

CROP PRODUCTION AND SOIL SCIENCE

PRELIMINARY OBSERVATIONS ON THE GEOGRAPHICAL DISTRIBUTION OF THE FALL ARMYWORM (*Spodoptera frugiperda*) (J.E.Smith, 1797) INFESTATION IN NIGERIA.

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ABSTRACT

Spodoptera exempta (black armyworm) and *S. littoralis* (cotton leafworm) were the only insect pests in the genus *Spodoptera* that have been reported in Nigeria. In March 2016, a reported case of larvae infestation of some insects observed to be from the Order Lepidoptera was reported on different farms in Ikenne and Ibadan. Incidence and severity of the insect pest observed in both locations were as high as 100%. Geo-referenced survey to determine the distribution of fall armyworm (FAW) (*Spodoptera frugiperda*) on maize in Nigeria were conducted in 2017/2018 in different agro-ecological zones of Nigeria following FAW identification and reports received nationwide from maize farmers. Early symptoms observed from 7-10 days were “window pane”, ragged edges and shot-holes on the leaves. Additional symptoms (frass, rolled leaves covering the larvae and damaged whorl) were observed later as the larvae matured. Larvae collected from fields were preserved in 70% ethyl alcohol, others were reared in the laboratory; pupae and adults resulting from the larvae were used to identify the pest as *S. frugiperda* using morphological characteristics as described by Todd and Poole (1980). The report of this survey indicates the presence of a new invasive pest identified as *Spodoptera frugiperda*, the Fall Armyworm in Nigeria.

Keywords: Fall Armyworm, maize, invasive species, geographical distribution.

INTRODUCTION

Maize is a staple food of great socio-economic importance in Nigeria and widely grown in most agro-ecological zones of the country. It is cultivated on an area of 6.8 million hectares with a yield of 11.84 metric tons per hectare in 2015 (Foreign Agricultural Service/USDA, 2016; FAOSTAT, 2021). Nigeria ranked among the largest producer of maize in Africa and the

world (IITA, 2012). Ripened maize is eaten roasted or boiled and processed into different food products. It is also a local cash crop on which many agro-based industries depend on as raw materials for production of various products in Pharmaceutical, textile and food beverages industries (Iken and Amusa, 2004; Ofor *et al.*, 2009; Badmus and Ariyo, 2011).

Maize production, despite its importance, is largely still at a subsistence level coupled

with the problem of pests. It is estimated that seventy percent of the farmers are smallholders accounting for 90 percent of the total farm output (Cadoni and Angelucci, 2013). Notable insect pests reported on maize in Nigeria include stem borer (*Busseola fusca* and *Sesamia calamistis*, *Eldana saccharina*), earworm (*Helicoverpa zea*) and variegated grasshopper (*Zonocerus variegatus*) among other pests that can wreak havoc on the crop (Iken and Amusa, 2004; Ofor *et al.*, 2009). In Nigeria, the production of maize is now seriously hampered by the newly introduced invasive pest, the fall armyworm (FAW) (Goergen *et al.*, 2016).

Fall Armyworm, *Spodoptera frugiperda*, is widely distributed in the tropical and subtropical regions of America (Andrews, 1988; Alves *et al.*, 2012; Plantwise, 2016) and has now spread to 28 African countries (Kiprop, 2017). Continental research centres and developmental organizations have indicated that FAW has become the most destructive pest in reducing maize production in Africa (Abrahams *et al.*, 2017).

Studies on genetic diversity and gene flow of fall armyworm populations from the North America and the Caribbean revealed the existence of two morphologically identical strains that differ in host preference, physiology, behaviour and pesticide susceptibility (Lynch *et al.* 1983; Pashley 1986, 1988; Pashley *et al.* 1995, Prowell *et al.* 2004). One strain was identified as corn (maize) strain that mainly feeds on maize, sorghum, and other large grasses; the other strain was called the rice strain and mainly feeds on rice, Bermuda grass [*Cynodon dactylon* (L.)], and other small grass species (Pashley, 1986). The two strains are morphologically identical and identification is largely dependent on molecular markers (Nagoshi and Meagher 2004).

After a long dry spell, between January and March, 2016 and first rain in Ogun and Oyo states of Nigeria, an unidentified insect pest infestation at that time was observed causing severe damage to maize in different farms in Ogun and Oyo states. Different larva stages of the insect were found on the maize, reared and identified as *Spodoptera frugiperda* (FAW). Further investigation in Nigeria revealed that several reports of a pest aligning with Fall Armyworm's morphological description have been received nationwide by Ministries, Departments and Agencies (MDAs) including Nigeria Agricultural Quarantine Service (NAQS). Thus, a nationwide survey was conducted to determine the presence, extent of spread and genetic diversity of FAW in some selected states of the country.

MATERIALS AND METHODS

Survey Area: Insect Collection and

Sampling method

Larvae were collected on the farms randomly from whorl of 20 stands of maize plants showing symptoms of damage. Three (3) farms each within three Local Government Areas per selected states in the agro-ecological zones of Nigeria were visited and personal interview were conducted with the farmers. (Figure 1). The farmers' fields were identified through the assistance of Nigeria Agricultural Quarantine Service (NAQS) and Agricultural Development Project (ADPs) staff. The geographical coordinates of each farm visited were recorded using a global positioning system (GPS Garmin eTrex Legend HCx) unit. The collected larvae samples were reared in the laboratory for identification and preserved in 70% ethyl alcohol for DNA sequencing.

Insect rearing and Morphological Identification

Larvae were reared in glass jars (35 cm diameter x 16 cm high) covered with a muslin sheet. Maize leaves (supplied daily after removal of frass) was put in the jar to observe the development of the larva. A thin layer of sterile top soil in a small container (10cm diameter x 1cm high) was placed inside the jar for pupation. The jars were left on the laboratory bench at room temperature (37°C) until adult emergence.

Maize seeds were also planted in small garden pots (40 cm diameter x 20 cm height) inside an insect rearing box. Three 2nd in-star larvae were transferred onto the 7 days old maize seedlings and observed for signs of larvae feeding, damage and adult emergence. The emerged adults were killed using fumes of ethyl acetate and mounted for taxonomic identification using morphological characters.

DNA Barcoding

Insect vials containing larvae samples labelled (A - J) (Table 1) collected randomly on maize plants from different locations across the country were preserved in 70% ethanol. The samples were sent to Centre for Agriculture and Bioscience International (CABI), United Kingdom for DNA Barcoding using method of Sambrook *et al.* (1989) stated below:

Molecular assays were carried out on each sample using nucleic acid as a template. A proprietary formulation [microLYSIS®-PLUS (MLP), Microzone, UK] was subjected to the rapid heating and cooling of a thermal cycler, to lyse cells and release deoxyribonucleic acid (DNA). Following DNA extraction, Polymerase Chain Reaction (PCR) was employed to amplify copies of the rDNA in vitro.

The PCR product was assessed by undertaking gel electrophoresis. PCR purification step was carried out to remove

unutilised dNTPs, primers, polymerase and other PCR mixture compounds and obtain a highly purified DNA template for sequencing. This procedure also allows concentration of low yield amplicons. Sequencing reactions were undertaken using BigDye® Terminator v3.1 kit from Applied Biosystems (Life Technologies, UK) which utilises fluorescent labelling of the chain terminator ddNTPs, to permit sequencing. Removal of excess unincorporated dye terminators was carried out to ensure a problem-free electrophoresis of fluorescently labelled sequencing reaction products on the capillary array AB 3130 Genetic Analyzer (DS1) DyeEx™ 2.0 (Qiagen, UK). Modules containing prehydrated gel-filtration resin were optimized for clean-up of sequencing reactions containing BigDye® terminators. Dye removal was followed by suspension of the purified products in highly deionised formamide Hi-Di™ (Life Technologies, UK) to prevent rapid sample evaporation and secondary structure formation. Samples were then loaded onto the AB 3130 Genetic Analyzer and sequencing undertaken to determine the order of the nucleotide bases, adenine, guanine, cytosine, and thymine in the DNA oligonucleotide. After sequencing, identifications were undertaken by comparing the reverse complement of the sequence obtained from Barcode of life Data system (BOLD) (Ratnasingham and Hebert, 2007)

RESULTS

Insect Development and Morphological Identification

Eggs (Plate 1) collected from the maize seedlings hatched within 3-4 days into the 1st-instar larva (neonates). The 1st-instar larvae that were cultured; adults' emergence was observed after 30-32 days. The larva passes through six instars within 15-21 days with the

first having green colour with black head. Subsequent instars had orange-brown head with progressive change in colour from pale green, greenish yellow, light brown to dark brown/reddish brown colour before pupation (Plate 2). The larvae had an inverted “Y” and four black dots arranged in a square near the end of the last abdominal segment (Plate 3). Duration of pupa stage was between 7 -14 days with the pupa being obdect, having reddish brown colour and black dots at the side of the abdomen (Plate 4). Male adult have grey/brown colour forewings with white spots near the tips and centre. The fore wings of the female were observed to have uniform greyish brown to a fine mottling of grey and brown (Plate 5).

Infestation and Damage

Spodoptera frugiperda infested all stages of maize but impact was more on maize of 2-6 weeks old (Plate 6). Damaged symptoms first observed on maize as soon as it emerged were shot-holes (7) and “window pane” (8) on the leaves as a result of the larva feeding on the epidermis/chlorophyll. As the larvae mature, more shot holes, frass, damaged whorl, resulting in the death of the growing point “Dead Heart” ragged leaves were observed with some of the plants stunted. Larvae hid within whorl or rolled leaves (Plate 9), unrolling the leaves or whorl revealed the larva. It was also observed that some maize plants as they matured compensated for damage by growing vigorously to recover from the attack.

Insect Genetic Diversity

All but three samples were successfully sequenced. The unsuccessful samples (B, F and H) were sequenced several times but failed repeatedly. The successfully sequenced samples were identified in the Barcode of Life Data System (BOLD) as

shown in **Table 1**. Samples E and I are *Spodoptera frugiperda* Haplotype 1 (Rice), whilst samples A, D, G and J are *Spodoptera frugiperda* Haplotype 2 (Maize), however sample C was identified as *Eldana saccharina*.

DISCUSSION

Extensive damage to maize plant was observed in several farms across the some agro ecological zones of Nigeria (Odeyemi *et al.*, 2016). Preliminary investigations between 2016, 2017 and 2018 indicated that the causal organism was armyworm larva, which was presumed to be African armyworm (*S. exempta*) naturally, since it was the only known armyworm attacking maize in Nigeria (Iken and Amusa, 2004; Ofor *et al.*, 2009). The level of damage attributed to the new invasive armyworm species and geographical spread after introduction in 2016 compared with *S. exempta* which had never been known to cause such massive destruction (Ofor *et al.*, 2009) is significantly alarming. Also, the “gregarious form” was black with yellow stripes which were not observed in all larvae collected from the field or cultured created the suspicion that this could be another species of armyworm. The larvae have an inverted “Y” and four black dots arranged in a square near the end of the last abdominal segment with the pupa having reddish brown colour (Bohnenblust & Tooker, 2012) which distinguished it from other species of armyworms. Also, the characteristics features of white spots near the tips of forewings from the emerged adults also confirmed that it is a different species. These features were used to confirm that the larvae causing much of the damage belong to that of the Fall Armyworm (*Spodoptera frugiperda*) (Plantwise, 2016, CABI, 2016).

The pathway of introduction of this moth into Nigeria is yet to be understood since it has not been reported in neighbouring countries even in other African countries before 2016. Reported cases of the pests migrating long distances on prevailing winds, breeding continuously in areas that are climatically suitable have been confirmed (CABI, 2017). DNA Barcoding of the samples in this present study revealed behavioural pattern that suggests that the rice strain of *Spodoptera frugiperda*, which mainly feeds on rice, Bermuda grass [*Cynodon dactylon* (L.) Pers.], and other small grass species (Pashley 1986); may have developed adaptive preference to feeding on corn (maize) in the Nigeria. It is plausible to conclude that subtle genetic changes (epigenetics and post-translational modification) of the *Spodoptera frugiperda* haplotype that were not researched in this study may have led to main host preference change. However, it may just be due to ecological adaption to abundance of maize and absence of rice in the environment given that maize production since the 1980s has exceeded rice production year on year in Nigeria (IRRI, 1995; FAOSTAT, 2020). The identification of a sample in this study as *Eldana saccharina* also suggests a behavioural change paradigm. *Eldana saccharina*, which is only a minor pest of maize (Assefa *et al.*, 2006), caused damage that were consistent with that of *Spodoptera frugiperda* (a major pest of maize).

In conclusion, further investigation will be required to confirm any of these suggestions. The present effort is the first report of Fall Armyworm (*Spodoptera frugiperda*) occurrence and distribution pattern attacking maize in Nigeria indicating the need to develop urgent baseline for appropriate management strategies to combat the damage and spread caused by the pest.

