



Susceptibility of Stored Cocoa Bean Seeds to Ochratoxigenic Fungi in Ondo State, Nigeria

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Aims: To determine the susceptibility of stored cocoa beans to ochratoxigenic fungi in Ondo State, Nigeria.

Place and Duration of Study: The study was carried out at Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria; and cocoa beans samples were collected from stalls in Ondo, Idanre, Oda-Akure, Oba-Akoko, Ile-Oluji, and Ikpenmen towns in Ondo State for a period of twelve months.

Methodology: The serial dilution method was used in plating and isolating the fungi in cocoa bean samples while identification was done conventionally. TLC was used to determine ochratoxin A production by toxin producers.

Results: Cocoa beans collected from stalls in Oba-Akoko had the highest mean fungal incidence of 195×10^5 cfu/g of cocoa bean while those from Idanre stalls had the lowest mean with 4.964×10^5 cfu/g of cocoa bean. Cocoa samples had high fungal incidence between July and September, 2014 compared to other months the study took. *Penicillium*, *Fusarium*, *Verticicladium*, *Neocosmospora*, *Mucor*, *Rhizopus*, *Beaveria*, *Alternaria*, *Sachharomyces*, *Phoma* were the genera isolated using Czapek Yeast Extract, and TLC screening showed that *A. niger*, *A. ochraceous*, *A. carbonarius*, *A. terreus* and *A. niger* are OTA producers. HPLC quantification showed that cocoa bean seeds collected from stalls in Idanre were the least contaminated by OTA while high concentration level of 32.09 ± 22.9 ng/g was detected in Oba-Akoko area of Ondo State.

Conclusion: The study showed that cocoa beans collected from different stalls in Ondo State were

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susceptible to ochratoxin A contamination, and generally high concentration of the toxin is produced in the wet season than in the dry season. The study further showed that ochratoxigenic moulds infesting stored farm produce such as cocoa bean seeds in Ondo State are majorly the *Aspergillus* species.

Keywords: Susceptibility; ochratoxigenic; ochratoxin A; Aspergilli.

1. INTRODUCTION

Cocoa (*Theobroma cacao*) is the highest foreign exchange earning agricultural produce in Nigeria [1] and farming of cocoa and its associated processes employs a sizable population of the Nigerian labour force directly and otherwise [2,3]. Cocoa farming and production occurs between 10° N and 10° S of the equator [4], and it is carried out by small holders who majorly practice subsistence farming [4]. In 2012, Ondo State produced about 50% of three hundred and eighty three million (383 m) tons Nigeria exported making the state the highest producer of the crop in Nigeria, and in 2013, production reached 300,000 tons with a 7% increase over the preceding year. Other cocoa producing states include Ogun, Oyo, Ekiti, Edo, Kogi, Abia, Osun, Edo, Kwara, Taraba, Delta, Cross-Rivers, Adamawa, and Akwa-Ibom [5] which all lies within the production zone of Nigeria.

Fungal deterioration of seeds, grains and feed stuff is a chronic problem in developing countries, and in Nigeria, fungal contamination of field and storage grains is a challenge [6]. Mycotoxic fungi survive under a range of climatic conditions on food crops and their products and in animals along the food chain [7] producing mycotoxins whose toxicity is determined by abiotic and biotic factors such as temperature, humidity, pest infestation, fungal strain, and storage conditions [8-10]. Food and Agriculture Organization (FAO) reported that, about 25% of world's crop harvest is re-contaminated yearly with mycotoxins [11] which result subsequently in mycotoxicosis; a disease condition associated with consumption of food contaminated by mycotoxin [12] as these toxins are stable under most food processing conditions [13]. These disease conditions could be neurotoxic, estrogenic, immunosuppressive, nephrotoxic, and carcinogenic.

