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Relationship between Meteorological Variables and Effective Earth Radius Factor over Auchi, Edo State, South-South, Nigeria

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Abstract: The effective earth radius factor; *k*-factor is a major parameter in the prediction of the local radio wave propagation conditions of the lower atmosphere from meteorological variables. In this study the *k*-factor values were calculated and analyzed from the measurements of air temperature, relative humidity and atmospheric pressure in Auchi area of Edo State, South-South, Nigeria using a self-implemented cost effective portable weather monitoring device for a period of one year; 2017, so as to determine the relationship between these meteorological variables and the *k*-factor values. The fixed measuring method by placing the weather monitoring device on 188 m height above sea level was employed for the measurements of the various meteorological variables at the administrative block of Edo University Iyamho. The results show that the calculated *k*-factor values range from 1.06 to 1.94 with an average value of 1.47 and were generally higher during the months with much rainfall compared to that of the months with lesser rainfall. The measured meteorological variables were having significant influences on the calculated *k*-factor values during all the months, and these influences were more pronounced during the months with much rainfall compared with the months with lesser rainfall. The influence of the air temperature was higher compared to the other meteorological variables. The results that are obtained from this study should be taken into account for enhancement and improvement purposes in radio communication systems.

Keywords: Lower atmosphere; Air temperature; Atmospheric pressure; Relative humidity; Radio wave

1.0 Introduction

In the prediction of the local radio wave propagation conditions of the lower atmosphere, meteorological variables are very useful because of their influence on radio wave communication links in the atmosphere [1, 2].

The electromagnetic waves that are propagated in the lower atmosphere are mainly affected by the different components that made up the atmosphere, this is as a consequence of the variations of some meteorological variables such as air temperature, atmospheric pressure and relative humidity and these variations made the refractive index of the air in the lower atmosphere to vary from place to place [1, 2, 3, 4]. The path bending of the electromagnetic waves as a result of the non-homogeneous spatial distribution of the refractive index of air causes harsh effects such as multipath fading and interference, attenuation due to diffraction on the terrain obstacles which is also known as the radio holes [3, 5]. The variation in refractivity in the lower atmosphere is a function of meteorological variables [1, 2, 3, 6].

In order to have a good communication link for radio wave, the transmission medium need to be considered in order to have a better signal from the radio communication network, since the radio wave communication links are influenced by meteorological variables [2, 7]. The attitude and phase scintillations, absorption, scattering of radio wave network signals and other numerous complex mechanisms that occur in the lower atmosphere are caused by the random changes in the surface and vertical refractivity which can cause transmission signals lost and co channel interference. The effect of interference as a result of refractivity difference in the lower atmosphere is much in the humid climate than in the temperate climate regions due to the occurrence of high intensity humid rainfall [3, 7].

Some commonly used format of referring the International Telecommunications Union recommendation, (ITU-R) is presently being used in planning of broadcasting services for frequencies greater than 30 MHz, the mostly used radio propagation wave formulae from this recommendation are obtained from measurements carried out in most temperate climate regions of the world like Europe, Asia and North America. Although, the sub-Sahara African climate is different from these temperate climate regions own, these formulae can still be used for the planning of radio wave propagation services in the sub-continent due to the scarcity of accurate data from these regions [2, 5, 7, 8]. The radio refractive index is an important parameter in determining the quality of radio signals [3]. To determine the characteristics of a radio channel, surface and elevated refractivity data are mostly needed. The surface refractivity is more important for the prediction of some propagation effects than the elevated refractivity. Local coverage, refractivity gradient and other statistics of refractivity provide the most crucial explanation of the likely occurrence of refractivity related influence needed for local radio wave prediction methods [2, 5, 7, 9, 10]. The assessment of these meteorological variables will definitely enhance a better local radio propagation conditions that will assist radio network service providers in enhancing their quality of services [3].

In this study the measurement results of air temperature, relative humidity and atmospheric pressure were made at 188 m height above sea level at the Administrative block of Edo University Iyamho, Auchi area of Edo State, South-

South, Nigeria using a self-designed cost effective portable weather monitoring device. The measured meteorological were used to derive and analyze the *k*-factor. To the best of our knowledge from existing literatures this study is unique not only for the fact that we are using a self-implemented inexpensive portable weather monitoring device for the measurements of the meteorological variables but also it is one of the most recent studies on the relationship between meteorological variables and *k*-factor specifically in Auchi area of Edo State, South-South, Nigeria.

2.0 Materials and Methods

2.1 The Weather Monitoring Device

The weather monitoring device was implemented in such a way that it can be used remotely and the readings are displayed on the user friendly LCD display in numerical digital values for atmospheric temperature (^oC), atmospheric pressure (mbar), relative humidity (%) and light intensity (lux) which can also be sent to computer via the programmed micro SD card or/and through the serial port; the Arduino SD card module. The user has the option of choosing how often the meteorological variables will be logged, measured, recorded, stored and displayed. The acquired meteorological variables are analyzed and the LCD displays the values respectively. In addition, the meteorological variables for each day are saved on the micro SD card in Excel format on a separate file with each file created with a file name that corresponds to the date and time when the meteorological variables were acquired. The users also have the option to stop the meteorological variables acquisition process at any given time by interrupting the routine. Details of the construction and implementation of the weather monitoring device including its validity is contained in [11].

2.2 Area and Method of Measurements

The measurements were done at the administrative block of Edo University Iyamho, Auchi area of Edo State, South-South, Nigeria which is located within Latitude 7.07°N and Longitude 6.27°E of the Greenwich Meridian at 188 m height above sea level. The area experiences the humid tropical climate, which is characterized by wet and dry seasons; the vegetation is that of the Savannah, with mostly open grassland and few scattered fire resistant trees. The topography is relatively undulating and it slopes from the north of the area to the south [2, 12].

The fixed measuring method by placing the weather monitoring device on 188 m height above sea level was employed for the measurements of the various meteorological variables at the administrative block of Edo University Iyamho, Auchi area of Edo State, Nigeria for continuous measurements. Although, the weather monitoring device measures four meteorological variables as stated earlier, only the daily records of air temperature (^oC), atmospheric pressure (mbars) and relative humidity (%) were used for this particular study. The records cover twenty four hours each day from 00 hour to 2300 hours local time at intervals of one hour of which the average values from each day are then copied from the micro SD card to the computer from the weather monitoring device. The measurements of the meteorological variables were made for a period of one year; January to December, 2017.

2.3 Theoretical Background

It has been shown that the electromagnetic waves that are passing

through the atmosphere bends, due to the various layers of the atmosphere and its permittivity, but it would have otherwise travel in a straight path if it was homogeneous; as a result of this spatial distribution of the refractive index of the air which causes hostile effects [2, 5]. The atmosphere's refractive index, n and the relative permittivity, $\mathcal{E}_r$ can be connected with [2, 7, 10]:

$$n^2 = \mathcal{E}_r \quad (1)$$

Since the value of the atmospheric refractive index is ≈ 1 and the variation is infinitesimal. A suitable parameter that can be used when modeling the variation of the atmospheric refractive index is the refractivity, N which is defined as;

$$N = (n - 1) \times 10^6 \quad (2)$$

N and meteorological variables such as the air temperature, atmospheric pressure, vapour pressured is connected by;

$$N = \frac{77.6}{T}\left(P + 4810\frac{E}{T}\right) \quad (3)$$

N can thus, be expressed as (ITU-R, 2004);

$$N = 77.60\frac{P}{T} + \left(3.73 \times 10^5 \frac{E}{T^2}\right) \quad (4)$$

The troposphere refractivity can be divided into two proportions, namely; the dry proportion and the wet proportion. The dry proportion contributes about 70% of the total refractivity in the troposphere. This dry proportion increases with increasing density of the gas molecules and changes with their distribution. It is normally stable and can be calculated from the measured air temperature and atmospheric pressure with an accuracy of about 20% [1, 2] using:

$$N_d = 77.60\frac{P}{T} \quad (5)$$

Where P is barometric pressure (millibars) and T is absolute temperature in Kelvin.

On the other hand, the wet proportion which is as a result of the polar nature of water molecules, contributes the main variation of refractivity in the atmosphere and can be calculated [1, 3] using:

$$N_w = 3.73 \times 10^5 \frac{E}{T^2} \quad (6)$$

Where E is partial pressure of water vapor (millibars) and can be calculated from;

$$E = \frac{RH}{100} \times E_s \quad (7)$$

Where RH is the relative humidity (%) and E_s which is the saturated vapour pressure (millibars) by:

$$E_s = 6.11 \times 10^{\frac{17.27\,(T - 273.15)}{T - 35.85}} \quad (8)$$

If the height; h of a ray above the earth's surface, the radius; r of the ray curvature and the vertical gradient of refractive index; dN/dh, the horizontal angle of the path; θ to a given point can be written as:

$$\frac{1}{r} = \frac{1}{N}\left(\frac{dN}{dh}\right) \cos\theta \quad (9)$$

According to [13], r may be connected to the relative earth radius; R in terms of the refractive index gradient:

$$\frac{r}{R} = k \quad (10)$$

Where k is the effective earth radius factor which can now be expressed as:

$$k \approx \frac{1}{1 + R\left(\frac{dN}{dh}\right)} \quad (11)$$

[14] in their work show that the *k-factor* can be used for categorizing the refractive conditions as normal refraction or standard atmosphere, sub-refraction, super-refraction and ducting as the case may be.

Recall that $R \approx 6370$ km. Thus, Eqn. (11) may now be expressed in terms of N as:

$$k \approx \frac{1}{1+(\frac{dN}{dh})/157} \quad (12)$$

Within the earth's surface, $dN/dh \approx$-39 N-units/km and this will give a *k*-factor value of 1.33; with such value we will have what is known as normal refraction or standard atmosphere. Here, radio signals are transmitted along a straight line path on the earth's surface and go into space unimpeded. If $1.33 > k > 0$, we will have sub-refraction; which implies that the radio waves propagate abnormally away from the earth's surface. But when $\infty > k > 1.33$ we will have super-refraction and this signifies that the radio wave signals spread irregularly towards the earth's surface; thus, extending the radio horizon and increasing path clearance thereby giving irregularly huge ranges above the line of view as a result of multiple reflections. But, if $-\infty < k < 0$, there will be ducting and this will make the radio waves to bend downwards with a curvature greater than the earth's own. The radio signals can become trapped between a layer in the lower atmosphere and the surface duct which is the earth's or sea's surface or between two layers in the lower atmosphere which is the elevated duct. In this wave guide-like propagation, very high radio signal strengths can be obtained at a very long range which is far above the line of view [2, 3, 7].

3.0 Discussion of Results

The analysis for this study was done procedurally via calculation of *k*-factor from the measured meteorological variables; air temperature, relative humidity and atmospheric pressure using Eqn. 1 to Eqn. 12 accordingly. The monthly calculated *k*-factor values for the period under consideration range from 1.06 to 1.94 with an average value of 1.47. Some statistical analysis was also done so as to determine their variability. Comparisons of the various measured meteorological variables and the calculated and the *k*-factor values were also respectively done graphically. The average monthly measured meteorological variables of each of the measured meteorological variables and the calculated *k*-factor are contained in Table I. The mean values for air temperature, relative humidity and atmospheric pressure were 25.46 ^{o}C, 73.08 % and 1004.56 mbar respectively for the period of the measurements; January to December, 2017.

Since the average value of the calculated *k*-factor is 1.47 and $\infty > k > 1.33$, we can inferentially say that the local radio wave propagation condition for Auchi, area of Edo State, South-South, Nigeria is predominantly super-refractive. This signifies that the radio wave signals spread irregularly towards the earth's surface, hence extending the radio horizon and increasing path clearance, thereby giving irregularly huge ranges above the line of view as a result of several reflections.

In Fig. 1 the *k*-factor values for the period under consideration; 2017 on monthly basis which were obtained from the monthly records is shown. A critical look at this figure revealed that the months with higher relative humidity (occasion with much rainfall); April, May, June, July, August, September and October have greater values compared to the ones with lower relative humidity (occasion with lesser rainfall); November, December, January, February and March, the values range from 1.20 to 1.94 during the months with high relative humidity;

while, the values for the months with lower relative humidity range from 1.06 to 1.62 for the period under consideration. This result again agrees very well with the results of [7, 15].

Fig. 2 shows the plot of the measured air temperature against the calculated *k*-factor values during the various months; January to December, 2017, Fig. 3 shows the plot of the measured relative humidity against the calculated *k*-factor values during the various months; January to December, 2017, while Fig. 4 shows the plot of the measured atmospheric pressure against the calculated *k*-factor values during the various months; January to December, 2017. It was observed that these measured meteorological variables were having significant influences on the calculated *k*-factor values during all the months in 2017, and these influences were more pronounced during the months with higher relative humidity which are; March, April, May, June, July, August, September and October; rainy season, compared with the months with lower relative humidity which are; November, December, January and February; dry season.

The measured air temperature was observed to have much influence on the calculated *k*-factor values all through the months in 2017 compared to the other two meteorological variables (relative humidity and atmospheric pressure), this again affirm that fact that air temperature have significant influence on other meteorological variables [3, 16, 17].

4.0 Conclusion

The measurements of air temperature, atmospheric pressure and relative humidity were made at 188 m height above sea level in the administrative block of Edo University Iyamho, Auchi area of Edo State, South-South, Nigeria for a period of one years; 2017 using a self-implemented inexpensive portable weather monitoring device so as to analyze the relationship between these meteorological variables and *k*-factor values. The results that were obtained from this study would assist in the enhancement and improvement of radio communication systems.

Deductively, the summarized results obtained from this study are:

The local radio wave propagation condition for Auchi area of Edo State, South-South, Nigeria is predominantly super-refractive.

The measured air temperature, relative humidity and atmospheric pressure were having significant influence on the calculated *k*-factor during all the months in 2017 and these influences were much during the months with higher relative humidity. The measured air temperature was having much influence on calculated *k*-factor all through the months in 2017.

