the relationship that exists among the twenty Bambara groundnut accessions. TVSu-119 was found to be related to a small group consisting of TVSu-275 and TVSu-361 showing that they are closely related. TVSu-129 and TVSu-179 were also found to be closely related and form a cluster with TVSu-356, which then form a larger cluster with TVSu-263 and TVSu-277 which form a smaller cluster. These also form another larger cluster group. Both of these clusters form another larger cluster. TVSu-2090 and TVSu-363 also form a small cluster group and so are closely related. They form a larger group with TVSu-261, then with TVSu-280, all of which then form a larger group with TVSu-365. Accessions TVSu-349 and TVSu-357 were found to be closely related and form a small cluster group. These two accessions are then related to a larger group consisting of TVSu-367 and TVSu-83 which are also related to TVSu-334 which does not have any closely related accession and TVSu-331 and TVSu-346 which are closely related.
### Table 3.7: Yield quality

<table>
<thead>
<tr>
<th>Accn</th>
<th>SdLth(mm) ±SD</th>
<th>SdWid(mm) ± SD</th>
<th>ShThk(mm) ± SD</th>
<th>SdWt(g) ± SD</th>
<th>Hdsdwt(g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TVSu-119</td>
<td>11.19 ± 0.78&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>9.26 ± 0.46&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.22 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23.83 ± 10.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.32&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TVSu-129</td>
<td>11.72 ± 0.57&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>9.14 ± 0.77&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>0.66 ± 0.16&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>62.88 ± 24.04&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>61.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TVSu-179</td>
<td>10.76 ± 0.62&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>8.99 ± 0.36&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>1.07 ± 0.35&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>66.28 ± 52.58&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>75.62&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TVSu-2090</td>
<td>11.21 ± 0.86&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>8.8 ± 0.13&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>0.66 ± 0.14&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>83.22 ± 10.81&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>60.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TVSu-261</td>
<td>11.3 ± 1.26&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>9.13 ± 0.49&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>0.64 ± 0.1&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>112.3 ± 30.4&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>59.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TVSu-263</td>
<td>9.23 ± 0.37&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.43 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.8 ± 0.19&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>51.22 ± 25.76&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>50.82&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TVSu-275</td>
<td>10.81 ± 1.28&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>9.1 ± 0.51&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>0.57 ± 0.17&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>87.57 ± 9.11&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>73.82&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TVSu-277</td>
<td>10.06 ± 0.65&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>8.36 ± 0.34&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>0.78 ± 0.16&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>32.15 ± 28.08&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>59.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TVSu-280</td>
<td>11.53 ± 0.26&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>9.8 ± 0.36&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>1 ± 0.1&lt;sup&gt;def&lt;/sup&gt;</td>
<td>93.68 ± 35.86&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>75.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TVSu-331</td>
<td>11.81 ± 1.04&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>9.89 ± 0.8&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>1 ± 0.1&lt;sup&gt;def&lt;/sup&gt;</td>
<td>63.78 ± 33.2&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>80.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TVSu-334</td>
<td>12.71 ± 1.22&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>9.91 ± 0.52&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>1.12 ± 0.24&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>62.73 ± 33.58&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>67.52&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TVSu-346</td>
<td>11.66 ± 0.35&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>9.82 ± 0.23&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>1.13 ± 0.28&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>81.4 ± 22.99&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>81.72&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>TVSu-349</td>
<td>11.51 ± 1.84&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>9.87 ± 0.91&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>0.86 ± 0.13&lt;sup&gt;bcdef&lt;/sup&gt;</td>
<td>88.8 ± 21.51&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>100.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TVSu-356</td>
<td>11.27 ± 1.52&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>9.28 ± 0.96&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>0.73 ± 0.25&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>94.77 ± 20.22&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>73.72&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>TVSu-357</td>
<td>11.56 ± 0.46&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>10.35 ± 0.91&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>1.09 ± 0.35&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>88.35 ± 17.58&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>94.62&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>TVSu-361</td>
<td>11.55 ± 1.03&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>8.96 ± 0.37&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>0.67 ± 0.27&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>50.83 ± 18.6&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>67.92&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>9.06 ± 0.39&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>0.97 ± 0.16&lt;sup&gt;bcdef&lt;/sup&gt;</td>
<td>88.65 ± 38.15&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>66.92&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>TVSu-365</td>
<td>10.58 ± 0.75&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>8.61 ± 0.37&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>0.53 ± 0.07&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>78.85 ± 29.31&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>46.62&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>TVSu-367</td>
<td>13.15 ± 1.34&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>10.47 ± 1.24&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.89 ± 0.11&lt;sup&gt;bcdef&lt;/sup&gt;</td>
<td>101.17 ± 23.03&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>79.12&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>TVSu-83</td>
<td>12.48 ± 1.22&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>9.9 ± 1.48&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>0.89 ± 0.16&lt;sup&gt;bcdef&lt;/sup&gt;</td>
<td>64.28 ± 64.53&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>115.62&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

CV 8.17 7.01 22.99 41.59 0

F value 0.004** 0.006** 0.0003** 0.09ns <.0001**

KEY: SdLth= Seed Length, SdWid= Seed Width, ShThk= Shell Thickness, SdWt= Seed Weight, Hdsdwt= 100-Seed Weight, SD= Standard Deviation

Means with the same letters are not significantly different at P≤0.05.
CONCLUSION

Bambara groundnut is a crop that is widely grown in Nigeria and other African countries and has become an important NUS. This legume, predominant in sub-Saharan Africa still exhibits immense untapped potentials. This paper presents research work on agro-morphological performance of 20 Nigerian accessions, with the aim of revealing the variability that exists within the selected accessions of this crop and selection for nutrition parameters and analysis. This will also further aid selection of elite materials in breeding and agricultural improvement programs (Atoyebi et al., 2016). Table 3.2 and 3.3 show the mean square variance between the characters of the accessions under study. Table 3.4 shows the qualitative growth and yield characters of the accessions while Tables 3.5 to 3.7 show the quantitative growth, seed and yield characters of the accessions.

All of the accessions were found to have a bunchy growth habit. TVSu-356 was found to have the highest value for yield/plant, and so can be said to be the best accession for its high yield properties. TVSu-277 was found to have considerable values for characters such as terminal leaflet length and terminal leaflet width. It had the highest values for plant height and petiole length, and so this accession can be said to have high vegetative/growth properties. TVSu-367 was found to have the highest values for characters such as pod width, seed length and seed width. It also had high value for seed weight, all of these indicating that it has good yield properties. TVSu-334 had the highest values for characters such as plant spread and peduncle length. It also had high values for pod length and seed length. It can then be said to have considerable vegetative and yield properties. TVSu-119 was found to have low values for terminal leaflet width, yield/plant and seed weight. It can be said to have low yield and vegetative properties.

Figure 1: The Tree’s Procedure- Ward’s Minimum Variance Cluster Analysis
Characterization and evaluation of Bambara groundnut germplasm and identification of the best parents are important for improvement of the crop. The genotypes in this study showed significant variation in phenotypic characters, indicating that the accessions had high genetic diversity which can be exploited for use in a breeding program, regardless that they are all from Nigeria. The genetic potential of the accessions as revealed in this study can assist in choosing suitable parental lines thereby maximizing the efficiency of a Bambara groundnut breeding program (Unigwe et al., 2016).

Bambara groundnut (Vigna subterranea (L.) Verdc.) has a large number of landraces throughout Africa where small-scale farmers have preserved its genetic diversity on-farm. To date, the full genetic diversity of the crop remains largely unexploited (Azam-Ali et al., 2001). Also there has being little work on its breeding for improved Bambara groundnut varieties at the moment, infact none presently in Nigeria. However, with the results of this work, which further states the importance of the crop as revealed by agro-morphological descriptors in the study, it serves to form a basis for selection in agricultural improvement programs and nutrition studies in Bambara groundnut (Atoyebi et al., 2016).

The result of this study agrees with the findings that despite the numerous potentials in the crop. There is however a need to improve upon its utilization and market potentials, especially in developing countries of Africa, like Nigeria (Atoyebi et al., 2016).

Bambara groundnut has the potential to be used to contribute to the climate change ready agriculture. The requirement for nitrogen fixing, stress tolerant legumes is clear, particularly in low input agriculture. However, ensuring that existing negative traits are tackled and demand is stimulated through the development of markets and products still represents a challenge to making greater use of this legume (Mayes et al., 2019). It could be a potential future crop to be exploited for food security, climate change mitigation and poverty alleviation, given that it is well adapted to various agro-ecological conditions and climate change (Temegne et al., 2018).

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SUL e-CONFERENCE – PAPER 06

MANAGEMENT OF VIRUS DISEASES IN A CHANGING CLIMATE: ROLE OF UNDERUTILIZED LEGUMES

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Abstract

The development of diseases in a host plant is dependent on three major factors such as a susceptible host, virulent pathogen and a conducive environment. This review highlighted the interactions of climate elements and cropping systems with plant viruses and their vectors as well as the impacts on crops. Extreme weather conditions could adversely affect viral disease emergence and resurgence. Climate change can exacerbate the susceptibility of a plant host to virus infection and as well increase the degree of virulence of the virus. The results of these interactions are increased incidence and severity of symptoms and resurgence/reemergence of virus diseases. All these are indicators for food shortage and food insecurity. In order to alleviate this occurrence, effective disease management strategies are required. Apart from breeding for resistance cultivars, which is an ideal means of disease control; the use of pesticides for the control of virus vectors is also good but it has environmental concerns. The integration of underutilized legumes in the cropping systems could be a laudable approach for the control of viruses in a changing climate. The use of the legumes in crop rotation, mixed cropping, intercropping as well as alley cropping results in pest, weed and disease control. These types of cropping systems increase soil nutrients and crop yield. Therefore, there is need to incorporate climate smart agriculture into our farming systems which has a profound effect in mitigating food insecurity. The overall effect is reduction in food shortage and increased farmers’ income.

Keywords: Cropping system, disease, food security, underutilized legumes, virus

INTRODUCTION

Climate is defined as the average weather pattern of a place over a long period of time (Salaudeen et al., 2016). Climate change occurs due to global warming resulting from the release of greenhouse gases (such as methane, carbon dioxide, nitrogen oxide) which trap heat in the atmosphere, hence making the earth temperature to be warmer than usual (Ayanwuyi and Akintonde, 2012). Africa is among the areas of the world that are exposed to global warming (Niang, 2014). Increase in global temperature due to climate change has led to a rise in sea levels and has changed the amount and pattern of precipitation and expansion of subtropical deserts (Oyerinde et al., 2013).

Agriculture is one of the most vulnerable sectors to climate change. Changes in climate have been estimated to reduce global agricultural production by 1-5% per decade for the past 30 years (Porter et al., 2014). Therefore, variations in climate have significant effect on global and regional food production especially; the common staple food crops (Kiprotich et al., 2015). Climate change is one of the many ways in which the environment can move in the long term from disease-suppressive to disease-conducive or vice versa (Perkins et al., 2011) and a significant growth in the body of literature has shown how climate change affects plant
diseases (Pautasso et al., 2011). Changes in environmental conditions are likely to exacerbate plant disease symptoms (McElrone et al., 2001) and are the cause of about 44% new disease emergence (Anderson et al., 2004). Plant diseases are caused by bacteria, fungi, nematodes and viruses but the focus of this review is on the effect of climate change on plant virus diseases and their management using underutilized legumes in cropping systems.

**Interaction between plants, viruses and vectors in a changing climate**

It is important to note that the response of plant diseases to different climatic factors varies (Eastburn et al., 2011). Alteration in environmental conditions associated with climatic change such as temperature regimes, atmospheric chemistry and drought have the ability to alter the incidence and severity of plant disease epidemics and disease pressures on natural and crop plant systems (Chakraborty, 2005; Crowl et al., 2008). The development of plant disease is as a result of three-way interaction between a susceptible host plant, a virulent pathogen and a favourable environment; this is referred to as the disease triangle (Fig. 1i) (Eastburn et al., 2011; Grulke, 2011). In case of viruses, transmission and survival of majority depend on biotic vectors (Fig. 1ii) (Whitfield et al., 2015). Insects are the largest class of vectors transmitting viruses; other classes include mites, nematodes and fungi. Among the best studied insect vectors are aphids, whiteflies and thrips other insects such as leafhoppers, planthoppers and beetles also transmit viruses (Bragard et al., 2013). The most economically important emerging viruses transmitted by the three important insects mentioned above are members of the poytviruses, begomoviruses and tospoviruses (Jones, 2009; Jones and Barbetti, 2012). Percentage yield losses experienced from viral attack on agricultural crops ranged between 10 and 100% depending on the virus-host-vector relationships and the prevailing epidemiological factors (Taiwo et al., 2007).

Fig. 1. Disease triangles for non-vector and vector-borne pathosystem scenarios. Source: Jones and Barbetti, 2012.
Effect of climate elements on crop response to virus diseases

Climate encompasses abiotic factors such as rainfall, temperature, sunshine, relative humidity and wind. These fundamental components of climate exert obvious impacts on crop production and yield per unit area, individually or through their interactions (Gautam et al., 2013). Environment is an important factor in the development of plant diseases. Therefore, any alteration in it can affect disease severity and losses due to it (Elad and Pertot, 2014).

Temperature is an important environmental factor which influences plant-pathogen interactions and can either increase or decrease disease resistance (Singh et al., 2018). It plays a key role in disease epidemiology (Daugherty et al., 2009). Increased temperature brings about pole ward shift of agro-climatic zones resulting in the spread of pathogens into new geographic areas where they come in contact with new potential plant host (Etterson and Shaw, 2001). Another impact of increased temperature is high transmission rate of invasive pathogens (Robinet et al., 2011). Multiplication, stability, survival, synergism, spread and perpetuation of viruses are either directly or indirectly dependent on elements of climate (Jones, 2009; Navas-Castillo et al., 2011). The effect of temperature on virus vectors cannot be overemphasized as elevated temperature often increases multiplication and spread of airborne virus vectors such as winged aphids (Raymundo and Bajet, 2000). The reason is that increased temperatures are usually related to increased fitness, higher survival rates, and shorter development times (Bale et al., 2002) thereby affecting disease incidence and severity (Whitfield et al., 2015). Robson et al. (2007) reported that the highest survival rate of the aphid species (*Pentalonia nigronervosa*) responsible for the transmission of Banana bunchy top virus (BBTV) occurs at the temperature of 25 °C. Similarly, the transmission of this virus is most efficient at this same temperature (Anhalt and Almeida, 2008). Therefore, the favourable temperature for the aphid vector could explain the spread of the virus in banana. The spread of Tobacco mosaic virus (TMV) or Turnip mosaic virus (TuMV) was facilitated at a higher temperature by weakening plant defense response (Prasch and Sonnewald, 2013). It should be noted that increased temperature does not always lead to increased virus disease transmission; at times decrease in temperature could result in high virus spread. Kido et al. (2008) reported that fall in temperature from 25 to 20 °C increased the expression of systemic symptoms of *Melon necrotic spot virus* while increased temperature from 20 to 25 °C decreased symptom expression.

Water availability is another climate element affecting plant, virus and vector interactions. It includes rain, high air humidity and high soil moisture, which all support the development of plant diseases in a changing climate. Rain and humidity greatly promote the virulence of pathogens which infect the aerial parts of plants (Velasquez et al., 2018). High relative humidity favours lush plant growth and spread of contact-transmitted viruses in crops and pastures. This is because it is easier for viruses to penetrate wounds on new flushes than the hard-leafed plants typical of low humidity conditions (Jones, 2004). However, extreme weather conditions such as heavy rainfall, drought or flood arising from climate change affect plant virus epidemics. Continuous heavy rainfall events wash away insect vectors from plants, thus, reducing the vector populations and consequently reducing the incidence of vector-borne viruses (Jones, 2016). Flooding which results in water logging could destroy the pupal stage of thrips vectors in the soil. Similarly, oviposition of *Bemisia tabaci* can be damaged by heavy rain, thereby, reducing the population of insect-vectors leading to reduced virus epidemics (Jones, 2016).