Mycotoxic fungi could be a field or a storage fungus. Bankole [14] explained that the field fungi causes ear rot disease, and toxin production before harvest requiring moisture of over 20%, high relative humidity between 70-90%, and

warm temperature (22°C to 30°C). The fungi genera implicated include *Fusarium*, *Cladosporium*, and *Alternaria* while the storage fungi are genera *Penicillium* and *Aspergillus* which are capable of growing at low water contents than the field fungi and they tend to contaminate grains in silos and other storage places [15]. Whittaker et al. [16] reported that, as a result of movement of food from one place to the other, no part or place can be considered mycotoxin-free because mycotoxins persist after the vegetative growth of fungi has occurred.

Ochratoxins are types of mycotoxin produced by *Aspergillus* and *Penicillium* species [17]; the *Aspergillus* species are *A. alutaceus*, *A. ostianus*, *A. quercins*, *A. sulphureus*, and *A. ochraceus* while the *Penicillium* include specie *P. verrucosum* - *P. verrucosum* chemotype I that produces ochratoxin A (OTA), verrucolon, and citrinin, and *P. verrucosum* chemotype II which produces only OTA and verrucolon. Cereal-borne *P. verrucosum* produces chemotype III [18,19]. OTA infestation of cocoa beans in African countries and in South America is reportedly caused by the black Aspergilli - *A. carbonarius* and *A. niger* aggregate [20-25].

OTA was classified into group 2B as possibly carcinogenic to human [26,27] while the National Toxicology Program (NTP) classified it as "reasonably anticipated to be a human carcinogen" [28], and COT [29] declared OTA a genotoxic carcinogen with immunosuppressive, teratogenic and mutagenic potentials. Ochratoxin A has been found in coffee bean, cereals, cocoa bean, pulses, dried fruits, wines, beans, spices, nuts, olives and figs [30-32] and recent studies on rice from markets in Lagos State, Nigeria showed the presence of OTA in samples analyzed and other farm products [33,34] while, 98% of rice samples from Niger State were contaminated by the toxin [35]. Ayejuyo and colleague [34] detected low levels of OTA (0.01- 2.18 ng g⁻¹), whereas Makun and colleagues [35], detected levels as high as 134 – 341 ug/kg. Cola nuts (*Cola nitida*) from Nigeria have been shown to contain OTA.

The presence and growth of fungi may lead to spoilage of food and detrimental alteration in food quality and quantity [6] therefore, the study sought to determine the susceptibility of stored cocoa beans to ochratoxigenic fungi in Ondo State, Nigeria considering the facts that Ondo State is the highest producer of the crop and because cocoa earns Nigeria more exchange than any other crop.

2. MATERIALS AND METHODS

2.1 Study Locations

The sample collection locations are cocoa major growing areas of the state which included Idanre, Ondo West, Akure South, Owo, Ile-Oluji local governments [36], and Akoko South West (the best in the Akoko area of the state). Samples were collected from stalls in the designated locations. In Owo local government area, samples were sourced in Ikpemen (a town). Major towns where samples were collected are Idanre town, Ile-Oluji, Oda-Akure, Oba-Akoko, and Ondo town. The local governments were all located within the tropics.

2.2 Collection of Cocoa Bean Samples

Cocoa beans were used for the study. Stored cocoa beans were collected from stalls between July, 2014 and June, 2015 (12 months) from six (6) local government areas of Ondo State. Seventy two (72) composite samples of cocoa beans were randomly collected, and each composite sample was made up of ten (10) sub-samples making a total of seven hundred and twenty samples accessed. Samples were stored cocoa bean ready for market. Two (2) to three (3) kilograms of cocoa beans were collected from each stall, properly labeled, and packaged in sterile polyethylene bags for laboratory analysis.

2.3 Isolation and Identification of Fungi

The cocoa beans were air-dried before proceeding with the isolation process. The modified methods of Makun et al. [35] were used. The serial dilution methods was carried out by surface sterilizing five (5) gram cocoa bean randomly picked from a pool of 2 or 3 kg sample, immersed in 70% ethanol for 1 minute with a little agitation then 50% NaOCl solution for 3 minutes with a little agitation and strained before submerging in 70% ethanol for 30 seconds. Finally, the sample was rinsed then blended with 195ml of sterile 0.1% peptone water. Serial

dilutions were done on the diluents and plated on Potato Dextrose Agar (PDA) and Sabourard Dextrose Agar (SDA). Also, five bean seeds were aseptically spaced in sterile Petri-dishes containing PDA and chloramphenicol (500 mg per litre). The inoculated petri dishes were incubated at 28°C for 5 - 7 days. All the samples were plated in triplicates.