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Table 1: Identity of larvae collected from maize plant in selected location in Nigeria using BOLD database

Isolate	Location	Coordinates	Identification	Sequencing Result
A	Kano	12.452616 ⁰ N 8.504850 ⁰ E	<i>Spodoptera frugiperda</i>	100% to voucher specimen sequences
B	Jigawa	1120.712 ⁰ N 951.523 ⁰ E	No ID	DNA failed to amplify even when repeated
C	Benue	07.24266 ⁰ N 008.17211 ⁰ E	<i>Eldana saccharina</i>	99% to voucher specimen sequences
D	Niger	09.31350 ⁰ N 008.61456 ⁰ E	<i>Spodoptera frugiperda</i>	100% to voucher specimen sequences
E	Anambra	06.20560 ⁰ N 007.03982 ⁰ E	<i>Spodoptera frugiperda</i>	100% to voucher specimen sequences
F	Imo	05.25348 ⁰ N 006.89038 ⁰ E	No ID	DNA failed to amplify even when repeated
G	Ogun	07.082003 ⁰ N 003.91167 ⁰ E	<i>Spodoptera frugiperda</i>	100% to voucher specimen sequences
H	Ondo	06.42288 ⁰ N 003.54578 ⁰ E	No ID	DNA failed to amplify even when repeated
I	Zamfara	556.605 ⁰ N 12.13491 ⁰ E	<i>Spodoptera frugiperda</i>	100% to voucher specimen sequences
J	Enugu	06.00892 ⁰ N 007.27839 ⁰ E	<i>Spodoptera frugiperda</i>	100% to voucher specimen sequences

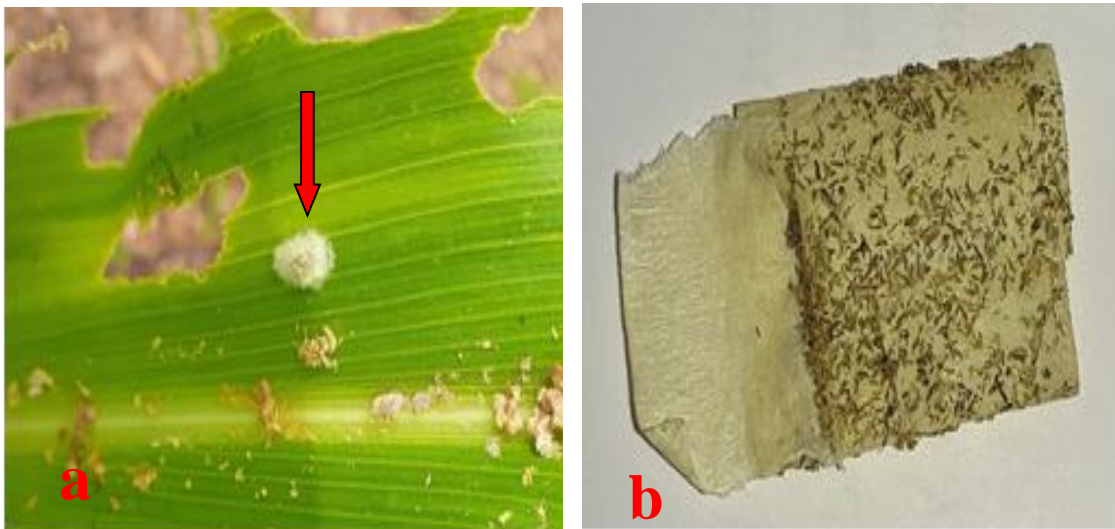


Plate 1: (a) Egg mass of *Spodoptera frugiperda* (arrowed). (b) FAW neonates

Preliminary Observations on the Geographical Distribution of the Fall Armyworm (*Spodoptera Frugiperda*)
(J.E.Smith, 1797) Infestation in Nigeria.

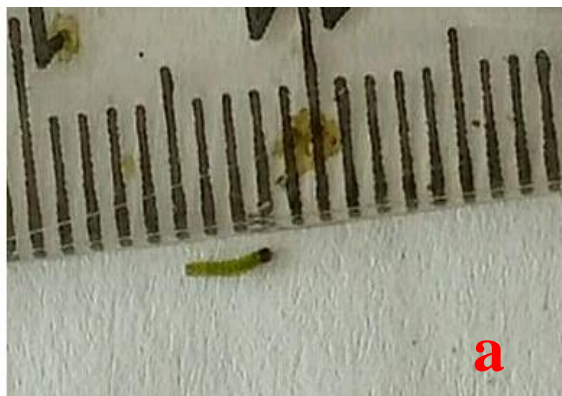


Plate 2: Fall armyworm (a) 1st (b) 2nd (c) 3rd (d) 4th (e) 5th and (f) 6th larva in-star stages



Plate 3: Matured *Spodoptera frugiperda* with arrowed inverted Y at the head region and the four (4) black dots. Photo credit. Ogunfunmilayo A. O. (2018).



Plate 4: Reddish brown colour pupa.(a) Anterior with black dots (arrowed) (b) Posterior view



Plate 5: Fall armyworm adults

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Plate 6: Damaged tattered maize leaves (arrowed).



Plate 7: "Window panes" on the leaves (arrowed)



Plate 8: Adult FAW resting on maize leaf (circled) leaf whorl

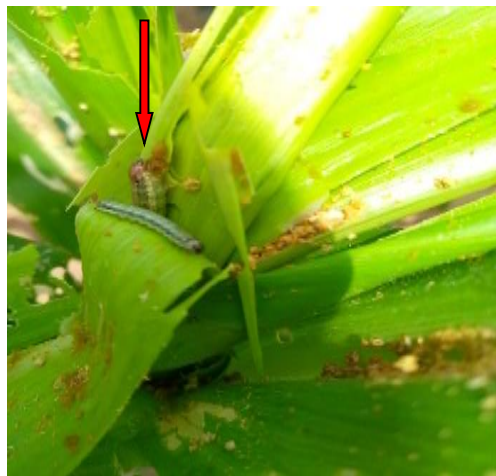


Plate 9: Fall army worm larvae in maize (arrowed)

EVALUATION OF GRAIN YIELD AND RESISTANCE TO FALSE SMUT AND BLAST DISEASES OF UPLAND RICE IN IBADAN, SOUTH-WESTERN NIGERIA

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ABSTRACT

Rice (*Oryza sativa* L.) is the most important staple food in the world today. Nigeria is the highest producers and importer of rice in the West Africa. Its production is threatened by several diseases causing grain yield losses of staggering dimension. Previous attempts to increase rice production through the use of improved varieties without proper regard for plant protection measures were unsuccessful. Hence, the objectives of this study were, to screening rice lines for resistance to false smut and blast (leaf and panicle blast) under natural conditions and determine yield loss to the diseases. The experiment was conducted at the Research Farm of National Cereal Research Institutes, Ibadan Out-Station in 2017 planting season. It was laid out in a randomized complete block design with three replications. Ten upland rice lines and 3 checks were screened, twenty plants were randomly selected from a sampling area of 1 m² quadrant. Leaf blast (LB), panicle blast (PB) and False Smut (FSm) incidence scoring was carried-out at 2-12 weeks after planting, using a visual scale of 0-9, The resistance level (RL) was also determined; following the standard evaluation scale (SES) of International Rice Research Institute (IRRI). All the data collected were analysed using ANOVA. The results indicated that ART3-8L14P3-2-B-2 (6.33) and FARO 63 (5.67) had significantly ($p < 0.05$) higher incidence of FSm than all other lines evaluated. ART16-9-29-12-1-1-1-B-1-1 (1.33) and FARO 64 (1.67) had lower incidence of PB than ART15-13-2-2-2-1-B-1-2 (6.33). ART16-9-29-12-1-1-1-B-1-1 (4066.7 kg/h) and ART16-16-7-15-1-B-1-B-1-1 (3966.7 kg/h) produced significantly higher grain yield than FARO 63 (1500.0 kg/h), ART3-8L14P3-2-B-2 (1800.0 kg/h) and ART16-10-2-32-1-B-1-B-1-1 (1750.0 kg/h) and these three lines were highly resistance to FSm, LB, and PB; and moderately resistance to FSm and PB; and resistance to LB, respectively. These resistant lines will be useful in breeding for disease resistant rice varieties.

Keywords: Rice, Nigeria, False Smut, Resistance, Fungus, Severity

INTRODUCTION

Rice (*Oryza sativa* L) constitutes one of the most important staple foods for over half of the world's population. This is because more than 50% of the world population depends on it. This situation has been demonstrated in

Asia and sub-Saharan Africa (Akpokodje *et al.*, 2001). Currently, rice ranks third after wheat and maize in production (Guimaraes, 2009; Ajah and Ajah, 2014). Over 90 percent of the world's rice is produced and consumed in the Asia-Pacific region, while Egypt and

Nigeria are the top producers in Africa (USDA, 2017).

Nigeria produces about 6.5 million metric tons of rice annually, with production area of about 1.7 million hectares. Rice per capital consumption is put at 40 kg/person/year, this is 10 kg less than the global annual (FAO, 2004). The country is the highest producer and importer of rice in the West Africa sub-region and the second largest importer in the World after China (Statista, 2011). It was estimated that Nigeria imports rice of about 356 billion naira worth annually (ATA, 2011). Rice growing ecologies in Nigeria include Mangrove Swamp which contributes 1 % to rice production in Nigeria, Deep water (5 %), Irrigated lowland (16 %), Rainfed lowland (inland valleys; 48 %) and Rainfed upland (30 %) (Africa Rice Center WARDA/FAO/ SAA, 2008).

Upland rice is one of the most popular cropping options in slope area under rainfed condition, and accounts for about 11% of global rice production (Tuhina-Khatun *et al.*, 2015). To meet the future rice demands, yield increase per unit of land and resistance to both biotic and abiotic stresses are seen as a key component to achieve self-sufficiency in rice production. However, rice is threatened by climatic and pedological unfavorable conditions such as, weeds, ravagers and parasites (Sarraf *et al.*, 2004; Kouassi *et al.*, 2005).

Rice blast is a problem almost everywhere rice is grown, Nigeria inclusive. It is one of the main pathological threats to rice crop production worldwide (Chadha and Gopalakrishna, 2005). The disease is caused by the fungus *Magnaporthe oryzae* (anamorph: *Pyricularia oryzae* Cavara) (Couch and Kohn, 2002). Disease symptoms appear on the aerial parts of the plant, which can consume the whole leaves, causing the death of the plant at any stage of growth. Leaf

blast, node blast, neck rot or neck blast or panicles blast, turn the leaves white and the plant die before being filled with grain. However, the most severe infection encountered is leaf blast. It is one of the most widespread and devastating biotic stresses causing yield reduction of 20 - 100% (Khush and Jena, 2009). Previous attempts to increase rice production through the use of improved varieties or fertilizers without proper regard for plant protection measures were unsuccessful due to severe losses from pests and diseases. Most of the available cultivars are hampered dramatically by this disease (Kihoro *et al.*, 2013).

The use of resistant varieties is the most economical and effective way of controlling rice blast mainly in resource poor farmers' fields. Unfortunately, the causal fungus is able to overcome this resistance (due to the high variability of the blast fungus) within two to three years after these plants are cultivated (Lavanya and Gnanamanickham, 2000). The solution to the problem therefore is the selection of rice varieties with multigenic (stable and horizontal) resistance. Green or false smut caused by *Ustilagoideae virens* (Cooke) Tak. (*Ustilago virens* (Cooke) Tak.), is a major disease of rice wherever it occurs. Awoderu (1972) reported a severe incidence of the disease at Ado-Ekiti, Ekiti state, on a plot of Ofada rice variety. Ahonsi *et al.*, (2000) observed a buildup of the disease due to late sowing and application of higher nitrogen doses.

One of the numerous approaches to verify the stability of genotypes is by evaluation of grain yields and diseases resistant under multi-environments (Acuña *et al.*, 2008). The devastating yield loss caused by these diseases necessitates evaluation for disease resistance lines and to be used in breeding for high-yielding disease resistant varieties to achieve self-sufficiency in rice production.

Hence, the objectives of this study were, to screen rice lines for false smut, leaf and panicle blast resistance under natural conditions and evaluate the yield loss caused by these diseases.

MATERIALS AND METHOD

Study Location

The experiment was conducted at the Research Farm of National Cereal Research Institutes (NCRI), Ibadan Out-Station of Latitude 7° 22'N and Longitude 3° 58'E with mean annual rainfall of 1150-1250mm. With textural class of loamy soil (1:2 soil/water) using USDA textural calculator. The study was conducted in 2017 planting season.

Source of Seeds

Ten (10) upland rice lines (ART3-8L14P3-2-B-2, ART16-9-29-12-1-1-1-B-1-1, ART16-10-2-32-1-B-1-B-1-1, ART16-9-4-18-3-2-1-B-1-1, ART16-12-25-23-1-1-3-B-1-2, ART16-12-12-2-23-2-B-1, ART15-13-2-2-2-1-B-1-2, ART16-9-33-2-1-1-1-B-1-1, WAB788-16-1-1-2-HB, ART16-16-7-15-1-B-1-B-1-1) and 3 checks (FARO 59, 63, 64) used in this study were sourced from NCRI Badeggi, Niger State.