Atmospheric CO₂ is also a climate element which has impact on virus and vector epidemiology. Global atmospheric CO₂ concentration has increased as a result of human
consumption of natural resources such as burning of fossil fuels and deforestation (IPCC, 2013). Positive impact of elevated CO$_2$ (eCO$_2$) to plant physiology include enhanced biomass and yield resulting from higher photosynthetic rates and water conservation due to increased stomatal closure (Li et al., 2004; Hikosaka et al., 2005). Elevated CO$_2$ can also alter the interactions between plant, insect and pathogen (Nancarrow et al., 2014; Trebicki et al., 2015). These altered interactions often result in serious and frequent outbreaks of insect pests and plant disease in agricultural fields (Percy et al., 2002). Elevated CO$_2$ leading to increased rate of photosynthesis has profound effect on the population size, population dynamics, feeding, fecundity and development of aphids (Dader et al., 2016; Trebicki et al., 2016). The population size and fecundity of bird cherry-oat aphid (Rhopalosiphum padi) was reported to increase under eCO$_2$ (Xing et al., 2003). Vassiliadis et al. (2016) reported that Barley yellow dwarf virus (BYDV)-infected wheat plants showed more severe symptoms and higher virus titers under eCO$_2$.

The effect of wind on plants is dependent on its speed, duration and the rate at which it can penetrate canopy layers (Burgess et al., 2016). Insect activity, development and dispersal of pests and diseases are affected by high winds (Shaw, 2012). Projected periodic increases in wind velocity associated with climate change are likely to increase dispersal of insect and mite vectors of viruses via wind currents (De Barro, 1995), leading to virus epidemics over wider areas. The interaction between environmental variables, plants and pathogens leading to disease formation is explained in Fig. 2 below while Table 1 explains the impact of climate elements on plant virus biological parameters.

![Fig. 2. Impact of environmental conditions on plant-pathogen interactions](image-url)
The interaction between the environment, plant host and pathogen is a tripartite relationship which operates within a continuum, from interactions fully conducive for disease (disease optima) to those that maintain healthy plants.

Source: Velásquez et al. (2018)

Table 1. Effect of climate change parameters at microclimate to regional climate scales on plant virus biological parameters

<table>
<thead>
<tr>
<th>Climate change parameters</th>
<th>Effect of parameters on vectors and hosts</th>
<th>Effect of parameters on viruses</th>
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</thead>
<tbody>
<tr>
<td>(a) Direct</td>
<td>Affects vector distribution</td>
<td>Ability to survive extreme weather events within plant hosts</td>
</tr>
<tr>
<td>Mean temperature</td>
<td>Affects vector abundance</td>
<td>Ability to survive desiccation and ultraviolet light outside plant hosts</td>
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<td>Maximum mean temperature (including heat waves)</td>
<td>Affects vector activity and behavior</td>
<td>Influence of greenhouse gases on virus multiplication within hosts</td>
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<tr>
<td>Minimum mean temperature (including freezing)</td>
<td>Methods of vector survival between growing periods</td>
<td>Entry via wounds</td>
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<tr>
<td>Mean rainfall and altered rainfall patterns</td>
<td>Ability of vector to survive extreme rainfall-related events</td>
<td>Soil-borne vector transmission</td>
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<tr>
<td>Extreme rainfall-related events (including monsoonal rain, hail, flooding, and drought)</td>
<td>Ability of vector to survive extreme rainfall-related events</td>
<td>Soil-borne vector transmission</td>
</tr>
<tr>
<td>Relative humidity (including leaf microclimates)</td>
<td>Ability of vector to survive extreme high winds</td>
<td>Transmission by contact</td>
</tr>
<tr>
<td>Wind speed and direction</td>
<td>Influence of increased greenhouse gases on vector populations</td>
<td>Transmission by wind-mediated contact transmission or water</td>
</tr>
<tr>
<td>Greenhouse gas concentration</td>
<td>Vector infestation of alternative cultivated or weed reservoir hosts</td>
<td>Transmission by seed, pollen, or vegetative propagation</td>
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<th>Climate change parameters</th>
<th>Effect of parameters on vectors and hosts</th>
<th>Effect of parameters on viruses</th>
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<tbody>
<tr>
<td>General climate instability</td>
<td>Alterations to host physiology affecting attractiveness to vectors</td>
<td>Importance of alternative cultivated plant or weed reservoir hosts</td>
</tr>
<tr>
<td>(b) Indirect</td>
<td>Alterations to host physiology affecting efficiency of vector transmission</td>
<td>Ability to persist and multiply inside or upon vectors</td>
</tr>
<tr>
<td>Altered ranges of cultivated plants grown</td>
<td>Alterations to plant morphology influencing attractiveness to vectors</td>
<td>Ability to multiply and spread within plant hosts</td>
</tr>
<tr>
<td>Alterations in regional areas cultivated</td>
<td>Alterations to plant morphology influencing direct virus infection</td>
<td>Changes in rates of systemic movement within plant hosts</td>
</tr>
<tr>
<td>Alterations in alternative cultivated or weed reservoir hosts</td>
<td>Alterations to host or vector phenology</td>
<td>Ability to evolve rapidly and invade new hosts</td>
</tr>
<tr>
<td>Changes in cultivation Systems</td>
<td>Alterations in vector activity due to the presence of another vector, a predator or a parasite/parasitoid</td>
<td>Generalist or specialist</td>
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<td></td>
<td>Alterations to temperature sensitivity of host resistance to vectors or viruses</td>
<td>Alterations in symptom expression and virus titer within single or mixed host infections</td>
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<td>Alterations in effectiveness of chemical control measures against vectors</td>
<td>Alterations in effectiveness of cultural control measures</td>
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<td></td>
<td>Alterations in effectiveness of cultural control measures against vectors</td>
<td>Alterations in effectiveness of phytosanitary control measures</td>
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<td></td>
<td>Alterations in effectiveness of biological control measures against vectors</td>
<td>Alterations in effectiveness of biological control measures against viruses (such as cross-protection)</td>
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Source: Jones, 2016
Role of underutilized legumes in increasing food security and virus disease management

Underutilized legumes (ULs) are a group of flowering plant species belonging to the family Fabaceae. They are named ULs because they are less known, neglected and less exploited food legumes. They are also referred to as orphan crops. Examples of ULs include African yam bean, pigeon pea, lima bean, winged bean, mung bean, jack bean, sword bean, e.t.c. Some of the significant roles played by ULs include being a source of quality protein, medicinal value, animal fodder, natural fertilizer, restoration of environment as well as soil enrichment property (Singh et al., 2007). Underutilized legumes have the ability to withstand abiotic stresses and climate change effect (Popoola et al., 2019). They are resilient and possess desirable traits which are useful for climate change adaptation (Mabhaudhi et al., 2019).

The use of eco-friendly approaches in disease management is important for sustainable crop production. There is need for the adoption of novel approaches to counter the resurgence of viral diseases under changed climatic scenario. Breeding and cultivation of resistant cultivars is the best solution to the problems of climate change on agricultural crops. However, integration of underutilized legumes in cropping systems such as crop rotation, intercropping and mixed cropping will go a long way in increasing food security and reducing virus occurrence on the field.

Alley cropping

Alley cropping is an agricultural practice involving the cultivation of leguminous shrub or tree species with annual crops (Marin et al., 2007). Residues from the leguminous trees serve as organic fertilizers which increase soil fertility (Oliveira et al., 2018). Alley cropping improves the biological, chemical and physical properties of soils by incorporating organic matter into the soil (Ekepu and Tirivanhu, 2016). Aihou et al. (2006) reported the use of pigeon pea in alley cropping for soil fertility restoration while Atachi et al. (2006) reported its use in pest management. The advantages of growing legumes with alternative crops include nitrogen fixation, insect pest reduction, disease prevention and increase in yield (Majumdar, 2011). Legumes play an important role by breaking the cycles of pests and diseases in many areas of the world (Jensen et al., 2010; Kopke and Nemecek, 2010).

Crop rotation

Crop rotation is the practice of growing different crops on the same piece of land at different times (Bybee-Finley and Ryan, 2018). It serves a dual purpose of improving soil quality and productivity (Zotarelli et al., 2007; Gan et al., 2015) as well as suppression of pests and pathogens (Zhu et al., 2000; Gurr et al., 2003). Legume-based rotations have been proved to be an effective approach in improving soil nitrogen and increasing water use efficiency and also reducing plant diseases (Govarrts et al., 2007; Kutcher et al., 2011).

Introduction of legumes into agricultural rotations helps in reducing the use of fertilizers, thereby, lowering the emission of greenhouse gases (GHG) such as carbon dioxide (CO₂) and nitrous oxide (N₂O) (Reckling et al., 2014). Other positive effect of grain legumes in rotation include improvements of soil organic matter and structure (Hernanz et al., 2009), phosphorus mobilization (Shen et al., 2011), retention and availability of soil water (Angus et al., 1991) and reduced pressure from diseases and weeds (Robson et al., 2002). Grain legumes also serve as break crops when used in rotation with other crops such as cereal crops. This is because they are not susceptible to the same pests and diseases which affect cereals (Zander et al., 2016). Grain legumes as break crops also help in weed control (Seymour et al., 2012). Faba bean in crop rotation disrupted pests and disease cycles (Stoddard et al., 2010).
**Intercropping**

Intercropping is defined as an agricultural practice involving the cultivation of two or more crops on the same piece of land at the same time (Matusso et al., 2014). Intercropping is grouped into different types which include growing crops in alternating rows (row intercropping), growing crops in alternating strips (strip intercropping), crops grown in relay (relay cropping) and mixed intercropping (He et al., 2019). It is an easy and effective agricultural practice which helps in reducing disease and pest infestations (Li et al., 2009; Ding et al., 2015). Intercropping minor legumes with other non-leguminous crops provide the following benefits: enhanced yield, better pest control (Lopes et al., 2016), pollution mitigation (Luo et al., 2016), competitive yields with reduced inputs (Monti et al., 2016), reduced disease occurrence and improved access to essential elements such as phosphorus (Tilman et al., 2002). Ememwa et al. (2017) reported that intercropping with a legume significantly reduced Cassava brown streak disease in Western Kenya. The use of ULs in intercropping system was reported by Midya et al. (2005) in which blackgram was used for the smothering of weeds in rice field. In addition, the use of wheat-chickpea intercrop for the biological control of pest was reported by Lopes et al. (2016). Intercropping groundnut with pigeon pea reduced the incidence of Peanut bud necrosis disease by 60.6% (Sunkad et al., 2005).

**Mixed cropping**

Mixed cropping is an effective approach involving the planting of two or more crops in a mixture. Its advantages include balancing the input and output of soil nutrients, weed and insect suppression, plant disease control and resisting of climate extremes leading to overall increase in productivity (Chhabra et al., 2018). Mixed cropping reduced the attack of pests and disease through enhanced biological control or direct control of pests (Gurr et al., 2003).

**Conclusion**

This review highlighted the interactions of climate elements with plant viruses and their vectors as well as the impacts on crops. Extreme weather conditions could adversely affect viral disease emergence and resurgence. Therefore, there is need to incorporate climate smart agriculture (CSA) into our farming systems. The role of underutilized legumes in reducing the incidence of viruses in agricultural crops in a changing climate has a profound effect in mitigating food insecurity. Waha et al. (2012) reported that multiple cropping systems appear to mitigate the effect of climate on crop failure when compared to single cropping systems. Due to the resilience nature of ULs, intercropping them with non-legume based crops would help in reducing pest and disease attack on the field and also lower the adverse effect of climate change in agriculture.

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Proximate and phytonutrients composition of selected Bambara groundnut (Vigna subterranea (L.) Verdc.) accessions

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Abstract

Bambara groundnut (Vigna subterranea (L.) Verdc.) is a member of the underutilized legumes cultivated in some parts of Africa and Asia for numerous purposes though yet to attain its full potential. In this study, five accessions obtained from the Genetic Resources Center, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria were analyzed for proximate and phytonutrients. Results for the proximate analysis showed ash ranged between 3.97% (TVSU-1636) to 3.83% (TVSU-1518); crude fat 5.91% (TVSU-1630) to 5.54% (TVSU-1649); crude protein 21.33% (TVSU-1630) to 19.74% (TVSU-1649) and crude fibre 4.52% (TVSU-1636) to 4.19% (TVSU-1649). Others are moisture 9.75% (TVSU-1636) to 9.88% (TVSU-1570), dry matter 90.26% (TVSU-1636) to 90.07% (TVSU-1649) and carbohydrate 45.47% (TVSU-1636) to 45.05% (TVSU-1518). The phytonutrients (%) composed of flavonoids, alkaloids and saponin. The flavonoids ranged from 0.009 % to 0.005% in TVSU-1636 and TVSU-1649 respectively. The alkaloid varies from 0.182% in TVSU-1649 to 0.248% in TVSU-1636 while the saponin content varies from 0.333% in TVSU-1649 to 0.387% in TVSU-1636. Statistically significant differences (p < 0.05) in all the parameters studied and the accessions. In conclusion, the result of this research suggests that the accessions could support objectives of dietary diversification using Bambara groundnut by providing low-cost protein and other beneficial nutrients for humans and animals in sub-Saharan Africa.

Keywords: Bambara groundnut, nutrients, antinutrients, food and income security, sub-Saharan Africa