2.4 Morphological Identification of Fungal Isolates

The fungal species were characterized based on their morphological and cultural characters using taxonomic guides and standard procedures according to Pitt [37], Kozakiewicz [38], Klich [39], and Matasyoh et al. [40]. The morphological characteristics evaluated included; colony growth and color. Microscopic fungal structures such as the fruiting structures and spores were examined using a compound microscope with magnification of 100X. Fungal mycelia were scrapped, stained with lactophenol blue or 0.03% lactofuschin and covered with cover slip then viewed using a compound microscope. Suspected isolates were sub-cultured to obtain single spore cultures.

2.5 Identification of Ochratoxigenic Fungi

2.5.1 Czapek yeast extract tests

The isolated fungi species were tested for the potential to produce ochratoxin using the modified methods of Costa and Scussel [41]. Plates containing already prepared and sterilized Czapek Yeast Extract Agar (CYA) were inoculated with 6 mm diameter of 6 day old colony of isolated fungi, incubated for 7 days at 28°C, and colony diameter, colour, and exudates production observed [42-44]. After incubation, the Petri dishes were exposed to ultraviolet light with wavelength of 336 nm in a dark cabinet to check fluorescence. The presence of green light gave positive result that the isolates were ochratoxigenic in nature.

2.5.2 TLC clean-up and test

Confirmation was done using the modified methods of Yazdani et al. [45] and Aroyeun and Adegoke [46]. Using 5mm diameter plug, fungal colony were scrapped off and three to four plugs picked into a test-tube containing 10 ml chloroform: acetone (85:15 v/v), shaken vigorously every 5 min for 20 min. The suspension were subsequently filtered using a Whatmann no 1 filter paper and filtrate

evaporated in a water bath at a temperature of 50°C till dryness. The residue was re-suspended in 0.5 ml chromatographic grade methanol and filtered using 0.2 µl syringe filter. The filtrate was kept for TLC analysis.

Thin Layer Chromatographic plates coated with silica gel developed in toluene: ethyl acetate: formic acid 90%: chloroform (7:5:2:5, v/v/v/v) were used. Chromatography plates were examined for fluorescence and the R_f compared to R_f of OTA standard. Confirmation of the presence of OTA was done using ammonium hydroxide, positive result give purple blue colour [46,47].

2.6 Analyses of OTA on Cocoa Beans

2.6.1 Extraction of ochratoxin a from cocoa beans

OTA extraction from cocoa beans was done using the modified methods of Jayeola et al. [25]. Ten gram of ground cacao powder was added to 200 ml of 1% (v/v) sodium bicarbonate/deionised water and blended at high speed for 2 minutes. Extract weres filtered through Whatman No.4 filter paper or centrifuged at 4,000 rpm for 10 minutes. Five milliliter of the filtrate (equivalent to 0.25 g sample) was diluted with 5 ml PBS (Phosphate-buffered saline (PBS) prepared by dissolving 1.16 g disodium hydrogen orthophosphate, 0.2 g potassium dihydrogen phosphate, 0.2 g potassium chloride, and 8 g sodium chloride in 1 L ultrapure water. Final pH was 7.4).

2.6.2 Immunoaffinity clean-up

The immunoaffinity column was kept at room temperature before use. The column was firmly attached to a glass syringe barrel using an adapter and placed on a clamp stand. The whole of the diluted extract was then applied by pouring into the Ochraprep (R-Biopharm, Rhone ltd, USA) column and allowed to pass through the immunoaffinity column either by gravity or at a flow rate of 2-3 ml/min. Twenty milliliter PBS was passed through the column to wash it at a flow rate of approximately 5 ml/min. The column was then dried by passing air through it.