Experimental Design and Seed Planting

The experiment was laid out in a randomized complete block design with three replications. Each entry was planted in plot size of 2 m x 5 m and separated by 1 m border. Fertilizer application was at 60 kg N per ha in two splits of 30 kg N per ha at seeding and 30 kg N per ha at 40 days after seeding (DAS), 30 kg P₂O₅ and 30 kg K₂O were also applied at seeding. Weeds control was carried out using standard management practices.

Disease Assessment

Twenty plants of each rice lines and varieties were randomly selected from a sampling area

of 1 meter square quadrant at the middle of each plot and tagged, and the following parameters were observed and recorded:

Blast scoring was carried out at 2 - 12 weeks after planting, and at 3 - 12 weeks after heading for neck and panicle blast respectively. Degree of infection was measured using a visual scale of 0 - 9 (0 = no infection, 1 = mild infection, (\leq 4%), 3 = moderate infection, (5-10%), 5 = high infection, (11-25%), 7 = severe infection, (26-50%) and 9 = very severe infection (> 50%). Scoring was based on the number of plants and leaves infected, lesions and sizes of lesion on the leaves, necks and panicles infested (WARDA, 1999). False Smut (FSm) incidence was determined at 3-12 weeks after heading, using the following scale: 0 = no disease observed, 1= less than 1%, 3= 1-5%, 5= 6-25%, 7= 26-50%, 9= 51-100%. All disease data were collected, using the standard evaluation scale (SES, 2013) of International Rice Research Institute (IRRI). Mean for each scores were calculated, by summation of all the scores divided by the number of observation; and used for final analyses.

Panicle blast severity (PBS) was determined using the formula below:

$$PBS = \frac{(10 \times N1) + (20 \times N3) + (40 \times N5) + (70 \times N7) + (100 \times N9)}{\text{Total no. of panicles observed}}$$

Where N1-N9 are the number of panicles with score 1- 9.

Determination of Resistance Level

The resistance level (RL) of each test lines to foliar disease was assessed based on the mean severity, by Junaid *et al.*, 2000, modified to 1 – 5 scale. 1 = (1.0 – 1.99) highly resistant (HR), 2 = (2.0 – 2.99) moderately resistant (MR), 3 = (3.0 – 3.99) moderately susceptible (MS), 4 = (4.0 – 4.99) susceptible (S) and 5 = (5.00 and above) highly susceptible (HS).

Agronomic Data Collection

Data were collected on the percentage seedling emergence, number of tillers/m², number of panicles/m², days to maturity and grain yield. The emerged seedlings were counted 6 days after planting (DAP) and expressed as percentage of total number of expected plants in each plot.

The total numbers of tillers for each replicate was determined by counting the population in 1 meter square quadrant at 4 WAP, and the mean recorded. Same procedure was adopted to determine number of panicles/m² in each plot. Days to maturity: This was determined by counting the number of days from seeding to grain ripening when 85% of grains on panicle have matured. One thousand seeds were counted and weighed from each replicate and the mean was recorded in grams.

All matured panicle were harvested at 14% moisture, weighed and recorded in kilogram per hectare, with at least two border rows discarded.

Data Analysis

All the data collected were subjected to analysis of variance (ANOVA), using SAS system 9.0 edition (2012) and means were separated and compared using Duncan's Multiple Range Test (DMRT) at 5 % level of significance.

RESULTS AND DISCUSSION

As shown in Table 1, ART3-8L14P3-2-B-2 (6.33) and FARO 63 (5.67) had significantly ($p < 0.05$) higher incidence of false smut than all other lines evaluated and were both moderately susceptible to the disease. FARO 63 (5.67) and ART15-13-2-2-2-1-B-1-2 (5.67) also recorded significantly ($p < 0.05$) higher incidence of leaf blast than all other lines, which correspond to 30% incidence

from the SES scale, and were moderately susceptible to the disease. ART16-9-29-12-1-1-1-B-1-1 (1.33) and FARO 64 (1.67) had lower incidence of Panicle blast than ART15-13-2-2-2-1-B-1-2 (6.33) (moderately susceptible) which correspond to 30% incidence from the SES scale, and were both highly resistance to the disease and the same trend was also recorded for the Panicle blast severity. Variability in rice germplasm in response to various diseases was also reported by Hossain and Srikant, 2001; and Castano *et al.*, (1990); they also categorized rice germplasm into different groups ranging from highly susceptible to highly resistant against various rice diseases. Nagaraju *et al.*, (1991) reported significant variability in rice genotypes against diseases. In a study on upland rice cultivars in Senegal, Mbodj *et al.*, (1989) observed high level of quantitative resistance to blast in some varieties while others were moderately resistant. Similar results have also been reported by Saifulla *et al.*, (1991) where 23 genotypes were screened during 1990 and 1991, and 19 genotypes were found to be highly resistance and 3 resistant to leaf and neck blast caused by *P. oryzae*.

As presented in Table 2, the percentage seed germination of the evaluated lines varied significantly. All the checks had significantly ($p < 0.05$) lower germination percentage: FARO 63 (37.20%), FARO 59 (51.87%) and FARO 64 (56.27%) than the set evaluated excepts for ART16-12-12-2-23-2-B-1 (44.40%), ART3-8L14P3-2-B-2 (47.87%), ART15-13-2-2-2-1-B-1-2 (53.87%) and ART16-12-25-23-1-1-3-B-1-2 (57.73%) that were not significantly different from each other. This could be attributed to their respective genetic make-up. FARO 64 (64.33) and ART16-12-12-2-23-2-B-1 (64.00) had significantly ($p < 0.05$) higher number of tillers/m² than ART15-13-

2-2-2-1-B-1-2 (31.67), ART16-9-33-2-1-1-1-B-1-1 (32.00) and FARO 59 (32.33). FARO 64 (72.00) equally had significantly ($p < 0.05$) higher number of panicles/m² than all the lines evaluated except for ART15-13-2-2-2-1-B-1-2 and FARO 59 that recorded 31.67 and 32.33, panicles/m² respectively (Table 2). This difference in number of tillers and panicles depends on the physiochemical properties which are influenced by the genotype of the varieties. This confirms the earlier reports of GRiSP (2013) and Adigbo *et al.*, (2007) who reported that plant tillering ability varies with variety and environmental conditions. Fageria *et al.*, (1997) and Rani *et al.*, (2006) also, reported that tillering characteristics among other factors are highly influenced by the genetic characteristics of the cultivars grown. Similarly, the significant variation observed in the number of panicles among the lines tested, could be due to the differences in the genetic potential of these lines, and this confirmed the report of GRiSP (2013) who stated that panicle or leaf initiation was influenced by the type of variety and the high level of management during the vegetative stage.

ART16-9-29-12-1-1-1-B-1-1 (4066.7 kg/h) and ART16-16-7-15-1-B-1-B-1-1 (3966.7 kg/h) produced significantly ($p < 0.05$) higher grain yield than FARO 63 (1500.0 kg/h), ART3-8L14P3-2-B-2 (1800.0 kg/h) and ART16-10-2-32-1-B-1-B-1-1 (1750.0 kg/h), while ART15-13-2-2-2-1-B-1-2 which recorded lowest 1000-seed weight (7.91 g) also had lowest grain yield of 266.7 kg/h

(Table 2). Since tillering is an essential factor when estimating yield (Jaffuel and Dauzat, 2005), it then implies that ART16-12-12-2-23-2-B-1 and FARO 64 are high yielding varieties while ART15-13-2-2-2-1-B-1-2, ART16-9-33-2-1-1-1-B-1-1 and FARO 59 are poor yielding. Thus, ART15-13-2-2-2-1-B-1-2 that recorded lowest seed weight and grain yield was moderately susceptible to the blast diseases.

CONCLUSION.

Findings from this study suggest that rice varieties with disease resistant status in one locality may show susceptibility in other localities. This may be due to variation in the virulence of the races of *P. oryzae* at the different locations.

The study shows that the evaluated rice lines have varying degree of resistance to false smut, leaf and panicle blast in favourable environments, these disease reduces grain yield and yield trait depending on the disease's severity and on the variety's genetic make-up. The study provided a useful information to plant breeders by providing sources of disease resistance that can be further evaluated in rice genetic improvement programmes, which can lead to increase in rice productivity and save the economy of the farmers. Further study is required to determine the type of resistance to the fungal diseases in the resistant rice lines; and transfer of resistance genes to susceptible high yielding and preferred rice varieties.

Table 1: Mean Scores for Disease Incidence, Severity and their Resistance Level to the Disease Evaluated, 2017 Cropping Season.

Lines Designation	FSM	Resistance level	LBI	Resistance level	PBI	PBS	Resistance level
ART3-8L14P3-2-B-2	6.33 ^a	MS	2.33 ^b	R	3.00 ^{bc}	9.00 ^b	R
ART16-9-29-12-1-1-1-B-1-1	0.67 ^d	HR	1.67 ^b	HR	1.33 ^c	2.33 ^c	HR
ART16-10-2-32-1-B-1-B-1-1	2.33 ^{cd}	R	2.33 ^b	R	3.67 ^{abc}	13.67 ^b	MR
ART16-9-4-18-3-2-1-B-1-1	3.00 ^{cd}	R	2.33 ^b	R	3.00 ^{bc}	6.00 ^b	R
ART16-12-25-23-1-1-3-B-1-2	3.00 ^{cd}	R	3.00 ^b	R	5.00 ^{ab}	20.00 ^{ab}	MS
ART16-12-12-2-23-2-B-1	2.33 ^{cd}	HR	3.00 ^b	R	3.00 ^{bc}	9.00 ^b	R
ART15-13-2-2-2-1-B-1-2	1.67 ^{cd}	HR	5.67 ^a	MS	6.33 ^a	39.33 ^a	MS
ART16-9-33-2-1-1-1-B-1-1	3.00 ^{cd}	R	3.67 ^{ab}	R	3.00 ^{bc}	9.00 ^b	R
WAB788-16-1-1-2-HB	3.00 ^{cd}	R	2.33 ^b	R	3.67 ^{abc}	10.67 ^b	MR
ART16-16-7-15-1-B-1-B-1-1	3.67 ^{bc}	MR	3.00 ^b	R	4.33 ^{abc}	20.33 ^{ab}	MR
FARO 59	1.67 ^{cd}	HR	1.67 ^b	HR	2.33 ^{bc}	4.33 ^b	R
FARO 63	5.67 ^{ab}	MS	5.67 ^a	MS	4.33 ^{abc}	20.33 ^{ab}	MR
FARO 64	2.33 ^{cd}	R	1.67 ^b	HR	1.67 ^c	2.67 ^c	HR

*Means with the same alphabet are not significantly different from each other in the same column by DMRT ($P>0.05$).

FSM = False smut, LBI = Leaf Blast Incidence, PBI = Panicle Blast Incidence, PBS = Panicle Blast Severity.

HR = Highly resistant.

R = Resistant.

MR = Moderately resistant.

MS = Moderately susceptible.

Table 2: Agronomic Parameters and Yield Performance of Evaluated Rice Lines in 2017 Cropping Season

Lines Designation	Germination Percentage	Number of tillers/m ²	Number of Panicles/m ²	Days to Maturity	1000 Seed weight (g)	Grain Yield (kg/ha)
ART3-8L14P3-2-B-2	47.87 ^b	41.00 ^{bc}	53.33 ^{bc}	92.00 ^d	15.09 ^{bc}	1800.0 ^{bc}
ART16-9-29-12-1-1-1-B-1-1	86.53 ^a	51.33 ^{ab}	54.00 ^{bc}	94.00 ^{cd}	24.95 ^{ab}	4066.7 ^a
ART16-10-2-32-1-B-1-B-1-1	80.67 ^a	35.00 ^{bc}	38.00 ^d	94.67 ^{cd}	26.35 ^a	1750.0 ^{bc}
ART16-9-4-18-3-2-1-B-1-1	89.07 ^a	51.00 ^{ab}	57.33 ^b	94.67 ^{cd}	24.32 ^{ab}	2800.0 ^{ab}
ART16-12-25-23-1-1-3-B-1-2	57.73 ^b	42.33 ^{bc}	44.00 ^{cd}	97.33 ^{bcd}	23.50 ^{ab}	2666.7 ^{ab}
ART16-12-12-2-23-2-B-1	44.40 ^b	64.00 ^a	59.00 ^b	94.67 ^{cd}	23.30 ^{ab}	3083.3 ^{ab}
ART15-13-2-2-2-1-B-1-2	53.87 ^b	31.67 ^c	53.33 ^{bc}	107.00 ^{ab}	7.91 ^c	266.7 ^c
ART16-9-33-2-1-1-1-B-1-1	83.20 ^a	32.00 ^c	34.33 ^d	104.33 ^{abc}	25.10 ^{ab}	2266.7 ^{ab}
WAB788-16-1-1-2-HB	90.93 ^a	41.33 ^{bc}	60.33 ^{ab}	114.67 ^a	23.01 ^{ab}	2600.0 ^{ab}
ART16-16-7-15-1-B-1-B-1-1	92.93 ^a	40.67 ^{bc}	42.00 ^{cd}	101.00 ^{bcd}	29.39 ^a	3966.7 ^a
FARO 59	51.87 ^b	32.33 ^c	62.00 ^{ab}	92.67 ^d	24.25 ^{ab}	2533.3 ^{ab}
FARO 63	37.20 ^b	47.67 ^{bc}	42.67 ^{cd}	92.00 ^d	24.48 ^{ab}	1500.0 ^{bc}
FARO 64	56.27 ^b	64.33 ^a	72.00 ^a	114.33 ^a	23.49 ^{ab}	2600.0 ^{ab}

*Means with the same alphabet are not significantly different from each other in the same column by DMRT ($P>0.05$).