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Month	Average Air Temperature (°C)	Average Relative Humidity (%)	Average Atmospheric Pressure (mbars)	Calculated *k*-factor
Jan	**28.20**	**42.70**	**1001.70**	**1.16**
Feb	**31.80**	**48.20**	**1002.50**	**1.30**
Mar	**30.10**	**68.70**	**1004.60**	**1.50**
Apr	**29.30**	**85.20**	**1007.10**	**1.40**
May	**28.40**	**92.10**	**1005.70**	**1.30**
June	**26.30**	**92.40**	**1005.90**	**1.58**
July	**25.00**	**95.30**	**1004.70**	**1.72**
Aug	**26.60**	**78.60**	**1003.90**	**1.88**
Sept	**25.50**	**78.40**	**1005.40**	**1.40**
Oct	**25.70**	**68.90**	**1005.60**	**1.44**
Nov	**27.80**	**68.20**	**1004.70**	**1.40**
Dec	**26.50**	**58.30**	**1002.90**	**1.52**
Mean	25.46	73.08	1004.56	1.47

Table I: Average Measured Meteorological Variables and Calculated k-factor for 2017

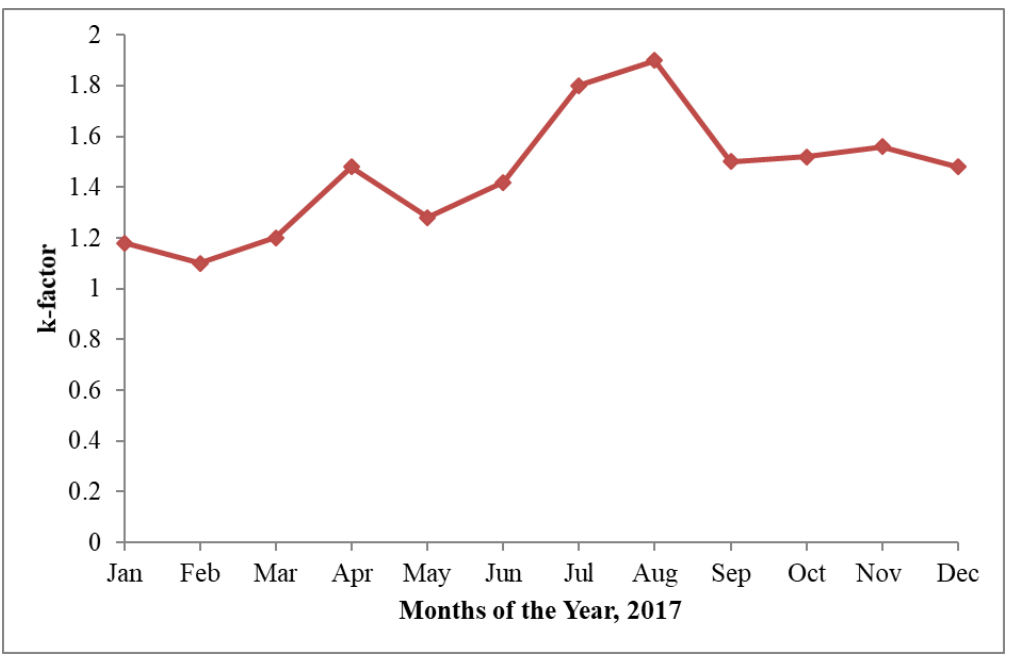

Fig. I: Monthly Variations of k-factor

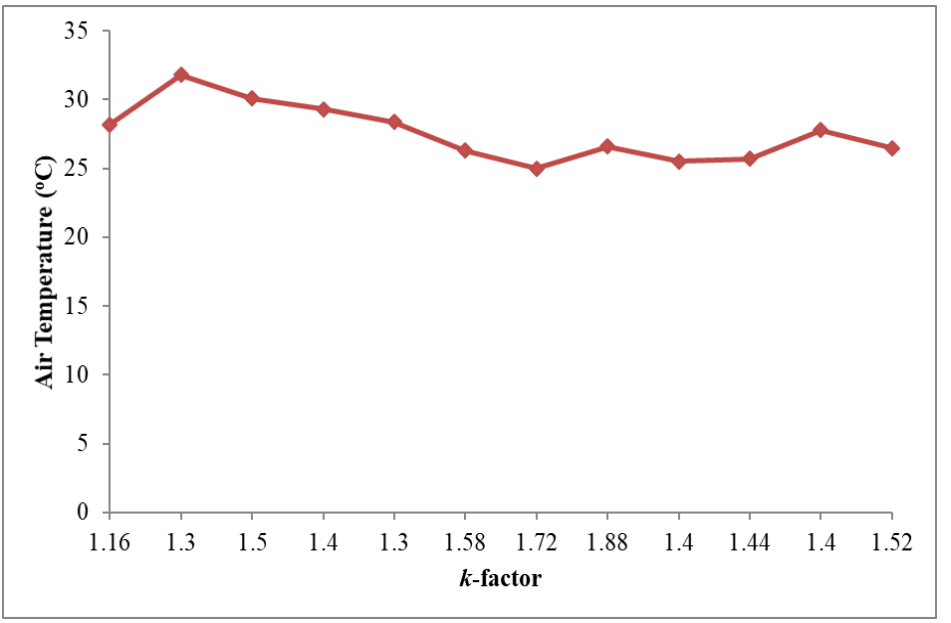

Fig. II: Air Temperature with the k-factor

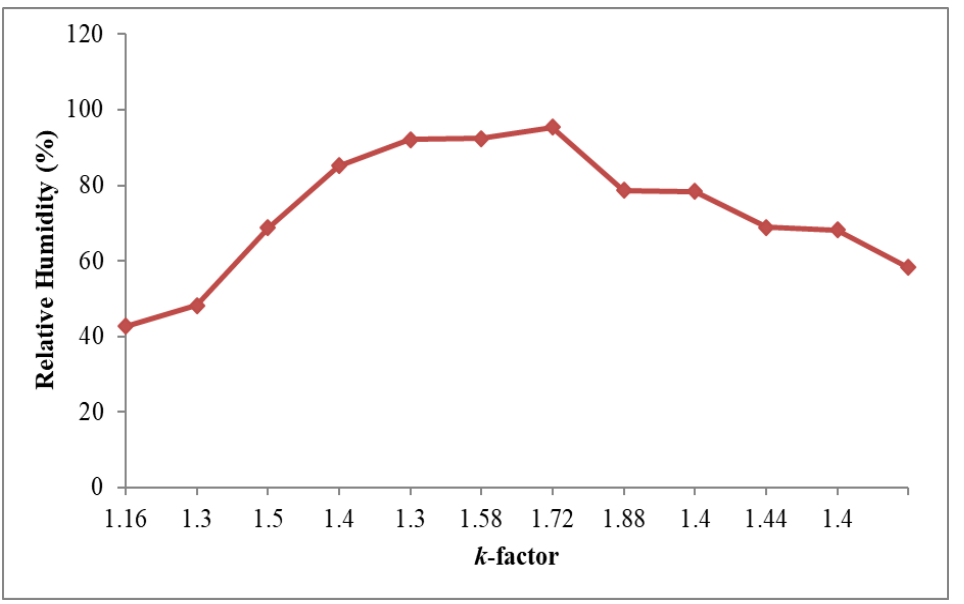

Fig. III: Relative Humidity with the k-factor

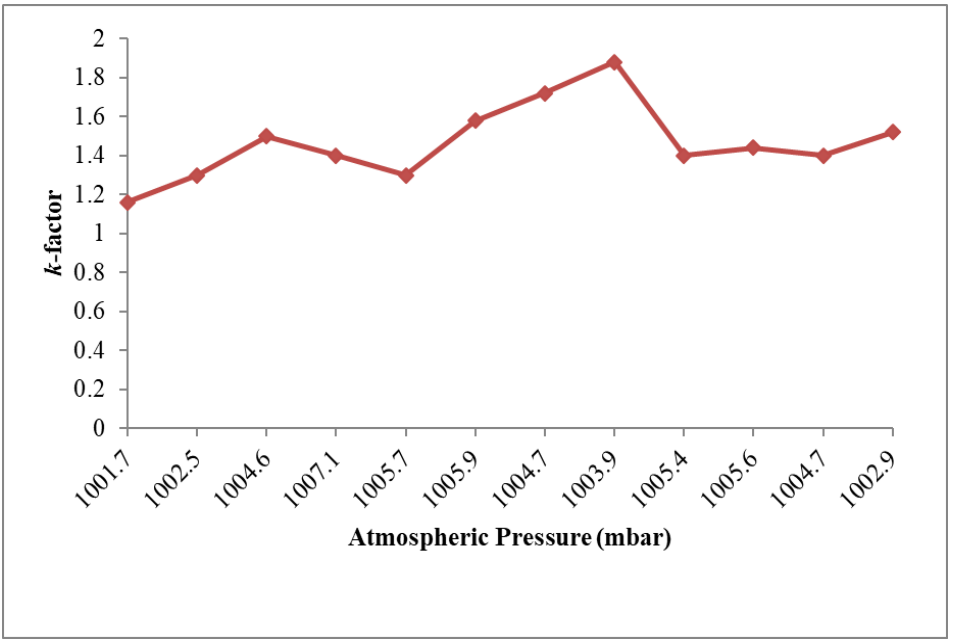

Fig. IV: Atmospheric Pressure with the k-factor

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Effect of Solid State Fungal Fermentation on the Chemical Composition of Adansonia digitata Seed

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Abstract: The seed of *Adansonia digitata* was fermented with the aim of producing additional plant protein food and feed. The seed was subjected to natural fermentation for 120 hours under laboratory condition. Nine moulds and two yeasts were isolated and characterized macroscopically and microscopically as *Aspergillus niger, Aspergillus flavus, Penicillium citrinum, Penicillium chrysogenum, Mucor racemosus, Mucor hiemalis, Rhizopus stolonifer, Alternaria tenuis, Scopuloriopsis brevicaulis, Saccharomyces cerevisiae and Schizosaccharomyces pombe*. Spores of isolated fungi were used as starter cultures in the fermentation of the seed using solid-state fermentation method for 120 hours. The fermented products were analyzed for proximate and anti-nutrient content using standard methods. The result showed a significant increase ($p<0.05$) in crude protein, total ash and carbohydrate but decreased significantly ($p>0.05$) in crude fat and crude fibre. The anti-nutrients in form of total tannins, saponins, oxalate and phytate content were significantly decreased after fermentation. From the findings in this research, it was concluded that fermented *A. digitata* seed can serve as additional plant protein food source in food and feed formulation for livestock.

Key words: *Adansonia digitata*, anti-nutrients, nutrients, fermentation, fungi.

Introduction

The growing concern for the acute food shortages for the world's expanding population has led to the exploitation of non-conventional food sources as potential alternatives [1]. The solution to the food problem must be sought through a combination of all available sources. Due to the increasing demand for protein and energy to support the ever-increasing world population, efforts are been directed at exploring new and nonconventional sources of food that grow in the arid and semiarid land regions of the world. Food and agricultural scientists are now beginning to screen wild and under-exploited native plants for possible potential sources of food in an attempt to widen the narrow food base [2, 3]. Several reports have also indicated that lots of lesser-known native crop species are high in nutrients and could possibly relieve critical food shortages if given adequate promotion and research attention [4, 5]. However, prior to utilization of such unconventional resources, data indicating the nutrient composition and toxic factors should be available. Toxicological evaluation of possible epidemiological response to the ingestion of novel food sources and the methods of processing that will enhance their utility as food or feed ingredient are all necessary in order to achieve optimal utilization [6]. Competition between man and his livestock for food sources has been recognized as a major cause of the increase in the cost of ingredients used in compounding livestock feed. This has been recognized to account for more than 70% of the total cost of animal production thus seriously reducing the return and marginal profit [7] in the developing countries essentially in Africa and particularly in Nigeria. Several methods have been employed to improve the nutritional quality of legumes, cereals and other form of seeds, essentially fermentation. Fermentation is one of the oldest methods and widely used process of producing and preserving food on local and industrial levels [8]. Fermentation for food production may either be anaerobic or aerobic or both. Fermentation changes the characteristics of the food by the action of the enzymes produced by the fermenting microbes whether bacteria, mould or yeasts. The term solid-state fermentation (SSF) has been variously defined as the cultivation of microorganisms on solid, moist substrates in the absence of free aqueous phase [9], or cultivation of microorganisms in the presence of a liquid phase at maximal solid substrate concentrations or on inert carriers supporting moist substrate [10]. It has several advantages over liquid state or submerged fermentation. *Adansonia digitata*, the baobab tree, is a member of the Bombacaceae family, which consists of around 20 genera and around 180 species [11]. Authors [12] reported that the acceptability and optimal utilization of *A. digitata* seed as a protein source is limited by the presence of inherent anti-nutrients such as protease inhibitors, tannins, phytic acid and amylase inhibitors. Phytochemicals (anti-nutrients) have been reported to be toxic at 5 g per serving [13]. The acceptability and optimal utilization of *A. digitata* seed as a protein source has been reported to be limited by the presence of anti-nutritional factors such as trypsin inhibitors, protease inhibitors, tannins, phytic acid, oxalate, alkaloids, phytate and amylase inhibitors [14, 15, 16]. [17] suggested that though processing techniques may rob a food item of some nutrients, processing systems may also enhance food nutritional quality by reducing or destroying the anti-nutrients present. The aim of this work therefore, is to evaluate the proximate and antinutrient content of *A. digitata* seed fermented with mono-culture fungi under

solid state techniques with a view to determining their nutritive potentials.

Materials and Methods

Collection and authentication of seed

Mature, dried *A. digitata* pods were collected from the premises of University of Ilorin, Ilorin, Kwara State, Nigeria was authenticated at the Department of Plant Biology with voucher number of *Adansonia digitata* (UIH 1048).

Preparation of seed

The pods were cracked manually to release the seeds. The seeds ebbed in pulp were washed with plenty of clean water to remove the pulp before drying to remove the wetness. The seeds were pulverized with an electrical grinder to rough particles sizes of about 2 mm in diameter, thereafter they were stored in airtight container for further use.

Isolation of organisms from naturally fermented *A. digitata* seed

The pulverized seeds were subjected to natural fermentation as follows. Precisely 250 g of the pulverized seeds were mixed with 250 ml of sterile distilled water in plastic fermentors of two litre capacities. The mixtures were stirred properly until a uniform mash was obtained, covered and allowed to ferment at room temperature (28±2 ^{o}C) in the laboratory for seven days [18, 19]. Fungi were isolated from the naturally fermented seeds through serial dilution and pour plate method using Potato- Dextrose agar into which 10 % Streptomycin has been added to inhibit bacteria growth. Culturing was done in duplicates.

Identification of the Isolates

The fungi isolated from the fermenting mixture were sub cultured until pure isolates were obtained. Morphological and microscopical analyses for the identification of the isolates were carried out and result obtained compared with literature to identify the organisms as described by [20]. Pure cultures of the fungal isolates were preserved on agar slant at 4 ^{o}C for further use.