INTRODUCTION

Bambara groundnut (Vigna subterranea (L.) Verdc.) is a popular member of the underutilized legumes positioned to support global food insecurity and malnutrition particularly in sub-Saharan Africa if appropriately promoted and utilized (Mayes et al., 2019). Crops classified as underutilized are those that have received less global attention in terms of global trade, cultivation, utilization and research (Stamp et al., 2012; Varshney et al., 2012), even though they have been reputed to serve as simple sources of food and income security among rural dwellers (Padulosi et al., 2013). They are often cultivated by women and smallholders farmers with less farming inputs such as fertilizers (Mabhaudhi et al., 2013; Naylor et al., 2004). The cultivation of underutilized legumes have been reported to have good impacts for subsistence farmers in the area of revenue generation (Durst and Bayasgalanbat, 2014), limiting reliance on major staples for food and animal feed (Chivenge et al., 2015) with the addition of less farming inputs when compared to usual farming systems in regions where they are planted (Stamp et al., 2012). In this study we analysed the proximate and phytonutrients available in bambara groundnut germplasm collected from the Genebank of the International Institute of Tropical Agriculture (IITA). We intend to provide additional
data on the contribution this crop could make in providing a valuable source of nutrition in developing regions. Crops of the legumes are known members of the Fabaceae, superfamily of over 15,000 plant species that have the capacity to fix Nitrogen through the process of biological Nitrogen fixation, have seeds and at times root tubers used as food for humans and feed for animals (Gupta et al., 2015). On the other hand, pulses are members of food legumes utilized majorly for seeds used when properly dried for various uses (Singh, 2017). In recent discussion, legume species used as vegetables such as green bean, those used for soil nutrition or animal feed such as alfalfa and those used for oil extraction such as soybean are not regarded as pulses (Asif et al., 2013; Duranti and Gius, 1997). However, soybean due to its numerous functions now assume a good source of plant-based protein and re-classified as pulse (Considine et al., 2017; Foyer et al., 2016; Vollmann, 2016). Common examples of pulses referred to as orphan, neglected or underutilized legumes are Bambara groundnut, African yam bean, winged bean, adzuki, jack and marama beans. Others are Kersting’s groundnut, mung and rice beans, grass and pigeon pea as well as lupin (Cullis and Kunert, 2017; Padulosi et al., 2013). Owing to reduced cultivation, (Foyer et al., 2016) and global utilization (Miller et al., 2017), they (pulses) do not make any significant impact to global nutrition intake when compared to staples such as rice (FAO, 2017). Even though, they play a very important role in rural populations where they provide affordable and nutritious meals (Asif et al., 2013; Graham and Vance, 2003; Heim et al., 2017; Mudryj et al., 2014). They also support improvement in human health conditions such as those who suffer from diabetes (Becerra-Tomás et al., 2018), hypertension (Polak et al., 2015), body weight management and dietary control (McCrary et al., 2010), and also contributing to reduced levels of fat (Bazzano et al., 2011). Bambara groundnut seeds have been utilized and processed in several ways for human and animal use. Mubaiwa et al. (2017) describes partly arid area of Zimbabwe, Southern Africa. In many West African countries such as Benin Republic, freshly harvested pods are boiled with salt and consumed with pepper to be eaten as snack. Flour can also be produced from the seeds when milled and put to several uses (Kaptso et al., 2015). From the fresh seeds, delicious steamed-paste popularly called moi moi or okpa (porridge made from the seeds) are usually produced and widely common in some parts of Nigeria (Okpuzor et al., 2010). In Nigeria, especially in the East and some part of Northern Nigeria, Bambara groundnut is an important food crop and can be used in traditional preparation of various meals. The seeds are sometimes roasted, boiled or milled and used in preparing soup (Adu-Dapaah and Sangwan, 2004) or roasted and chewed with palm kernel. Animals also benefit by the use of Bambara groundnut seed haulms for feeding of livestock and poultry (Anchirinah et al., 2001). According to Mayes et al. (2019), flour from Bambara groundnut seeds can be used to make confectionary products such as cakes and bread. Milk obtained from bambara groundnut competes well to that from soybean with bambara groundnut milk containing up to 20 % protein compared with about 4% protein in the soy milk (Adu-Dapaah et al. 2016) and mostly preferred to that from other pulses due to its flavour and colour (Goli 1997). In Indonesia, a deep-fried Bambara groundnut snack made from the immature seed is highly sought after. Known as ‘Kacang Bogor’ (‘Bogor nut’), it fetches high prices in supermarkets and even in specialist food shops in Europe. In appearance, it is similar to dry roasted peanut, but is drier (less oil) and more strongly flavoured (Sri Redjeki, pers. comm.). Nutritionist have developed several recipes using bambara groundnut as substitutes for other ingredients in Indonesia, Malaysia (Crops For the Future) and recently at the Food and Nutrition Sciences Laboratory, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria (Feldman et al., 2019).
MATERIALS AND METHODS

Materials: The five accessions of Bambara groundnut used in this research were obtained from the Genetic Resources Center, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. All chemicals used were of analytical grade using standard laboratory procedures.

Proximate analysis: The moisture content of the accessions was determined after drying at 105°C until a constant weight was attained (Indudhara Swamy et al., 1971). The micro-Kjeldahl method was employed to determine the crude protein (N×5.95) (International, 2005). Crude lipids were extracted with petroleum ether, using a Soxhlet apparatus and ash contents were determined based on methods outlined in AOAC (2000). Total carbohydrate was calculated by the difference method (adding the values of moisture, crude protein, ash and crude fat and subtracting the sum from 100) (McDonald, 1973). Samples were analyzed chemically for crude fibre according to the official methods of analysis described by the Association of Official Analytical Chemist (International, 2005). The dry matter content was determined according to AOAC official method reference no 967.08 while ash was determined according to AOAC official method 942.05. All analysis was carried out in duplicate.

Antinutrient analysis: Alkaloids determination analysis was done using spectrophotometric method as described by Arntfield et al. (1985). While Saponins and Flavonoids were determined according to Association of Official Analytical Chemist standard referenced procedures (Chemists, 1990).

Statistical analysis: The analysis was carried out in duplicates for all determinations and the results expressed as mean ± SE. SAS 9.4 was used for the Analysis of Variance (ANOVA). Significance of the differences was defined as p<0.05 for ANOVA. The difference in mean was compared using the Duncan’s new Multiple Range test (Duncan, 1955).

RESULTS AND DISCUSSION

RESULTS

Proximate Composition. All proximate components in the seed (Tables 1) were highly significant (P < 0.0001). Protein content varied from 21.13 % in TVSU-1518 to 19.75% in TVSU-1570. Crude fat content ranges from 5.91 (TVSU-1630) to 5.54 % (TVSU-1649); crude fibre contains 4.19 % in TVSU-1649 and 4.52 % in TVSU-1630. Ash values ranges from 3.81% in TVSU-1518 to 3.97% in both TVSU-1630 and TVSU-246; moisture content varies from 9.745% in TVSU-1630 to 9.93% in TVSU-1649, dry matter ranges from 90.12% in both TVSU-1570 and TVSU-246 to 90.25% in TVSU-1630 and carbohydrate varies from 45.05% in TVSU-1518 to 43.25%. The phytonutrients such as flavonoids, alkaloids and saponin. Flavonoids values varies from 0.0094% (TVSU-1630) to 0.00505 (TVSU-1649); alkaloids; 0.2135% (TVSU-1518) and 0.182% (TVSU-1649) and saponin 0.387% (TVSU-1630) to 0.3325 % (TVSU-1649) (Table 1).
Table 1: Proximate composition (%) of selected Bambara groundnut accessions

<table>
<thead>
<tr>
<th>Accession</th>
<th>CP ± SE</th>
<th>C fat ± SE</th>
<th>C fibre ± SE</th>
<th>Ash ± SE</th>
<th>M ± SE</th>
<th>DM ± SE</th>
<th>CHO ± SE</th>
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<tr>
<td>TVSU-1518</td>
<td>21.13 ± 0.0082&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.81 ± 0.0057&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.47 ± 0.008&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.81 ± 0.0035&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9.815 ± 0.0065&lt;sup&gt;c&lt;/sup&gt;</td>
<td>90.185 ± 0.0065&lt;sup&gt;b&lt;/sup&gt;</td>
<td>45.05 ± 0.0083&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>TVSU-1570</td>
<td>19.93 ± 0.0082&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.69 ± 0.0057&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.25 ± 0.008&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.80 ± 0.0035&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.875 ± 0.0065&lt;sup&gt;b&lt;/sup&gt;</td>
<td>90.125 ± 0.0065&lt;sup&gt;c&lt;/sup&gt;</td>
<td>43.53 ± 0.0083&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>TVSU-1630</td>
<td>21.33 ± 0.0082&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.91 ± 0.0057&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.52 ± 0.008&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.97 ± 0.0035&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.745 ± 0.0065&lt;sup&gt;d&lt;/sup&gt;</td>
<td>90.255 ± 0.0065&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45.47 ± 0.0083&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TVSU-1649</td>
<td>19.74 ± 0.0082&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5.54 ± 0.0057&lt;sup&gt;e&lt;/sup&gt;</td>
<td>4.19 ± 0.008&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3.87 ± 0.0035&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.93 ± 0.0065&lt;sup&gt;a&lt;/sup&gt;</td>
<td>90.07 ± 0.0065&lt;sup&gt;d&lt;/sup&gt;</td>
<td>43.26 ± 0.0083&lt;sup&gt;e&lt;/sup&gt;</td>
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<tr>
<td>TVSU-246</td>
<td>20.12 ± 0.0082&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.77 ± 0.0057&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.30 ± 0.008&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.91 ± 0.0035&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.88 ± 0.0065&lt;sup&gt;b&lt;/sup&gt;</td>
<td>90.12 ± 0.0065&lt;sup&gt;c&lt;/sup&gt;</td>
<td>43.98 ± 0.0083&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>CV</td>
<td>0.05</td>
<td>0.14</td>
<td>0.27</td>
<td>0.12</td>
<td>0.09</td>
<td>0.01</td>
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<td>F Value</td>
<td>&lt;.0001*</td>
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Key: CP= Crude protein; C fat=Crude fat; M=Moisture; DM= Dry matter; CHO=Carbohydrate; CV= Coefficient of variation, SE= Standard Error; TVSU=Tropical Vigna sub Vigna subterranea * Significant level at $P < 0.0001$. Means with the same subscript along the column are not significantly different ($P<0.05$).
Carbohydrates, proteins, fat and fibre are often regarded as proximate composition which typically describes the major constituents of any food products analysis (Finglas et al., 2014; Greenfield and Southgate, 2003). The proximate analysis also explained the moisture (or dry matter) as well as ash in a food product which further differs from the macro and micro elements found in any food products. In nature, pulses are reputed to have high levels of protein (up to about 40%) and fibre (20% less or more) than commonly consumed cereals and usually low levels of fat (often below 10%) when compared with oilseeds (Gepts et al., 2005). Consequently, the amount of these proximate are different from pulses or within species due to several factors such as variation due to genetic and environmental interactions (Amarteifio et al., 2010; Medic et al., 2014). Studies have shown that bambara groundnut has higher mean composition of these proximate but lower concentration of dietary fibre when matched with chickpea. However, when matched within the genus Vigna, it has mean reported concentrations at levels similar to those found in cowpea and mungbean, both of which have benefited from special breeding programs over the 20 years to improve their nutritional and food quality (Boukar et al., 2016; Ebert, 2014; Kim et al., 2015; Muñoz-Amatriain et al., 2017; Shanmugasundaram et al., 2009).

**Carbohydrate composition**

It has been reported that polysaccharides play an important role in human nutrition, with starch representing a major source of calories providing a mutual support with resistant starch to improve gut health (Chibbar et al., 2010), and the management of of common diseases (Smith et al., 2012; Tosh and Yada, 2010). The higher amylose content in pulse starch compared with cereal or tuber starches contributes to a lower rate of starch digestion in the human gut. This has led to the classification of pulse-derived starches as slowly digestible and resistant starches (McCrorry et al., 2010), resulting in a low glycaemic index (GI), suitable for inclusion into diets for management of diabetes (Birt et al., 2013; Curran, 2012). The carbohydrate component of Bambara groundnut includes a high proportion (up to 53%) of starch (Yao et al., 2015), comparable to levels reported for chickpea, and over four times the maximum levels reported for soybean (Stevenson et al., 2006). The values reported in this study was however between TVSU-1518 (45.05%) and in TVSU-1649 (43.26%). The starch amylose component of bambara groundnut seed is comparable to related Vigna crops, cowpea and mungbean, suggesting a generic level substantially higher than the mean of 15–30% starch reported for pulse carbohydrates (Hoover et al., 2010).

**Protein**

It is widely known that pulse seeds contain between 20–40% protein (Pandey et al., 2016), at least twice that found in cereal grains (Asif et al., 2013). Although bambara groundnut has been highly regarded as tropical rich high protein crops (Goudoum et al., 2016; Hillocks et al., 2012; Massawe et al., 2002), several reported values for protein concentrations are well below 25% (Boateng et al., 2013; Mubaiwa et al., 2017; Mwale et al., 2007). The result from this current study also corroborates these claims. Bambara groundnut (V. subterranea) has a higher maximum concentration of crude protein in comparison to other pulses. Due to this, protein reported in the bambara groundnut global gene pool reported to date varies from 9.6–30.7% and there appears to be the opportunity for increasing the protein content through well planned breeding programme. When this approach is adopted, there could be the transformation of a currently underutilized crop to one that potentially could make a more significant contribution to food and nutritional security especially in areas they are cultivated according to Mayes et al. (2019). On the other hand, protein quality of foods is usually judged based on the digestibility of the proteins and quantification of essential amino acids in relation to nutritional requirements (Vaz Patto et al., 2015).
Corrected Amino Acid Score (PDCAAS) has been used as a measure for assessing protein quality for the more than two decades (Hughes et al., 2011), with bambara groundnut protein having a reported score of 32.8 (Mune et al., 2011). This is much lower than reported for other pulses, such as lentil (51–63), chickpea (52), or soybean (100) (Boye et al., 2012; Vaz Patto et al., 2015). The protein digestibility of pulses can be improved by utilisation of domestic processes such as soaking, autoclaving, and cooking (Boye et al., 2010).

**Fatty acids**
Bambara groundnut has low levels of fat when compared with other oilseeds crops such as groundnut and soyabean, and is consistent with the reported values established for pulses (Mudryj et al., 2014), and classifies it as a legume species not used for oil extraction (Asif et al., 2013; Vollmann, 2016). However, bambara groundnut has a higher mean concentration of total fat in comparison to chickpea, cowpea and mungbean. The concentration and specific composition of bambara groundnut fatty acids is likely to represent a significant nutritional source, beneficial for human health in diets otherwise lacking readily available sources of animal or vegetable oils. Some studies have reported predominant fatty acids in bambara seed oil are oleic acid, linoleic acid, palmitic acid, linolenic acid and stearic acid. Based on reported data to date, there exists large variation for concentrations of oleic, linoleic and linolenic acids within the bambara groundnut genepool. Some studies (Adeleke et al., 2018; Minka and Bruneteau, 2000) have reported oleic and linoleic acid concentrations each higher than 40% of the total seed fatty acid content. Of particular interest to human health are the essential dietary fatty acids linoleic acid (omega-6), and linolenic acid (omega-3). Bambara seed oil has a concentration of linoleic acid in a similar range to other Vigna crops cowpea and mungbean, and higher linolenic acid concentration than soybean and chickpea. Both linoleic and linolenic acids appear to have important human health benefits in their unmodified form, as clinical trial data suggest that intake can reduce the likelihood of hypercholesterolemia and improve cardiovascular function (Halimi et al., 2019; Mensink et al., 2003; Wanders et al., 2010). According to Mayes et al. (2019) since bambara groundnut has a similar fatty acid profile, there is a window to substitute the related Vigna cowpea or mungbean crops in some production systems where their cultivation may be favorable.

**Crude Fibre**
The concentrations of total, insoluble and soluble dietary fibre reported for bambara groundnut are approximately half that reported for chickpea, but comparable with other Vigna species, and follow the general pattern established for pulses. However, these data are not consistent with the claim that Bambara groundnut has the highest amount of soluble dietary fibre amongst grain legumes (Adeyeye et al., 2015; Murevanhema and Jideani, 2013; Olaleye et al., 2013). Unfortunately, experimental data for bambara groundnut insoluble dietary fibre and soluble dietary fibre concentrations are limited, with data available only from a single study (Yao et al., 2015). As a result, the extent of within species variation for these fibre fractions is not known globally. The availability of such data could help resolve uncertainty in recommending inclusion of Bambara groundnut within diets for management of diabetes and other cardiovascular conditions. Crude fibre contains 4.19 % in TVSU-1649 and 4.52 % in TVSU-1630 and similar to other previous studies on Bambara groundnut proximate values.

**Moisture Content**
It has been reported that the optimum moisture content for pulses is in the range of 9–12% to avoid the production of mycotoxins and for safe storage. At a moisture level below 10%,
respiration in most food grain almost stops, increasing grain storage life (Sujeetha et al., 2014). The moisture content of the Bambara groundnut accessions analyzed was less than 10% and fell within the optimum moisture content range for safe storage of pulses. (i.e low moisture content analyzed explains why Bambara groundnut seeds obtained from pods harvested during the dry season (without any additional drying after harvest) can be stored in polyethylene bags for several months at room temperature without microbial growth and deterioration (Baiyeri et al., 2018). The values obtained in this study were less than the results reported by Ojuederie and Balogun (2017) for African yam bean (11.3 to 12.6%).