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ASSESSMENT OF AFRICAN BUSH-MANGO (*IRVINGIA SPP*) PRODUCTION IN SOUTHERN NIGERIA

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ABSTRACT

African Bush mango (*Irvingia spp*) is a tree crop commonly found in the wild lowland forests of Central and West Africa. To effectively sustain the production of this important tree crop and its economic value, there is a need to assess production challenges of *Irvingia* producers/collectors in Southern Nigeria. Purposive and Snowball sampling technique was used to select 43 producers. Data were collected with the aid of interview schedule; and analyzed using descriptive statistics. Most African bush mango producers/collectors were mostly (60%) male, 69.8% of respondents belong to a group/ association of farmers, 37.2% have farm size of 0.4-2.0 hectares. Mixed cropping is prominent in the study area with 46.5% of the producers planting root and tuber crops. Most of the African bush mango producers (53.5%) have access to extension service on fortnight basis, while 86% of them have not participated in any training related to the crop. Also 53.1% of the producers/collectors pick matured fully ripe fruits during harvest which are mostly sold to wholesalers. The most prominent production challenge faced by *Irvingia* producers is pest and disease infestation (60.5%), other challenges include limited productive land, lack of improved *Irvingia* seedlings and poor post-harvest handling. It is recommended that producers/collectors should be empowered and their capacity built for improved knowledge and skill in African bush mango production.

Keywords: collectors, production challenges, improved seedlings, pest and disease infestation

INTRODUCTION

African bush mango is a tree crop of about 15-40m height commonly found in the wild lowland forests of Central and West Africa (Orhevba *et al*, 2013). It belongs to the family *Irvingiaceae* and it is also known as bitter bush mango, dry season bush mango or “dika nut” (Asaah, *et al*. 2003). *Irvingia gabonensis* and *Irvingia wombolu* are well known species of the *Irvingiaceae* family of plants. *Irvingia gabonensis* tree is used to curtail environmental degradation and mitigate

deforestation (Leakey *et al*, 2003). *Irvingia gabonensis* is the edible type which produces sweet flesh. This is eaten fresh while the stone is usually split to reveal its kernel that is used for soup thickening. The *Irvingia wombolu* is the type that has non-edible flesh. The flesh is sour and the fruit stone is also extracted to produce the kernel known as ‘ogbono’. There are no much differences between the two species except for fruit sweetness and some tree morphological differences. The pulps of the two varieties have different properties but the kernels have

similar characteristics and because they are not differentiated in the market, the two types of kernels are mixed together for sale (Awe *et al.*, 2012). *Irvingia spp* ranks high among non-timber forest products in usefulness. It is a commercially and socially important tree that is fully utilized by native African tribes who make use of the bark, leaves, stems, fruits and seed kernels. Also, the wood is a strong and durable material for construction (Festus and Nwala, 2012).

Tchoundjeu and Atanga, (2007) reported that *I. gabonensis* is cultivated for commercial production in Southern Nigeria and Southern Cameroon. Fruits are sold locally while kernels are widely traded domestically from the forest zone to savanna zone and between West and Central Africa region. According to them, kernels are also exported to Europe with Cameroon as the major exporter to the tune of 260,000 USD per year for 107tonnes. In Nigeria, estimated demand for African bush mango per year was 78.8million kilogrammes with 80% of this being from southern Nigeria (Larinde and Omokhua, 2015). Considering its importance in current and future international markets as a potential source of income, there is the need to ensure sustainable production. The study therefore assessed production challenges among producers in order to identify the entry point for appropriate intervention for improved livelihood of relevant stakeholders. The general objective of the study is to identify the information and training needs as well as production constraints of *Irvingia* producers in Nigeria.

The specific objectives are to:

- i. ascertain the socio- economic characteristics of producers;
- ii. assess farming attributes of African bush mango producers/collectors;

- iii. examine access of producers/collectors to capacity building opportunities;
- iv. assess their information and training needs; and
- v. Identify production challenges of the producers/collectors.

METHODOLOGY

The study was conducted in Southern Nigeria. Major crops grown in this part of the country are banana, plantain, maize, vegetables, cassava, rice and yam. Cash crops that are found in the region are oil palm, rubber, cashew, cocoa and kolanut (Okezie *et al.*, 2012). Prominent horticultural crops being produced are vegetables, melon, pineapples, mango, pepper, guava and pawpaw; while other cash crops include African bush mango (RSMoA, 2015).

Purposive sampling technique was used to select Southern Nigeria because *Irvingia* is grown mostly in that part of the country. Three States of Southern Nigeria namely; Imo, Abia and Rivers where *Irvingia* is mostly produced or collected from the wild were purposively selected. Agricultural Extension Agents of the selected states' Agricultural Development Programmes were consulted to identify senatorial districts and prominent communities with *Irvingia* producers and collectors.

All identified senatorial districts where *Irvingia* producers and collectors exist were purposively sampled. List of *Irvingia* producers and collectors was not available in the states, hence, Snowball sampling technique in which the first respondent assist in identifying the next was used to select respondents. A total of 43 producers and collectors were sampled in the three states as shown on Table 1. Structured interview schedule was used to elicit information from the producers on all aspects of the objectives

while data were analyzed using descriptive statistics.

Table 1: Sampling frame

States	Selected Senatorial Districts	Selected communities	Sampled producers/collectors
Abia	3	6	12
Imo	3	6	23
Rivers	2	4	8

Source: Field survey, 2017

RESULTS AND DISCUSSION

Socio-Economic Characteristics of African Bush Mango Producers/Collectors

In the study area, it was observed that African bush mango is a tree crop that could be deliberately planted or found growing and collected in the wild. The results as shown on table 2 revealed that most of the African bush mango producers/collectors were males (60.5%); married (90.7%) with 65.1% in the 40-59 years age group. This result implies that male play dominant role in *Irvingia* kernel production as reported by Adisa and Okunade (2011). Males are considered for land inheritance, coupled with the fact that the crop is a perennial and permanent crop that involves complete land ownership. Furthermore, the finding of Ike, (2008) in Nsukka Agricultural Zone of Enugu state revealed that males (91%) dominated the ownership of tree crops.

Most of the respondents had 6-10 household members (53.5%). About fifty-six percent of the African bush mango producers possess tertiary education (55.8%); this is in line with the finding of Elah, (2010) which showed that most of the *Irvingia spp* producers and collectors had one form of education or the other (at least primary education) in both east and southwestern region of Cameroon. Also, all the producers were Christians (100%) and

44.2% have farming as their main occupation. Most of those who do not have farming as main occupation (53.5%) were civil servants (65.4%) and traders (23.1%) among others.

Furthermore, more than one-third of the producers had 1-5 years of experience (37.2%) in African bush mango production/collection. Also, 69.8% of the producers/collectors was members of a group/association; out of which 53.3% was members of cooperative societies and 30.0% belong to farmers' association. This is contrary to the findings of Elah, (2010) which stated that only 3.7% and 24% of producers in South west and East regions of Cameroon were members of one form of association or the other.

Farming Attributes of African Bush Mango Producers/Collectors

About three-quarters of the respondents (74.4%) have African bush mango produced on less than 2.0 hectares of land (Table 3). The indication of the little acreage used for *Irvingia* production could be due to the assumption that *Irvingia* trees are part of forest trees that has been retained in farmer's field and not been felled or burnt during clearance for shifting cultivation, or for cocoa farms. Limitation of land in Southern Nigeria is a key issue coupled with the threat of soil erosion reducing available land for production (Ladipo, *et al.*, 1996, Atangana *et al.*, 2001).

The most prominent farming systems practiced in the study area was mixed cropping (46.5%) and mixed farming (27.9%). In addition to African bush mango, producers plant fruits (plantain, banana, pineapple, and pawpaw), food crops (cassava, maize, and yam), tree crops (breadfruit, Kola, Cocoa), vegetables (okra, amaranthus, cucumber, and fluted pumpkin),

and spices (ginger). They also rear animals such as poultry, sheep and goat, fish, snail, rabbits and pigs. Involvement in farming system activities is an indication that the producers/collectors explore other income generating activities to compliment *Irvingia spp* business enterprise. It is a common occurrence among small scale producers to be involved in other income generating activities for economic survival and sustenance. Moreover during harvest, 53.1% of the producers/collectors pick matured fully ripe fruits which are sorted and mostly sold to wholesalers (37.8%) with low grade fruits attracting lower price (39.5%).

Access of Producers/Collectors to capacity building opportunities

Eighty-six percent of the producers/collectors have not participated in any training (Table 4). However 7% of the respondents who have participated in training attested to the fact that the training covered aspects of field management and production.. Thus, organized capacity building programs for respondents have inherent potentials to greatly enhance current level of production. Most of the African bush mango producers (53.5%) have access to extension service on a fortnight basis (65.2%). Regular interaction with extension service delivery can also be explored using a train the trainer approach. Some of the information sources explored on production by African Bush Mango producers are friends and extension agents (23.3%) respectively as well as relatives (20.9%) while none sourced information from GSM (Table 4). This finding is in line with Ugwumba *et al.*, 2013 which observed that sufficient access to information in favour of modern production and processing equipment and storage facilities through trained extension agents and even

experienced friends will ensure enhanced income and better life for the producers.

Information and Training Needs of African Bush Mango Producers/Collectors

Most of the producers/collectors (67.4%) need information on credit/loan as well as nursery preparation techniques and marketing channels (55.8%) respectively (Table 5). This result implies that producers/collectors are willing to move from just gathering of fruits in the wild to cultivating in the field so as to ensure sustainability. Also, most of them need training on storage of seeds/fruits (65.1%); processing (60.5%) as well as on health and nutrition issues (48.8%). The most important part of *Irvingia spp* to the rural people of Nigeria in general is its nutritious seeds which have also been found useful in the reduction of cholesterol and body weight in obese patients (Ngondi *et al.* 2005). This may be one of the reasons why producers/collectors require training on storage of *Irvingia spp* fruits/seeds, processing, as well as health and nutrition issues.

Challenges in African Bush Mango production

The most prominent production challenge faced by the producers is pest and disease infestation (60.5%) while 58.1% also experience high cost of farm chemical procurement (Table 6). Seeds are infested by larvae of merchant grain beetle (*Oryzaephilus mercator*) according to PROTA. The pests and disease infestation among respondents could be due to variation in weather condition during storage of seeds on the field or at home. Moreover, non-availability of improved planting materials and inadequate post-harvest handling practices were also opined as challenges by

Assessment of African Bush-Mango (*Irvingia Spp*) Production in Southern Nigeria

55.8% of the producers. Inadequate post-harvest handling was another notable challenge faced by respondents. This could

be due to nature of the harvested fruit which grows moldy under inadequate storage within a short time.