Fungal spore preparation and monoculture fermentation of seed

Fungal spore suspension of actively growing mid log phase culture of the fungal isolates were prepared according to the method described by [21]. An agar slant of four days old pure culture of each of the organisms was used. Sterile distilled water (10 ml) was added to the slant and shook well to wash the spores. The spore suspension was counted using the Neubauer counting chamber. A spore suspension of about $5x10^4$ spore/ml was used in each case for inoculation. Twenty grams (20 g) of the seed samples were measured separately into 250 ml Erlenmeyer flasks, plugged with cotton wool, wrapped with aluminium foil and sterilized in the autoclave at 121 ^{o}C for 15minutes. The sterile samples were mixed with 20ml of sterile distilled water and stirred properly until uniform mashes were obtained in each case. Two millilitre (2 ml) from each of the monoculture suspension was used as fermentation starter to inoculate each of the samples in the fermentors. The mixtures were allowed to ferment for 120 hours at room or ambient temperature (28±2^{o}C) [18, 19]. Fermented samples were taken daily, dried at 60 ^{o}C in the oven for 4hours to safe moisture content and used for analysis of proximate (moisture content, crude fibre, crude protein, crude fat, ash content, carbohydrate and glucose), phytochemical (saponins, tannins, phytates and oxalates) analysis were done on fermented products after 120 hours.

Determination of proximate content of the seed

The crude protein content of the samples was determined following the Kjeldahl method.

The moisture content, total ash, crude fibre, crude fat (Soxhlet extraction

method) content of the seed were determined following [22]. The total carbohydrate or Nitrogen Free Extract (NFE) was determined by the difference method.The reducing sugar content of the seed wasdetermined quantitatively by the 3,5- Dinitrosalicylic Acid (DNSA) as described by [22]. The water holding capacity of the seed was determined according to the method described by [23].

Determination of the some anti-nutritional factors in the seed

The antinutrient content of the seed was determined quantitatively. Total saponins was determined by the method of [24], oxalates by the method of [25], total soluble tannin by the method described by [26] and the phytate content was determined by the method of [27].

3. Results

Isolation of Fungi

Nine moulds were isolated and identified during fermentation as *Aspergillus niger, Aspergillus flavus, Penicillium chrysogenum, Penicillium citrinum, Rhizopus stolonifer, Mucor racemosus, Mucor hiemalis, Alternaria tenuis, Scopulariopsis brevicaulis, Saccharomyces cervisiae and Schizosaccharomyces pombe* (Table 1).

Proximate content of fermented products

The effect of monoculture fungal fermentation on *A. digitata* seed at ambient temperature for 72 hours is presented in Table 2. Fermentation with each of the fungus has various effects on the proximate composition of the seed. The results obtained are significantly ($p<0.05$) different from the unfermented sample. Crude protein, carbohydrate and total ash were significantly ($p<0.05$) increased after fermentation while the crude fat and crude fibre were significantly ($p<0.05$) decreased. The highest increase in protein (31.29%) was recorded in sample fermented with *Penicillium citrinum* while the lowest (28.80%) was recorded in sample fermented with *Mucor racemosus* (Table 2).

Reducing sugar (glucose) content of fermented seed

The effect of fermentation on the reducing sugar content of monoculture-fermented seed is as presented in Figure 1. The value of reducing sugar increased significantly ($p<0.05$) in all the fermented products compared to the unfermented sample. The highest increase (16.5 mg/g) was recorded in sample fermented with *Shizosaccharomyces pombe* while the value was insignificantly reduced ($p<0.05$) to 2.5mg/g recorded in sample fermented with *Rhizopus stolonifer.*

Effect of monoculture fermentation on some anti-nutrients in the fermented seed.

The effect of monoculture fermentation on saponins, oxalates, tannins and phytates content of *A. digitata* seed is presented in Figures 2 to 5. The values of the phytochemicals significantly ($p<0.05$) reduce after fermentation.

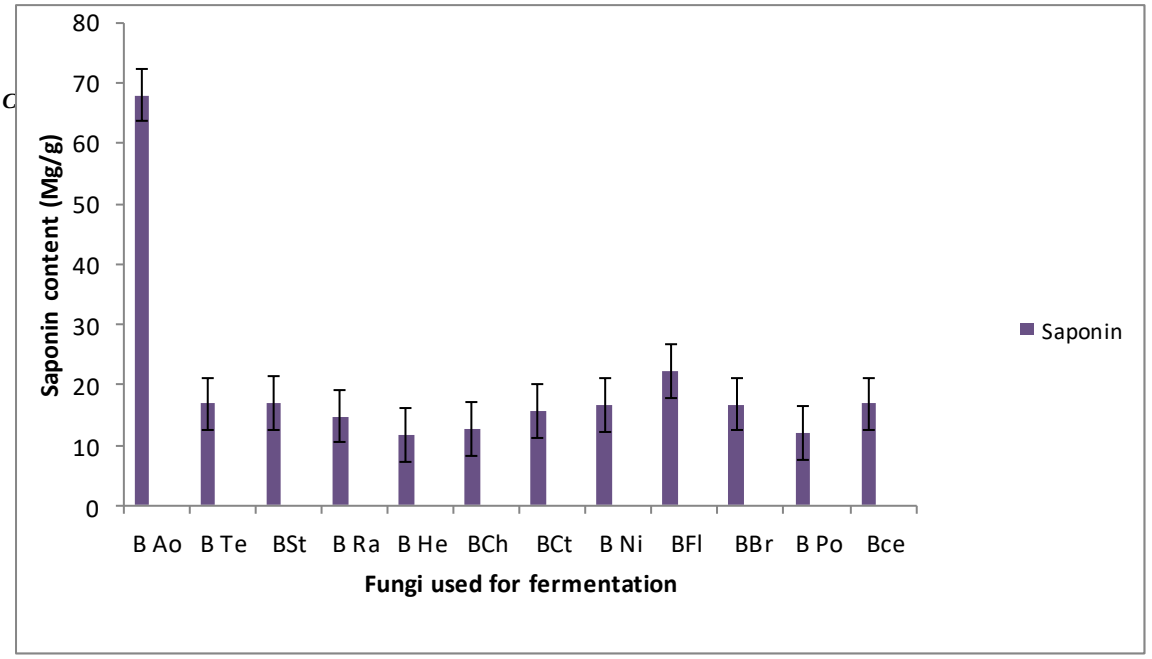
Macroscopy and microscopy description of isolates	Fungal isolates
Filaments were white fast spreading with brown-black spores at the tip	*Aspergillus niger*
Colonies were greenish yellow with rough edges, flat and fast spreading	*Aspergillus flavus*
The filaments were bluish green or pale with broad white margin	*Penicillium chrysogenum*
Filaments were whitish on top but leathery and orange yellow underneath	*Penicillium citrinum*
Colony appeared fluffy white but turned black with age	*Rhizopus stolonifer*
Filaments appeared gray, fluffy white but turn dirty white with age	*Mucor racemosus*
Mycelia were grayish or slightly yellowish turning grayish brown with age	*Mucor hiemalis*
Colony has blackish aerial mycelium; spores were dark, brown borne in chains	*Alternaria tenuis*
Colony has brownish loose hyphae carrying lemon shaped spores	*Scopulariopsis brevicaulis*
Colonies were creamy with rough edges; cells were ovoid or ellipsoidal	*Saccharomyces cervisiae*
Colony was round and smooth at the edge; cells were cylindrical	*Schizosaccharomyces pombe*

Table 1: Fungal isolates from naturally fermented pulverized *A. digitata* seed

Organisms	MC	TA	PRO	FAT	FIB	CHO
BTe	6.98±1.39^{ab}	4.32±0.22^{ab}	31.50±2.06^{c}	11.92±0.64^{a}	4.46±0.7^{ab}	40.84±0.88^{ab}
BSt	5.87±0.00^{a}	3.96±0.00^{a}	29.43±0.00^{ab}	12.57±0.00^{ab}	4.96±0.00^{ab}	43.21±0.00^{bc}
BRa	7.43±1.28^{bc}	4.45±0.40^{ab}	28.80±1.12^{ab}	11.85±0.13^{a}	4.88±0.21^{ab}	42.6±0.24^{bc}
BHe	6.29±0.05^{ab}	4.44±0.05^{ab}	28.92±0.33^{ab}	11.91±0.60^{a}	4.48±0.62^{ab}	43.98±1.55^{bc}
BCh	8.20±3.34^{c}	6.75±2.02^{b}	30.83±0.34^{bc}	11.77±0.46^{a}	3.74±1.90^{a}	38.76±7.40^{a}
BCt	8.27±3.29^{c}	6.61±2.25^{ab}	31.29±2.60^{c}	12.25±0.41^{a}	3.45±1.34^{a}	38.14±7.21^{a}
BNi	7.25±1.89^{bc}	5.52±0.97^{ab}	30.36±2.04^{bc}	13.31±0.89^{ab}	3.71±1.91^{a}	39.87±1.95^{a}
BFl	7.91±3,10^{bc}	3.89±0.74^{a}	30.60±1.68^{bc}	12.59±0.21^{ab}	3.65±1.91^{a}	41.36±3.39^{bc}
BCe	7.50±0.00^{bc}	5.00±0.00^{ab}	31.00±0.00^{c}	13.50±0.00^{ab}	3.50±0.00^{a}	40.20±0.00^{ab}
BPo	8.00±0.00^{c}	5.00±0.00^{ab}	30.00±0.00^{bc}	13.00±0.00^{ab}	3.00±0.00^{a}	40.10±0.00^{ab}
BAo	7.64±2.11^{bc}	4.39±1.61^{ab}	19.86±1.30 ^{a}	18.60±8.46^{b}	8.87±5.31^{b}	40.01±1.35^{ab}

Table 2: Proximate composition of *Adansonia digitata* fermented with mono-culture of fungal isolates.

Data are mean of two replicate ± SEM. Mean within the same row carrying different superscripts are significantly different at ($p<0.05$). (MC= moisture content, TA= total ash, CPRO= crude protein, CFAT= crude fat, CFIB= crude fibre and CHO= total carbohydrate.)
(BSt= baobab fermented with *R.stolonifer*, BTe= baobab fermented with *A. tenuis*, BRa= baobab fermented with *Mucor racemosus*, BHe= baobab fermented with *M. hiemalis*, BCh= baobab fermented with *P. chrysogenum*, BCt= baobab fermented with *P. citrinum*, BNi= baobab fermented with *A. niger*, BFl= baobab fermented with *A. flavus*, BPo= baobab fermented with *S. pombe*, BCe= baobab fermented with *S. cerevisiae* and BRo= unfermented baobab seed)



Data are mean of two replicate ± SEM.

Figure 1: Glucose content of monoculture fungal fermented *Adansonia digitata*

(BAo: unfermented baobab; BTe:Moringa fermented with *Alternaria tenuis*; BSt: baobab fermented with *Rhizopus stolonifer; B*RA: baobab fermented with *Mucor racemosus;* BHi: baobab fermented with *Mucor hiemalis;* BCh: baobab fermented with *Penicillium chrysogenum;* BCt: baobab fermented with *Penicillium citrinum;* BNi: baobab fermented with *Aspergillus niger;* BFl: baobab fermented with *Aspergillus flavus;* BBr: baobab fermented with *Scopuloriopsis brevicaulis;* BPo: baobab fermented with *Schizosaccharomyces pombe;* BCe: baobab fermented with *Saccharomyces cerevisiae*) Data are mean of two replicate ± SEM.

Organisms	MC	TA	PRO	FAT	FIB	CHO
BTe	6.98±1.39^{ab}	4.32±0.22^{ab}	31.50±2.06^{c}	11.92±0.64^{a}	4.46±0.7^{ab}	40.84±0.88^{ab}
BSt	5.87±0.00^{a}	3.96±0.00^{a}	29.43±0.00^{ab}	12.57±0.00^{ab}	4.96±0.00^{ab}	43.21±0.00^{bc}
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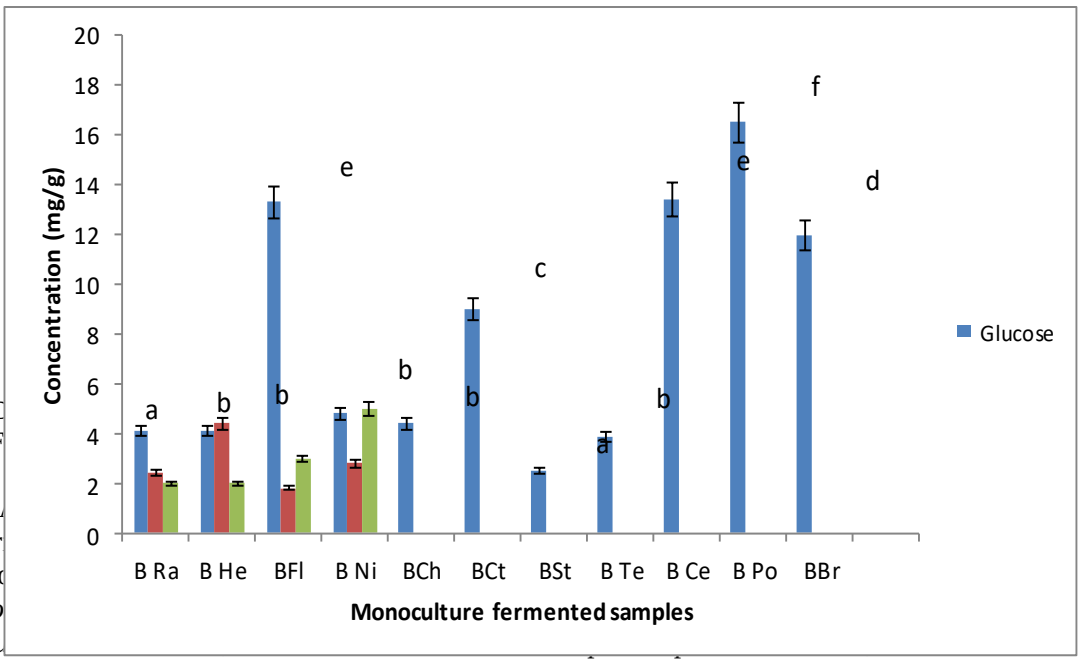
BHe	6.29±0.05^{ab}	4.44±0.05^{ab}	28.92±0.33^{ab}	11.91±0.60^{a}	4.48±0.62^{ab}	43.98±1.55^{bc}
BCh	8.20±3.34^{c}	6.75±2.02^{b}	30.83±0.34^{bc}	11.77±0.46^{a}	3.74±1.90^{a}	38.76±7.40^{a}
BCt	8.27±3.29^{c}	6.61±2.25^{ab}	31.29±2.60^{c}	12.25±0.41^{a}	3.45±1.34^{a}	38.14±7.21^{a}
BNi	7.25±1.89^{bc}	5.52±0.97^{ab}	30.36±2.04^{bc}	13.31±0.89^{ab}	3.71±1.91^{a}	39.87±1.95^{a}
BFl	7.91±3,10^{bc}	3.89±0.74^{a}	30.60±1.68^{bc}	12.59±0.21^{ab}	3.65±1.91^{a}	41.36±3.39^{bc}
BCe	7.50±0.00^{bc}	5.00±0.00^{ab}	31.00±0.00^{c}	13.50±0.00^{ab}	3.50±0.00^{a}	40.20±0.00^{ab}
BPo	8.00±0.00^{c}	5.00±0.00^{ab}	30.00±0.00^{bc}	13.00±0.00^{ab}	3.00±0.00^{a}	40.10±0.00^{ab}
BAo	7.64±2.11^{bc}	4.39±1.61^{ab}	19.86±1.30 ^{a}	18.60±8.46^{b}	8.87±5.31^{b}	40.01±1.35^{ab}

Table 2: Proximate composition of *Adansonia digitata* fermented with mono-culture of fungal isolates.