Figure 1: % Composition of phytonutrients in selected Bambara groundnut accessions

Phytonutrients composition
The presence of anti-nutritional factors or phytonutrients in pulses, including enzyme inhibitors, flatulence factors, tannins, phytic acid and saponins (Rochfort and Panozzo, 2007) hinder their wider utilization and consumption (Soetan and Oyewole, 2009). The antinutrients can reduce protein digestibility, affecting bioavailability of amino acids by up to 50% (Gilani et al., 2012), as well as lowering digestibility and bioavailability of other nutrients (Sandberg, 2002). Their removal is considered a breeding target for most pulses, as this could also
potentially increase digestibility and improve taste (Wang et al., 2003). Cooking and thermal treatment of pulse seeds causes inactivation several types (Bora, 2014). Soaking followed by cooking of bambara groundnut seeds has been shown to reduce tannin and phytic acid content with a concomitant increase in in-vitro protein digestibility (Mazahib et al., 2013). Processing methods such as soaking, germination, fermentation, and treatment with phytase have been successful in reducing phytic acid content of chickpeas, sorghum and millet (Gupta et al., 2015). The results of phytoneutrients or anti-nutritional factors of the five accessions were shown in figure 1. It was observed that the accessions had comparable results with previous results on African yam bean by Ajibola and Olapade (2016) and were lower than those obtained from (Nwosu, 2013) and these values fall within the permissible limit based on (Ndidi et al., 2014).

Conclusion

Underutilized pulses such as bambara groundnut can make a positive contribution to food and nutritional security at both the regional and global level. The role of bambara groundnut as a subsistence crop for predominantly female farmers in sub-Saharan Africa supports its potential to provide income and food security in developing countries where the crop is grown. Further improvement of the nutritional properties of this crop appears attainable and would elevate its status as a nutritious crop, enabling it to be promoted in Africa and Southeast Asia for wider inclusion into human diets.

References


HEPATO PROTECTIVE ACTIVITIES OF CAJANUS CAJAN SEED EXTRACT ON STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Abstract

Cajanus cajan (L.) Millsp. belonging to the botanical family leguminosae are considered to be beneficial to human health. This study investigated the hepato-protective activities of Cajanus cajan extract in streptozotocin-induced diabetic rats. Sixteen physiologically normal rats were assigned to group I to serve as normal control and the diabetic rats (48) were randomly grouped into groups II, III, and IV. Group II animals were left untreated and served as diabetic control while groups III, and IV were respectively treated orally with Cajanus cajan seed extract and glibenclamide (standard antidiabetic drug). Four animals per group were sacrificed weekly and parameters such as glucose and liver function markers were estimated after the treatment. The Alanine Aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP) and γ-glutamyl transaminase (GGT) assay were used to assess the effect of Cajanus cajan on the liver of rats. Data obtained were statistically analyzed with one-way analysis of variance and results were considered statistically significant at p < 0.05. The extract reversed the plasma concentration of AST, ALT, ALP and GGT in the diabetic rats which were disrupted in the streptozotocin-induced diabetic rats. Cajanus cajan ameliorates the effects of streptozotocin in diabetic rats.

Keywords: Cajanus cajan, liver function, diabetes

INTRODUCTION

Cajanus cajan (L.) Millsp. belonging to the botanical family leguminosae known as “fio fio” in Igbo, ‘otii’ in Yoruba, ‘waken-masar’ in Hausa and pigeon pea in English (Aiyeloja and Bello, 2012), is native to India which is the world’s largest producer. Chemical constituents’ investigations have indicated that pigeon pea leaves are rich in flavonoids and stilbenes, which are considered to be responsible for the beneficial efficacies of pigeon pea leaves on human health (Parrotta, 2001).

Chronic hepatic disease is one of the foremost health problems worldwide, with liver cirrhosis and drug induced liver injury accounting for the ninth leading cause of death amongst the western and developing countries population (Saleem et al., 2010). It is, therefore, necessary to explore the herbal options in the management of drug induced liver damage to replace the currently used drugs of low efficacy and safety. This study investigated the hepato-protective activity of Cajanus cajan extract in streptozotocin-induced diabetic rats.
MATERIALS AND METHODS

Experimental Design

Sixteen physiologically normal rats were assigned to group I to serve as normal control and the diabetic rats (48) were randomly grouped into groups II, III, and IV. Group II animals were left untreated and served as diabetic control while groups III, and IV were respectively treated orally with *Cajanus cajan* seed extract and glibenclamide (standard antidiabetic drug).

Four animals per group were sacrificed weekly and parameters such as glucose and liver function markers were estimated after treatment.

The animals were allowed to fast for 12 hrs after which they were sacrificed. Liver and plasma from each rats were collected for biochemical assays (ALP, ALT, AST and GGT). Data obtained were statistically analyzed with one-way analysis of variance and results were considered statistically significant at p < 0.05.

RESULTS AND DISCUSSION

Significant increases (p < 0.05) were observed in the activities of plasma AST, ALT, ALP and Malondialdehyde (MDA) of the streptozotocin only challenged group (negative control) when compared to the treated groups and normal control group. On the contrary, a significant decrease (p < 0.05) in the activities of these enzymes were observed in the liver. The *Cajanus cajan* seed extract was able to reverse the abnormality caused by the exposure to STZ. The extract was able to reduce elevated fasting blood glucose level in the Streptozotocin-induced diabetic rats as observed in Figure 1. Plasma AST, ALP, ALT and MDA were also reduced as observed in Figures 2, 3, 5 and 6 respectively. This decrease was comparable to the normal control and Glibenclamide treated rats. Aja *et al.* (2015) reported significant (P < 0.05) decrease in ALT, AST and ALP levels in alloxan induced diabetic albino rats treated with *C. cajan* and ethanol leaf extracts in dose dependent manner.

Liver injury is the result of oxidative stress induced by hepatotoxic agent introduced into the liver. Phytochemical constituents of the *C. cajan* extract are likely responsible for the inhibition of lipid peroxidation indicated by reduced levels of malondialdehyde in the treated rats. This study further confirms the findings of Ilesanmi *et al.* 2014, that *C. cajan* seed extract possess phytochemicals with significant antioxidant properties, a factor which may be responsible for hepatoprotective effect of the plant. The natural protective mechanisms of the liver which might have been compromised with the STZ administration was ameliorated as observed in the levels of liver marker enzymes and histological observations (Plate 1).
1. Effects of methanol extract of *C. cajan* seeds on fasting blood glucose level in rats. Values were expressed as Mean ± SEM, (n=16).

2. Effects of methanol extracts of *C. cajan* seeds on liver and plasma Aspartate aminotransferase (AST). Values were expressed as Mean ± SEM, (n=9). Bars with different alphabets are significantly different (p < 0.05).
Figure 3: Effects of methanol extracts of *C. cajan* seeds on plasma alkaline phosphatase (ALP). Values were expressed as Mean ± SEM, (n=16). Bars with different alphabets are significantly different (p < 0.05).

Figure 4: Effects of methanol extracts of *C. cajan* seeds on liver γ-glutamyl transaminase (GGT). Values were expressed as Mean ± SEM, (n=16). Bars with different alphabets are significantly different (p < 0.05).
Figure 5: Effects of methanol extracts of *C. cajan* seed extract on liver alanine amino transaminase (ALT). Values were expressed as Mean ± SEM, (n=16). Bars with different alphabets are significantly different (p < 0.05).

Figure 6: Effects of methanol extracts of *C. cajan* seeds extract on Malondialdehyde (MDA) levels in the plasma, kidney and liver of rats. Values were expressed as Mean ± SEM, (n=16). Bars with different alphabets are significantly different (p < 0.05).
CONCLUSION

*Cajanus cajan* extract was able to reverse liver damage in the STZ-induced rats, thereby preventing leakage of liver enzymes into system, acting as hepato-protective and hepato-curative agents. These findings validate the traditional use of this plant as liver tonic.

References


PROXIMATE CONSTITUENTS AND MINERAL COMPOSITIONS OF Moringa oleifera LEAF AND Cajanus cajan SEED EXTRACTS

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2 Department of Biochemistry, College of Biosciences. Federal University of Agriculture, Abeokuta, Nigeria.

ABSTRACT

This study was conducted to investigate the proximate constituent and mineral composition of Moringa oleifera and Cajanus cajan seed extracts and infer their possible implications as anti-diabetic seed extract. Proximate compositions of methanol extracts of M. oleifera leaf and C. cajan seeds are shown in Figure 1. Moisture content, free fatty acid, carbohydrate and ash contents in M. oleifera leaves were lower than C. cajan but not significantly different (p < 0.05), while M. oleifera leaves had significantly (p < 0.05) higher protein (49 %) and crude fibre (68 %) than C. cajan seeds. The free fatty acid value for C. cajan was 4.36 mg NaOH /g and M. oleifera was 3.20 mg NaOH /g. The mineral elements estimated in M. oleifera leaf and C. cajan seeds were magnesium, calcium, potassium, sodium, manganese, iron, zinc, nickel, lead, chromium and phosphorus as shown in Table 1. It was observed that M. oleifera contained higher quantity of calcium (94 %) than C. cajan. However, very low concentration of manganese, selenium and nickel were present in both plants. The lead and chromium levels were not detected in both plant samples. Further mineral elements estimated in C. cajan seeds were magnesium, calcium, potassium, sodium, manganese, iron, zinc, nickel, lead, chromium and phosphorus. However, very low concentration of manganese, selenium and nickel were present in the plants. The lead and chromium levels were not detected in both plant samples. The appreciable amount of mineral elements detected in the two plant extracts could have given credence to their anti-diabetic properties.

Keywords:...
2014; Nahar *et al.*, 2014). Moreover, in tradedicine, treatments of diseases or ailments usually involve a combination of two or more plant extracts with certain distinctive individual pharmacological relevance (Shohawon and Mahomoodally, 2013). Many herbs have been recommended for treating diabetes (Adeneye *et al.*, 2014; Akomas *et al.*, 2014; Ezike *et al.*, 2010). According to World Health Organization (WHO, 2016), 65–80 % of the World's population relied on traditional remedies for their primary health care needs. However, there has been increasing interest in exploiting the therapeutic efficacy of herbs / plants, due to their natural origin, cost effectiveness and lesser side effects (Naik *et al.*, 2003).

This is what informed the focus of this study which is to evaluate the nutritional potentials of *Moringa oleifera* and *Cajan cajan* in terms of proximate constituents and mineral compositions and their possible indications as anti-diabetic seed extracts.

2.0 MATERIALS AND METHODS

2.1.1 Plant Source, Collection and authentication of plant materials

The leaves of *Moringa oleifera* were collected from a biological garden at Onireke, Ibadan, Oyo state, Nigeria and *Cajan cajan* seeds were purchased from Bodija market in Ibadan. The plant materials were identified and authenticated by plant taxonomists (Mr. Ugbo O.A. and Mr. Shosanya O. S.). Voucher Specimen number FHI. 108486 was assigned to the *Moringa oleifera* leaf and FHI. 112436 was assigned to *Cajan cajan*. The herbarium specimens were deposited at Forestry Research Institute of Nigeria, Ibadan, Nigeria.

2.1.2 Preparation of plant extracts

*C. cajan* seeds and *M. oleifera* leaves were freed of unwanted materials (stones and dirt) and were pulverized to powder using stainless steel laboratory blender. The methanol extract of each plant sample was obtained by soaking 100 g of the dried powdered samples in 1 litre of 80 % methanol (1:10) for 48 hours, with intermittent stirring to facilitate extraction. The extracts were filtered using Whatman filter paper (No.1), and the resultant filtrate was concentrated through rotary evaporation at 40°C. The extracts obtained were stored at 4 °C in sterile air tight container prior to use. The average percentage yield of the extracts were calculated using the formula:

\[
\text{Percentage Yield (\%) = } \frac{\text{Weight of extract obtained}}{\text{Weight of plant material}} \times 100
\]

2.2 Methods

Proximate Analysis (i.e., Ash, moisture, crude fat, crude fibre, crude fat, crude protein) were carried out according to standard methods as shown below:

2.2.1 Moisture Content determination

Moisture content determination was carried out using the air oven method as described in AOAC (2010). Crucibles were washed and dried in an oven. They were allowed to cool in the desiccator and weight was noted. A known weight of samples was then transferred into the crucibles and dried at a temperature between 103-105 °C. The dry
samples were cooled in a desiccator and the weight noted. They were later returned to the oven and the process continued until constant weights were obtained.

Calculation:  Moisture content % = \( \frac{\text{Weight Loss}}{\text{Weight of Sample}} \times 100 \)

### 2.2.2 Determination of Ash content

Ash percentage was determined by using the method described in AOAC (2010). Briefly, a known weight of finely milled sample was weighed into clean, dried pre-weighed crucible with lid (W1). The sample was lighted over a low flame to char the organic matter with lid removed. The crucible was then placed in a muffle furnace at 600 °C for 6h until it turned to ashes completely. It was then transferred directly to desiccators, cooled and weighed immediately (W2).

\[ \text{Ash} \% = \left( \frac{W_2 - W_1}{\text{Weight of Sample}} \right) \times 100 \]

### 2.2.3 Determination of Crude Fat

The soxhlets extraction method used to determine the crude fat percentage as described in AOAC (2010). Briefly, a known weight of sample was weighed into a weighed filter paper and folded neatly. This was put inside pre-weighed thimble (W1). The thimble with the sample (W2) was inserted into the soxhlets apparatus and extraction under reflux was carried out with petroleum ether (40 °C – 60 °C boiling range) for 6hrs. At the end of extraction, the thimble was dried in the oven for about 30 minutes at 100 °C to evaporate off the solvent and thimble was cooled in a desiccator and later weighed (W3). The percentage weight of fat extracted was calculated:

\[ \% \text{ Fat} \ (w/w) = \left( \frac{W_2 - W_3}{W_2 - W_1} \right) \times 100 \]

### 2.2.4 Crude Protein Determination in the plant samples

The crude protein content was determined using micro Kjeldahl method as described in AOAC (2010). Sample (0.2077 g) was weighed into a long necked Kjeldahl flask. One tablet of Kjeldahl catalyst was added to the sample in the flask with 25 cm³ of concentrated \( \text{H}_2\text{SO}_4 \). The flask was vortexed and heated in a fume cupboard. The heating continue until a clear solution was obtained. The clear solution was cooled, poured into a 100 cm³ volumetric flask and made up to mark with distilled water. Ten mL of the resulting mixture was measured into the distillation set through the funnel. Boric acid (5 cm³) was pipetted into a 100 cm³ conical flask and placed at the receiving end of the distillation apparatus. The conical flask was placed such that the delivery tube dipped completely into the boric acid inside the flask. \( \text{NaOH} \) (40 %) was used to liberate ammonia out of the digest under alkaline condition during the distillation. Two drops of methyl orange were added to the round bottom flask containing the digested sample before 40 % \( \text{NaOH} \) was added. As soon as the contents became alkaline, the red colour changed to yellow showing \( \text{NaOH} \) to be in excess. Steam was then generated into the distillation set using a steam chest. The liberated ammonia was trapped in the boric acid solution and about 50 cm³ of the solution
collected into a conical flask. The solution in the flask was titrated against 0.1 M HCl until the first permanent colour change was observed.

\[ \% \text{N} = \left( \text{Molarity of HCl} \times \text{Sample titre} - \text{Blank titre} \right) \times 0.014 \times \text{DF} \]

(Weight of sample used) \times 100

\% N was converted to the percentage crude protein by multiplying by 6.25. where DF is dilution factor, \% N is percentage nitrogen, 6.25 is the nitrogen: protein conversion factor.

**2.2.5 Crude Fibre**

Two hundred (200 mL) freshly prepared 1.25 % (1 M) H\textsubscript{2}SO\textsubscript{4} was added to a known weight of the residue obtained from fat extraction was boiled for 30 minutes. The mixture was filtered and residue washed until it was free from acid. The residue was transferred quantitatively into a digestion flask, 1.25 % (0.1 M) NaOH was added and boiled for 30 minutes. The mixture was filtered and residue washed with water to remove alkali. The residue was drained and transferred to a silica dish for ignition at 600 °C. The dish and its content were dried to constant weight at 105 °C. The organic matter of the residue was burnt by igniting for 30 minutes in a muffle furnace at 600 °C. The residue was cooled and weighed. The weight difference before and after ignition was reported as crude fibre (AOAC, 2010).