Table 2: Socio-Economic Characteristics of African Bush Mango Producers/Collectors

Variable	Frequency	Percentage
Sex		
Male	26	60.5
Female	17	39.5
Marital status		
Married	39	90.7
Single	2	4.7
Widowed	1	2.3
No response	1	2.3
Age (in years)		
30-39	7	16.3
40-49	13	30.2
50-59	15	34.9
60 and above	7	16.3
No response	1	2.3
Family size		
1-5	13	30.2
6-10	23	53.5
11-15	3	7.0
>15	2	4.7
Educational status		
No formal education	1	2.3
Primary education	6	14.0
Secondary education	10	23.3
Tertiary education	24	55.8
No response	2	4.6
Religion		
Christianity	43	100.0
Farming as main occupation		
Yes	19	44.2
No	23	53.5
Undisclosed	1	2.3
Years of experience in African bush mango production/collection (in years)		
1-5	16	37.2
6-10	6	14.0
11-15	12	27.9
>15	8	18.6
Undisclosed	1	2.3
Membership of group/association		
Yes	27	62.8
No	13	30.2
No response	3	7.0

Source: Field survey, 2017

Table 3: Farming Attributes of African Bush Mango Producers/Collectors

Variable	Frequency	Percentage
Farm size (in hectares) for <i>Irvingia spp</i> production		
<0.4	15	34.9
0.4-2.0	17	39.5
2.4-4.0	1	2.3
4.4-6.0	-	-
>6.0	2	4.7
Undisclosed	8	18.6
Farming system		
Sole cropping	4	9.3
Mixed cropping	20	46.5
Mixed farming	12	27.9
Intercropping	1	2.3
Integrated farming	1	2.3
Others	1	2.3
Undisclosed	4	9.3
Animals reared*		
Poultry	20	37.7
Fish	3	5.7
Snail	1	1.9
Goat/sheep	23	43.4
Pigs/rabbits	6	11.3
Harvesting stage for <i>Irvingia spp</i>		
Mature unripe fruits	5	12.2
Mature ripe fruits	13	31.7
Mature fully ripe fruits	22	53.7
Unripe fruits	1	2.4
Sale of produce after harvest		
Wholesaler	14	32.6
Retailer	11	25.6
Processor	2	4.6
Consumers	2	4.6
Self	8	18.6
Undisclosed	6	13.9

Source: Field Survey, 2017 *-multiple response

Table 4: Access of producers/collectors to capacity building opportunities and extension

Variable	Frequency	Percentage
Participation in training on African Bush Mango		
Yes	3	7.0
No	37	86.0
Undisclosed	3	7.0
Access to extension service delivery		
Yes	23	53.5
No	19	44.2
Undisclosed	1	2.3
Frequency of extension visit		
Fortnightly	15	65.2
Monthly	4	17.4
Others	4	17.4
Information sources		
Friends	10	23.3
Relatives	9	20.9
Radio/television	2	4.7
Newspapers	-	-
Extension agents	10	23.3
Internet	1	2.3
Research Institutes	3	7.0
GSM (Global Systems for Mobile Communications)	-	-
Others	8	18.6

Source: Field Survey, 2017

Table 5: Information and Training Needs of African Bush Mango Producers/Collectors

Variables	Information needs		Training needs	
	Frequency	Percentage	Frequency	Percentage
Storage of seeds/fruits	20	46.5	28	65.1
Nursery preparation techniques	24	55.8	20	46.5
Land preparation and planting	18	41.9	19	44.2
Weed management	17	39.5	16	37.2
Weather information	21	48.8	16	37.2
Safe use of agrochemicals	17	39.5	22	51.2
Harvesting	18	41.9	16	37.2
Processing	19	44.2	26	60.5
Marketing channels	24	55.8	14	32.6
Credit/loan	29	67.4	18	41.9
Health and nutrition	20	46.5	21	48.8

Others (specify)	4	9.3	5	11.6
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Source: Field Survey, 2017

Table 6: Challenges in African Bush Mango production

Production challenges	Frequency	Percentage
Cost of planting materials	23	53.5
High transportation cost	23	53.5
Pest and disease infestation	26	60.5
Timely availability of improved seeds	21	48.8
Non-availability of improved planting materials	24	55.8
Inadequate post- harvest handling practices	24	55.8
High cost of farm chemical procurement	25	58.1
Adulteration of farm chemicals	19	44.2
Inaccessibility in fertilizer procurement	22	51.2
Inadequate market information	16	37.2
High labour cost	19	44.2
Glut	10	23.3
Limited productive land	22	51.2
Pilfering	21	48.8
Extension services	8	18.6
Harvest failure	15	34.9
Climate change	21	48.8

Source: Field Survey, 2017

CONCLUSION AND RECOMMENDATION

Male play dominant role in *Irvingia* production, acreage is low and few participated in training. Information is mostly needed in loan sourcing and nursery preparation techniques as well as marketing channels. Moreover, producers/collectors need training on storage of seeds/fruits and processing. Pest and disease infestation as well as high cost of farm chemical procurement, lack of improved *Irvingia spp* seedlings and post-harvest handling of *Irvingia spp* kernels are major constraints identified. There is the need for *Irvingia spp* producers to have access to propagation techniques that can be used to increase domestication of *Irvingia* species. This can be achieved through capacity building and

empowerment of farmers which is advocated to improve knowledge and skill in *Irvingia spp* production in the study area.

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ANTIOXIDANT ACTIVITY AND PHYSIOCHEMICAL PARAMETERS OF ORANGE AND GRAPE JUICES AS A FUNCTION OF VARIETIES.

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ABSTRACT

The presence of diverse antioxidant compounds like ascorbic acid (vitamin C), total carotenoid, polyphenols and total flavonoid in fruit juices make it to have protective effect against degeneration diseases such as malignant cells growth and others related diseases. In this study, the antioxidant activity of three orange varieties (Agege Sweet, Campbell Valencia and Cater Navel) and grape genotypes (sweet, white and pink) were investigated. After processing to juices, vitamin C, total phenolic and total carotenoid contents were determined, and juice antioxidant capacity was measured by two *in vitro* methods (DPPH scavenging activity and reducing power). The physicochemical results showed Cater Navel to have the highest juice percentage, soluble solid, acid sugar ratio and pH, with 51.22% , 12.75 °brix, 17.53 and 3.8, respectively. The vitamin C contents ranged from (45.66 – 68.50) mg/100 ml. For the orange varieties, Cater Navel had the highest (66 mg/100 mL) and white grape have the highest (68.50 mg/100mL) for the grape varieties. Total carotenoids in oranges, ranged from (2.63 – 7.19) mg/L with Campbell Valencia having the highest value. Total carotenoids were not detected in the grape varieties with exception of the pink genotype with 3.96 mg/L. All the variety values ranged of total phenolic were from 513.41 to 598.28 gallic acid equivalents per litre. Reducing power ranged from 126.97 to 440.86 mg ascorbic acid equivalent per litre, while DPPH radical scavenging activity showed white grape among the variety to inhibit the 100% control to the lowest percentage activities.

Keywords: *Orange juices, grape juices, antioxidant activities, carotenoids, vitamin C*

INTRODUCTION

Fruit juice can be considered to assume the position of a functional food in view of the vast epidemiological factors for its reducing risk of definite types of diseases especially tumors growth called cancer (Bhattacharjee *et al.*, 2011; Ceymann *et al.*, 2012; Tajik *et al.*, 2017). It is a reservoir of various phytochemical called antioxidant molecules, such as ascorbic acid, carotenoids, flavonoids

and phenolic acids that are accountable for it. The disease-preventing potential of a fruit juice is a result of a several such compounds which may show some synergistic relationship. Ascorbic acid and carotenoids, the biomolecule of concern, have been shown to have strong scavenging activity and shows the highest physical quenching rate constant with singlet oxygen (Prior and Cao 2000; Tiwari *et al.*, 2008b). Considering the relatively high consumption rates of orange

and grape juices; as sources of vitamin C and polyphenolic compounds, vitamin C is considered as a most important water-soluble antioxidant.

In biological systems, vitamin C acts as a protection of molecules in extracellular and intracellular spaces and decrease tocopherol radicals back to their active forms at the cellular membranes (Tajik *et al.*, 2017; Tiwari *et al.*, 2008a; Kaur and Kapoor, 2001). It can directly quenched singlet oxygen, hydrogen peroxide, superoxide radical and hydroxyl radical. Presently ascorbic acid is the most commonly used vitamins supplement globally. Based on available information on epidemiological, biochemical and clinical studies, the current recommended daily acceptance (RDA) dose for ascorbic acid is 100-120 mg/day needed to attain optimum risk reduction of heart diseases and cellular saturation, stroke and cancer in healthy folks (Abeyasingbe *et al.*, 2007; Wang *et al.*, 2007). In orange juices, the vitamin C content varies from 150 to 450 mg/L, and about one glass of orange juice (200 mL) can supply about 30-80% of recommended daily intake of vitamin C (Gil-lzquierdo *et al.*, 2001; Khandare *et al.*, 2011). Research have shown epidemiologically correlation in dietary intake of phenolic acids with reduced threat of prostate cancer and it has been established to be powerful than carotenoid in scavenging cell proliferation in various human epithelial cancer cell lines (Akusu *et al.*, 2016; Burin *et al.*, 2010; Gil *et al.*, 2001). Nutritional indicators of healthy life styles and good dietary habits have been linked to consumption of fruit juice. Recently, some research reports the presence of flavonoids in citrus juices, which are also vital in conferring antioxidative health benefits (Fragose *et al.*, 2012; Jensen *et al.*, 2011; Pulido *et al.*, 2000). Thus, grape juice is a good source of flavonoids and other

phenolics in the human diet (Burin *et al.*, 2010; Ceyman *et al.*, 2012). It has been reported by many authors that, the intake of grape juice is beneficial and improves the endothelial function, fortification of low-density lipoproteins (LDLs) against oxidation, reduction of native plasma protein oxidation, intensification of the serum antioxidant capacity, and decreases the platelet aggregation (Ganha~o *et al.*, 2011; Gorinstein *et al.*, 2001).

Although, when compared the phenolic content in orange juices and grape juices with other potential sources like spices, their high consumption rate in human diet makes them a good source of phenols. In recent time many research studies on orange and grape juices have been analyzed for their polyphenols, antioxidant activities and ascorbic acid contents and there has been little report on the disparity of phenolic content, physiochemical properties and antioxidant activity in different genotypes. This study reports the variations in the contents of the antioxidants potentials, such as total carotenoids, ascorbic acid, and polyphenols in some selected orange and grape varieties and their input to their antioxidant activity. This research work will insight that enable to define their quality in relationship to their antioxidant components and physiochemical parameters of these fruit juices.

MATERIALS AND METHODS

Orange fruits (Agege sweet orange, (ASO)(*Citrullus. sinensis*); Cater navel (*C. caracara*) (CN); Campbell Valencia (*C. changshanensis*) (CV) and grape fruits (*C. paradisi*) of Sweet grape (SG); White grape (WG) and Pink grape (PG) hue varieties were collected from the citrus orchard of National Horticultural Research Institute (NIHORT),

Ibadan. The fruits were sorted to remove the spoiled ones, washed and allowed to dry. Each variety was weighed (M1), further peeled and processed into juices with automated machine at the pilot plant. The juice extracted from each variety was also weighed (M2) according to the method of Xu *et al.* (2007).

Physiochemical Analysis

Titrate acidity was calculated by titrated 0.01 M sodium hydroxide against 10 ml of fruit juice and to measure the volume 0.01 M NaOH needed to raise the pH of the juice to value of 8.3. Before the titration was carried out, the pH meter was standardized with buffers of pH 4.0 and 7.0. The pH values were recorded with a digital pH meter (PHS-25, Precision scientific Instrument Co., Ltd., Shanghai, China). The total soluble solid (TSS) contents were measured with a refractometer at 20°C, and was used to express as °brix the value. The juice percentage was calculated $(M1 / M2) * 100$ following the method of Xu *et al.* (2007). M1 is the weight of the puree after processing. M2 is the initial weight of the whole fruits used for processing.

Determination of ascorbic acid content by HPLC

The procedure used for the quantification of ascorbic acid in the fruit juice was according to Gardner *et al.* (2000), a reversed-phase High Performance Liquid Chromatography (HPLC) process. A combination of 1 g of sample and 5 mL of acetonitrile–acetic acid–water (75:2:33 v/v) transferred into centrifuge tube and stirred for 10 min at ambient temperature and centrifuged at 8000 rpm for 15 min at 4°C. Twice the residues were extracted with the same extraction mixture. Supernatants were pooled together and made up to 25 mL with the extraction solvent. The sample solution was filtered

through a 0.45 µm membrane filter before injected. Standard of Ascorbic acid with concentration ranged from 10 to 50 µg/mL was prepared with acetonitrile–acetic acid–water (75:2:33 v/v). Standard was procured from Sigma-Aldrich (St. Louis, MO, USA) and prepared at concentrations of 10 to 50 µg/mL. For eluted of the standard and samples flow rate of 1 mL/min of acetonitrile–acetic acid–water (75:2:33 v/v) was used as the mobile phase and absorbance read at 254 nm.

Total Polyphenol, Flavonoid, and Carotenoid Contents

Total polyphenol of the fruit juice was determined by using 1% HCl in 80% methanol as the extraction solvent. Accurately pipette 2 mL of the extraction mixed solvent were added to 200 mg of sample in a centrifuge tube, extracted on a shaker at 200 rpm for 2 h at room temperature, and centrifuged at 10,000 rpm for 15 min at 4°C. The residue was extracted twice by adding 2 mL of the extraction solvent. All supernatants were pooled and made up to 10 mL with extraction solvent. Modified method by Singleton and Rossi (1965) was used to determine the concentration of total polyphenol. A volume of 1 mL of the extract was pipetted into a test tube along with 4.5 mL of water; 2.5 mL of 0.2 N Folin-Ciocalteu reagents, and after 5min, 2 mL of Na₂CO₃ was added and mixed well. The mixture was kept for 2 h at room temperature for colour development. Absorbance was measured at 765 nm. Total polyphenol was expressed as gallic acid equivalents. Gallic acid was bought from Sigma-Aldrich (St. Louis, MO, USA).