Data are mean of two replicate ± SEM. Mean within the same row carrying different superscripts are significantly different at ($p<0.05$). (MC= moisture content, TA= total ash, CPRO= crude protein, CFAT= crude fat, CFIB= crude fibre and CHO= total carbohydrate.)
(BSt= baobab fermented with *R.stolonifer*, BTe= baobab fermented with *A. tenuis*, BRa= baobab fermented with *Mucor racemosus*, BHe= baobab fermented with *M. hiemalis*, BCh= baobab fermented with *P. chrysogenum*, BCt= baobab fermented with *P. citrinum*, BNi= baobab fermented with *A. niger*, BFl= baobab fermented with *A. flavus*, BPo= baobab fermented with *S. pombe*, BCe= baobab fermented with *S. cerevisiae* and BRo= unfermented baobab seed).



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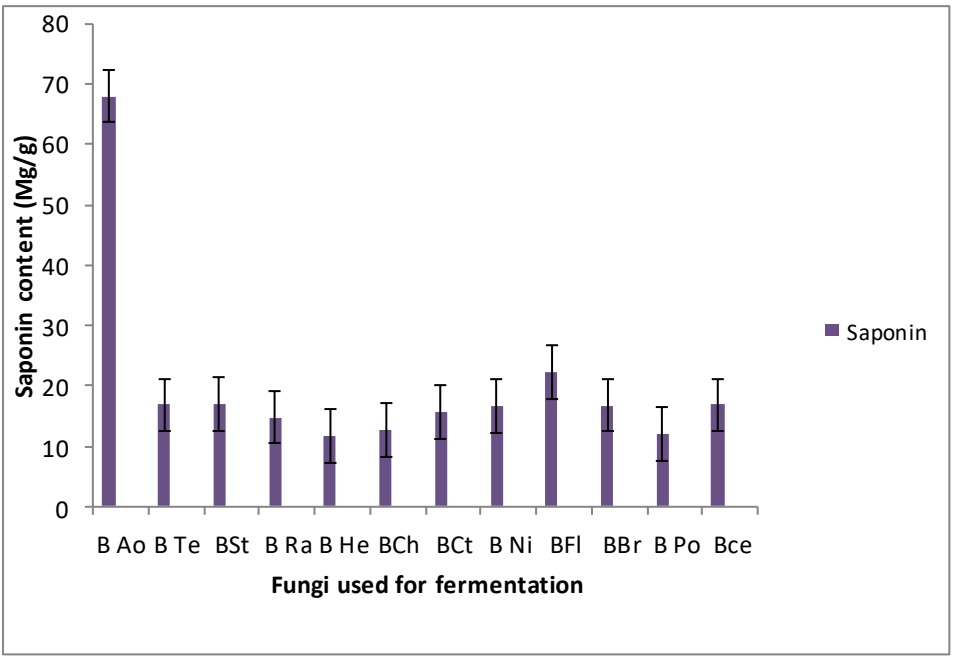
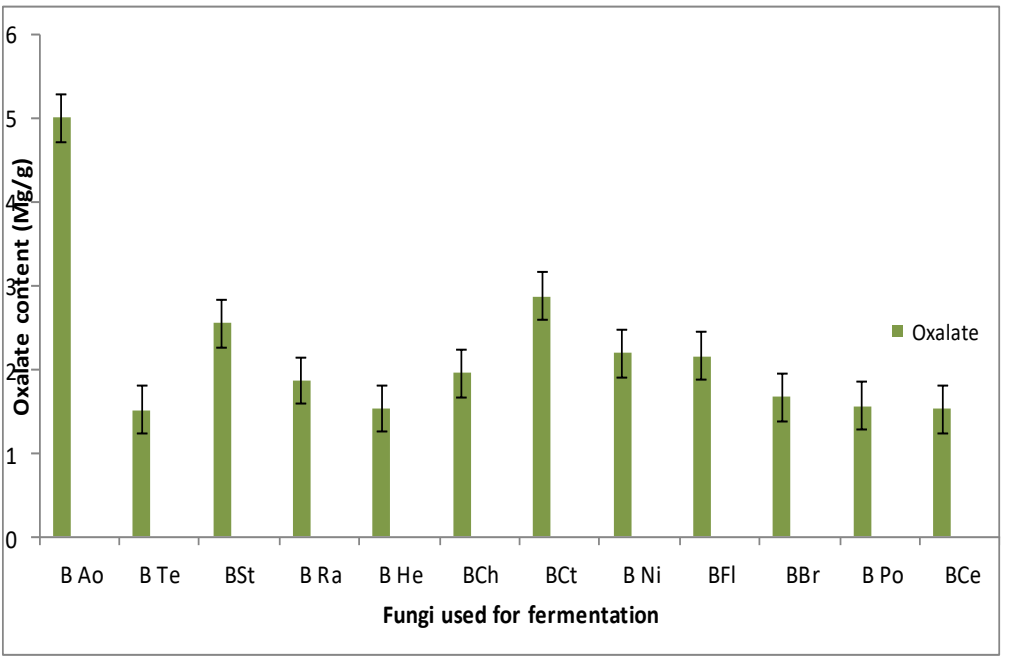
fermented with *Mucor hiemalis;* BCh: baobab fermented with *Penicillium chrysogenum;* BCt: baobab fermented with *Schizosaccharomyces pombe;* BCe: baobab fermented with *Saccharomyces cerevisiae*)

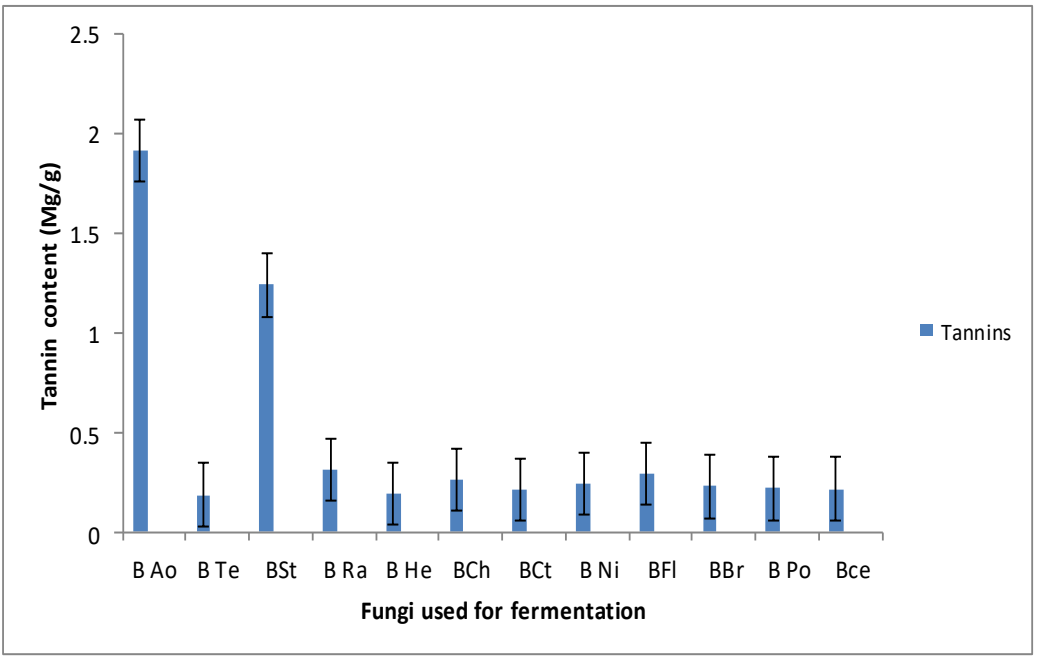


(BAo: unfermented baobab; BTe:Moringa fermented with *Alternaria tenuis*; BSt: baobab fermented with *Rhizopus stolonifer; B*RA: baobab fermented with *Mucor racemosus;* BHi: baobab fermented with *Mucor hiemalis;* BCh: baobab fermented with *Penicillium chrysogenum;* BCt: baobab fermented with *Penicillium citrinum;* BNi: baobab fermented with *Aspergillus niger;* BFl: baobab fermented with *Aspergillus flavus;* BBr: baobab fermented with *Scopuloriopsis brevicaulis;* BPo: baobab fermented with *Schizosaccharomyces pombe;* BCe: baobab fermented with *Saccharomyces cerevisiae*)



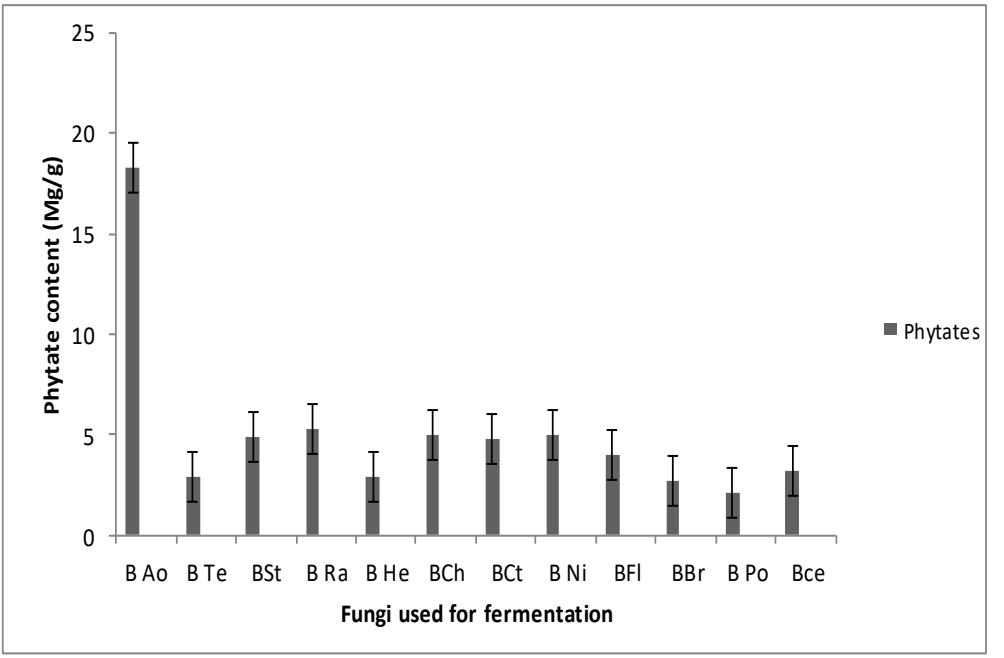
Data are means of two replicates ± SEM.
Figure 3: Tanin content of mono-culture fungal fermented *Adansonia digitata*

(BRO: unfermented baobab; BTe:Moringa fermented with *Alternaria tenuis*; BSt: baobab fermented with *Rhizopus stolonifer; B*RA: baobab fermented with *Mucor racemosus;* BHi: baobab fermented with *Mucor hiemalis;* BCh: baobab fermented with *Penicillium chrysogenum;* BCt: baobab fermented with *Penicillium citrinum;* BNi: baobab fermented with *Aspergillus niger;* BFl: baobab fermented with *Aspergillus flavus;* BBr: baobab fermented with *Scopuloriopsis brevicaulis;* BPo: baobab fermented with *Schizosaccharomyces pombe;* BCe: baobab fermented with *Saccharomyces cerevisiae*)



Data are means of two replicates ± SEM.
Figure 4: Tanin content of mono-culture fungal fermented *Adansonia digitata*

(BAo: unfermented baobab; BTe:Moringa fermented with *Alternaria tenuis*; BSt: baobab fermented with *Rhizopus stolonifer; B*RA: baobab fermented with *Mucor racemosus;* BHi: baobab fermented with *Mucor hiemalis;* BCh: baobab fermented with *Penicillium chrysogenum;* BCt: baobab fermented with *Penicillium citrinum;* BNi: baobab fermented with *Aspergillus niger;* BFl: baobab fermented with *Aspergillus flavus;* BBr: baobab fermented with *Scopuloriopsis brevicaulis;* BPo: baobab fermented with *Schizosaccharomyces pombe;* BCe: baobab fermented with *Saccharomyces cerevisiae*)



Data are means of two replicates ± SEM.