2.6.3 Elution of ochratoxin A

Bound ochratoxin from the column were slowly eluted using 1.5 ml of desorption solution (the desorption solution consist of acetic acid:methanol (2:98 v/v) both HPLC grade. This

was allowed to pass through the column by gravity and collected in a sample vial. During the elution, backflushing (or reversing the direction of flow) of desorption solution 3 times was done to ensure complete elution of the Ochratoxin A. Distilled water 1.5 ml was passed through the immunoaffinity column and collected in the sample vial to give a 3 ml total volume and quantified using HPLC. A Shimadzu HPLC machine that had mobile phase of acetonitrile, water (40:60) was used for quantification. The apparatus had a fluorescent detector, a C 18 column 3.9 x 150 mm, 5 µm and an injection system made of 100 µL loop. The HPLC pump was set at 1 mLmin⁻¹ flow rate.

2.7 Statistical Analysis

Tukey-Kramer Honestly Significant Difference (HSD) tests were conducted to compare colony counts of cocoa bean seeds for the different location. The threshold for statistical significance was set at a P value of = 0.05. The analysis was conducted using JMP in Version 5.1; SAS Institute 1992–1998.

3. RESULTS

3.1 Fungal Population

Sampling was carried out between July, 2014 peak of the raining season and June, 2015 (12 months). Colony forming unit per gram of cocoa beans were calculated on a monthly basis and then averaged for the wet season that started April to October and the dry season from November to March. Cocoa samples had the highest incidence of fungal count in August, 2014 with 8.19×10^5 cfu/g of cocoa bean and the lowest was recorded in March, 2015 to be 1.58×10^5 cfu/g of cocoa bean (data not shown). Cocoa beans collected from Oba in Akoko South West LG area had the highest average fungal population of 195×10^5 cfu/g of cocoa bean (Fig. 1) for the wet season and 115×10^5 cfu/g during the dry season while cocoa from Idanre had the lowest average with 4.964×10^5 cfu/g of cocoa bean dry season and 8.643×10^5 cfu/g cocoa bean for the wet season. Samples had high fungal incidence between July to September, 2014 but, the values reduced drastically as the dry season sets in. Samples from Idanre recorded generally very low fungal population with the highest value in August, 2014 at 2.55×10^4 cfu/g of cocoa bean (data not shown). The fungal counts in Oba were significantly different from other collection sites.

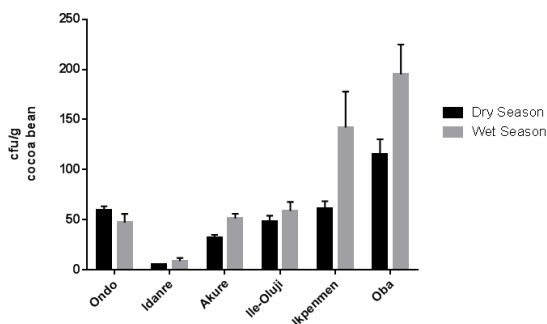


Figure 1. Average fungal count for the dry and wet seasons in cocoa producing areas of Ondo State

3.2 Isolated Fungi

The results on isolation of fungi from cocoa bean samples indicated that a total of thirty (30) fungal species were obtained (Table 2). The genera include *Aspergillus* which was most common, *Penicillium*, *Fusarium*, *Verticicladium*, *Neocosmospora*, *Mucor*, *Rhizopus*, *Beaveria*, *Alternaria*, *Sachharomyces*, *Phoma*. *Aspergillus* had fourteen (14) different species, genera *Penicillium* – 3 species; *Fusaria* are 3 and the others, one each.

Table 1 showed sites where different fungi species isolated were collected. *Aspergillus flavus*, *Aspergillus ochraceus*, *Penicillium citrium*, *Penicillium stecki* were found in samples collected from all the sites. *A. alutaceus* and *A. granulosis* were isolated only found in samples from Ikpenmen in Owo local government area of Ondo State. Samples from Idanre and Ondo had the least of different fungal strain infestation and colonization.