**2.2.6 Estimation of Carbohydrate**

The carbohydrate content was estimated by difference.

\[ \% \text{CHO} = 100 - \text{Sum of the percentages of moisture, ash, fat, protein and crude fibre}. \]

**2.2.7 Determination of free fatty acid**

Free fatty acid concentration was determined using the method described by Okpuzor et al. (2009). Normal hexane was used to extract fat from the plants powdered samples. Each oil sample (1.0 g) from *M. oleifera* leaves and *C. cajan* seed was weighed and dissolved with 50 mL ethanol in a conical flask. Two drops of phenolphthalein indicator were added and titrated with 0.1 M sodium hydroxide solution (NaOH), to give a pink coloured end point (which persisted for 15 minutes).

\[ \text{Acid value} = 40 \times T \times C \]

\[ W \]

Where 40 is the equivalent weight of NaOH, T is the average titre value of the NaOH used, C is concentration of NaOH solution used (0.1 M); W is weight in grams of the test sample.

**2.3 Mineral element Analysis**

Mineral element compositions were determined using Atomic Absorption Spectrophotometer after acid digestion as described by Iwuji et al. (2013). Briefly, 1.0 g of *M. oleifera* leaf and *C. cajan* seed were weighed each into a 150 mL beaker and 10 mL of
concentrated HNO₃ was added to each sample in the beaker and allowed to infuse thoroughly. After which 3 ml of 60 % HClO₄ and H₂SO₄ (1:1) was added and the mixtures were digested using HNO₃. The digestes were allowed to cool and 10 ml concentrated HCl was added and transferred to 50 ml volumetric flask. The volume of the solutions was made up to the mark with distilled water, and then transferred to a bigger flask. The solutions were further diluted to 100 ml with distilled water. Potassium and sodium were measured by flame photometer analyzer. Concentrations of other elements in the digest were measured using atomic absorption spectrophotometer (AAS – unicam 939/959 model). Measurements were taken and reported as mg/100 g.

3.0 Results and discussions

The result of the Proximate compositions of methanol extracts of M. oleifera leaf and C. cajan seeds are shown in Figure 1. Moisture content, free fatty acid, carbohydrate and ash contents in M. oleifera leaves were lower than C. cajan but not significantly different (p < 0.05), while M. oleifera leaves had significantly (p < 0.05) higher protein (49 %) and crude fibre (68 %) than C. cajan seeds. The free fatty acid value for C. cajan was 4.36 mg NaOH /g and M. oleifera was 3.20 mg NaOH /g.
Figure 1: Proximate composition of *M. oleifera* and *C. cajan*. Each bar represents the mean ± SEM of triplicate determinations. Bars with different alphabets are significantly different from each other at p < 0.05

The result of the mineral elements composition of *M. oleifera* and *C. cajan* were presented in the Table one (1) below: The mineral elements estimated in *M. oleifera* leaf and *C. cajan* seeds were magnesium, calcium, potassium, sodium, manganese, iron, zinc, nickel, lead, chromium and phosphorus as shown in Table 1. It was observed that *M. oleifera* contained higher quantity of calcium (94 %) than *C. cajan*. However, very low concentration of manganese, selenium and nickel were present in both plants. The lead and chromium levels were not detected in both plant samples.
Table 1: Mineral elements composition in *M. oleifera* and *C. cajan* seed extracts

<table>
<thead>
<tr>
<th>Mineral elements (mg/100 g)</th>
<th><em>M. oleifera</em></th>
<th><em>C. cajan</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium</td>
<td>55.00 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.00 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Calcium</td>
<td>26.70 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.50 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Potassium</td>
<td>88.30 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.30 ± 0.18&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sodium</td>
<td>52.50 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.10 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>248.30 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>121.70 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.04 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.013 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Selenium</td>
<td>0.02 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.003 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lead</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Nickel</td>
<td>0.033 ± 0.00</td>
<td>ND</td>
</tr>
<tr>
<td>Iron</td>
<td>13.30 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.3 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.64 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.43 ± 0.02</td>
</tr>
<tr>
<td>Chromium</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Parameters were measured in triplicates and values expressed as Mean ± standard deviation. Values along the same row with different superscripts are significantly different at p<0.05.

Where;

ND means not detected.

Further mineral elements estimated in *C. cajan* seeds were magnesium, calcium, potassium, sodium, manganese, iron, zinc, nickel, lead, chromium and phosphorus as shown in Table 1. However, very low concentration of manganese, selenium and nickel were present in the plants. The lead and chromium levels were not detected in both plant samples. The appreciable amount of mineral elements detected in the two plant extracts could have given credence to their anti-diabetic properties.

For instance, Magnesium is involved in the release of insulin and maintenance of the pancreatic β-cells. Manganese is essential for glucose metabolism and its deficiency may result in glucose intolerance similar to diabetes mellitus in some animal species. Zinc plays vital roles in insulin biosynthesis, storage, and secretion.

Thus, zinc deficiency is related to a number of metabolic disruptions including insulin degradation, impaired glucose tolerance and reduced pancreatic insulin content. Zinc is also postulated to improve glycaemia and a sufficient zinc status in type 2 diabetics may counteract the deleterious effects of oxidative stress, thereby helping to prevent complications associated with Diabetes mellitus (Piero et al., 2012). The mineral elements obtained in the
extracts may be a contributory factor in the antidiabetic properties displayed by the extracts. In addition, some active compounds including phenol, 2-methoxyl-5-(1-propenyl), pentadecanoic acid, 1- (+) ascorbic acid, 8,11-octadecadienoic, methyl ester, identified in the extracts have been associated with different biological activities such as antimicrobial, antioxidant and antidiabetes effects (Madkour et al., 2017; Aja et al., 2015; Sánchez-Machado et al., 2010).

The results of the proximate compositions of methanol extracts of M. oleifera leaf and C. cajan seeds are shown in Figure 1. Moisture content, free fatty acid, carbohydrate and ash contents in M. oleifera leaves were lower than C. cajan but not significantly different (p < 0.05), while M. oleifera leaves had significantly (p < 0.05) higher protein (49 %) and crude fibre (68 %) than C. cajan seeds. The free fatty acid value for C. cajan was 4.36 mg NaOH /g and M. oleifera was 3.20 mg NaOH /g.

Conclusion

Proximate compositions and the mineral constituents of methanol extracts of M. oleifera leaf and C. cajan seeds evaluated shows appreciable amount of nutritional constituents and mineral elements which could have given credence to their anti-diabetic properties.

REFERENCES


PROXIMATE AND ANTINUTRIENT COMPOSITION OF SELECTED BAMBARA GROUNDNUT (VIGNA SUBTERRANAEA (L.) VERDC.) ACCESSIONS

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Abstract

Underutilized legumes have gained recent prominence among various stakeholders (researchers and industries alike) globally but particularly in the sub-Saharan African region. Bambara groundnut (Vigna subterranea (L.) Verdc) is a member of the underutilized legumes cultivated in several communities in Africa and Asia for numerous purposes even though its utilization hasn’t yet attained full potential. In this study, five accessions obtained from the genebank at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria were analyzed for proximate and anti-nutrients. Results for the proximate analysis showed ash ranged between 3.97% (TVSU-1636) to 3.83% (TVSU-1518); crude fat 5.91% (TVSU-1630) to 5.54% (TVSU-1649); crude protein 21.33% (TVSU-1630) to 19.74% (TVSU-1649) and crude fibre 4.52% (TVSU-1636) to 4.19% (TVSU-1649). Others are moisture 9.75% (TVSU-1636) to 9.88% (TVSU-1570), dry matter 90.26% (TVSU-1636) to 90.07% (TVSU-1649) and carbohydrate 45.47% (TVSU-1636) to 45.05% (TVSU-1518). The antinutrients analyzed (%) composed of flavonoids, alkaloids and saponin. The flavonoids ranged from 0.009 % to 0.005% in TVSU-1636 and TVSU-1649 respectively. The alkaloid varies from 0.182% in TVSU-1649 to 0.248% in TVSU-1636 while the saponin content varies from 0.333% in TVSU-1649 to 0.387% in TVSU-1636. Significant statistical differences (p < 0.05) were observed in all the parameters studied and the accessions. The outcome of the study indicated that the accessions could support objectives of dietary diversification using Bambara groundnut by providing low-cost protein and other beneficial nutrients for humans and animals in sub-Saharan Africa.

Keywords: Bambara groundnut, nutrients, antinutrients, food and income security, sub-Saharan Africa

INTRODUCTION

Vigna subterranea (L.) Verdc popularly called Bambara groundnut (BG) is a member of the underutilized legumes positioned to support global food insecurity and malnutrition particularly in sub-Saharan Africa with the right awareness for its promotion and utilization (Mayes et al., 2019). Crops classified as underutilized are those that have received less global attention in terms of global trade, cultivation, utilization and research (Stamp et al., 2012; Varshney et al., 2012), even though they have been reputed to serve as simple sources of food and income security among rural dwellers (Padulosi et al., 2013). They are often cultivated by women and smallholders farmers with less farming inputs (Mabhaudhi et al., 2013;
The cultivation of underutilized legumes have good impacts for subsistence farmers in the area of revenue generation (Durst and Bayasgalanbat, 2014), limiting reliance on major staples for food and animal feed (Chivenge et al., 2015) with the addition of less farming inputs when compared to usual farming systems in regions where they are planted (Stamp et al., 2012). In this study, we analysed the proximate and antinutrients of selected Bambara groundnut from the germplasm conserved at the Genebank of the International Institute of Tropical Agriculture (IITA). The availability of data on the contribution this crop could make Bambara groundnut a valuable source of nutrition in developing regions. BG like other known legumes have the capacity to fix atmospheric Nitrogen and nodulates very well (Gupta et al., 2015). In recent discussion, green bean (used as vegetables), soybean (used for oil extraction) and alfalfa (reputed for soil nutrition and animal feed) are regarded as pulses (Asif et al., 2013; Duranti and Gius, 1997). A very good and affordable means of obtaining protein is with the use of soybean (Considine et al., 2017; Foyer et al., 2016; Vollmann, 2016). Prominent pulses referred to as orphan, neglected or underutilized legumes are Bambara groundnut, African yam bean, winged bean, adzuki, jack, Kersting’s groundnut and marama beans amongst others (Cullis and Kunert, 2017; Padulosi et al., 2013).

Due to reduced planting activities (Foyer et al., 2016) and global utilization (Miller et al., 2017), they do not make any significant impact to national nutrition intake when compared to staples such as rice (FAO, 2017) despite playing significant roles in rural populations where they provide income, affordable and nutritious meals (Asif et al., 2013; Graham and Vance, 2003; Heim et al., 2017; Mudryj et al., 2014). They also support improvement in various human health status such as those who affected by high sugar level (Becerra-Tomás et al., 2018), hypertension (Polak et al., 2015), issues about obesity as well as diet and weight management (McCrorry et al., 2010) to fat reduction interventions (Bazzano et al., 2011). BG seeds have been used and processed in several ways for human and animal use as described by Mubaiwa et al. (2017) in the arid area of Zimbabwe, Southern Africa. In many West African countries such as Benin Republic, freshly harvested BG seeds are boiled and consumed with or without pepper. Flour can also be produced from the seeds when milled and put to several uses (Kaptsuo et al., 2015). From the fresh seeds, delicious steamed-paste popularly called moi moi or okpa (porridge made from the seeds) are usually produced and widely common in some parts of Nigeria (Okpuzor et al., 2010).

In Nigeria, especially in the East and some part of Northern Nigeria, BG is an important food crop and can be used in traditional preparation of various meals. The seeds are sometimes roasted, boiled or milled and used in preparing soup (Adu-Dapaah and Sangwan, 2004) or chewed with palm kernel. Animals also benefit by the use of BG seed for feeding of livestock and poultry (Anchirinah et al., 2001). According to Mayes et al. (2019), flour from BG seeds can be used to make several confectionaries. BG derived milk competes well to that from soybean with BG milk containing more than 19% protein compared with about less than 5% protein in the soy milk (Addo et al., 2016; Adu-Dapaah and Sangwan, 2004) and preferred by many due to its flavour and colour (Goli et al., 1991). In Asia for instance and particularly in Indonesia, a fried BG snack made from the immature seed is highly sought after. Known as ‘Kacang Bogor’, which are sold in exorbitant prices in supermarkets and even in specialist food shops in Europe. In appearance, it is similar to dry roasted peanut, but is drier (less oil) and more strongly flavoured. Nutritionist have developed several recipes using BG as substitutes for other ingredients in Indonesia, Malaysia (Crops For the Future) and recently at the Food and Nutrition Sciences Laboratory, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria (Feldman et al., 2019).
MATERIALS AND METHODS

Materials: The BG accessions used for this study were obtained from the Genebank, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Standard laboratory procedures were employed using chemicals of analytical grade for sample analysis.

Proximate analysis: Standard laboratory procedures as described by (Indudhara Swamy et al., 1971) was used for moisture content determination. Micro-Kjeldahl method was used to determine the crude protein (International, 2005). According to the AOAC method, crude lipids and ash were extracted with petroleum ether, using a Soxhlet apparatus AOAC (2000). Total carbohydrate was calculated by the difference method (McDonald, 1973). Samples were analyzed chemically for crude fibre according to the official methods of analysis described by the Association of Official Analytical Chemist (International, 2005). The dry matter content was determined according to AOAC official method reference no 967.08 while ash was determined according to AOAC official method 942.05. All analysis was carried out in duplicate.

Antinutrient analysis: Spectrophotometric method as described by Arntfield et al. (1985) was used in determining the alkaloids while saponins and flavonoids were determined according to Association of Official Analytical Chemist standard referenced procedures (Chemists, 1990).

Statistical analysis: Statistical Analysis Software (SAS, 9.4) was used in data analysis. Result was recorded in duplicates for all determinations and the results expressed as mean ± SE. Significance of the differences was defined as p<0.05 for ANOVA. The difference in mean was compared using the Duncan’s new Multiple Range test (Duncan, 1955).