Total flavonoid concentrations were measured using method outlined by Gorinstein *et al.*, 2001. One mL of diluted juice in ratio 1:5 with water was transferred

in a 10 mL glass tube. Four ml distilled water was added follow by 0.3 mL of 5% NaNO₂ was also added. After 5 min, 0.3 mL of 10% AlCl₃ was added. After another 10 min, 2 mL of 1 M NaOH was added. Furthermore, the solution was diluted to a total volume of 10 mL with distilled water. The absorbance of the solution was read at 510 nm and flavonoid concentration was measured by using a catechin standard for calibration curve.

Total carotenoid determination was carried out using spectrophotometric method described by Lee (2001). 2 mL juice was mixed with 10 mL of mixture of solvent in this proportion hexane:acetone:ethanol, (2:1:1, v/v/v), (extracting solvent) agitated, and centrifuged for 15 min at 1500 rpm. The supernant layer of hexane containing the carotenoids was carefully recovered and placed into a 25 mL volumetric flask. Volume of recovered hexane was then adjusted to 25 mL with hexane and was measured using a spectrophotometer (JENWAY 6400) at 450 nm. Total carotenoid contents of the fruit juices were determined as mg b-carotene per liter by using an extinction coefficient of 2505.

ANTIOXIDANT ACTIVITY.

Determination of the fruit juice radical – scavenging activity by 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) .

Total free radical scavenging activities of the juice extracts were determined and compared to vitamin E, vitamin C, and butylated hydroxytoluene (BHT) according to the method reported by Yu (2001) using the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). Furthermore, the juice was centrifuged at 6000 g for 10minutes at 40^oC and 0.2 ml of the supernatant was added to 7.8 mL of methanolic DPPH solution (100 mM). Absorbance at 517 nm was determined

after 30 min. and the percent inhibition activity was calculated. Radical DPPH scavenging capacity was estimated without antioxidant (control) as 100% and ability to inhibit with antioxidant lower than the control. The lower the percentage DPPH remaining, the more powerful the scavenging activities. All tests were conducted in triplicate.

Determination of Reducing Power

The reducing power of the extract was evaluated according to the method of Kaur and Kapoor (2001). A volume of 1 mL of the juice extract was prepared in 1.5 mL distilled water and thoroughly mixed with the mixture of 0.2 mM phosphate buffer (2.5 mL) at pH 7.4 and after 1% K₃Fe(CN)₆ (2.5 mL). The resulting compound was incubated at 50 ^oC for 20 min, followed by the adding 10% TCA (2.5 ml) and later centrifuged at 3000 rpm for 10 min. The supernant layer of the solution was collected and mixed with distilled water (2.5 mL) and follow by the addition of 0.1% ferrous chloride (0.5 mL). The absorbance was read at 700 nm against a blank sample. The more the absorbance value of the reaction mixture shown, the higher the reducing power of the juice extract and expressed in mg ascorbic acid equivalent/100 mL.

RESULTS AND DISCUSSION

Biochemical Parameters of Citrus Fruit Juices

Physiochemical parameters (juice percentage, pH, titratable acidity, sugar acid ratio and total soluble solids) in the juices were measured. The composition of the fruits juice biochemical properties of the study is shown in Table 1. Differences in chemical composition of juices can be attributed to genetic and physiological factors (Akusu *et*

al., 2016; Gorinstein *et al.*, 2001; Xue *et al.*, 2008). Dietary quality is important for determining the value of orange and grape varieties. Therefore, total soluble solids (TSS), titratable acidity (TA) and the ratio of soluble solid to acid (TSS/TA) were determined which are important factors for evaluating fruit quality. In this experiment, TSS, TA and the ratio of TSS/TA and pH and juice percentage were examined in three oranges and three grape varieties in ripened stage and the data obtained are shown in Table 1. Differences in values were found among the varieties in TSS content of citrus fruits. The highest amount of TSS was obtained from White grape (WG) which also had a high amount of TA followed by SG and CN (Table 1).

The pH of the citrus varieties varied significantly ($p < 0.05$) (3.1–3.80) (Table 1). Among the varieties, results indicated significant differences ($p < 0.05$) in the juice pH values. The pH value was highest in Cater Navel with value of 3.8, but lowest in White grape with 3.1 as shown in Table 1. The ratio of sugars to organic acids (TSS/TA) is correlated to flavor quality for different fruits and determines the optimum time for harvesting, because it is measured to be an index of quality (Cordenunsi *et al.*, 2002; Prior and Cao, 2000). Gardner *et al.* (2000) reported that the sugar to organic acid ratio is a most important parameter of citrus fruit when it comes to taste and may be more significant for quality and perceived sweetness by a sensory panel than soluble solids alone. As shown in Table 1 the highest TSS to TA ratio was detected in CN with 17.53 ± 0.41 , whereas the lowest was in WG with value of 8.81 ± 0.49 . The TA content of citrus did not vary significantly ($p = 0.05$), while the TSS/TA values were statistically significant ($p < 0.05$) probably due to the

variation of TSS contents among the varieties.

Soluble solids contents is an imperative fruit juice quality parameter, from both a sensory flavour perspective and fruit juice to fruit juice concentrate perspective. Citrus juice flavor is generally measured by the content of soluble solids, acid (measured as percentage brix), and Jones and Scott (1984) reported that flavor is correlated to total sugar and acid. Soluble solids are significant not only in terms of their contribution to flavor, but also as mentioned previously, in terms of their relationship to processing prerequisite. It is necessary to produce citrus higher in soluble solid contents because they will require less energy to evaporate water to a target final brix content. Titratable acidity levels were highest in white grape, which may combine with the high brix levels to contribute satisfactorily to flavour influence. The ratio of sugar to acid is something that may be used to indicate general flavor quality, and the fact that Cater Navel (CN) in this study had the highest soluble solids and high titratable acidity indicates that it may have better flavour quality to process into juice concentrate. The juice percentage is an important parameter to processing requirement in fruit juice industry, its relating the volume of juice that can be produce from mass of weight fruits. Cater Navel (CN) has the highest juice percentage 48.96 (Table 1) merits considerable attention for commercial processing into fruit juice. The lowest juice percentage was Sweet Grape (SW) 19.13% (Table 1) of the total fruit weight. Gould (1992) proposed that fruit varieties with high total soluble solids in superfluous of 5.5%, preferably rising to 8.5% and acidity in the array of 0.35–0.55% are appropriate for commercial juice production. Therefore, both the cater navel and pink grape are suitable for juice production in the fruit juice industries.

Total Polyphenol, Flavonoid, and Carotenoid Contents

Different concentration levels were found in the total phenolics, total flavonoid contents and the two antioxidant activity assayed among the various varieties. Research findings highlight an important role of polyphenolic constituents of higher plants that may act as antioxidants or via other mechanisms contributing to the anti-tumor growth or heart protective agent (Ceyman *et al.*, 2012; Yadav *et al.*, 2013).

The concentration of total polyphenol, total flavonoid and total carotenoid in the fruit juice portions of the six varieties of citrus fruits were presented in Table 2. Total polyphenol varied from 513.07 ± 7.61 to 585.75 ± 10.02 mg/litre (gallic acid equivalents). White grape contained the highest polyphenol (585.75 ± 10.02 mg/litre [gallic acid equivalents]), followed by Pink grape (577.89 ± 9.18 mg/litre [gallic acid equivalents]); the levels in Campbell Valencia, Sweet grape, Cater Navel and Agege sweet orange ranged from 564.63 ± 8.97 to 513.07 ± 7.61 mg/litre (gallic acid equivalents). Total flavonoid varied from 434.60 ± 7.79 to 105.17 ± 5.78 mg/litre (rutin equivalents). White grape and Campbell Valencia had the highest levels (434.60 ± 7.79 and 393.51 ± 6.97 mg/litre [rutin equivalents], respectively). Pink grape, and Sweet grape had moderate levels (288.57 ± 5.94 to 215.37 ± 6.10 mg/litre [rutin equivalents]), while Cater Navel and Agege sweet orange had the lowest (195.52 ± 5.87 and 105.17 ± 5.78 mg/litre [rutin equivalents], respectively). Total carotenoid content was much lower in amount than those of total polyphenol and flavonoid and varied from 6.38 ± 0.84 to 2.63 ± 0.17 mg/litre. For the orange genotype, Cater Navel showed the highest carotenoid content, followed, in value of decrease order, by Campbell Valencia, and

Agege sweet orange (4.68 ± 0.91 and 2.63 ± 0.17 mg/litre [β-carotene equivalents], respectively). Total carotenoids were not detected in the grape varieties with exception of the pink genotype with 3.96 ± 0.27 mg/litre. Gorinstein *et al.* (2001) reported that the polyphenol content in peeled lemon (*C. limon*) and orange (*C. sinensis*) were 164 ± 10.3 and 154 ± 10.2 mg/100 g fresh fruit (chlorogenic acid equivalents), respectively. In another finding, total polyphenol content in Valencia late juice was 48.8 ± 1.97 mg/100 ml (ferulic acid equivalents). Gorinstein *et al.* (2001) found that total polyphenol content in orange fruits varied from 50 to 100 mg/100 g fresh sample. In other reports of three commercially available orange juices (orange, Jaffa orange, and Florida orange), total polyphenol varied from 50.4 ± 1.0 to 75.5 ± 1.8 mg GAE/100 ml and total carotenoid varied from 0.30 ± 0.11 to 0.83 ± 0.20 mg/100 ml apart from for Florida orange which had no carotenoid detection (Gardner *et al.*, 2000). In this study, the total polyphenol and total carotenoid levels in both orange genotype and grape genotype were concurred to Gardner *et al.*, (2000) in the references mentioned above, but lower in the amount mentioned by Gorinstein *et al.* (2001).

Citrus juices are a major source of vitamin C, which is a vital antioxidant in juices (Gardner *et al.*, 2000; González-Molina *et al.*, 2010; González-Molina *et al.*, 2012). Concentration of vitamin C is a substantial indicator of citrus juice quality, and it may function as an indicator that all processes, which ensure a high quality of the product, have been applied in the production processes (Gardner *et al.*, 2010; Lee and Castle 2001). In both juices varieties analyzed in this study, the vitamin C content varied from the highest to the lowest values of 68.19 ± 3.05 to 45.58 ± 3.21 mg/100 g (Table

1). Table 1 shows ascorbic acid levels of the Grape varieties which did not vary much (68.19 ± 3.05 to 53.60 ± 3.09 mg/100 g), while the Orange and the Campbell Valencia varieties had the highest ascorbic acid and there were no significant differences from the values obtained among the Grape varieties. Cater Nater and Agege sweet orange were 46.35 ± 2.78 and 45.58 ± 3.21 , respectively. It has been reported that vitamin C levels in *C. sinensis* and *C. limon* were 47.7 and 51.3 mg/100 g fresh sample, respectively (Gorinstein *et al.*, 2001; Kaur and Kapoor, 2001). Both results confirmed citrus fruit juices as a major content of ascorbic acid, with 100 mL approximately equal to the recommended daily allowance (RDA) of 60 mg.

Free radical scavenging activity

Over a decade, there has been a plethora of research findings on the determination of phenolic content in fruits and vegetables (Girone's-Vilaplana *et al.* 2012a; Girone's-Vilaplana *et al.*, 2012b; Yadav *et al.*, 2013; Yang *et al.*, 2012; Ademoyegun *et al.*, 2013). They are being indicated as "star nutrients" because of the antioxidant effects of certain phenols, like epicatechin, often outstanding that of ascorbic acid and tocopherol (Stadler, 2001).

Fruit juice antioxidant activity has been tested using a wide range of procedures. Antioxidant activity is the ability to inhibit auto-oxidation of free-radical mediated oxidation of the substrate when existing in low concentration Ademoyegun *et al.* 2013 and Lee (2001). In this study, antioxidant activity was measured by free radical scavenging assay using (DPPH).

The result of antioxidants on DPPH radical scavenging was as an effect of their hydrogen donating capacity. DPPH is a stable free radical and accepts an electron or hydrogen

radical to become a stable diamagnetic molecule (Pulido *et al.*, 2000). The decrease in potential of DPPH radicals was measured by the reduction in its absorbance at 517 nm induced by antioxidants. In this study, the radical DPPH scavenging capacities of the fruit juices were measured and compared with tocopherol, vitamin C and BHT showed in Fig 1. The blank DPPH radical without antioxidant indicate 100% stable radical and stronger radical quenching agent results in a lower percentage DPPH remaining (Fig. 1). The fruit juices varieties significantly differed in their DPPH quenching activities. The White grape varieties had the highest potential to directly react and quench DPPH radicals, while the Agege sweet orange had the weakest scavenging activity against DPPH radicals. The fruit juices varieties were also compared to vitamin E, vitamin C, and BHT for the DPPH radical quenching capacities (Fig. 1). All the fruits juices at a level of 0.2 mL showed weaker radical quenching activity than 50 mg/mL (0.2 mL) of L-ascorbic acid. The Agege sweet orange per quenched at 80.49%, Cater Navel quenched at 45.94%, Campbell Valencia quenched at 51.57%; the grape genotype quenched at 45.94% for Pink grapes, quenched at 42.15% for Sweet grapes and at 39.79% inhibition for White grapes. The radical quenching capacity of the fruit juices were compared to 50 mg/mL vitamin E, vitamin C, and BHT with quenching capacity of 56.54, 29.45, and 65.97% DPPH radical, respectively. Fig. 1 illustrates the DPPH radical scavenging ability of various fruit juice extracts to quench the 100% control to the lowest percentage activities. Extract from the white grape showed a stronger DPPH scavenging activity than that of other citrus varieties.