3.3 Ochratoxigenic Isolates

Aspergillus ochraceus, *A. niger*, *A. niger* aggregate, *A. carbonarius*, *A. aculeatus*, *A. terreus*, *A. vesicolor* and *A. flavus* all gave

positive blue green fluorescence light under UV light at 365 nm when the culture plates were viewed. Further analysis with TLC revealed that *A. niger*, *A. ochraceous*, and *A. carbonarius* all gave bright blue green coloration under UV light while *A. terreus* and *A. niger aggregate* gave dull coloration as shown in Table 3.

3.4 Ochratoxin A in Cocoa Samples

Average OTA concentration on a monthly basis for samples collected in Idanre had the lowest in November and December, 2014 and January, 2015 as no OTA were detected but highest value of 1.92 ± 0.98 ng/g cocoa bean in May, 2014. All the other locations/sites had OTA detected in samples from those locations. Oba had very high values of 32.09 ± 22.9 ng/g in January, 2015 and lowest was in May, 2014 (13.73 ± 10.02).

Comparison of average ochratoxin A concentrations of cocoa samples collected from stalls in the dry and the wet seasons in Ondo town were 0.456 ± 0.107 ng/g and 0.54 ± 0.05 ng/g respectively, Idanre samples recorded 0.012 ± 0.008 ng/g dry season and 0.506 ± 0.31 ng/g in the wet season, and stalls in Ikpenmen had 6.054 ± 1.143 ng/g (dry season) and 9.18 ± 0.88 ng/g (wet season) cocoa bean seeds OTA concentration.

4. DISCUSSION

About 720 cocoa bean seeds were collected for microbial quantification and the samples recorded very high fungal incidence in some places and low concentration of fungal infestation in other places. Spoilage and storage fungi of the genera *Aspergillus* and *Penicillium* were found in the cocoa bean samples collected from Ondo State. *Aspergillus*, *Fusarium*, *Penicillium*, *Phoma*, *Alternaria*, *Rhizopus*, *Mucor* genera found in cocoa samples were in agreement with the report of Makun et al. [35].

Table 1. Sample collection sites and location

Name	Description	Vegetation	Latitude	Longitude	Soil type
Ondo	City	Tropical rain forest	6°58' 25.20 "	4°50' 54 "	Sandy soil
Idanre town	Town	Tropical rain forest	7°10' 38.60 "	5°09'	Sandy soil
Oda-Akure	Settlement	Tropical rain forest	7°09' 45.70 "	5°14' 44 "	Laterized soil
Oba-Akoko	Town	Tropical rain forest	7°22' 02.20 "	5°43'23 "	Laterized soil
Ikpenmen	Town	Tropical rain forest	7° 15' 04.90"	5°35' 57"	Laterized soil
Ile-Oluji	Town	Tropical rain forest	7° 09'45"	4°50' 54"	Sandy/Laterized

Samples were collected for a period of twelve months (the wet and dry seasons)