Figure 5: Phytate content of mono-culture fungal fermented *Adansonia digitata*

(BAo: unfermented baobab; BTe:Moringa fermented with *Alternaria tenuis*; BSt: baobab fermented with *Rhizopus stolonifer; B*RA: baobab fermented with *Mucor racemosus;* BHi: baobab fermented with *Mucor hiemalis;* BCh: baobab fermented with *Penicillium chrysogenum;* BCt: baobab fermented with *Penicillium citrinum;* BNi: baobab fermented with *Aspergillus niger;* BFl: baobab fermented with *Aspergillus flavus;* BBr: baobab fermented with *Scopuloriopsis brevicaulis;* BPo: baobab fermented with *Schizosaccharomyces pombe;* BCe: baobab fermented with *Saccharomyces cerevisiae*)

Discussion

The valuable nutrients in the fermented seed such as crude protein, mineral ash and total carbohydrate were increased after fermentation. This improvement can be attributed to the amylolytic and proteolytic activities of the fungi involved during growth in the process of fermentation. These enzymes were involved in breaking down complex organic molecules into simpler ones such as glucose and amino acids. The product of the enzymes is also utilized by the organisms for their own metabolic activities [28]. Moreover the noticeable increase in crude protein in the mono-culture fermented seed could also be attributed to the addition of mycoprotein (single cell protein), non-protein nitrogen amide and nucleic acid synthesized by fungal cells during growth [19]. The improvement observed could be due to the breaking down of the complex organic carbon compound in the seed hence the increase in glucose and decrease in crude fiber and fat content [29]. However the

available carbohydrate and lipid in the fermented seed was still sufficient to meet the daily requirement for animal feed, also they are now in a readily accessible form upon consumption by animals when incorporated into food and feeds items. The increase in carbohydrate content may also be attributed to the reduction in the crude fibre content. During fermentation polysaccharides including cellulose, pectin, lignocellulose and starch are broken down by microorganisms thereby reducing the fibre content of such seed. This result was in contrast to that obtained by earlier author on carbohydrate content of fermented *A. digitata* seed [30, 31] but was similar to that obtained by [19, 32, 33] on effect of fermentation on *Mangifera indica* and pigeon pea seed. The improvement in total ash recorded can be attributed to the breakdown of organic complexes like protein tannin complex or calcium oxalate complex in the seed to release the minerals into biologically available form [34]. The inherent antinutrients content was lowered significantly ($p<0.05$) after fermentation. The decrease in tannin, saponins, oxalates and phytate content of *A. digitata* seed could be due to degradation by microbial enzymes that are secreted by the fermenting fungi during growth and metabolism in the process of [35, 31]. These antinutrients are known to occur in complex compounds (protein-tanin complexes, calcium- oxalate complex, phytate and saponin protein complexes and other forms of complexes) in plants where they occur. The formation of these complexes binds protein and minerals in the seed and renders them biologically not available to plants after consumption. The breaking down of these complexes by various amylolytic, proteolytic and lipolytic enzymes produced by these fungi probably led to the reduction in anti-nutrients. Tannins are known to reduce the availability of proteins, carbohydrates and minerals by forming indigestible complexes with the nutrients. The reduction in tannin level due to fermentation could improve the availability of nutrients in the seed [36, 37, 31]. However, this result is contrary to the report of [30] on tannin content after fermentation of *A. digitata* seed. Fermentation has been reported as a means to improve the nutritional quality and drastically reduced anti-nutritional factors to safe level because the process produces enzymes that break down protein-tannin complexes to release free tannins [36]. Similarly the reduction in phytic acid is attributable to increase in the activities of phytase during fermentation. According to earlier report, the series of enzymatic actions terminating with the formation of inositol and phosphoric acid releases certain metals to increase their availability and caused subsequent decrease in phytate and increase in total ash [31, 38]. Moreover, reduction of phytic acid content of some plant products after processing has been reported [39, 40]. Phytic acid has been reported to form complexes with proteins (protein-phytate complex) [41] and chelates essential dietary minerals such as iron, zinc, calcium and magnesium, thus decreasing their utilization.

Furthermore, saponins has been shown to affect animal nutrition, performance and metabolism through erythrocyte haemolysis, depression of growth rate, bloat (ruminants), inhibition of smooth muscle activity, alter cell wall permeability and therefore produce some toxic effects when ingested, cause enzyme inhibition and reduction in nutrient absorption if it occurs beyond certain level in feed. [42]. Saponin content of *A. digitata* seed was significantly ($p<0.05$) decreased after fermentation. The finding was similar the report of [43, 38].

High occurrence of oxalates in feeds, has been previously shown to bind minerals like calcium and magnesium and interfere with their metabolism, cause muscular weakness and paralysis, gastrointestinal tract irritation, blockage of the renal tubules by calcium oxalate crystals, development of urinary calculi and hypocalcaemia [44]. Oxalates (22 fermented *A. digitata* seeds reduced significantly after fermentation with the isolated fungi. Fermentation and other methods of processing have been reported to decrease the oxalate content of food [45]. [19] however reported an increase in oxalate content of mango kernel cake fermented by *A. niger*.

Conclusion

Monoculture fungal fermentation has desirable effects on the seed of *A. digitata*. Fermented seed could therefore be considered as an alternative, supplement or additional protein source for propounding animal feed. This will tremendously reduce the cost of feeding in animal production and also reduce the competition between man and animal for the available staple food. However only fungi without history of poisoning or production of harmful metabolites should be employed for the fermentation.

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Marker Assisted Foreground Selection for Identification of Striga Resistant Backcross Lines in Sorghum bicolor

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Abstract: Striga is a major constraint to sorghum production causing high yield loss due to increasing infestation. Locally-adapted cultivars with resistant genes/QTLs could be an effective control strategy for Striga. Marker-Assisted Foreground Selection was used to select backcross lines possessing Striga resistance QTLs from N13. Marker polymorphism was conducted for the donor parent N13 and 10 recurrent parents using 10 Simple Sequence Repeat (SSR) markers. Recurrent parents with SSR alleles, polymorphic to the donor parent allele were selected. F1 lines were developed by making a cross between the selected recurrent parent and the donor. The F1 were confirmed for heterozygosity using SSR markers. Selected heterozygote F1s were backcrossed to their recurrent parent to develop backcross populations (BC1F1 and BC2F1). BC1F1 and BC2F1 populations were genotyped using SSR markers flanking the Striga resistant QTLs in N13. Forty two DANYANA-N13 BC2F1 lines (with 4 QTLs in 3 lines, 3 QTLs in 10 lines and other 28 lines having 1 to 2 QTLs) were selected for the presence of N13 QTLs. Forty three SAMSORG39-N13 BC2F1 lines (with 3 QTLs in 2 lines while 41 lines had 1 to 2 QTLs) were also selected for the presence of N13 QTLs. Although, selected lines will be genotyped for the recovery of recurrent parent background and evaluated to identify elite genotypes for possible release as varieties, the successful introgression of Striga resistance QTLs using Marker Assisted Selection suggests that in developing superior sorghum varieties, breeders could make use of molecular marker technologies to speed up breeding programmes.

Keywords: Backcross, Marker Assisted Selection, Striga, Sorghum, Simple Sequence Repeats (SSR).

1. Introduction

Sorghum is grown in the savanna and the semi-arid regions of Nigeria and it is daily used as staple food by millions of people in this region and also in other parts of the nation [1]. Foods produced from sorghum grains possesses high nutritive value and are also high in energy as such, they are recommended for infants, pregnant and lactating mothers, the aged and sick individuals [2]. Thus, the production of sorghum in Nigeria has a significant benefit to Nigerian farmers and also important for national food security. However, the production of sorghum is hampered with lots of biotic and abiotic constraints. A major biotic constraint with high economic importance is Striga hermonthica. This weed which attaches itself and penetrates the root of its host for water and nutrient is known to cause up to a 100% yield loss [3] under highly endemic situation and affects the majority of farmers in the northern part of the country. The issue of Striga is becoming more prevalent mostly in field where wild sorghum is found and where continuous mono-cropping is practiced. It is also getting more serious as a result of erratic rainfall pattern, low soil fertility in regions where farming is on the high side and agricultural input is low thereby affecting resource-poor subsistence farmers [4, 5].

Due to the intrinsic host-parasite interaction, the huge amount of seed produce and the length of seed viability in soil, Striga problem have become more complex over the years [6]. However, there are various measures that have been put in place to combat the scourge of Striga. Despite all efforts, these measures in most cases have not been economically possible or successful [7]. It has thus been accepted that a most durable and accessible technology would be an integrated management strategies with host plant resistance as the focal point. Though Striga resistant sorghum varieties could contribute to the reduction in reproductive activity of Striga and to the number of seeds in the soil, these varieties are not always adapted to the local environment and are also not superior varieties. Thus, an important aspect on the integrated management strategy is the use of resistant, farmer-preferred varieties [8]. To fight the scourge of Striga through integrated strategy of using resistant farmer preferred varieties with no environmental influence on selection, molecular marker based selection technique must be employed.

According to Collard et al., [9], marker assisted or marker based selection (MAS), involves the use of molecular markers to select individual plants possessing genomic regions which influence the expression of certain traits of interest. With MAS, the major goal of crop improvement can be enhanced when dealing with important traits that are difficult to study due to environmental influences [10]. As compared with conventional plant breeding methods, same breeding progress that will be achieved over a long period will be achieved within a short period of time when MAS is employed [11]. Thus, at early stage of a plant, without the loss of identity, traits can be transferred into preferred plant varieties, gene pyramiding can also be achieved and the efficiency of breeding can be increased [12, 13]. Significant headway has been made in the development of molecular markers and in the use of these makers in detecting QTLs [8] and tagging of genes [14] influencing Striga resistance in sorghum. Consequently, those molecular markers that are tightly linked with Striga resistance are thus being used to increase the value of conventional breeding through indirect selection of plants which possess the desirable gene in segregating populations [15].

The transfer of these QTLs/genes into elite sorghum background through marker assisted backcrossing (MABC) will provide a solid foundation to improve Striga resistance in farmers preferred lines. Allard

[16], describes back crossing (BC) as a breeding technique which is used to introduce one or a few genes into an adapted variety. With the use of marker assisted back crossing (MABC) tools, lines which will be used as parents in next generation are selected [17]. This is made possible with the aid of molecular markers that are closely linked or flanking to already detected and validated QTLs.

The objective of this research activity therefore is to (i) develop backcross populations from crosses involving recurrent and donor parent (ii) conduct foreground selection for presence of Striga resistant QTLs using reported SSR markers.

II. Materials and Methods

A. Plant materials for generation of backcrosses

N13 sorghum line with 5 Striga resistance QTLs [8] obtained from ICRISAT, Mali, was used as donor parent, for introgression of resistance QTLs into farmers' sorghum varieties. Farmers' varieties, which included SAMSORG17, SAMSORG40, SAMSORG43, SAMSORG14, SAMSORG39, SAMSORG41, DANYANA, CRS-01 and CRS-02, were obtained from the Institute for Agricultural Research (IAR), Samaru, Nigeria. All these were used at the initial stage for the development of F1s. Due to the polymorphism result; this was then narrowed down to SAMSORG39 and DANYANA as recurrent parents for the transfer of N13 alleles.

B. Development of Backcross population

All the accessed genotypes were selfed to ensure the use of pure lines in the crossing program. The selfed seeds were planted out on the crossing block at IAR, Samaru and N13 was used as pollen parent on all the farmers' varieties to produce F1 seeds. The F1 seeds were sown under irrigated environment at the National Center for Genetic Resources and Biotechnology (NACGRAB). DNA was extracted from individual F1 plants at the molecular biology laboratory of NACGRAB, Nigeria and was shipped to ICRISAT, India. The DNA samples were screened using SSR markers for F1 hybridity confirmation. Selected F1 were then used as pollen parents on their respective recurrent parent for development of backcross populations (BC1F1 and BC2F1).

C. Genotyping of backcross (BC1F1 and BC2F1) population

At every backcross stage, DNA was extracted from the population using CTAB protocol and samples were shipped to ICRISAT, India for genotyping. Twenty five SSR markers for N13 QTLs were selected and used for genotyping and only those markers which revealed polymorphism between the donor parent (N13) and the respective recurrent parents were used in selecting lines with the N13 Striga resistant QTLs.

Polymerase Chain Reaction (PCR) for amplification of regions of interest was done on the Gene Amp PCR systems 9600 (PE-Applied Biosystems). After PCR reaction process, a few of the amplicons from each SSR marker were randomly selected to confirm proper amplification and product concentration. After confirming the primers amplification on agarose gel electrophoresis, primers with good amplification profile were selected for subsequent PCR analysis on the entire population (BC1F1 and BC2F1). Samples with good amplification were subjected to capillary electrophoresis to determine their sizes via fragment analysis by using the ABI 3730xl DNA analyzer which is a fluorescent fragment detection system. Sizing of the PCR fragments and allele scoring was carried out by using the Gene-Mapper version 4.0 software.

Results

Confirmation of F1 generations

Eight different crosses were made using N13 as donor with 8 farmers preferred

varieties (SAMSORG17, SAMSORG40, SAMSORG43, SAMSORG14, SAMSORG39, SAMSORG41, CRS-01, CRS-02 and DANYANA) obtained from IAR, Zaria. For each cross, 10 F1s lines were produced and were genotyped to confirm heterozygosity with 10 SSRs marker of which 6 were polymorphic between the parental lines. Out of the 80 F1 lines screened, 6 were selected and only two were advanced to the next generation (Table 1) to reduce the amount of crosses and for easy handling and tracking.

Foreground selection of BC1F1 progenies

Foreground selection was carried out to identify the BC1F1 lines having the Striga QTLs from N13 using polymorphic markers that were tightly linked to the respective QTL.

Out of the 49 BC1F1 plants were genotyped for the N13 Striga resistant QTLs, only 9 were found to be having one to two QTLs introgressed. These 9 were heterozygous for the introgressed QTLs on chromosomes 1, 2 and 5. Four lines had only one QTL introgressed. The other five were heterozygous for two QTLs each from chromosomes 2 and 5. Out of the 9 selected lines, only 3 were advanced to BC2F1 (Table 2).

Foreground selection of BC2F1 progenies

A total of 143 lines were genotyped of which 71 were for the cross between N13 and SAMSORG39 while 73 were for the cross between N13 and DANYANA. A total of 43 BC2F1 plants were selected with about 1 to 3 QTLs being introgressed for the cross between N13 and SAMSORG39. While for the cross between N13 and DANYANA, 42 BC2F1 plants were selected with about 1 to 4 QTLs being introgressed. Result of marker assisted foreground selection is summarized in Table 2.

Table 1: List of F1 advanced to BC1F1

Crosses	F1 Genotypec	Hybrid	Hybrids advanced t BC1F1
Samsorg39 x N13	10	4	1
Danyana x N13	10	2	1

TABLE 2: Summary of Marker assisted foreground selection

Crosses	F1 hybrid advanced t BC1F1	BC1F1 plants genotyped	BC1F1 advanced to BC2F1	BC2F1 selected
SAMSORG39 : N13	1	35	1	43
DANYANA : N13	1	14	2	42

III Discussion

Backcross breeding method is basically used to transfer favorable alleles from a donor genotype, which has mostly poor agronomic properties, into a recipient elite genotype [16].

There is the possibility of accelerating the process of QTLs or genes introgression and also recovering of the recurrent parent genome when selection is made via the use of markers flanking or linked to the QTLs and also uniformly spaced markers from

other chromosomes of the recurrent parent [9].

In this research work foreground selection was made, and such kind of selection is made only for the marker alleles of the donor parent mainly at the target locus so as to ensure that the target locus is in heterozygous state till the last backcross is done. At the end of the backcross program, the selected plants are then selfed and those with homozygous donor parent alleles for the selected markers are then harvested for further evaluation and release.