Reducing Power

Fe (III) reduction is often used as an indicator of electron- donating activity, which is a main mechanism of reduction antioxidant action, and can be powerfully relationship with other antioxidant properties (Su and Silva, 2006). Fig. 2 shows the concentration for the reducing powers of the fruit juice extracts ranged from 126.97 to 440.86 mg ascorbic acid eqv/ 100mL. The reducing power of the juice extract and standards, which is directly proportional to the absorbance, increased with increasing concentration. WG exhibited the highest reducing ability, followed by CV, while the lowest reducing activity was found in A. However, A exhibits the lowest value of 126.97 mg ascorbic acid eqv/100 ml (Fig. 2), implying the less potent redundancy among the test substances. Like the total polyphenols, reducing power was significantly higher in grape juice than the oranges juice. Among fruit juices, the reducing power was significantly higher in grape varieties than in orange fruit juice ($P<0.05$). It has been reported that the greater the total polyphenolic content, the higher is the antioxidant activity (Yang *et al.*, 2012; Ademoyegun *et al.*, 2013). Therefore, the total polyphenol contents of the studied fruit juices were compared with their reducing antioxidative capacities. It was found that the highest content of total polyphenols was in white grape and the same highest reducing power was found in WG. Therefore, this investigation concur to the findings of Yang *et al.* (2012) that the greater the total polyphenolic content, the higher is the antioxidant potential. White grape and Cater Navel had the highest contents of antioxidants (ascorbic acid, antioxidant activity and total phenolics) in the grape and orange genotypes and represent valuable genotypes for increasing the status of

nutritive antioxidants. Among the citrus varieties, Cater Navel can be considered to merit great attention for commercial processing into fruit juice because of its high juice percentage and high total soluble solids.

CONCLUSION

Total solids, titrable acidity, acid sugar ratio and juice percentage have imperative indicator in the fruit juices processing production. Fruit juice varieties with high total soluble solids in superfluous of 5.5%, if possible up to 8.5%, acidity in the array of 0.35–0.55% and high juices percentages as desirable qualities for processing in juices production factory. In conclusion, cater navel genotype with high TSS (12.75 brix) and acidity 0.83% is appropriate orange varieties for industrial processing. This study also shows the capacity of white grape variety; contain highest levels of antioxidants activity, the highest content of polyphenol for represent valuable varieties for successful increase the status of dietary antioxidants. Also the highest antioxidant potential of white grape makes its fruit juice desirable for dietary prevention of non-communicable diseases.

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Table 1: physicochemical parameters of citrus fruit juice

Fruit juice samples	Titratable acidity %	pH	Total soluble solid	Sugar acid ratio	Juice percentage %
A	0.82 ± 0.03	3.70 ± 0.13	10.00 ± 0.45	10.53 ± 0.38	28.80 ± 2.12
CN	0.83 ± 0.03	3.80 ± 0.15	12.75 ± 0.32	17.53 ± 0.41	48.96 ± 1.94
CV	0.88 ± 0.07	3.3 ± 0.09	10.50 ± 0.35	10.10 ± 0.43	41.67 ± 1.97
PG	0.96 ± 0.08	3.57 ± 0.14	12.50 ± 0.47	10.05 ± 0.39	37.43 ± 2.14
SG	1.14 ± 0.05	3.53 ± 0.16	12.33 ± 0.39	9.07 ± 0.39	19.13 ± 2.15
WG	1.15 ± 0.08	3.10 ± 0.20	12.33 ± 0.41	8.81 ± 0.49	33.63 ± 1.99

Table 2: Antioxidant Analysis

Sample	Antioxidant nutrients		Star Antioxidant	
	Ascorbic acid mg/100ml	Total Carotenoid mg/L	Total phenolic content mgGAE/L	Total Flavonoid content mg/L
A	45.58 ± 3.21	2.63 ± 0.17	513.07 ± 7.61	105.17 ± 5.78
CV	66.34 ± 2.98	4.68 ± 0.91	564.63 ± 8.97	393.51 ± 6.97
CN	46.35 ± 2.78	6.38 ± 0.84	533.09 ± 9.09	195.52 ± 5.87
PG	64.21 ± 2.89	3.96 ± 0.27	577.89 ± 9.18	288.57 ± 5.94
SG	53.60 ± 3.09	ND	561.79 ± 8.98	215.37 ± 6.10
WG	68.19 ± 3.05	ND	585.75 ± 10.02	434.60 ± 7.79

ND = Not detected. GAE = gallic acid equivalent

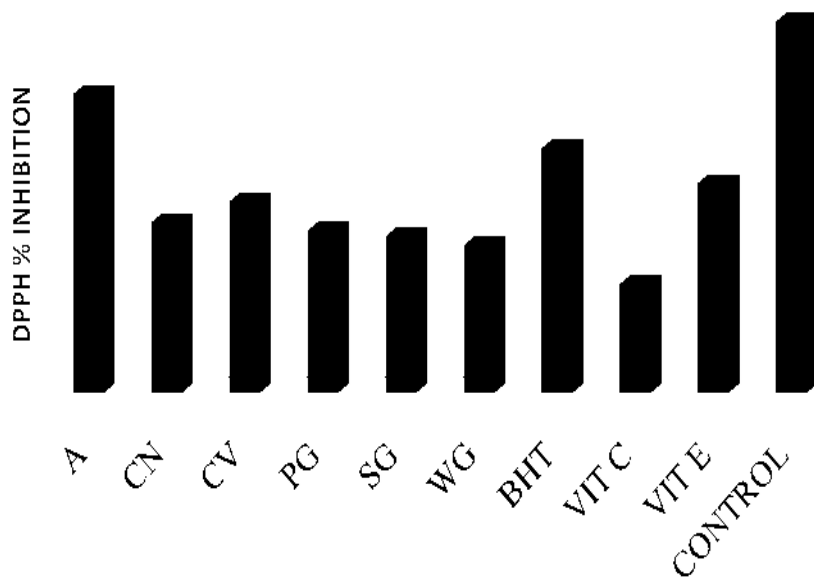


Figure 1—Radical DPPH scavenging activity of the citrus extracts.

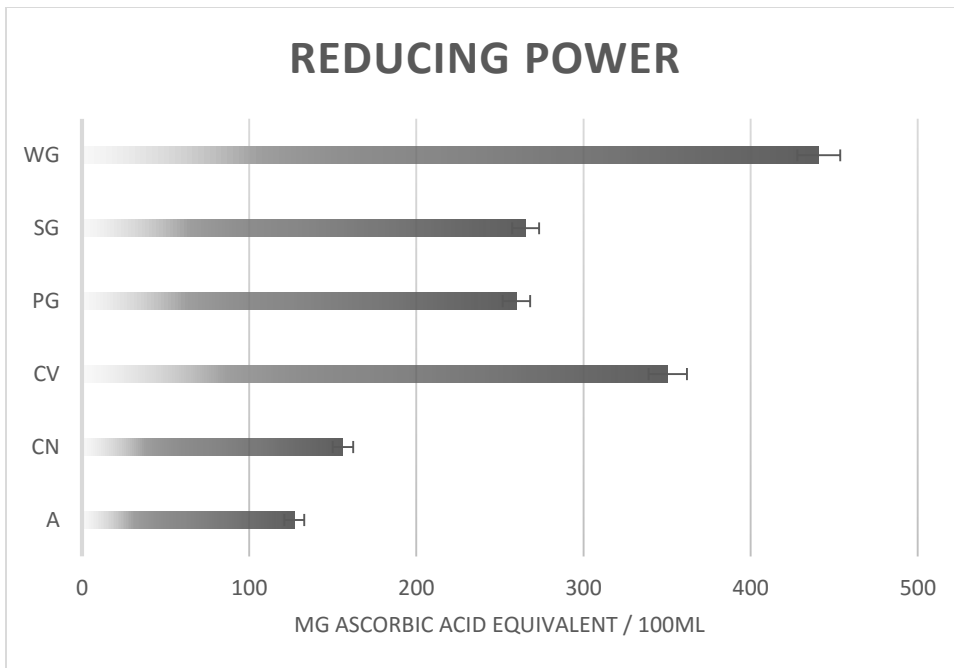


Fig 2: Reducing antioxidant power assay of the citrus juice genotype.

INFLUENCE OF PHOSPHORUS FERTILIZER ON THE YIELD AND YIELD COMPONENTS OF FOUR SOYBEAN VARIETIES AT LAFIA, SOUTHERN GUINEA SAVANNA OF NIGERIA

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ABSTRACT

Field trials were conducted during 2018 and 2019 cropping seasons at the Teaching and Research Farm of the Faculty of Agriculture, Nasarawa State University Keffi, Shabu-Lafia Campus to study the yield performance of soybean varieties under different phosphorus (P) fertilizer levels. The study area fell within the southern Guinea savanna zone of Nigeria, located on latitude 6° 15' and 9° 30' N and Longitude 6° 30' and 11° 0' E. The treatments consisted of the four P fertilizer levels (0, 13, 26 and 39 kg P₂O₅ ha⁻¹) and four varieties of soybean (TGX 1448-2E, TGX1987-62F, TGX 1989-19F and TGX 1835-10E). The treatment combinations were laid out in a randomized complete block design (RCBD) and replicated three times. The yield and yield components were significantly (P<0.05) affected by the treatments and each increase in P application resulted in increased number of pods plant⁻¹, number of seeds pod⁻¹, seed yield plant⁻¹, 100 seed weight and seed yield hectare⁻¹ in both cropping seasons. The results also showed that application of 39 kg P₂O₅ ha⁻¹ produced significantly (P<0.05) highest in all the yield parameters measured while control plots produced lowest in both cropping seasons respectively. The results further revealed that the varietal effect of soybean on all the yield and yield components were significant (P<0.05) with TGX 1987-19F variety produced consistently highest number of pods plant⁻¹, number of seeds pod⁻¹, 100 seed weight and seed yield hectare⁻¹ while TGX 1835-10E variety produced the lowest yield. In view of the results, this study, therefore recommended that soybean farmers in Lafia and its environs should apply P fertilizer at 39 kg P₂O₅ ha⁻¹ and adopt TGX 1989-19F variety for profitable soybean production.

Key words: Lafia-southern Guinea savanna, phosphorus fertilizer, soybean.

INTRODUCTION

Soybean (*Glycine max.* L. Merrill) is one of the most widely grown leguminous crops in the world (Adeyeye, 2009). Increasing demand for the crop due to its nutritional and medicinal values for humans, animals and

industrial usage has provided many incentives for farmers and producers around the world to increase their production to meet the rising demand (Kwon *et al.*, 2007). Soybean performs well in the southern and northern Guinea savanna of Nigeria, where rainfall is more than 700 mm. It is produced

mostly in the middle belt of Nigeria with Benue State accounting for about 65%-70% of the total production in the country. Soybean requires well drained and fertile loamy soils with high organic matter and a pH range between 6.0 and 7.5 (Dugje *et al.*, 2009). It contains more than 36% protein, about 30% carbohydrates and 20% oil. It is an excellent source of dietary fibre, Vitamins and Minerals (Atli, 2019). The world average yield of soybean is about 4 tons ha⁻¹ but less than 1.2 tons ha⁻¹ in most tropical soils (Wandile, 2018). The low yield could be due to poor agronomic practices and P deficient of about 70 % of tropical soils (Dugje *et al.*, 2009). Phosphorus is a major limiting factor for soybean production in many soils in the tropics (Abdulkadir, 2006). When soil P level is low, soybean cannot grow and produce normally, or tolerate stresses as they should and thus becomes a principal yield-limiting nutrient (Pereira and Bliss, 1989). The productivity of soybean can be fully realized by adopting suitable agronomic practices such as application of nutrients especially P fertilizer at optimum level and use of suitable variety that is well adapted to agro-climatic condition of the area. So many researchers in different parts of the world recorded increased grain yield of soybean with the application of P in different varieties (Adams *et al.*, 2014 and Morshed *et al.*, 2008). Information on yield performance of soybean varieties under different P levels in the study area are lacking, hence, the need of this study. Therefore, the aim of this study is to investigate the effect of P levels of application on the yield of soybean varieties.