Table 2. Isolated fungi at different collection location

S/N	Isolated fungi	Ondo	Idanre	Akure	Ile-Oluji	Ikpenmen	Oba-Akoko
1	<i>Aspergillus flavus</i>	x	x	x	x	x	X
2	<i>A. fumigatus</i>	x	x	x	x	x	X
3	<i>A. nidulans</i>	x	-	x	x	x	X
4	<i>A. niger</i>	x	x	x	x	x	X
5	<i>A. glaucus</i>	-	-	x	x	x	X
6	<i>A. niger aggregate</i>	x	-	x	-	x	X
7	<i>A. versicolor</i>	x	x	x	x	x	X
8	<i>A. terreus</i>	-	-	x	x	x	-
9	<i>A. aculeatus</i>	x	-	x	x	-	-
10	<i>A. carbonarius</i>	x	x	x	x	x	X
11	<i>A. alutaceus</i>	-	-	-	-	x	-
12	<i>A. granulosis</i>	-	-	-	-	x	-
13	<i>A. ustus</i>	-	x	-	x	-	-
14	<i>A. ochraceous</i>	x	x	x	x	x	X
15	<i>Penicillium citrinum</i>	x	x	x	x	x	X
16	<i>P. steckii</i>	-	-	x	x	-	X
17	<i>P. chrysogenum</i>	x	x	-	-	x	X
18	<i>P. verrucosum</i>	-	-	x	x	-	X
19	<i>Verticicladium sp</i>	-	-	x	x	x	X
20	<i>Neocosmospora vasinfecta</i>	-	-	-	x	x	X
21	<i>Fusarium verticillioides</i>	x	x	x	x	x	X
22	<i>F. oxysporum</i>	x	x	x	x	x	X
23	<i>F. graminearum</i>	x	-	x	-	x	-
24	<i>Mucor mucedo</i>	-	-	-	x	x	X
25	<i>Rhizopus stolonifer</i>	x	x	x	x	x	X
26	<i>Beaveria bassiana</i>	-	x	-	-	x	X
27	<i>Alternaria alternata</i>	x	-	-	-	x	X
28	<i>Phoma spp</i>	-	-	x	-	x	X
29	<i>Torula spp</i>	-	x	-	-	x	X
30	<i>Saccharomyces cerevicea</i>	x	-	-	x	x	X

KEY: x shows fungi isolated at the location, - shows it is absent

Table 3. Suspected ochratoxigenic fungi species

S/N	Suspected OTA producer	Bright coloration	Dull coloration
1	<i>Aspergillus ochraceous</i>	x	-
2	<i>Aspergillus alutaceus</i>	-	-
3	<i>Aspergillus niger</i>	x	-
4	<i>Aspergillus niger aggregate</i>	-	X
5	<i>Aspergillus carbonarius</i>	x	-
6	<i>Aspergillus aculeatus</i>	-	-
7	<i>Aspergillus terreus</i>	-	x
8	<i>Aspergillus flavus</i>	-	-
9	<i>Aspergillus vesicolor</i>	-	-

KEY: X, present and -, absent

The rainy season normally commence from April to October with double maxima rainfall and slight dry season between November and March [48]. The result of fungal colonization recorded in Oba and Ikpenmen were generally higher for the wet season than the dry season, which could be the result of poor fermentation and post fermentation procedures engaged in by farmers in the Akoko and Oba areas of the state. In a bid to beat the markets, the farmers do not allow proper fermentation to take place, so that, the weight of the bean seeds do not decrease but unfortunately, the resultant effects are black and clustered seed [49] which prevent evaporation and encourage mould infestation. Tagro et al. [49] explained that high moisture content above 8% after drying, clustered beans, black beans increases mould counts which is the opposite of brown bean seeds found in Idanre, Ondo, and Akure with lower fungal counts. Poor farming practices, ignorance and unhygienic drying methods cum poor storage conditions could predispose cocoa beans to fungal colonization.

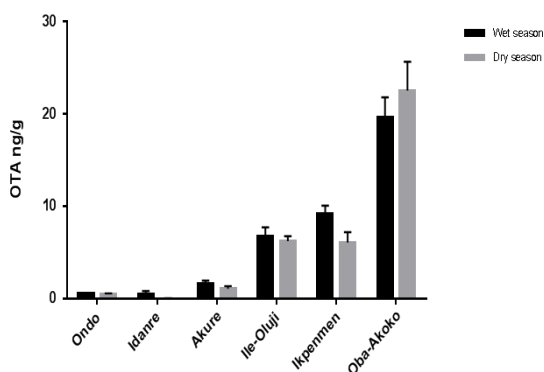


Figure 2. Average OTA concentration in ng/g cocoa sample between the dry and wet seasons in Ondo State

Cocoa beans from Akoko and Owo areas of Ondo State were usually graded poorly as a result of black and clustered beans, slaty and mouldy seeds. All these cumulate to making the region prone to higher fungal infestation.