RESULTS AND DISCUSSION

RESULTS

Proximate Composition

In food product analysis, carbohydrates, fat, fibre, proteins are usually referred to as constituents of the food. The proximate composition also consists of the moisture and ash contents. Tables 1 shows the results obtained from the present study which indicated highly significant (P < 0.0001) proximate composition. Protein content varied from 21.13 % in TVSU-1518 to 19.75% in TVSU-1570. Crude fat content ranges from 5.91 (TVSU-1630) to 5.54 % (TVSU-1649); crude fibre contains 4.19 % in TVSU-1649 and 4.52 % in TVSU-1630. Ash values ranges from 3.81% in TVSU-1518 to 3.97% in both TVSU-1630 and TVSU-246; moisture content varies from 9.745% in TVSU-1630 to 9.93% in TVSU-1649, dry matter ranges from 90.12% in both TVSU-1570 and TVSU-246 to 90.25% in TVSU-1630 and carbohydrate varies from 45.05% in TVSU-1518 to 43.25%. The phytonutrients such as flavonoids, alkaloids and saponin. Flavonoids values varies from 0.0094% (TVSU-1630) to 0.00505 (TVSU-1649); alkaloids; 0.2135% (TVSU-1518) and 0.182% (TVSU-1649) and saponin 0.387% (TVSU-1630) to 0.3325 % (TVSU-1649) (Table 1).
Table 1: Proximate composition (%) of selected Bambara groundnut accessions

<table>
<thead>
<tr>
<th>Accession</th>
<th>CP ± SE</th>
<th>C fat ± SE</th>
<th>C fibre ± SE</th>
<th>Ash ± SE</th>
<th>M ± SE</th>
<th>DM ± SE</th>
<th>CHO ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>TVSU-1518</td>
<td>21.13 ± 0.0082b</td>
<td>5.81 ± 0.0057b</td>
<td>4.47 ± 0.008b</td>
<td>3.81 ± 0.0035d</td>
<td>9.815 ± 0.0065c</td>
<td>90.185 ± 0.0065c</td>
<td>45.05 ± 0.0083b</td>
</tr>
<tr>
<td>TVSU-1570</td>
<td>19.93 ± 0.0082d</td>
<td>5.69 ± 0.0057d</td>
<td>4.25 ± 0.008d</td>
<td>3.80 ± 0.0035c</td>
<td>9.875 ± 0.0065b</td>
<td>90.125 ± 0.0065c</td>
<td>43.53 ± 0.0083d</td>
</tr>
<tr>
<td>TVSU-1630</td>
<td>21.33 ± 0.0082a</td>
<td>5.91 ± 0.0057a</td>
<td>4.52 ± 0.008a</td>
<td>3.97 ± 0.0035a</td>
<td>9.745 ± 0.0065d</td>
<td>90.255 ± 0.0065a</td>
<td>45.47 ± 0.0083a</td>
</tr>
<tr>
<td>TVSU-1649</td>
<td>19.74 ± 0.0082e</td>
<td>5.54 ± 0.0057e</td>
<td>4.19 ± 0.008e</td>
<td>3.87 ± 0.0035c</td>
<td>9.93 ± 0.0065a</td>
<td>90.07 ± 0.0065d</td>
<td>43.26 ± 0.0083e</td>
</tr>
<tr>
<td>TVSU-246</td>
<td>20.12 ± 0.0082c</td>
<td>5.77 ± 0.0057c</td>
<td>4.30 ± 0.008c</td>
<td>3.91 ± 0.0035b</td>
<td>9.88 ± 0.0065b</td>
<td>90.12 ± 0.0065c</td>
<td>43.98 ± 0.0083c</td>
</tr>
<tr>
<td>CV</td>
<td>0.05</td>
<td>0.14</td>
<td>0.27</td>
<td>0.12</td>
<td>0.09</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>F Value</td>
<td>&lt;.0001*</td>
<td>&lt;.0001*</td>
<td>&lt;.0001*</td>
<td>&lt;.0001*</td>
<td>&lt;.0002*</td>
<td>&lt;.0002*</td>
<td>&lt;.0001*</td>
</tr>
</tbody>
</table>

Key: CP= Crude protein; C fat=Crude fat; M=Moisture; DM= Dry matter; CHO=Carbohydrate; CV= Coefficient of variation, SE= Standard Error; TVSU=Tropical Vigna sub Vigna subterranea * Significant level at P < 0.0001. Means with the same subscript along the column are not significantly different (P<0.05).
Carbohydrates, proteins, fat and fibre are often regarded as proximate composition which typically describes the major constituents of any food products analysis (Finglas et al., 2014; Greenfield and Southgate, 2003). The proximate analysis also explained the moisture (or dry matter) as well as ash in a food product which further differs from the macro and micro elements found in any food products. In nature, pulses are reputed to have high levels of protein (up to about 40 %) and fibre (20% less or more) than commonly consumed cereals and usually low levels of fat (usually less than 10%) when compared with oilseeds (Gepts et al., 2005). Consequently, the amount of proximate are different from pulses or within species due to several factors such as variation due to genetic and environmental interactions (Amarteifio et al., 2010; Medic et al., 2014). Studies have shown that BG contain greater number of proximate though with low level of fibre. The proximate composition in BG is close to what has been previously reported for cowpea, mungbean. These legumes have benefitted from several crop improvements programmes which BG is still tailed towards nutritional and food quality improvements (Boukar et al., 2016; Ebert, 2014; Kim et al., 2015; Muñoz-Amatriain et al., 2017; Shanmugasundaram et al., 2009).

**Carbohydrate composition**

Carbohydrates play very significant role in human and animal nutrition as a major supplier of energy (Calories) providing mutual support to improve health (Chibbar et al., 2010), and the management of of common diseases (Smith et al., 2012; Tosh and Yada, 2010). In this study, the carbohydrate composition ranges from 43.53 % in TVSU-1570 to 45.47 % in TVSU-1630. This is lower when compared to the values of 63.37 % reported by Alhassan et al. (2015); 67.55 % by Tchiotsa et al. (2004) with estimated high proportion of starch proportion up to 60% in BG seeds (Yao et al., 2015). However, the outcome of this study is similar to other previous results (Adebowale et al., 2011; Amarteifio and Moholo, 1998; Annan et al., 2003; Ijarotimi and Olopade, 2009).

**Protein**

Studies have reported legume seeds considerable less than 40% protein, (Pandey et al., 2016), a multiple of what is obtainable in cereals (Asif et al., 2013). For BG, several research reports indicates its position as a protein rich crop due to the amount (Goudoum et al., 2016; Hillocks et al., 2012; Massawe et al., 2002), above 20% protein level (Boateng et al., 2013; Mubaiwa et al., 2017; Mwale et al., 2007). The result from this current study also corroborates these claims. BG is reported to have huge crude protein unlike other pulses. Globally, BG protein varies from 9.6 % to 30.7 % providing an avenue for crop improvement to a carefully structured breeding system. Should this be successful, the journey to the agricultural transformation of the crop which have started with sole aim of making substantial contribution to reducing malnutrition and food security (Mayes et al., 2019). In other related studies, the protein composition was higher to those of Abu-Salem and Abou-Arab (2011), 17.70%; Tchiotsa et al. (2004) 18.83 % and Alhassan et al. (2015) 18.83%. However, the results obtained in this study is similar to the report by Olaley et al. (2013) 15.2-22.2 %; Amarteifi and Moholo (1998) on BG seeds as well as Chinedu and Nwinyi (2012); Soetan (2017) (26,60%). and Adegboyega et al. (2020) on African yam bean (32.40%) and Adegboyega et al. (2019) on winged bean (31.13-28.43%).

**Fatty acids**

BG is highly reputed for low level in the amount of fat when compared with soybean and groundnut (Mudryj et al., 2014), and essentially not too suitable for oil production (Asif et al., 2013; Vollmann, 2016). According to Mayes et al. (2019) due to BG’s relative similarity in fatty
acid structure with cowpea and related family member, there could be an opportunity to change the related vigna family in areas where BG is cultivated.

Crude Fibre

Crude fibre contains 4.19 % in TVSU-1649 and 4.52 % in TVSU-1630 and similar to other previous studies on Bambara groundnut crude fibre constituents (Alhassan et al., 2015; Alozie et al., 2009; Amarteifio and Moholo, 1998; Rocha-Guzmán et al., 2006).

Moisture Content

For most pulses, moisture content ranges between 9-12 % which assures reduction of microbial attack and storage safety thereby improving germplasm shelf life (Sujeetha et al., 2014). In the current study, the moisture content of the BG accessions analyzed were less than 10% and fell within the recommended values which implies the seeds can be stored at room temperature without microbial attack for a number of months (Baiyeri et al., 2018). The values obtained in this study were less than the results reported by Ojuederie and Balogun (2017) for African yam bean (11.3 to 12.6%). Other similar studies on BG confirmed outcome of this current report (Adebowale et al., 2011; Annan et al., 2003; Ijarotimi, 2008; Koné et al., 2011).
Antinutrients composition

Antinutritional factors in pulses includes but not limited to tannins, phytic acid and saponins (Rochfort and Panozzo, 2007) which reduces premium placed on orphan legume utilization (Soetan and Oyewole, 2009). The antinutrients can reduce protein bioavailability and digestion by over 45 % (Gilani et al., 2012), as well as lowering circulation of other nutrients (Sandberg, 2002). Their removal is considered a breeding target for most pulses (Wang et al., 2003). The results antinutrients of the five accessions were shown in figure 1. Flavonoids ranges from 0.006 % in TVSU-1570 to 0.009 % in TVSU-1630. Alkaloids varies from 0.190 % in TVSU-1570 to 0.248 % in TVSU-1630. Lower than values reported by Soetan 2017. Saponins ranges also varies from 0.333 % in TVSU-1649 to 0.387 % in TVSU-1630. These results are similar to the works of Soetan (2017) and Mubaiwa et al. (2018). Additional, the accessions had comparable results with previous results on African yam bean by Ajibola and Olapade (2016) and were lower

Figure 1: % Composition of antinutrients in selected Bambara groundnut accessions

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than those obtained from (Nwosu, 2013) and these values fall within the permissible limit based on (Ndidi et al., 2014).

Conclusion
BG contains desirable nutritional properties that could support human and animal consumption and may support global dietary diversification, infant weaning and other levels of utilization. Concerted efforts must be made to increase awareness on its nutritional importance for food and income security. Targeted breeding programmes could further assist to eliminate identified antinutrients and increase consumption strategies.

References


SUL e-CONFERENCE – PAPER 11
EFFECT OF AFRICAN YAM BEAN (SPHENOSTYLIS STENOCARPA) SEED AND TUBER FLOUR MEALS ON SOME KEY TISSUES IN HEALTHY MALE WISTAR RATS

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Abstract
African yam bean (AYB) is an underutilized legume with immense potentials as a food security crop. The effect of AYB seed and tuber meals on the tissues of the kidney, liver, and testis of healthy male Wistar rats was investigated. Rats fed on standard pelletized rat chow were used as control. The consumption of 100% unprocessed AYB seeds caused liver and kidney damage in rats due to increased levels of aspartate aminotransferase (5.04±1.62 U L⁻¹), alanine aminotransferase (8.46±2.43 U L⁻¹) and lipid peroxidation (0.27±0.02 unit mg⁻¹ protein). AYB tubers were innocuous to Wistar rats investigated. Proper processing of AYB seeds which has more antinutrients, is required for safe consumption by humans and livestock. This study has shown that tubers of AYB are safe for human consumption and should be utilized in meals as it contains fewer antinutrients and had no significant effect on the tissues examined in Wistar rats.

Keywords: Anti-nutritional factor; Aspartate aminotransferase; Catalase; Glutathione reductase; Histopathology; Lipid peroxidation; Oxidative stress; Underutilised legume

Introduction
The diet of most households in sub-Saharan Africa is deficient in protein and essential nutrients, especially among the rural poor. Some individuals cannot afford to purchase animal protein (fish, meat and poultry products) to include in their diets. Hence, they seek an alternative source in legumes. Legumes are used as staple food at the subsistence level in nearly all parts of the world, and it is often eaten together with cereals and used as a substitute source of protein and calorific value for man and livestock (Ojuederie & Balogun 2017). The primary staple crops have received much focus over the years in enhancing food security with the disregard for underutilized crops with immense potentials as a substitute protein source and nutritional security crop (Aremu et al., 2019). African yam bean (AYB) Sphenostylis stenocarpa, (Hochst ex. A. Rich) Harms a neglected and underutilised legume common to Africa, is one of such crops (Ogunsola, Ojuederie, & Emmanuel, 2016). Sphenostylis stenocarpa belongs to the Fabaceae family, and it is the most economically important species amongst the seven species of Sphenostylis (Ojuederie, Balogun, Akande, Korie, & Omodele, 2015; Potter & Doyle, 1992; USDA, 2009). The AYB produces two essential products, the underground root tuber and the edible yam bean seeds which grow in pods above the ground (Olasoji, AK, & Owolade, 2011). The crop is cultivated mostly at the subsistence level, and only a little percentage of the grains produced is sold (Osuagwu & Nwofia, 2014). The genetic resources of AYB need to be preserved for use in genetic improvement/breeding programme as it ranks well among neglected crops with high potentials as a food security crop (Adewale et al., 2012). The plant can produce root nodules and also fix atmospheric nitrogen effectively in the soil (Oganale, 2009). The AYB seeds are rich in protein with high levels of
essential amino acids especially lysine and methionine as well as carbohydrate, lipid and minerals making it a crop with remarkable nutritional potentials equivalent with other known grain legumes (Adewale, Daniel, & Onye, 2013; Ndidi, Olagunju, Muhammad, Billy, & Okpe, 2014; Ojuederie and Balogun, 2017). Nutritionally, the seed contains between 2–5% fat, 20–29% protein, (Adewale et al., 2012; Aremu et al., 2019; Potter et al., 1992) as well as 2-4% fibre, and 50-62% carbohydrate (Ojuederie and Balogun, 2017). The tubers when cooked taste like a potato but unlike potato which has just 5% protein content, AYB tubers have 11-19% protein on a dry weight basis (NRC, 2006; Ojuederie & Balogun, 2019). It could be used as an excellent alternative to cowpea, and it is highly consumed by the farmers who maintain the crop's genetic resources (Adewale et al., 2012; Adewale et al., 2013; Ogunsola et al., 2016; Ojuederie, Balogun, Fawole, Igwe, & Olowolafe, 2014). AYB could be used as a substitute protein source for livestock and poultry are given that a small amount AYB served as a substitute for soya bean in the meal of weaner rabbits. The weights of white Leghorn layers, when fed with AYB meal, increased after proper processing (Hussein, Urge, & Animut, 2016). Besides being used as a dietary protein, neglected grain legumes like AYB have comparatively higher free radical scavenging ability as well as the ability to reduce cholesterol; hence, it could be considered as a functional food (Oke, Obowale, & Ogunlakin, 2013).

Nonetheless, underutilized legumes contain anti-nutritional factors, such as trypsin inhibitors, and phytate which hinders protein digestibility (Attia, Millot, Di-Poi, Bégout, Noble, Sanchez-Vazquez, et al., 2011), and mineral availability due to their toxic nature, thereby decreasing the overall nutritional value in legumes (Ndidi et al., 2014). African yam bean contains high concentrations of antinutrients such as trypsin inhibitor, phytate, tannin, lectin, Saponins, oxalate, and alkaloids (Adewale, Daniel, & Onye, 2013; Ajibade, Balogun, Afolabi, Ajomale, & Fasoyiro, 2005; Fasoyiro, Ajibade, Omore, Adeniyan, & Farinde, 2006; Ojuederie, Balogun, & Abberton, 2016). This attribute coupled with the hardness of the seed coat increases the time taken for cooking and processing the beans which are often consumed either alone or with yam, maize, and rice (Eneh, Orjionwe, & Adindu, 2016). These antinutrients have discouraged the utilization and acceptability of AYB by consumers and subsistent farmers. The safety of humans and livestock can still be achieved through appropriate and efficient processing, and cooking of the bean seeds of AYB before consumption to reduce the anti-nutritional factors present (Adewale et al., 2013). Notwithstanding the high nutritional worth and economic importance of AYB, it is still neglected with regards to research for crop improvement (Ogunsola et al., 2016; Popoola, Adegbite, Obembe, Adewale, & Odu, 2011). Some researchers have reported the pharmacological properties of AYB seeds for the treatment of gout, arthritis and high blood pressure (Eneh et al., 2016; Okoye et al., 2017). Aqueous extracts of the seed of AYB reduced the lipid profiles and sodium (Na+) concentration in the serum of experimental Wistar rats, with 3 ml of aqueous extract of AYB reducing cholesterol and triglyceride levels more than the control (2.11 ± 0.01 vs control 2.20 ± 0.12 mmol/l and 0.40 ± 0.01 vs control 0.46 ± 0.11 mmol/l) respectively (Okoye et al., 2017).

In Nigeria and most West African countries, the seeds are consumed. Still, the tubers if harvested are discarded due to the belief that tubers of AYB are toxic to man and livestock despite its rich nutritional content. However, tubers are eaten in Central Africa. The manner in which the tubers are utilized in Africa is not fully known, however, its South American counterpart the Mexican yam bean (*Pachyrhizus erosus*) is eaten in several ways. The tubers could be sliced and soused with onion and chili to make a common snack, or a tasty milk prepared from boiled milk having grated tubers (NRC, 2006). Additionally, the tubers could be sliced and added to Oriental stir-fries or eaten either alone, or with other vegetables (NRC, 2006). Research on the effects of consumption of the tubers of AYB by Wistar rats will give us a better understanding of the possible effects it would have on man and livestock. Thus, we
investigated the impact of AYB seed and tuber meals on the liver, kidney, and testis of healthy male Wistar rats, to determine the level of toxicity if any, when consumed.