In N13, the target regions used for foreground selection were basically 5 stable QTLs for the area under Striga number progress curve (AUSNPC) which was common to 2 sets of recombinant inbred line populations derived from N13 [8]. These 5 QTLs were located on linkage group SBI-01, SBI-02, SBI-05(two QTLs) and SBI-06. The selected QTLs explain approximately 11- 45% of the phenotypic variation. Thus SSRs markers flanking these QTLs were used for foreground selection at each backcross generation. The use of tightly linked flanking markers ensures an effective foreground selection. As such, there is a high selection reliability when flanking markers are used. Also, there is the reduction of linkage drag when markers that flank a target gene is used [18].

Using the marker assisted backcrossing foreground selection method, we were able to introgress genomic regions from the popular Striga resistant donor parent N13 into the genetic backgrounds of elite farmers' varieties SAMSORG39 and DANYANA. According to Morris et al., [19], marker assisted selection has the possibility of tremendously reducing the time needed for selecting desirable genotypes possessing traits of interest.

At BC1F1, only nine plants were selected with the N13 QTLs alleles of which two were from SAMSORG39 with just one QTL introgressed (SBI-01-1(QTL1)) and the other 7 were from the recurrent parent DANYANA with 2 QTLs on linkage group SBI-02-1 and SBI-05-2 introgressed into 5 of the lines. The selection of very few lines with only one to two QTLs introgressed could be attributed to the extremely low DNA quality due to degradation in transit as a result of long distance between Nigeria and India. This resulted in some of the samples being completely lost and most of the selected SSRs markers not being able to amplify the samples. Although for a PCR analysis with SSR markers, the DNA requirement in terms of quantity is small and in the terms of quality is medium [17] but in this case it was not applicable. With good quality DNA of high absorbance ratio from 1.8 to 2.0%, [20] genotyped and reported 12 BC1F1 lines with one to three Striga resistant QTLs introgressed.

Taking a look at the number of lines with the introgressed Striga resistant QTLs, for the cross between N13 and DANYANA, only 14 BC1F1 plants were genotyped out of which 7 were selected with 5 having 2 QTLs introgressed and two of the selected lines having one QTL introgressed. This result is somewhat really encouraging because if the population size would have been increased to the required, then there is the possibility that more QTLs would have been captured. This is almost similar to what Mehtre, [21] got in his research. Out of thirty BC1Fl plants in a backcross population, he reported about 14 heterozygous plants detected by 11 SSR primer pairs that flanked four shoot fly QTLs. Also in another BC1F1 backcross population, out of thirty plants screened at seedling stage with 11 SSR marker loci linked with shoot fly resistance traits, Nineteen BC1F1 plants that were heterozygous for one or more targeted QTLs were identified [21].

A number of authors have reported varying population sizes ranging from hundreds to thousands that are ideal for a 99%

probability of introgressing about 1 to 5 QTLs at any backcross generation [22, 23, 24]. Which was actually not visible in the case of the cross between N13 and DANYANA due to less seed production and also poor germination. Out the 159 BC1F1 lines genotyped with 2 markers used in the monitoring of each QTL in the research of Niyibigira et al.,[20], 12 lines were selected as having the Striga resistant QTLs introgressed, one of the selected 12 was seen to be heterozygous for 3 QTLs, 8 were heterozygous for 2 QTLs while the other 3 were heterozygous for one QTL. In the case of the cross between N13 and DANYANA with only 14 BC1F1 lines genotyped, about 4 or more markers were used to monitor each of the QTLs. Although most of the markers did not amplify the DNA samples due to the level of degradation. However, for the QTL on SBI-02-1, 4 of the markers used had a successful amplification which also aided the selection of the 5 lines with 2 introgressed QTLs. Looking at the cross between N13 and SAMSORG39, out of 35 BC1F1 lines that were genotyped, only 1 was advanced with just one QTL from SBI-01. According to Collard et al., [9], a single QTL selected for, through marker assisted selection is very useful in plant breeding as long as such QTL is stable over varying environments and accounts for the largest quota of phenotypic variance for the trait.

At BC2F1, 42 lines of DAYANA had 1 to 4 N13 Striga resistant QTLs introgressed with 4 QTLs being introgressed into 3 lines, 3 QTLs being introgressed into 10 lines and others with 1 to 2 QTLs. While 43 lines of SAMSORG39 had 1 to 3 QTLs introgressed at BC2F1 also with 3 QTLs being introgressed into 2 lines and others with 1 to 2 QTLs being introgressed. Similar result has been reported by Mehtre [21] in his research in which he genotyped 224 BC2Fl plants obtained from five backcross populations with 10 SSR marker loci associated to QTLs influencing shoot fly resistance traits in four linkage groups. Out of the 224, about 100 plants were heterozygous for one or more targeted QTL. In a computer simulation in which tomato was used as a model, Tanksley et al [25], explained that it is very possible to recover the genome of the recurrent parent in two generations for every best plant selected out of the total of 30 plants per generation. Thus, if a background selection is made, there is the possibility that out of the selected BC2F1 plants, there are those with high percentage of the recurrent parent genome.

IV. Conclusion

The results of this study has proven the possibility of using a popular donor germplasm to improve locally adapted lines via marker-assisted backcrossing.

One up to four Striga resistances QTL were introgressed successfully in the SAMSORG39 and DANYANA sorghum variety grown in Nigeria. Nonetheless, it is very important to conduct a background selection for the recovery of the recurrent parent genome. Also, more advanced backcross, if possible up to BC3F1, is needed in order to stabilize the Striga resistance QTL. The introgressed lines are expected to be useful for improving sorghum productivity in the Striga prone areas of Nigeria if the introgressed resistance QTLs are actually effective against S. hermonthica populations in those regions.

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Mixed-Culture Fungal Fermentation for Protease and Amylase Production from Adansonia Digitata Seed Through Solid State Technique

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Abstract: Production of amylase from the seed of A. digitata by mixed-culture fungal fermentation was examined. Six fungi were isolated from naturally fermented A. digitata seed in the laboratory and identified as Mucor racemosus, Mucor hiemalis, Penicillium citrinum, Penicillium chrysogenum, Alternaria tenuis and Rhizopus stolonifer. Spores obtained from the fungi were randomly mixed for protease and amylase production using solid-state technique and the enzyme activity was monitor for 120 hours incubation period. The highest protease and amylase activity was obtained at 72 hours (100.8 and 53.60 µmol/mg respectively) by a mixed-culture of P. citrinum, R. stolonifera, A. tenuis and M. racemosus. While the lowest activity (22.2 and 4.0 Umol/mg) was obtained at 120 and 24 hours of fermentation by a mixed-culture of A. tenuis, M. racemosus, M. hiemalis and M. racemosus respectively. The optimum pH for amylase production was pH 5.5 at 28 ± 2 oC. This study revealed that mixed-culture of these fungi especially A. tenuis and M. racemosus were able to produce amylase, hence may be considered as a source for the production of industrial amylase from A. ditata seed.

Keywords: amylase, mixed-culture, enzyme activity, A. digitata, fermentation.

Introduction

Mixed-culture fermentation refers to a process in which the inoculums always consist of two or more organisms [1]. It may also be a case of fermentation under natural or uncontrolled environment. The cultures can consist of known species to the exclusion of all others, or they may be composed of mixtures of unknown species. Mixed-cultures may be of one microbial

group that is, consists of only bacteria or fungi, and sometimes they may consist of a mixture of fungi and bacteria or fungi and yeasts or other combinations in which the components are quite unrelated. Many important biochemical processes are usually accomplished by co-culturing two or more microorganisms for better performance [2]. Among microorganisms, various types of synergistic effects have been noticed. Compared to pure culture fermentation, mixed culture fermentation has some absolute advantages and some new substances are usually produced with various desirable characteristics [3, 4, 5]. The application of pure cultures dominates in biotechnological processes but through the application of mixed cultures their combined metabolism results in considerable higher yield of ethanol, enzymes, acetic acid and enrichment of the vitamin content of fermented foods [2]. Special attention has been given to the mixed cultures used for increasing biological enzyme production, such as amylase, inulinase, xylanase, endoglucanase and β-glucosidase [1]. Solid-state fermentation involves the cultivation of microorganisms on a solid substrate, such as grains, rice and wheat bran. This method is an alternative to the production of enzymes in liquid by submerged fermentation. SSF has many advantages over submerged fermentation. These include, high volumetric productivity, relatively high concentration of product, less effluent generated and simple fermentation equipment. Fermentation with microorganisms, like bacteria, yeast and moulds is used to produce enzymes [6, 7]. Filamentous group of fungi constitutes a storehouse of economically important enzymes, alcohols, organic acids, and pharmaceuticals. Enzymes like amylases, glucoamylases, and invertase used in the biodegradation of starch, cellulase, active degrader of cellulose, are produced from various species of moulds and bacteria respectively [8]. Amylases and proteases are the major enzymes involve in biodegradation of plants and other organic molecules. Fungi secrete the enzymes to break down complex organic molecules into simpler ones, which they can easily absorb during metabolism.

Amylases are groups of enzymes that has been defined as a group of hydrolases that can specifically cleave the O-glycosidic bonds in starch. The important groups of amylases are glucoamylase and α-amylase. The alpha-amylases can be derived from various sources such as plants, animals and microorganisms due to the wide distribution in nature [9, 10]. However, fungal and bacterial amylases have predominant applications in the industrial sector. Major advantage of using fungi for the production of amylases is the economical bulk production capacity and ease of manipulation. Many species of Aspergillus and Rhizopus are used as a source of fungal α-amylase [10, 11].

Protease is a very important industrial enzyme whose production is estimated to be approximately about 60% of the total world-wide enzyme production [12]. They occupy key position with respect to their applications in both physiological and commercial products [8]. Fungal proteases have an advantage over bacterial protease as mycelium can be easily removed by filtration. Their areas of applications include industries such as detergent, food, pharmaceuticals and de-hairing of leather, tanning, dairy, baking, surgical cleaning, brewing and medical applications [13, 14, 15].

Adansonia digitata, (Linn) the baobab tree is a member of the Bombacaceae family. This deciduous tree was originally located in South Africa, Botswana, Namibia, Mozambique and Zimbabwe [16], but can be found in most countries within the African continent. African A. digitata is a

very long-lived tree with local multipurpose uses [17]. Currently, the seed has no industrial importance and it is grossly underutilized. This research sought to produce protease and amylase from the seed through mixed-culture fungal fermentation as previous effort on enzyme production has always been concentrated on mono-culture fermentation technique.

Materials and Methods

Collection and authentication of plant materials:

Adansonia digitata (Baobab) seeds were collected from the premises of University of Ilorin, Ilorin, Kwara State and were authenticated at the Herbarium unit of the Department of Plant Biology, University of Ilorin Adansonia digitata (UIH 1048).

Preparation of seed

A. digitata pods were cracked open manually and washed with clean water to remove the pulp from the seeds. They were dried at 60 0C in the oven for four hours to remove the wetness. The seeds were pulverized with an electrical grinder to rough particle sizes of about 01 to 0.01 mm in diameter and stored in air tight container for further uses.

Determination of pH of seed

The pH of the seed was determined by using a Crison micro pH meter (Model 2000). Ten grams of the seed was mashed separately into a 100 ml beaker; 40 ml of distilled water (pH 7.0) was added and the mixture was left undisturbed for 30 minutess followed by occasional stirring with a glass rod before the pH of the seeds was measured [18].

Determination of the Water Holding Capacity (WHC) of the seed

The water holding capacity of the seed was determined according to the method of [19]. This was done to determine the quantity of water required for SSF of the seed.

Isolation of organisms

Fungi were isolated from the naturally fermented naturally fermented seed through serial dilution. Culturing was done by pour plate method using Potato Dextrose Agar (PDA) into which 10% Streptomycin solution has been added to inhibit bacteria growth. The plates were incubated at room temperature (28±2 0C) for 48 hours. Visible fungal colonies were isolated and further sub-cultured to obtain pure cultures. The pure isolates were maintained on agar slant and kept in the refrigerator at 4 0C for further studies [20]; [21]; [22].

Identification of the Isolates

Morphological and microscopical analysis for the identification of the isolates were carried out and compared with literature as described by [23].

Selection of fungal isolates for mixed-culture fermentation

Six of the fungal isolated were used after they were identified as Penicillium chrysogenum, Penicilliuim citrinum, Mucor racemosus, Mucor hiemalis, Rhizopus stolonifer, and Alternaria tenuis.. Each of the organisms was used to prepare spore suspension used as starter cultures for the fermentation of seed mashes before incubation at 28 ±2 0C for 120 hours [21], selected fungi were randomly mixed to obtain six mixed cultures.

Fungal spore preparation

Fungal spore suspension of actively growing mid log phase culture of the fungal isolates were prepared according to the method described by [24]. An agar slant of four day old pure culture of each of the organisms was used. Sterile distilled water (10 ml) was added to the slant and shaken to wash the spores. The spore suspension was counted using the Neubauer counting chamber. A spore suspension of about 5x104 spore suspension/ml was used in each case for inoculation.

Preparation of inoculum

The inoculums used for the study were prepared as highlighted:

CtCh: Penicillium citrinum (5 ml containing about 5 x 104 spore/ml suspension) mixed

with Penicillium chrysogenum (5 ml containing about5 x 104spore/ml suspension);

CtSt: Penicillium citrinum (5 ml containing about 5 x 104 spore/ml suspension) mixed with Rhizopus stolonifer (5 ml containing about 5 x 104 spore/ml suspension);

HeTe: Mucor hiemalis (5 ml containing about 5 x 104spore/ml suspension) mixed with Alternaria teneus (5 ml containing about 5 x 104 spore/ml suspension);

HeRa: Mucor hiemalis (5 ml containing about 5 x 104spore/ml suspension) mixed with Mucor racemosus (5 ml containing about 5 x 104 spore/ml suspension);

StTe: Rhizopus stolonifer (5 ml containing about 5 x 104 spore/ml suspension) mixed with Alternaria teneus (5 ml containing about 5 x 104 spore/ml suspension) and

CtRa: Penicillium citrinum (5 ml containing about 5 x 104 spore/ml suspension) mixed with Mucor racemosus (5 ml containing about 5 x 104 spore/ml suspension) [24].

Fermentation

Twenty grams (20 g) of the seed was measured into 250 ml Erlenmeyer flasks and 20 ml of water was added and sterilized in the autoclave at 121 0C for 15minutes. The sterile samples were mixed and stirred properly until uniform mashes were obtained in each case. Two millilitres (2 ml) of the spore suspension was used as fermentation starter to inoculate each of the samples in the fermentors. The mixtures were allowed to ferment for 120 hours at 28±2 0C [21]; [22].