MATERIALS AND METHODS

The experiments were conducted during the rainy seasons of 2018 and 2019 at the Teaching and Research Farm of the Faculty

of Agriculture, Nasarawa State University, Keffi, Shabu- Lafia campus The study area was located on latitude 6° 15' and 9° 30' N and Longitude 6° 30' and 11° 00'E, which fell within the southern Guinea savanna zone of Nigeria. The experiment consisted of four levels of phosphorus fertilizer in the form of single super phosphate (0, 13, 26 and 39 kg P₂O₅ ha⁻¹) and four varieties of soybean (TGX 1448-2E, TGX 1987-62F, TGX 1989-19F and TGX 1835-10E). The sixteen treatment combinations were laid out in a randomized complete block design (RCBD) and replicated three times to form (48) plots. The gross plot size was 6 m² (3m x 2 m) while the net plot size was 3 m² (1.5 m x 2 m). A composite soil sample of the study area from 0-15 cm and 15-30 cm depth was taken randomly at six locations for physical and chemical analyses. The field was cleared, four ridges per plot were constructed manually. The site was marked into plots and replications. One meter unplanted alley was maintained between plots while 2.0 m unplanted alley was maintained between replications. The four levels of P fertilizer were incorporated into the ridges based on the treatment combinations. Four seeds were sown at a recommended spacing of 0.75 m between rows and 0.05 m between plants and thinned to two plants at 2 weeks after sowing (WAS). Hoe weeding was done at 3, 6 and 9 WAS to keep the plots weed free. Harvesting was done when the leaves turned yellowish. Five randomly selected plants were tagged and used for the following variables measured are;

Number of pods per plant of the five tagged plants was counted and mean recorded. Number of seeds per pod counted by randomly selecting 10 pods from each plot, seeds counted and the mean recorded. Seed yield per plant (g) was done by harvesting and removing all the pods on five sampled plants

in each plot and sun-dried, threshed and winnowed separately to obtain a clean seed, weighed and the mean recorded. A hundred seed weight (g) was determined by counting 100 seeds from each plot and weighing. This was repeated twice and the mean recorded. Seed yield per plot (g) was determined from the net plot harvested plants were put in labelled sacks and keep in a safe environment. Each sample was sun-dried for 10 days and the then threshed, winnowed and the clean seeds were weighed with sensitive weighing scale and recorded. The weight of the seeds obtained from the net plot was converted to kilogram per hectare. All data collected were subjected to analysis of variance and standard error of means (SE \pm) was used to compare treatment means at 5% probability level using SAS statistical package (SAS, 2020).

RESULTS

Table 1 shows the results of the physical and chemical properties of the study area. The results obtained indicated that soil pH were slightly acidic ranged from 5.94 to 6.07. The soil pH is within the preferably range for soybean production. The percent organic carbon, organic matter and total nitrogen values that ranged from 1.01 to 1.09, 3.03 to 3.09 and 0.21 to 0.35 were very low (Loveland and Webb, 2003). The available P was deficient with values less than the critical value of 20 ppm (Landon, 1991). Exchangeable K, Ca, Mg and Na values that ranged from 0.15 to 0.25, 2.40 to 3.09, 1.38 to 1.87 and 0.10 to 0.21 Cmol kg⁻¹ respectively were generally high with values higher than the critical values of 0.12, 1.6, 1.0 and 0.03 Cmol/kg respectively (Landon, 1991). The higher values of exchangeable bases could be attributed to the texture and structure of the soils of the study area. The

poor soil physical and chemical properties of the study area may be as a results of excessive leaching, losses of organic and inorganic minerals due to soils larger pores spaces and nutrients uptake by the plants with no replenishment.

Grain Yield

The results obtained indicated that yield and yield components of soybean were significantly ($P < 0.05$) different among the treatments (Tables 2 and 3). Each increase in the rate of phosphorus applied significantly increased number of pod plant⁻¹, seed yield plant⁻¹, 100 seed weight and seed yield ha⁻¹ in both years respectively. This indicates the efficient and positive role of phosphorus on the yield of soybean. In 2019 cropping season, plots that were applied with 26 and 39 kg P ha⁻¹ produced statistically similar number of seeds pod⁻¹ but significantly greater than 13 kg P ha⁻¹ and control respectively. Application of 39 kg P₂O₅ ha⁻¹ of P fertilizer produced significantly the highest grain yield of soybean compared to other rates of P applied. The lowest grain yield was produced by the control plots in both years. Varietal effect on grain yield of soybean were significant ($P < 0.05$). In both years, TGX 1989-19F variety produced consistently higher number of pod plant⁻¹, 100 seed weight and seed yield ha⁻¹ as compared to other varieties. In 2018 and 2019 cropping seasons, TGX 1987-62F and TGX 1989-19F recorded similar number of seeds pod⁻¹ and seed yield plant⁻¹ respectively while TGX 1448-2E and TGX 1835-10E produced significantly ($P < 0.05$) lower. However, TGX 1987-62F and TGX 1989-19F produced significantly the highest seed yield plant⁻¹ and number of seed pod⁻¹ compared to other varieties evaluated in both cropping seasons respectively. The interaction between phosphorus and variety

on the number of pod plant⁻¹, number of seed pod⁻¹, seed yield plant⁻¹ and 100 seed weight were not significant while seed yield ha⁻¹ was significant in both the years at 5% level of probability respectively.

Table 4 shows a significant interaction between phosphorus and variety on the seed

yield per hectare of soybean. The results indicated that TGX 1989-19F variety produced significantly (P<0.05) the highest seed yield of 1597.3 to 1637.3 kg ha⁻¹ followed by TGX 1987-62F of 1556.0 to 1613.3 kg ha⁻¹ while TGX 1835-10E produced the lowest seed yield of 1181.7 to 1221.7 kg ha⁻¹ in 2018 and 2019 respectively.

Table: 1 Physical and Chemical Properties of Soils of the Study Area

Sampling Depth (cm)	pH (H ₂ O)	O.C	O.M (%)	N	Avail P (PPM)	K	Ca (Cmol kg ⁻¹)	Mg	Na
0-15	5.97	1.01	3.03	0.21	2.43	0.16	2.40	1.38	0.12
15-30	6.07	1.08	3.09	0.28	2.78	0.23	3.11	1.87	0.18
0-15	5.94	1.02	3.09	0.28	2.52	0.20	2.58	1.47	0.16
15-30	6.01	1.05	3.03	0.21	2.49	0.15	2.49	1.43	0.10
0-15	6.03	1.03	3.09	0.35	2.73	0.24	3.09	1.81	0.21
15-30	6.05	1.09	3.09	0.35	2.76	0.25	3.04	1.83	0.19

Table 2. Effects of Phosphorus and Variety on the Yield Parameters of Soybean in Lafia, 2018 cropping season.

Treatment	Number of pod Plant ⁻¹	Number of seeds Pod ⁻¹	Seed yield Plant ⁻¹ (g)	100 seeds weight (g)	Seed yield (Kg ha ⁻¹)
Phosphorus (P, kg P ₂ O ₅ ha ⁻¹)					
0	35.8 ^d	2.3 ^d	8.6 ^d	10.0 ^d	949.0 ^d
13	41.8 ^c	2.4 ^c	12.3 ^c	10.8 ^c	1068.7 ^c
26	51.5 ^b	2.8 ^b	19.2 ^b	12.2 ^b	1289.0 ^b
39	52.8 ^a	2.9 ^a	24.7 ^a	12.9 ^a	1561.3 ^a
SE ±	0.19	0.03	0.12	0.13	0.46
Variety (V)					
TGX 1448-2E	40.0 ^c	2.6 ^b	14.1 ^c	11.7 ^c	949.3 ^c
TGX 1987-62F	54.7 ^b	2.7 ^a	21.2 ^a	12.2 ^b	1543.3 ^b
TGX 1989-19F	58.2 ^a	2.7 ^a	20.8 ^b	12.6 ^a	1591.7 ^a
TGX 1835-10E	37.3 ^d	2.5 ^c	12.9 ^d	10.3 ^d	786.7 ^d
SE ±	0.14	0.04	0.10	0.16	0.56
P × V	NS	NS	NS	NS	*

Means of different letter(s) in each column of treatment group are significant at 5% level of significance; NS = Not significant

* = Significant at 5 % level of significance

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Table 3. Effects of Phosphorus and Variety on the Yield Parameters of Soybean in Lafia, 2019 cropping season.

Treatment	Number of pod Plant ⁻¹	Number of seeds Pod ⁻¹	Seed yield Plant ⁻¹ (g)	100 seeds weight (g)	Seed yield (Kg ha ⁻¹)
Phosphorus (P, kg P ₂ O ₅ ha ⁻¹)					
0	40.9 ^d	2.3 ^c	11.4 ^d	9.8 ^d	958.0 ^d
13	48.2 ^c	2.4 ^b	13.9 ^c	10.6 ^c	1110.0 ^c
26	54.9 ^b	2.8 ^a	18.3 ^b	11.9 ^b	1306.0 ^b
39	60.0 ^a	2.8 ^a	19.2 ^a	12.6 ^a	1606.7 ^a
SE ±	0.16	0.03	0.13	0.11	0.42
Variety (V)					
TGX 1448-2E	48.7 ^c	2.6 ^b	14.3 ^b	10.7 ^c	952.0 ^c
TGX 1987-62F	53.0 ^b	2.6 ^b	19.1 ^a	11.4 ^b	1526.7 ^b
TGX 1989-19F	63.7 ^a	2.8 ^a	19.2 ^a	11.7 ^a	1624.0 ^a
TGX 1835-10E	42.1 ^d	2.5 ^c	12.6 ^c	9.8 ^d	803.0 ^d
SE ±	0.19	0.06	0.12	10.6 ^c	0.51
P × V	NS	NS	NS	11.9 ^b	*

Means of different letter(s) in each column of treatment group are significant at 5% level of significance.

NS = Not significant

* = Significant at 5 % level of significance

Table 4. Interaction between Phosphorus and Variety on the Seed Yield per Hectare of Soybean in Lafia, 2018 and 2019 cropping seasons.

Variety (V)	2018				2019			
	Phosphorus (P, kg P ₂ O ₅ ha ⁻¹)							
	0	13	26	39	0	13	26	39
TGX 1448-2E	848.7 ^o	1066.0 ^k	1189.3 ^f	1306.7 ^c	846.7 ^o	1094.0 ^k	1213.3 ^g	1327.3 ^c
TGX 1987-62F	941.3 ⁿ	1136.0 ⁱ	1280.0 ^e	1556.0 ^b	960.7 ⁿ	1141.3 ⁱ	1308.0 ^e	1613.3 ^b
TGX 1989-19F	963.3 ^m	1147.3 ^h	1298.3 ^d	1597.3 ^a	971.7 ^m	1162.7 ^h	1320.0 ^d	1637.3 ^a
TGX 1835-10E	780.7 ^p	940.0 ^l	1086.7 ^j	1181.7 ^g	813.3 ^p	984.0 ^l	1132.0 ^j	1221.7 ^f
SE ±		1.04				1.13		

Means having the same letter(s) are not significantly different at 5% level of significance.

DISCUSSION

There was a significant ((P<0.05)) variation due to different levels of phosphorus fertilization on the number of pod plant⁻¹, number of seeds pod⁻¹, seed yield plant⁻¹ and 100 seed weight of soybean plant. These results are in corroboration with the findings

of Dixit *et al.* (2011) who reported significant (P<0.05) differences in number of seeds pod⁻¹, seed yield plant⁻¹ and 100 seed weight of soybean due to P fertilizer application.

The highest seed yields of 1597.3 and 1637.3 kg ha⁻¹ were respectively recorded for TGX 1989-19F variety with a fertilizer P application of 39 kg P₂O₅ ha⁻¹ in both

cropping seasons (Tables 4). This result is in agreement with those of Pauline *et al.* (2010) and Asei *et al.* (2015) who found that seed yield ha⁻¹ of soybean was greatest following application of 30-45 kg P ha⁻¹. Similar findings were also reported by Jahan *et al.* (2009) reported that increase in P application resulted in increased seed yield ha⁻¹ up to 45 kg ha⁻¹. The response of soybean to the highest rate of P applied could be due to the poor soil fertility status of the study site as shown by the soil analysis results (Table 1). TGX 1989-19F variety performed better than all other varieties in this study. The outstanding performance in the yield and yield components of this variety TGX 1989-19F could be attributed to the genotypic differences as suggested by Ahmed *et al.* (2010) as the yield differed significantly in all varieties.

The higher grain yield obtained from TGX 1989-19F variety is an indication that they are highly adaptable and may possess high ability to respond well to treatments applied more than other varieties evaluated. The lower grain yield obtained from these varieties TGX 1987-62F, TGX 1448-2E and TGX 1835-10E is an indication that the variety have lower yielding ability and may not be adaptable to this agro-climatic condition of the study area.

CONCLUSION AND RECOMMENDATION

From the results of this study, it can be concluded that the application of 39 kg P₂O₅ ha⁻¹ of P fertilizer produced a significantly (P<0.05) higher number of pods plant⁻¹, number of seeds pod⁻¹, seed yield plant⁻¹, 100 seed weight and seed yield hectare⁻¹ in both cropping seasons while TGX 1989-19F variety of soybean performed better than the other varieties evaluated. Therefore, this

study recommended that soybean farmers in Lafia and its environs should apply phosphorus fertilizer at 39 P₂O₅ kg ha⁻¹ and adopt TGX 1989-19F variety for profitable soybean production.

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