**Materials and Methods**

**Location of study**

This research was conducted in the Laboratory of the Department of Chemical and Food Sciences, College of Natural and Applied Sciences, Bells University of Technology, Ota, Ogun State, and the Veterinary Anatomy Department of the University of Ibadan, Nigeria.

**Source of plant materials and animals**

The seeds of AYB used for this study were obtained from the International Institute of Tropical Agriculture Ibadan (IITA); TSs 107 (mosaic seeds) and TSs 140 (mosaic seeds) and the Institute of Agricultural Research and Training moor plantation Ibadan (IAR&T) AYB 45 (mosaic seeds) and AYB 57 (brown seeds). Thirty healthy adult male Wistar rats (45 days old) of about 200g weight used in this study were obtained from the Department of Physiology, University of Ibadan, Nigeria.

**Preparation of samples**

Tubers of the four AYB accessions (AYB57, AYB45, TSs107, and TSs140) were prepared using the method of Ogunlakin, Oke, Babarinde, and Olatunbosun (2012) and dried in an oven at 80°C for 24h utilising the technique of Odebunmi, Oluwaniyi, Sanda, and Kolade (2007). The dried samples were milled into a fine flour using an electric blender, passed through 250nm stainless sieve and packaged in air-tight containers labelled for analysis (Ogunlakin et al., 2012; Ojuederie & Balogun 2017). African yam bean seeds of the same accessions were cleaned to remove extraneous matter, oven-dried and milled to a fine flour in the same way as the tubers for anti-nutritional analysis. The seed and tuber flours of the four accessions were made into separate composite meals for the Wistar rats.

**Determination of antinutrients in African yam bean seed and tuber**

The anti-nutritional factors; tannin, phytate, saponin, oxalate, and lectin, were determined following standard procedures (Ojuederie 2016). Trypsin inhibitor and cyanogenic glycosides were identified using the procedures of Edeogu, Ezeonu, Okaka, Ekuma, and Elom (2007).

**Treatment of Animals**

Six albino rats were randomly placed into five groups and raised at the Department of Biochemistry's animal house at the Bells University of Technology, Ota, Nigeria. Before the commencement of the treatments, the albino rats were fed on a standard diet of pelletized rat chow for two weeks, with water given *ad libitum* at room temperature with a 12h light and dark cycle (Ajiboye, Erukainure, Okoro, & Asieba, 2016). The Animals Ethics Committee of the Institute of Medical Research and Training, University College Hospital, University of Ibadan gave the approval for the experimental procedure used in this study. The five test groups were:
Group-I Wistar rats fed on a standard pelleted rat chow (control group) + water \textit{ad libitum}.

Group-II Wistar rats fed on 50\% African yam bean seed meal + water \textit{ad libitum}.

Group-III Wistar rats fed on 100\% African yam bean seed meal + water \textit{ad libitum}.

Group-IV Wistar rats fed on 50\% African yam bean tuber meal + water \textit{ad libitum}.

Group-V Wistar rats fed on 100\% African yam bean tuber meal + water \textit{ad libitum}.

Tender care was given to the experimental rats following the guidelines for the Care and Use of Laboratory Animals (NRC, 2011). Seventy-two hours (72 h) after administration of the treatments, the rats were sacrificed using light ether anaesthesia.

\textit{In–vitro enzymatic and non-enzymatic antioxidant assays}

The organs harvested from the rats were homogenized in 50Mm Tris HCL buffer (pH 7.4) which contained 1.15\% KCL and the homogenate centrifuged at 10,000g for 15min at 4\( ^\circ \)C (Akintunde et al., 2013). The supernatant was collected, and catalase (CAT) activity estimated using hydrogen peroxide as a substrate (Akintunde et al., 2013)

\textit{Superoxide Dismutase}

The method of Radovanović, Borković-Mitić, Perendija, Despotović, Pavlović, Cakić, et al. (2010) was used to determine the SOD activity by calculating the inhibition of autoxidation of epinephrine at pH10.2 and a temperature of 30\( ^\circ \)C. The amount of total protein produced was established by the Lowry’s method (Soetan, Adedara, & Farombi, 2016) and reduced GSH determined at 412nm (Akintunde et al., 2013; Farombi, Ugwuezunmba, Ezenwadu, Oyeyemi, Ekor, & pathology, 2008).

\textit{Lipid Peroxidation assay}

Lipid peroxidation (LPO) was determined at 532nm using a spectrophotometer by thiobarbituric acid reactive substances, and malondialdehyde (MDA) estimated in mmol g\(^{-1}\) tissue (Soetan et al., 2016).

\textit{Assay of Serum alanine aminotransferase and aspartate aminotransferase activity}

Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured using the method of Obiezue et al., (2011). ALT was recorded by checking the concentration of pyruvate hydrazones produced with 2, 4 dinitrohydrazine. One unit/L of AST or ALT was defined as the liberation of 1mMol of pyruvate per minute at 37\( ^\circ \)C incubation per litre of serum.

\textit{Histopathological analysis}

The liver, kidney and testis harvested from the Wistar rats were sliced in bits and prepared following the method of Akintunde et al. (2013) by fixing in Bouin’s fixative for 6 h, then transferred to formalin,
sectioned, and stained routinely with hematoxylin and eosin for histopathological examination (Akintunde et al. 2013). A light microscope was used to produce stable photomicrographs.

**Statistical Analysis**

Data were analysed using a one-way analysis of variance and significantly different means separated at P < 0.05 using the Least Significant Difference. The anti-nutritional compositions of AYB seed and tuber used for meal formulation were compared using a paired t-test. The results are expressed as Means± SD of six rats in each group. The analysis was carried out using the Statistical Analysis System version 9.4 (SAS, 2010)

**Results and Discussion**

This study investigated the effects of AYB seed and tuber flour meals on the liver, kidney, and testis of healthy male Wistar rats. The enzymatic activity of alanine aminotransferase and aspartate aminotransferase were studied to assess liver malfunctions. The analysis of variance revealed that AST and ALT concentrations were significantly higher in both kidney and liver of rats fed with 100% seed flour meal compared to the other meal types. The levels of AST and ALT were significantly lower in the organs of rats fed with 50% and 100% tuber flour compared to rats fed with 50% and 100% seed flour (Fig 1).

![Figure 1: Effect of African yam bean seed and tuber meal on Plasma AST and ALT level in Wistar rats. All values are expressed as Means ± SD (n=6). Means followed by the same letters down the columns are not significantly different at p<0.05 using Least Significant Difference.](image)

This study established that the consumption of unprocessed 100% AYB seed meal caused liver and kidney damage in Wistar rats with elevated serum concentrations of the biomarkers aspartate aminotransferase (AST) and alanine aminotransferase (ALT) observed, compared to other meal types. The cellular integrity of the liver is affected by the plasma levels of ALT, AST and total protein which also determines its functionality (Gowda et al., 2009). Estimation of AST and ALT levels in the serum is often used as a quantifiable biomarker for the detection of hepatocellular damage and liver malfunction (Akintunde et al., 2013). Rats fed on 50% and 100% tuber flour meals had reduced levels of AST and ALT in the serum, which meant that there was no damage to the liver. In contrast, higher values obtained for AST and ALT could be due to severe damage to kidney and liver in rats fed with 50% and 100% seed flour meal. Antioxidant enzymes are fundamental to maintaining homeostasis within living organisms by
stabilizing or deactivating free radicals which causes imbalance in living systems. The foremost antioxidant enzymes involved in this process include superoxide dismutase, catalase, and glutathione reductase, which were all investigated in this study.

Hundred percent seed meal generated oxidative stress in the kidney of rats. There were moderate renal cortical congestion and mild necrosis (100% tuber meal) and mild renal cortical congestion (50% tuber meal) in the kidneys of the rats investigated (Fig 2a). No visible lesion was observed in control (Fig 2c). Severe diffuse degeneration and necrosis of renal tubules in the kidney of rats were observed in rats fed on 100 % seed meal (Fig 2d). Protein casts were also found in the tubular lumen of fed rats. Moderate renal cortical congestion and mild necrosis were observed in the kidney of rats fed with 50% seed meal (Fig 2e). No visible lesions were found in control fed with rat chow (Fig 2f).

Histopathological kidney sections of rats fed with rat chow revealed typical renal architecture. In contrast, severe diffuse degeneration and necrosis of renal tubules and protein casts in the tubular lumen were observed in the kidney of 100% seed meal fed rats possibly due to generation of oxidative stress in the organ of the fed Wistar rats. The effect of AYB seed and tuber meals on the concentration of SOD, LPO, GSH, CAT and total protein in the kidney of healthy Wistar rats 72 h after administration of the treatments is presented in Table 1. The levels of SOD, CAT, and total protein had no significant effect on the kidney as in the control group (Table 1). However, the results revealed that there was significant reduction in LPO (MDA) levels in the kidney of rats fed on 50% tuber and 50% seed meals compared to 100% seed meal which had the highest value (Table 1). GSH levels were not significantly different in groups II to V, but the control group (Group I) fed with rat chow had significantly lower GSH levels (Table 1).
Table 1: Effect of African yam bean seed and tuber meal on superoxide dismutase (SOD), lipid peroxidation (LPO), reduced glutathione (GSH), Catalase and total protein on the kidney of healthy wistar albino rats 72 hours after administration

<table>
<thead>
<tr>
<th>Meals</th>
<th>SOD (unit mg⁻¹ protein)</th>
<th>GSH (mmoles min⁻¹ mg⁻¹ protein)</th>
<th>LPO (unit mg⁻¹ protein)</th>
<th>CAT (mmoles min⁻¹ mg⁻¹ protein)</th>
<th>Total Protein (mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat Chow</td>
<td>0.28±0.03a</td>
<td>66.00±10.79b</td>
<td>0.22±0.10ab</td>
<td>1004.38±75.09a</td>
<td>0.24±0.01a</td>
</tr>
<tr>
<td>50% Seed</td>
<td>0.31±0.02a</td>
<td>77.87±2.31a</td>
<td>0.05±0.00c</td>
<td>1010.36±52.19a</td>
<td>0.23±0.00a</td>
</tr>
<tr>
<td>50% Tuber</td>
<td>0.29±0.01a</td>
<td>79.63±3.79a</td>
<td>0.05±0.00c</td>
<td>1027.43±14.65a</td>
<td>0.23±0.00a</td>
</tr>
<tr>
<td>100% Seed</td>
<td>0.31±0.01a</td>
<td>76.26±8.53a</td>
<td>0.27±0.02a</td>
<td>1041.71±3.26a</td>
<td>0.24±0.01a</td>
</tr>
<tr>
<td>100% Tuber</td>
<td>0.29±0.03a</td>
<td>78.72±10.34a</td>
<td>0.14±0.13b</td>
<td>1038.97±9.45a</td>
<td>0.23±0.00a</td>
</tr>
<tr>
<td>Mean</td>
<td>0.30</td>
<td>75.70</td>
<td>0.15</td>
<td>1024.57</td>
<td>0.23</td>
</tr>
<tr>
<td>LSD (α=0.05)</td>
<td>0.03</td>
<td>10.24</td>
<td>0.09</td>
<td>58.69</td>
<td>0.01</td>
</tr>
</tbody>
</table>

All values are expressed as Mean±SD of six rats per group. Means followed by the same letters down the columns are not significantly different at p<0.05. SOD (unit mg⁻¹ protein), GSH (mmoles min⁻¹ mg⁻¹ protein), LPO (unit mg⁻¹ protein), Catalase (mmoles min⁻¹ mg⁻¹ protein), Total protein (mg protein)

The effect of AYB seed and tuber meals on the concentration of SOD, LPO, GSH, CAT and total protein in the liver of healthy Wistar rats 72 h after administration of the treatments is presented in Table 2. Lipid peroxidation was significantly (p<0.01) increased in rats fed on 100% seed meal compared to the other meal types as well as the control group. Moreover, there was also a significant increase in liver catalase activity in rats fed on 100% seed meal which was not significantly different from rats in Group V and Group II fed on 100% tuber and 50% seed meals, respectively. The control group fed with rat chow had the least catalase concentration (891.6±54.4) followed by Group IV rats fed on a 50% tuber meal (Table 2). The 50% and 100% tuber flour meals did not affect the liver of the treated rats.
Table 2: Effect of African yam bean seed and tuber meal on superoxide dismutase (SOD), lipid peroxidation (LPO), reduced glutathione (GSH), catalase and total protein on the Liver of healthy wistar albino rats 72 h after administration

<table>
<thead>
<tr>
<th>Meals</th>
<th>SOD</th>
<th>GSH</th>
<th>LPO</th>
<th>CATALASE</th>
<th>TOTAL PROTEIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat Chow</td>
<td>0.33±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>54.68±1.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.48±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>891.57±54.40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.20±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>50 % Tuber</td>
<td>0.34±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.11±2.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.49±0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>945.88±87.16&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.23±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>50 % Seed</td>
<td>0.34±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.19±1.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.51±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1006.86±9.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.23±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>100% Tuber</td>
<td>0.35±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60.67±13.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.54±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1004.00±7.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.24±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>100% Seed</td>
<td>0.36±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.45±4.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.61±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1011.43±19.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.26±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean</td>
<td>0.34</td>
<td>56.22</td>
<td>0.53</td>
<td>971.95±12.85</td>
<td>0.23</td>
</tr>
<tr>
<td>LSD (α=0.05)</td>
<td>0.04</td>
<td>9.24</td>
<td>0.06</td>
<td>69.53</td>
<td>0.06</td>
</tr>
</tbody>
</table>

All values are expressed as Mean±SD of six rats per group. Means followed by the same letters down the columns are not significantly different at p<0.05. SOD (unit mg<sup>-1</sup> protein), GSH (mmoles min<sup>-1</sup> mg<sup>-1</sup> protein), LPO (unit mg<sup>-1</sup> protein), Catalase (mmoles min<sup>-1</sup> mg<sup>-1</sup> protein), Total protein (mg protein)

Severe portal and central venous congestion were, however, observed in the liver of rats fed with 100% seed meal (Fig. 3a). Rats fed with 50% seed meal had mild periportal hepatic necrosis and severe periportal infiltration by mononuclear cells (Fig. 3b). No visible lesions were observed in the control (Fig. 3c).

**Oxidative stress elevated the concentration of lipid Peroxidase through decreasing the levels of the antioxidant enzymes glutathione. Comparable observations were made in the liver of rats treated with tetracycline (Chandra, Salman, Mohd, Sweety, & Ali, 2015) and in kidney tissues (Bhattacharya, 2015). No lesions were seen in the testis of Wistar rats for all the treatments (Fig 4).**

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The effect of AYB seed and tuber meals on SOD, LPO, GSH, CAT and total protein on the testis of healthy Wistar albino rats 72 h after administration is presented (Table 3). The results indicated that SOD levels in the testis were significantly (p<0.01) reduced in rats fed on 50% tuber meal compared to the other treatments. Nonetheless, no significant differences were observed in SOD levels in rats fed on rat chow (control), 50% seed, 100% seed, and 100% tuber meals.