Enzyme extraction

One gram of the fermented substrate was extracted with 100 ml of distilled water on a rotary shaker at 200 rpm for 30 minutes at 28±2, the mixture was filtered with several (16) layers of cheese cloth and the filterate was used as the crude enzyme extract for the assay.

Protease assay

Protease activity was determined using spectrophotometric method [25]. Enzyme solution (2 ml) was incubated with 2 ml of 1.0% (w/v) casein in phosphate buffer (50mM, pH 7.0) at 37 0C for 30 minutes. The mixture was filtered with Whatman No 1 filter paper. To 2 ml of the filterate was added 2ml of Biurret reagent was added to each tube and mixed thoroughly. (Biurret reagent was prepared by mixing 0.5 ml of 1% cupric sulphate with 0.5 ml of 2% sodium potassium tartrate, followed by the addition of 50 ml of 2% sodium carbonate in 0.1 N NaOH). The mixture was then allowed to incubate at room temperature for 10 minutes prior to the addition of 0.2 ml of 1.0 N Folin-Ciocalteu's reagent. The resulting solution was incubated for 30 min at room temperature to develop the blue colour and the absorbance was read at 650 nm using a tyrosine standard. A separate blank was set up as standard. One unit of enzyme activity was defined as the amount of enzyme required to release one µg of tyrosine per min with Bovine Egg Albumin (BEA) as standard under the assay condition.

Amylase assay

Amylase activity was determined according to [26]; [27]. The reaction mixture contained 2 ml of crude enzyme and 2 ml of 0.1M phosphate buffer (pH 7.0) containing 1% (w/v) of soluble starch. The mixture was incubated at 37 0C for 30 minutes. After 30 minutes of incubation, then filtered, to the filtrate was added 2 ml of DNSA solution containing 1 g of DNSA dissolved in 20 ml of 1 M NaOH, to which 30 g of sodium potassium tartarate and water were added to make it 100 ml. The amount of reducing sugar released into the mixture after enzyme reaction was determined by reading the absorbance at 540 nm using the UV visible spectrophotometer.

Amylase activity was defined as the amount of enzyme that released 1µm of the reducing sugar (glucose) per minute under standard assay condition.

Results

Enzyme activities

The enzyme activities of protease and amylase are presented in Figures 1 and 2 respectively. The peak of activities occurred at the 72 hours of fermentation for both proteases and amylases generally. The maximum protease activity occurred at 72 hours of fermentation as 100 Umol/mg in substrate fermented with mixed-culture of P. citrinum and M. racemosus while the minimum was 22.2 Umol/mg at 120 hours. The highest amylase activity was 53.6 Umol/mg in substrate fermented with mixed-culture of A. tenuis and M. racemosus while the lowest activity was 4.0 Umol/mg at 24 hour. Protease and amylase activity generally reduced after 72 hours of incubation.

pH

The pH range obtained during fermentation was found to fall within 5.4 to 7.6. The highest activities of protease and amylase was observed at pH 7.6 after which the pH began to decrease.

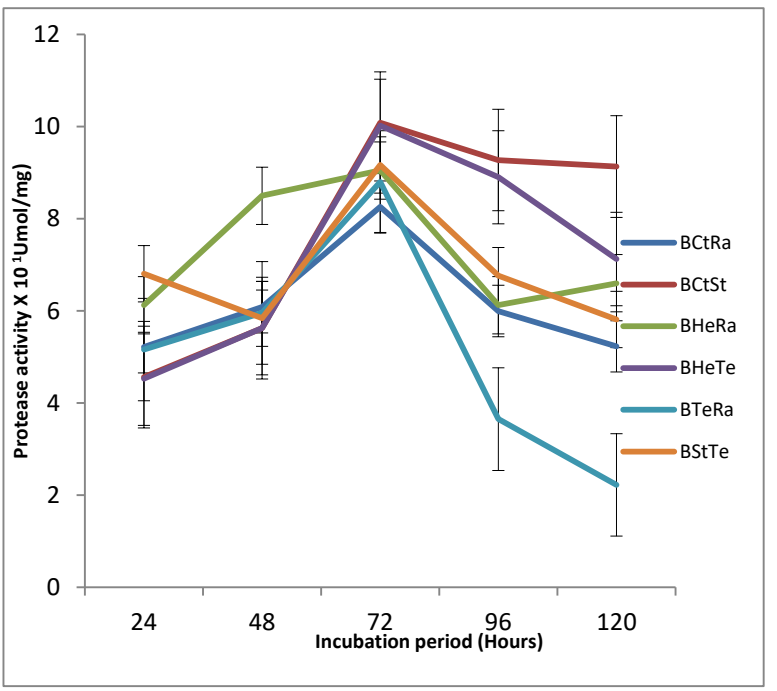

Figure 1: Protease activity during mixed-culture fungal fermentation of the seed of A. digitata Data are mean of two replicates ± SEM.

BCtRa= A. digitata seed fermented with mixed-culture P. citrinum and M. racemosus; BHeTe= A. digitata seed fermented with M. hiemalis and A. tenuis; BHeRa= A. digitata seed fermented with M. hiemalis and M. racemosus; BStTe= A. digitata seed fermented with R. stolonifer and A. tenuis; BCtSt= A. digitata seed fermented with P. citrinum and R. stolonifer; BTeRa= A. digitata fermented with A. tenuis and M. racemosus.

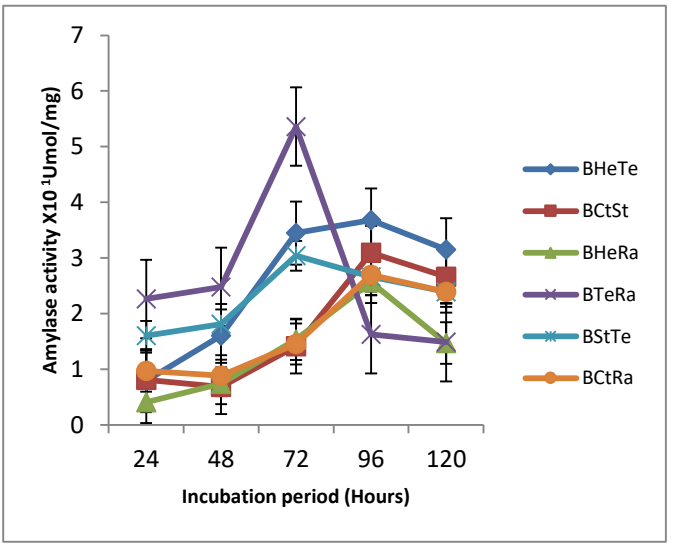

Figure 2: Amylase activity during mixed-culture fungal fermentation of the seed of A. digitata Data are mean of two replicates ± SEM.

BCtRa=A. digitata seed fermented with mixed-culture P. citrinum and M. racemosus; BHeTe= A. digitata seed fermented with M. hiemalis and A. tenuis; BHeRa= A. digitata seed fermented with M. hiemalis and M. racemosus; BStTe= A. digitata seed fermented with R. stolonifer and A. tenuis; BCtSt= A. digitata seed fermented with P. citrinum and R. stolonifer; BTeRa= A. digitata fermented with A.tenuis and M. racemosus.

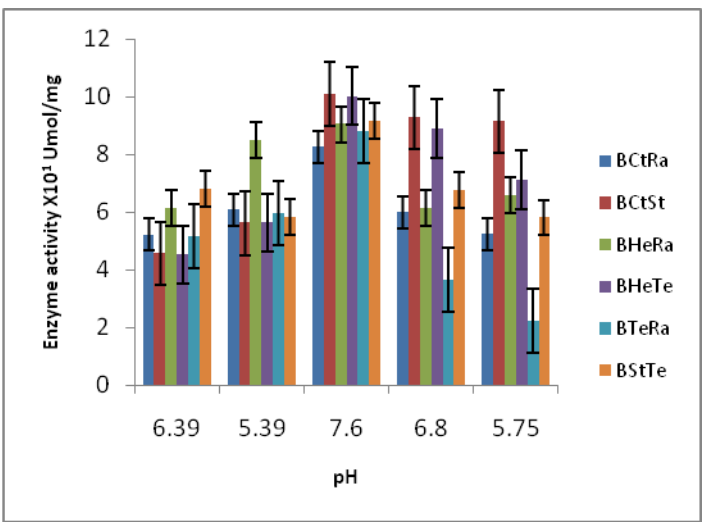

Figure 3: Changes in pH during fermentation
Data are mean of two replicates ± SEM.

BCtRa= A. digitata seed fermented with mixed-culture P. citrinum and M. racemosus; BHeTe= A. digitata seed fermented with M. hiemalis and A. tenuis; BHeRa= A. digitata seed fermented with M. hiemalis and M. racemosus; BStTe= A. digitata seed fermented with R. stolonifer and A. tenuis; BCtSt= A. digitata seed fermented with P. citrinum and R. stolonifer; BTeRa= A. digitata fermented with A.tenuis and M. racemosus.

Discussion

Protease and amylase activity increased steadily during the fermentation of the seed from 24 hours. Activity was found to increase steadily as incubation period increased. The maximum protease activity was recorded in substrate fermented with a mixed-culture of P. citrinum and M. racemosus while the highest amylase activity was recorded in substrate fermented with mixed-culture of A. tenuis and M. racemosus. Other mixed-culture also recorded various amount of enzyme activities. Generally the activities followed a similar trend. The peak of enzyme production was within the late exponential to early stationary phase of growth, beyond which the activity dropped (72 to 96 hours of fermentation). This may be due to depletion in nutrients and accumulation of toxic metabolites/wastes as a result of the increase in the population of the fungi. The incubation time for achieving the maximum enzyme level is governed by the characteristics of the culture and is based on growth rate and enzyme production. Maximum accumulation of amylase has been said to occur during stationary phase and further increase in incubation period beyond this decreased the production of amylase. This might be due to the deficiency of nutrients, accumulation of toxic substances and proteolysis of amylase as suggested by many workers [28]; [29]; [30]. The activities of enzymes achieved in this research were higher than those observed when Aspergillus species (a known enzyme producing organism) were used in pure culture for fermentation [31].

Microbial fermentation of organic compounds is usually accomplished through the enzymatic breakdown of complexes. The metabolic activities of fungi that result in fermentation and its subsequent effect is often through the secretion of enzyme into the substrate upon which they are growing. Fungi are saprophytes which normally release extra-cellular enzymes into their surroundings. The enzymes are usually organic catalysts that aid the breaking down of the organic complexes which are later absorbed by the fungi for growth and synthesis of various products. [31] recorded a similar result during a research carried out to produce enzyme.

The incubation time for achieving maximum enzyme yield has been reported to be governed by the characteristics of the culture and depended on growth rate and enzyme production [32];[33]. The findings in this study indicated that enzyme was secreted early in active growth phase and reached maximum towards the end of exponential growth phase. Time course has been reported to play a very crucial role in fungal metabolic activity and growth. The incubation time necessary for optimal biosynthesis varied between different enzymes produced from one substrate [34]; [24]; [35]; [36].

The decrease in enzyme production after 72 hours of incubation period could also be due to the inactivation of protease by other constituent enzymes. This suggested that the production of amylases and proteases by these microorganisms was fully correlated with their growth.

Enzyme production by microbial strains depends on the extra-cellular pH because culture pH strongly influences many enzymatic processes and transport of various components across the cell membranes, which in turn support the cell

growth and fermentation systems product production [37]. In this research, the pH of the medium ranges between weakly acidic to weakly alkaline condition. Maximum activities of amylase and protease were recorded at pH around neutral value (7.0). The range of pH from 5-9 has been reported for maximum amylase and protease activity [24];[37]; [36].

Recommendation and conclusion

In view of potential applications of amylase and protease enzymes and economy of production, attempt could be made to optimize culture parameters for their production from these two seeds.

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Biosynthesis of silver nanoparticles in improved strain of Auricularia polytricha -an edible mushroom from Nigeria and its antimicrobial activities.

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Abstract: Nano materials and their application are of great use in research because of their size range. In this study, Auricularia polytricha (EW1) collected from Benin in Southern Nigeria was subjected to mutation by exposure to UV-light resulting in a mutant (EW1M1). EW1 and EW1M1 were evaluated for biosynthesis of silver nano-particles. The anti-microbial properties of both strains were also evaluated. Absorption spectra of silver nano-particle (AgNPs) of mutant exhibited a strong broad peak at 420 nm while wild type absorption peak was obtained at less than 420 nm. The mutant was further characterised. DLS showed a monodispersion with diverse sizes, morphology and shapes. TEM micrograph revealed a monodispersed formation of the nano-particles, with uniform size at 10 – 20 nm. FTIR study revealed the absorption bands at 3380, 2921, 2839, 1658, 1083 and 610 cm^{-1} respectively showing the functional groups reducing the silver nitrate to silver ion. There was formation of zone of inhibition on all the microorganisms that were used for the study but the control showed no zone of inhibition. The mushroom extract of mutant strain exhibited higher anti microbial activity than the wild type.

Keywords: Auricularia polytricha, Silver Nano-particle (AgNPs), UV- Vis Spectra, Antimicrobial Activities.

Introduction

Auricularia species is a soft, jelly like edible mushroom. It is hunted and consumed by the Benin people in Southern area of Nigeria [1]. In Ghana, the mushroom is used as blood tonic [4]. It is a popular ingredient in many Chinese dishes, and medicines. Medically, the

polysaccharides extracted from the mushroom, help stimulate the immune system in humans, because the production of interferon and interleukins that stop the proliferation of cancer cells [17]. Other medical properties of Auricularia species include its antitumor, hypoglycemic, anticoagulant and cholesterol lowering properties [5]. Mushrooms are good antioxidants [6]. It also possesses antiviral and anti-cancer properties [20].

Nanotechnology is concerned with the synthesis of nanoparticles of variable sizes, shapes, chemical composition and controlled disparity. Nanoparticles are particulate or dispersions of solid particles with size range of 10 to 1000 nm [18]. They possess physical, chemical, electronic/electricalmechanical, thermal, dielectric, optical and biological properties that are different from the bulk material of the same element [18]. The optical, physicochemical and electronic properties of nanoparticles vary with difference in their size, shape and crystalline formation. The field of nanotechnology has gained great importance because of its potential applications in various fields such as chemical and textile industries, medicine/drug gene delivery and computer etc.