Dano et al. [50] and Bastide et al. [51] explained that microorganisms are introduced into the bean seeds at the stage of fermentation, these microbes could be precursor for colonization of other fungal species resulting in high incidence of fungal strains. Munjounpou et al. [21] collaborated the finding that high fungal incidence are recorded after fermentation. Copetti et al. [23] added that drying increases fungal contamination which will result in high

incidence of fungal infestation but was contravened by Dano et al. [50] who posited that the type of drying method do not significantly affect the colonization or incidence of fungi in cocoa samples. To this end, FAO/WHO/UNEP [52] recommended fast drying to curtail fungal infestation and multiplication during drying.

Bean seeds from Idanre and Ondo were observed to be brown and not clustered. Samples from the two towns recorded low fungal counts and OTA contaminations. Brown cocoa bean seeds in high proportion in sample showed that cocoa were properly fermented thus biochemical and enzymatic reactions took place leading to expulsion of some polyphenolic compound and hydrolysis of anthocyanins [49]. These chemical changes, the author explained is responsible for colour change to brown. Brown cocoa seeds are smooth with hard skin so making them impervious to fungal colonization.

Tagro et al. [49] explained that closeness to sea was responsible for stagnant humidity which inadvertently resulted in more contamination, which was contrasted by our findings as the two (2) locations (Oba-Akoko and Ikpenmen) with the highest fungi contaminations respectively were further away from the sea than other locations that recorded lower fungi contaminations.

The presence of *Aspergillus* and *Penicillium* species and other storage moulds showed that these fungi are post harvest contaminants. *A. niger*, *A. ochraceus*, *A. carbonarius* have been implicated to be OTA producers [21]. The study was in consonance with Mounjounpou et al. [21] who posited that *A. ochraceus* and *A. carbonarius* were isolated from OTA contaminated cocoa bean seeds. The presence of *A. niger*, *A. flavus*, *A. ochraceus* in cocoa samples were corroborated by Aroyeun and Adegoke [46].

The report on OTA concentration were in agreement with earlier report on contamination of cocoa in Nigeria and other African countries [21,53]. High OTA concentration recorded in Ikpenmen and Oba-Akoko were as a result of poor fermentation management. Clustered bean, black bean seeds have been implicated to harbor pockets of moisture; Aroyeun and Adegoke [46] explained that high moisture leads to OTA formation. The authors opined that high relative humidity is a precursor to spoilage of stored produce as a result of mould growth and

mycotoxin production hence higher OTA contamination during the wet season.

During the wet seasons, fungi thrive better as a result of high humidity and hot environment which led to high incidence of fungal colonization recorded in the wet season. The report was in agreement with the position of Makun et al. [54] that most of the conditions favoring fungal growth are prevalent in the wet season.

The study suggests high infestation of cocoa bean seeds by saprophytic fungal that subsequently contaminate the bean seeds with ochratoxin A. OTA has been classified as possibly carcinogenic so, prompt action needed be taken to further supervise and monitor post harvest management practices of farmers in Ondo State, Nigeria. Reports indicated that contamination start at the point of breaking pods to storage locations where increased OTA production takes place [50,55].

OTA concentration reported in the study were generally higher above the 2ng/g recommended as tolerable level for cocoa and its products [56], which corroborated reports earlier presented by Dongo et al. [53] for cocoa in Cote d'Ivoire with average 0.25 ng/g as samples collected in Nigerian cocoa for samples from Idanre and Ondo and, average of 40 µg/kg in other places which were comparable with those of Oba-Akoko in the present study.

5. CONCLUSION

The problems of climate change, pest proliferation, poor agricultural management practices, old cocoa plants, dearth of labour force, and unsteady government policies have been reported to bring about reduced cocoa production and quality in respect of sales and export. The menace of contamination by mycotoxins and especially ochratoxin A had not previously been seen as a challenge by cocoa farmers in Nigeria. The high concentration of ochratoxin A recorded in cocoa samples from Ondo State showed that post-harvesting operations such as fermentation, drying, packaging, transportation, and storing within the state needs improvement as these are the major avenues through which fungal colonization and subsequent production of toxin take place.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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