Table 3: Effect of African yam bean seed and tuber meal on superoxide dismutase (SOD), lipid peroxidation (LPO), reduced glutathione (GSH), catalase and total protein on the Testis of healthy Wister albino rats 72 h after administration

<table>
<thead>
<tr>
<th>Meals</th>
<th>SOD</th>
<th>GSH</th>
<th>LPO</th>
<th>CATALASE</th>
<th>TOTAL PROTEIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat Chow</td>
<td>0.31±0.05</td>
<td>60.43±4.34</td>
<td>0.22±0.10</td>
<td>859.77 ± 61.33</td>
<td>0.29±0.02</td>
</tr>
<tr>
<td>50 % Tuber</td>
<td>0.27±0.01</td>
<td>59.62±1.60</td>
<td>0.21±0.03</td>
<td>917.05 ± 83.80</td>
<td>0.24±0.01</td>
</tr>
<tr>
<td>50 % Seed</td>
<td>0.34±0.02</td>
<td>61.32±2.45</td>
<td>0.23±0.04</td>
<td>994.38 ± 1.25</td>
<td>0.26±0.00</td>
</tr>
<tr>
<td>100% Tuber</td>
<td>0.34±0.02</td>
<td>59.32±5.34</td>
<td>0.25±0.03</td>
<td>1109.86 ± 64.21</td>
<td>0.24±0.01</td>
</tr>
<tr>
<td>100 % Seed</td>
<td>0.34±0.02</td>
<td>61.92±4.38</td>
<td>0.26±0.02</td>
<td>1130.03 ± 88.48</td>
<td>0.27±0.02</td>
</tr>
<tr>
<td>Mean</td>
<td>0.32</td>
<td>60.52</td>
<td>0.23</td>
<td>1002.22</td>
<td>0.26</td>
</tr>
<tr>
<td>LSD (α=0.05)</td>
<td>0.03</td>
<td>5.43</td>
<td>0.07</td>
<td>68.16</td>
<td>0.01</td>
</tr>
</tbody>
</table>

All values are expressed as Mean ± SD (n=6). Means followed by the same letters down the columns are not significantly different at p < 0.05. SOD (unit mg-1 protein), GSH (mmoles min⁻¹ mg⁻¹ protein), LPO (unit mg⁻¹ protein), Catalase (mmoles min⁻¹ mg⁻¹ protein), Total protein (mg⁻¹ protein)
Likewise, there was no significant variation in the levels of GSH and LPO in the testis. Catalase concentration was however elevated in rats fed on 100% seed and 100% tuber meals by 31.43% and 29.09% respectively than in the control treatment. Nevertheless, there was a considerable reduction in catalase concentration in rats fed on rat chow and 50% tuber meals. Total protein level was also significantly reduced by 20.83% in rats fed on 50% and 100% tuber meals than the control, and by 12.50% than in rats fed on 100% seed meal where it was significantly (p<0.001) increased.

Hundred per cent seed meal generated an imbalance between the free radicals produced and the ability of the liver and kidney to detoxify the adverse effects through neutralization by the antioxidant defence mechanisms. Oxidative stress is a pathological condition caused by the disparity between the development and removal of reactive oxygen species (ROS) derived from free radicals and the potential of a biological system to get rid of the reactive intermediates and return the order to its regular state (Bhattacharya, 2015; Chandra et al., 2015). Oxidative stress causes base damage by the formation of ROS such as O$_2^-$ (superoxide radical), OH$^-$ (hydroxyl radical) and H$_2$O$_2$ hydrogen peroxide as well as fragmentation of the DNA molecule (Chandra et al., 2015). Superoxide dismutase, catalase, glutathione peroxidase as well as non-enzymatic compounds such as tocopherol ascorbic acid and glutathione give cells protection against oxidative stress by reducing free radical concentrations (Bhattacharya, 2015; Mao et al., 2011). Under conditions of oxidative stress, activities of these enzymes are elevated. Soetan et al. (2016) also made such observations on the liver and kidney of rats fed with Lablab purpureus seeds. Reduction in the capacity of antioxidant systems leads to an increase in ROS concentrations which perpetually distorts the permeability of the mitochondria and induces the release of enzymes that activate cell death (Wang et al., 2013). The different treatments of Sphenostylis stenocarpa seed and tuber flour meals fed to Wistar rats did not have any significant effect on renal SOD and hepatic SOD activities but 100% seed meal increased CAT and GSH activities in both liver and renal tissues when compared to the control. Comparable results were also obtained by Soetan et al. (2016). Lipid peroxidation and liver catalase activity was also elevated in rats fed on 100% seed meal compared to 50% and 100% tuber meals.

Studies have revealed that catalase is a highly adaptive antioxidant enzyme that protects cells under oxidative stress (Ozkurt-Borazan et al., 2011; Soetan et al., 2016). The elevated level of CAT activity, therefore, could be attributable to an adaptive response to high concentrations of H$_2$O$_2$ during the treatment with a 100% seed meal of AYB. Free radicals produce H$_2$O and O$_2$ from the breakdown of H$_2$O$_2$ by catalase or by superoxide dismutase during the elimination of superoxide anions by superoxide dismutase (Akintunde et al. 2013). Rats fed on 100% seed and 100% tuber meals exhibited higher levels of catalase than the control which could be due to the production of excess H$_2$O$_2$ arising from another intermediary metabolism in the kidney and liver when rats were fed on 100% seed and tuber meals. Lipid peroxidation is induced by free radicals resulting in reactive molecules such as malondialdehyde (MDA), used as a biomarker for lipid peroxidation. Lipid peroxidation (LPO) disrupts the integrity of plasma membranes and causes damage to the tissues. In this study, there was a significant increase in levels of malondialdehyde in the liver and kidney of rats fed with 100% seed meal which may be attributed to the increased generation of the ROS and an altered antioxidant defence system. No damage was observed in the testis of rats investigated. Thus, the histological changes observed cannot be transferred via sexual reproduction. The increase in total protein level by 50% and 100% seed meals is indicative of a rise in the globulin portion of the complete protein functioning to combat infections in the rats which could be an indication of damage to some organs.

The analysis of the tubers and seeds of the four AYB accessions TSs107, TSs140, AYB45 and AYB57 revealed that anti-nutritional factors: phytate, oxalate, and tannin were more in seeds (Fig 5a) compared to tubers (Fig 5b).
Nevertheless, saponin content was higher in the tubers of AYB45 (480 mg 100g⁻¹) and AYB57 (540 mg 100g⁻¹) compared to the seeds (350 mg 100g⁻¹). Higher levels of oxalate were obtained in the seeds of AYB45 (310.0 mg 100g⁻¹), AYB57 (240.0 mg 100g⁻¹) and TSs140 (250.0mg 100g⁻¹) but lower in both seed (80 mg 100g⁻¹) and tuber (30 mg 100g⁻¹) of TSs107. Phytate also followed the same trend as that of oxalate concentration with TSs107 having the same concentration in both seed and tuber. On the other hand, tannins had higher concentrations in the tubers of AYB45 and AYB57 but the lower concentrations in the tubers of TSs107 and TSs140. Lectin concentration was almost the same in both seed and tuber of AYB45, slightly higher in the grains of AYB57 but only present in higher levels in the seeds of TSs107 (58.4 Lu mg⁻¹) and TSs140 (61.2 Lu mg⁻¹) and absent in the tubers. The paired t-test revealed considerable differences in the concentrations of antinutrients in the seeds and tubers of AYB with the seeds generally having higher levels for most antinutrients than tubers (Table 4).
Table 4: Paired t test comparison of anti-nutritional composition of African yam bean seeds and tubers used in meal formulation for wistar rats

<table>
<thead>
<tr>
<th>Accessions</th>
<th>Antinutrient</th>
<th>Seed (mg 100g⁻¹)</th>
<th>Tuber (mg 100g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSs107</td>
<td>Oxalate</td>
<td>80.0</td>
<td>30.0</td>
</tr>
<tr>
<td>TSs140</td>
<td>Oxalate</td>
<td>250.0</td>
<td>60.0</td>
</tr>
<tr>
<td>AYB45</td>
<td>Oxalate</td>
<td>310.0</td>
<td>80.0</td>
</tr>
<tr>
<td>AYB57</td>
<td>Oxalate</td>
<td>240.0</td>
<td>115.0</td>
</tr>
<tr>
<td>TSs107</td>
<td>Phytate</td>
<td>205.0</td>
<td>17.5</td>
</tr>
<tr>
<td>TSs140</td>
<td>Phytate</td>
<td>550.0</td>
<td>14.8</td>
</tr>
<tr>
<td>AYB45</td>
<td>Phytate</td>
<td>460.0</td>
<td>275.0</td>
</tr>
<tr>
<td>AYB57</td>
<td>Phytate</td>
<td>485.0</td>
<td>240.0</td>
</tr>
<tr>
<td>TSs107</td>
<td>Tannin</td>
<td>660.0</td>
<td>11.0</td>
</tr>
<tr>
<td>TSs140</td>
<td>Tannin</td>
<td>200.0</td>
<td>8.0</td>
</tr>
<tr>
<td>AYB45</td>
<td>Tannin</td>
<td>189.5</td>
<td>480.0</td>
</tr>
<tr>
<td>AYB57</td>
<td>Tannin</td>
<td>215.0</td>
<td>540.0</td>
</tr>
<tr>
<td>TSs107</td>
<td>Saponin</td>
<td>440.0</td>
<td>3.0</td>
</tr>
<tr>
<td>TSs140</td>
<td>Saponin</td>
<td>330.0</td>
<td>5.0</td>
</tr>
<tr>
<td>AYB45</td>
<td>Saponin</td>
<td>351.0</td>
<td>275.0</td>
</tr>
<tr>
<td>AYB57</td>
<td>Saponin</td>
<td>350.0</td>
<td>225.0</td>
</tr>
<tr>
<td>TSs107</td>
<td>Trypsin inhibitor (TIU mg⁻¹)</td>
<td>34.6</td>
<td>0.0</td>
</tr>
<tr>
<td>TSs140</td>
<td>Trypsin inhibitor (TIU mg⁻¹)</td>
<td>34.9</td>
<td>0.0</td>
</tr>
<tr>
<td>AYB45</td>
<td>Trypsin inhibitor (TIU mg⁻¹)</td>
<td>34.5</td>
<td>34.1</td>
</tr>
<tr>
<td>AYB57</td>
<td>Trypsin inhibitor (TIU mg⁻¹)</td>
<td>37.1</td>
<td>33.8</td>
</tr>
<tr>
<td>TSs107</td>
<td>Lectin (Lu mg⁻¹)</td>
<td>58.4</td>
<td>0.0</td>
</tr>
<tr>
<td>TSs140</td>
<td>Lectin (Lu mg⁻¹)</td>
<td>61.2</td>
<td>0.0</td>
</tr>
<tr>
<td>AYB45</td>
<td>Lectin (Lu mg⁻¹)</td>
<td>57.6</td>
<td>57.2</td>
</tr>
<tr>
<td>AYB57</td>
<td>Lectin (Lu mg⁻¹)</td>
<td>59.1</td>
<td>56.7</td>
</tr>
<tr>
<td>TSs107</td>
<td>Cyanogenic glycoside (mg 100g⁻¹)</td>
<td>6.1</td>
<td>0.9</td>
</tr>
<tr>
<td>TSs140</td>
<td>Cyanogenic glycoside (mg 100g⁻¹)</td>
<td>6.9</td>
<td>0.9</td>
</tr>
<tr>
<td>AYB45</td>
<td>Cyanogenic glycoside (mg 100g⁻¹)</td>
<td>6.9</td>
<td>6.4</td>
</tr>
<tr>
<td>AYB57</td>
<td>Cyanogenic glycoside (mg 100g⁻¹)</td>
<td>6.8</td>
<td>6.3</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>204.3</td>
<td>91.9</td>
</tr>
<tr>
<td>CV (%)</td>
<td></td>
<td>92.8</td>
<td>159.1</td>
</tr>
</tbody>
</table>

\( t (0.05) \) for comparing the two means = 2.88
The concentration of ANFs oxalate, phytate, tannin, and saponin were higher in the seeds of AYB used for meal formulation compared to the tubers, which may be responsible for the harmful effect of the seed flour meal on the rats. Oxalate if consumed in large quantities blocks the renal tubules by forming calcium oxalate crystals and development of urinary calculi (Soetan and Oyewole, 2009), which can lead to renal failure and hence death in vulnerable individuals. Oxalic acid affects the bioavailability of minerals, especially calcium as it forms insoluble calcium oxalate with calcium which is not absorbed in the body (Nwaogu and Udebuani, 2010). Phytate binds to minerals, making them unavailable to the animal while tannin causes a decrease in feed consumption, palatability, and reduced growth in animals (Abioye et al., 2015). Phytate chelates ions thereby reducing the availability of calcium and inhibit the absorption of iron because of calcium phytate complexes produced (Abioye et al., 2015). Higher concentration of phytate was observed in the seeds of accessions TSs140 (550mg 100g\textsuperscript{-1}), AYB45 (460mg 100g\textsuperscript{-1}) and AYB57 (485mg 100g\textsuperscript{-1}) used for formulation of the seed meal compared to the tubers; TSs140 (14.8mg 100g\textsuperscript{-1}) AYB 45 (275mg 100g\textsuperscript{-1}) and AYB57 (240mg 100g\textsuperscript{-1}). Tannin was highest in the seeds of accession TSs107 (600mg100g\textsuperscript{-1}) compared to the tuber (11mg100g\textsuperscript{-1}). The high concentration of tannins had undesirable effects on the digestive tract of rats due to the harmful nature of the metabolites which could have resulted in the albino rats eating less of the seed meal compared to consuming all the tuber meal.

The increasing presence of saponin in legume seeds reduces methane production in the rumen of livestock by suppressing protozoa which influences butyrate production during rumen methanogenesis. Consequently, it leads to energy loss derivable from a feedstuff by the animal. Saponins cause cytotoxic permeabilization of the intestines through its biological activities depending on the structure (Soetan et al., 2009). Elevated levels of saponin were obtained in the seeds of unprocessed AYB accessions TSs107 (440mg 100g\textsuperscript{-1}), TSs140 (330mg 100g\textsuperscript{-1}), AYB 45 (351mg 100g\textsuperscript{-1}) AYB57 (350mg 100g\textsuperscript{-1}) compared to the tubers (TSs107 (3mg 100g\textsuperscript{-1}) TSs140 (5mg 100g\textsuperscript{-1}) AYB45 (275mg 100g\textsuperscript{-1}) AYB57 (225mg 100g\textsuperscript{-1}) respectively. Unprocessed seeds of AYB studied, had higher anti-nutritional contents which can be eliminated or reduced by efficient processing methods. Roasting eliminates or reduces the amount of phytate in seeds more efficiently than boiling; while boiling eliminates oxalate from seeds much better than roasting (Ikhajiagbe and Mensah, 2012). Recent studies by Ojuederie and Balogun (2019) revealed that the tubers of the AYB accessions used for the meal formulation were rich in Magnesium and potassium (1.67gkg\textsuperscript{-1} and 10.09gkg\textsuperscript{-1}) and proximate components especially accession TSs107 and TSs140 which had low antinutrients in this study, having high protein (15.9% and 15.4%) and carbohydrate (68.7% and 67.9%) contents respectively (Ojuederie and Balogun, 2019). Thus, the tubers of AYB which were highly consumed by wistar rats in this study, could serve as a suitable source of protein and carbohydrate for human consumption, and lessen protein and nutritional deficiency in children if utilised in their diets.

**Conclusion**

Consumption of 100% *Sphenostylis stenocarpa* seed meal-induced hepatorenal toxicity in rats investigated in this study, due to induction of oxidative stress and lipid peroxidation by free radicals which increased the levels of malondialdehyde. The tubers of AYB are safe for human consumption and could be used in animal feeds as they did not trigger a toxic effect in the tissues and organs of Wistar rats investigated. Tin order to utilize the seeds of African yam bean in animal feeds, appropriate processing methods need to be carried out to reduce the levels of anti-nutritional factors which were found to be more concentrated in the seeds. This study will help researchers to unearth the knowledge gap in the antinutrient composition of AYB tubers that many researchers have not explored. Thus, the benefits of eating the tubers of AYB may be achieved.
Acknowledgements

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