Silver nanoparticles are known to possess strong antimicrobial properties and that is one of the major reasons for the development of nanosilver containing products. Out of over 1000 consumer products that contain nanomaterials, about 25 % are believed to contain silver nanoparticles. Some of the consumer products that contain nanosilver particles include food contact materials (such as cups, bowls, cutting boards, etc.), odour resistance textiles, electronics and other household appliances [8, 19] The AgNPs has been reported to have antimicrobial activity against human pathogens [12] and it has been used to solve many problems which are related to harmful and toxic by-products from artificially made products [11]. The use of AgNPs helps as resistance by some pathogenic microorganisms in multidrugs is eliminated because of their small size over their large surface area which increases their antibacterial efficiency [18, 19]. Thus synthesising nanotechnology in cosmetics, ATM buttons, medical, household appliances, baby toys, sport equipment and clothing the products to have antibacterial properties [3, 11, 8]. The silver nanoparticle process is biosafe, harmless to the surrounding cells of the body and eco-friendly over conventional antibiotics because they help destroy all pathogenic microorganisms which no organism has been reported to develop resistance to [2, 19]. In this work, we have used extract of edible Nigeria mushroom Auricularia polytricha which is also known as Wood ear mushroom for the synthesis of bio functional silver nanoparticles.

II Materials and Methods

Sample collection

Fresh sample of Auricularia polytricha growing on dead wood was collected from Benin Southern Nigeria (Benin 6 º20’0’’N, 5º3720’’E). Both wild and mutant strain were taken to the laboratory and cultured to obtain pure culture. Potato dextrose agar was used for the isolation of the pure culture. Preparation of the medium was according to the protocol of the manufacturers of the medium.

Mutation Induction

The actively growing culture (7 day old) of A. polytricha (EW1) was exposed to UV- light of 210nm (UV sterilizer, Millipore xx63 70000, USA) for 90 min to induce mutation. The mutant (EW1M1) was sub cultured on a PDA medium, supplemented with 5% yeast extract agar (YEA) and incubated at 25º C for 7days.

Biosynthesis of silver nanoparticles

Two grams of fresh sample of both the wild strain (EW1) and the mutant (EW1M1) of the A. polytricha were washed thoroughly with double distilled water, boiled for 10 minutes and filtered through whatman No1 filter paper using the method of [14] with slight modification. The extract was stored at 4 oC for further use.

The filtrate was used as reducing and stabilizing agent for 1mM of AgNO3 (99.9% Sigma- Aldrich). 1µl of the mushroom extract at a time was added to 100 ml of 103AgNO3 aqueous solution (prepared in deionized water), boiled and incubated at 37ºC until the colour changed to brown. Mushroom extract without the aqueous solution of AgNO3 was boiled in deionized water and served as the control experiment.

UV visible spectroscopy analysis [14]

The process of reaction between metal ions (silver ions) and biosynthesis of silver nanoparticle by the mushroom species (Auricularia polytricha) was monitored by UV visible spectroscopy of the aqueous solution. UV visible spectrophotometer (Spectroquant ® Pharo 300 M, UV) with a resolution power of 2.0 nm at between 200 to 600 nm, possessing a scanning speed of 300 nm / minutes was used for the analysis.

Fourier transmission infrared spectroscopy measurements (FTIR) [14]

Fourier transmission infrared spectroscopy measurements (FTIR) were carried out to determine the different functional groups present in the resulting silver nanoparticles. This was done following the method of [14] The residual solution after the biosynthesis reaction was centrifuged at 10,000 rpm for 15 minutes three times to purify the suspension by removing proteins /enzymes. The sample was completely dried at 60ºC. Finally, the dried nanoparticles were analoged by FTIR The Fourier transform infrared spectroscopy (FT-IR) spectrsum of the sample was recorded on Perkin Elmer spectrum 100 FT-IR spectrometer using KBr. (Thermo Nicolet nexus 670 spectrometer of resolution 4cm -1). Two grams of fresh sample of both the wild strain (EW1) and the mutant (EW1M1) of the A. polytricha were washed thoroughly with double distilled water, boiled for 10 minutes and filtered through whatman No1 filter paper using the method of [14] with slight modification. The extract was stored at 4 oC for further use.

The filtrate was used as reducing and stabilizing agent for 1mM of AgNO3 (99.9% Sigma- Aldrich). 1µl of the mushroom extract at a time was added to 100 ml of 103AgNO3 aqueous solution (prepared in deionized water), boiled and incubated at 37ºC until the colour changed to brown. Mushroom extract without the aqueous solution of AgNO3 was boiled in deionized water and served as the control experiment.

UV visible spectroscopy analysis [14]

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Dynamic Light Scattering (DLS)

The DLS was used to determine the average size distribution of the synthesized AgNPs. The reaction mixture was filtered several times and the resulting pure suspension put in an Ependoff tube. The particle distribution in the liquid was studied in a computer-controlled particle analyser (ZETA sizer Nanoseries Malveron instrument Nano Zs).

Transmission Electron Microscopy (TEM)

The transmission electron microscopy analysis was used to measure the size, morphology and chemical composition of the silver nanoparticles produced. The sample to be analysed were prepared by several layers of coating and allowed to dry. TEM images were obtained at an accelerating voltage of 300 kV.

Test Organisms

Two human pathogenic bacteria (Staphylococcus aureus and Escherichia coli) and two species of fruit rotting fungi (Aspergillus flavus and Penicillum notatum) were chosen as test organisms to determine the antimicrobial activity of the resulting AgNPs. Pure cultures of these organisms were obtained from the Microbial Biotechnology laboratory of the Department of Biotechnology (DBT-Centre) of Assam Agricultural University (AAU), Jorhat, India. The bacterial strains were maintained on nutrient agar (NA) medium at 35ºC until they were used, while the fungi species were maintained on Potato Dextrose Agar (PDA) (M-Lab) at 25ºC.

Antibacterial Activity [9]

The antibacterial activity of the synthesized silver nanoparticle, mushroom extract and the control were tested against the selected bacterial strains mentioned above using the method of [9]. Twenty millilitres (20 ml) of sterilized nutrient agar medium was poured into sterile petri plates and allowed to solidify. The test bacterial cultures were evenly spread over the medium by using a sterile cotton swab. A well of 0.5cm was made in the medium using a sterile cork borer. 200 µl of synthesized silver nanoparticle, mushroom extract and the control were each filled into a well on the plate and the culture was incubated for 24 hrs. At the end of the incubation period the plates were checked for clear zone of inhibition around each well.

Antifungal Activity

The antifungal activity of the synthesized silver nanoparticle and mushroom extract were tested against selected fungal strains mentioned above. The agar well method as described by [9] was employed in the assay.

Results

Biosynthesis of silver nanoparticles

There was a gradual visible colour change from light brown colour to reddish brown, when the mushroom extract was subjected to different concentrations of aqueous solution of 1mM of silver nitrate. This indicated the formation of silver nanoparticles in the mushroom extract.

UV vis spectroscopy analysis

Metal nanoparticles such as AgNPs had free electrons, which gave rise to a surface plasmon resonance (SPR) absorption band. The absorption spectra of AgNPs exhibited a strong broad peak at 420 nm, and observation of this band was attributed to surface plasmon resonance of

the particles which confirmed the presence of silver (Figure 1).

Fourier transmission infrared spectroscopy measurements (FTIR)

FT-IR study revealed the absorption bands at 3380, 2921, 2839, 1658, 1083 and 610 cm̄1 showing O-H(stretching), C-H(stretching), Aldehyde, C=O(stretching) Amide, N-H(stretching) Amide, C-Cl Alkylhalide respectively indicating the functional groups reducing the silver nitrate to silver ion.

Dynamic Light Scattering (DLS)

The particle size distribution analysis revealed that particle size of the silver nanoparticles produced in this study was between 5-50 nm (Figure 3).

Transmission Electron Microscopy (TEM)

Transmission Electron Microscopy (TEM) image analysis conducted showed that the morphology of AgNP2 produced was spherical with a size of 5nm (Figure 4). The nanoparticles produced were monodispersed.

Antibacterial/Antifungal Activity

Both the ordinary mushroom extracts and the synthesized nanoparticles exhibited antimicrobial properties. The synthesized silver nanoparticles of the mutant strain (EWIMI) elicited a higher antimicrobial activity against the test organisms strain (EWI) (Table 1).

Discussion

The gradual colour change from white/creamy to brown observed when the mushroom extract was treated with silver nitrate indicated the formation of silver nanoparticles. The observed colour change was as a result of excitation of surface plasmon resonance in the synthesized silver nanoparticles. [18] gave similar reports when they studied the biosynthesis of silver nanoparticles by Agaricus bisporus. A similar finding was also reported by [19] using Agaricus bisporus, Calocybe indica, Pleurotus florida and P. platypus extract.

The synthesized AgNPs of A. polytricha were characterized by UV-Vis Spectroscopy. There was a strong broad band absorption spectrum at 420 nm. This result is in agreement with the reports of [19] who studied the synthesis of silver nanoparticles from edible mushroom (Agaricus bisporus). Similar observation was also reported by [10] in their study of extracellular nanoparticles synthesis using Pseudomonas aeruginosa. This shows that mushrooms could be veritable tool in the synthesis of silver nanoparticles which have been shown to possess strong antimicrobial properties against some pathogenic organisms. The DLS shows the average size of synthesized AgNPs distribution analysis.

TEM micrographs of the nanoparticles obtained in this study show that the synthesized AgNPs are spherical in shape with average size of 5-50 nm. The AgNPs were monodispersed and well distributed within the solution. [16] in their study of AgNPs synthesis using Pleurotus florida reported the synthesis of AgNPs of average size of 20 nm that was polydispersed. However, [15] recorded the AgNPs of size range of 5 to 50 nm using Pleurotus sajo-caju

In this study, our FTIR investigation of the resulting AgNPs revealed absorption bands at 3380, 2921, 2839, 1658, 1083 and 610 cm1¯ representing Hydroxyl group O-H stretching, aldehyde

C-H stretching , C=Ostretching, amide I group and amide

II groups respectively. Similar findings have been reported by [7]. The peak at 1534cm-1 is attributed to amide II vibrations of proteins [13]. Amide II bands along with amide I bands were major regions of the protein infrared spectrum. The major absorbance peak at 1083 cm-1is attributed as structures in

chitin, a major structural polysaccharide in mushrooms; and this absorbance at 1083 cm-1may also arise from primary alcohol structures due to alcohol functional group detected [21]. The peak band at 610 cm-1 corresponds to the presence of alkyl halides.

The antimicrobial activity of the silver nanoparticle of A. polytricha was investigated against some pathogenic organisms using well diffusion technique. The synthesized silver nanoparticles recorded higher antimicrobial activity than the mushroom extract. This shows that the silver nanoparticle could be used as antimicrobial agents against the human pathogens that have been tested. Similar findings have been reported by [19]

Conclusion

The results of this study show the improved performance of mutant strain of Auricularia polytricha EM1W1 over its wild type EW1. Absorption spectra of silver nanoparticle (AgNPs) of mutant exhibited a strong broad peak at 420 nm while wild type absorption peak was obtained at less than 420 nm. The formation of zone of inhibition on all the microorganisms that were used for the study shows the usefulness of AgNPs in medicine while the control showed no zone of inhibition. The mushroom extract of mutant strain exhibited higher anti microbial activity than the wild type.

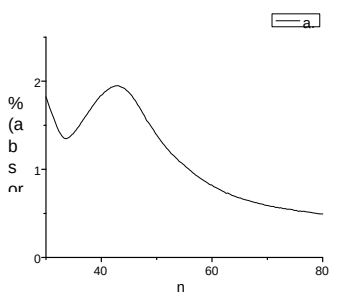

Fig 1: The *Auricularia* *(EW1*

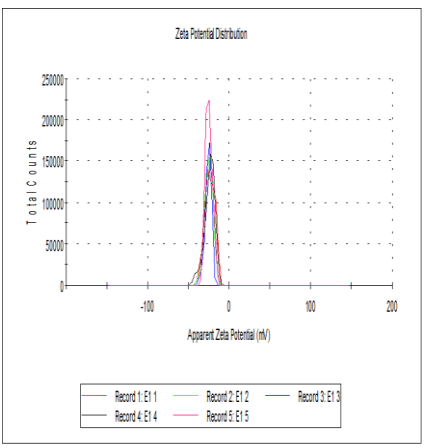

Fig 3: The DLS of *A. polytricha* showing peak

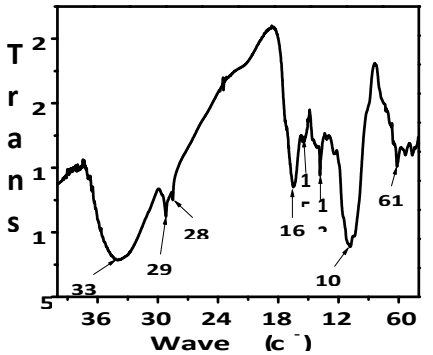

Fig 2: The FTIR from silver synthes*Auricularia*

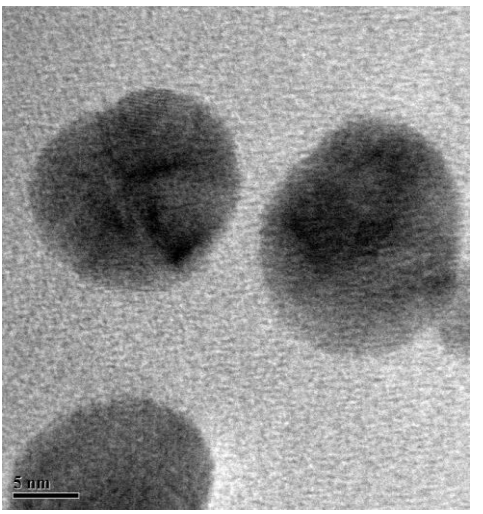

Fig 4: The TEM analysis of silver nanoparticles synthesized by Auricularia polytricha

Table 1: The mean zone of inhibition (mm) of Auricularia polytricha isolates.

Tested organisms	Zone of inhibitions (mm)				
	Synthesized silver nanoparticles		Mushroom extract		Control
	EW1	EW1M1	EW1	EW1M1	
P. acidiovorans	9.0	11.0	8.0	8.6	-
Escherichia coli	3.5	5.0	2.5	2.0	-
Staphylococcus aureus	2.0	3.5	0.5	0.0	-
Bacillus cereus	9.0	12.0	7.0	7.9	-
Aspergillus flavus	4.0	6.0	2.0	2.0	-
Penicillum notatum	1.0	2.0	0.5	0.9	-

Key – means no zone of inhibitions

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