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REGULAR ARTICLE

FUNGI ASSOCIATED WITH SPOILAGE OF DRIED COCOA BEANS DURING STORAGE IN EKTI STATE OF NIGERIA

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ABSTRACT

Fungi associated with cocoa beans during storage were surveyed in some stores in Ado, Ise, Emure and Ikere in Ekiti State of Nigeria during July-December 2010. The following fungi were consistently isolated from mouldy cocoa bean samples, namely; *Aspergillus niger*, *Aspergillus flavus*, *Botryodiplodia theobromae*, *Fusarium* spp., *Mucor* spp., *Neurospora* spp., *Penicillium* spp., and *Phytophthora palmivora*. The various fungi were isolated using washing, direct and dilution plate methods respectively. At Ado, the following fungi; *Aspergillus flavus*, *Aspergillus niger*, *Botryodiplodia theobromae*, *Mucor* spp., *Neurospora* spp. *Penicillium* spp., *Phytophthora palmivora*, *Rhizopus* spp. were occasionally isolated from stores that were not properly ventilated. At Ise, *Phytophthora palmivora*, *Mucor* spp. and *Penicillium* spp. were commonly isolated, while *Aspergillus flavus*, *Aspergillus niger* and *Rhizopus* spp. were occasionally isolated in stores where the bags were kept on the bare floor. At Emure, *Aspergillus* spp. and *Phytophthora palmivora* were commonly isolated, while *Aspergillus* spp., *Rhizopus* spp., *Neurospora* spp., *Botryodiplodia theobromae, Fusarium* spp. and Mucor spp., were occasionally isolated from stores with leaking roofs. At Ikere, *Aspergillus niger, Aspergillus flavus, Phytophthora palmivora*, *Fusarium* spp., *Mucor* spp. and *Penicillium* spp. were consistently isolated while *Rhizopus* spp., and *Botryodiplodia theobromae* were occasionally isolated. Some of these fungi gain access to the beans during fermentation, drying, storage and shipment to the foreign countries. Some of these isolated

fungi have been reported by many workers to produce toxic substances which have serious health implications in both man and animals.

Keywords: Survey, fungi, spoilage, storage, cocoa bean

INTRODUCTION

Cocoa belongs to the genus *Theobroma* in the family of the *Sterculiaceae*. But recently, with the application of molecular marker, *cacao* was classified to belong to the family *Malvaceae* (**Alvenson *et al.,* 1999**). According to **Opeke, (1992)** *Theobroma cacao* is cauliflorous and semi-deciduous and about 20 species of *Theobroma* are recognized.

Cocoa is a low-attitude crop. It grows from sea level up to an attitude of 700 metres. In Nigeria and in other African countries where cocoa is grown a minimum of 25 percent of clay is required for cocoa to do very well and healthy (**Opeke, 1992; Aigbekaen, 2004; Sanusi and Oluyole, 2005**).

The cocoa pods attain maturity between 110 to 130 days depending on the variety, from pollination to pod ripening (**Opeke, 1992; Sanusi and Oluyole, 2005**). To ensure the production of quality beans, it is essential that only matured and ripe pods are harvested and processed promptly (**Hamzat, 2005**). In West Africa there are two pods production seasons, the main season – July to December and light crop season – January to April (**Motamayor, 2002**). Harvesting exercise is carried out regularly and frequently to prevent immature ripening. For effective storage of excess cocoa pods, the use of modified packaging of cocoa pods in transparent polythene films conserved the commercial qualities of cocoa beans, such as cocoa butter, percentage moisture content, reduced growth and severity of decay (**Aroyeun *et al.,* 2006**).

Fermentation of cocoa beans is carried out in order to obtain a proper taste, colour; flavour associated with cocoa products and also to kill the embryo to forestall germination. There are several methods of fermentation. These include, heap fermentation, basket, sweat, box and tray fermentation method. The most popular and frequently used is the tray method (**Hamzat, 2005; Aroyeun *et al*., 2006**).

Stored cocoa is often damaged by insect, pests and most especially by moulds (**Hamzat, 2004; Aroyeun *et al*., 2006**). This type of damage mostly results from the failure to dry beans

properly. Production of good quality cocoa will therefore not only depend on proper fermentation but also on correct drying methods. Various methods of drying the fermented cocoa beans are used and these include sun drying and artificial drying. Most commonly used method in Nigeria is sun drying and this depend on the climatic conditions (**Motamayor, 2002**).

The quality of commercial beans depends very largely on how well the fermentation has been carried out (**Hamzat, 2004**). The period of fermentation of the cocoa bean determines the quality of the product that will be produced by the bean. On the other hand, microorganisms such as mould whose presence is detrimental to the quality of cocoa beans gain access to them during fermentation (**Hamzat *et al*., 2006**).

ICCO (2004) reported the isolation of several fungi growing on fermenting cocoa beans. Those of West Africa (Ghana) origin are *Aspergillus fumigatus, Aspergillus tamarii* and *Mucor pusillus*. With increasing length of fermentation therefore cocoa beans have a greater chance of being penetrated by mould which grows externally on them (**Opoku *et al*., 2007**).

The extent to which internal mouldiness occur in stored cocoa bean by fungi is inestimable. The cotyledons of the beans which is the mesh filament are affected and results into colour change from cream to green, yellow, black, brown or white in the cocoa beans (**Opeke, 1992; ICCO, 2004**).

These forms of structure are known as mould or microscopic fungi (**Krasauskas *et al.*, 2006**). Moreover, their presence in a bean could be readily seen with the naked eye especially when they are advanced in growth. Mould increase the free fatty acid content of cocoa butter and make referring of the fat essential before it can be used. In addition moulds do cause an actual loss in weight of cocoa beans (**Hamzat, 2004**).

The aim and objective of this study was to isolate the fungi associated with the spoilage of stored dried cocoa beans.

MATERIAL AND METHODS

Collection of Mouldy cocoa beans

Samples of mouldy cocoa beans were collected from early July to December 2010, in some cocoa stores in Ise, Emure, Ado and Ikere. A total of 20 cocoa stores were visited together in these towns.

Fifty beans were picked randomly from cocoa bags from each store. A total of 1000 beans were picked. Immediately upon collections, all samples were further dried to stop further mould growth (**Lillehoj *et al.*, 1975**). The samples were kept in insect free bags, labeled and transferred to the laboratory. The mouldy beans were separated from non affected beans.

Isolation of Microorganisms from Mouldy Cocoa Beans

Direct Plating Method

From each bag 50 beans were examined randomly for internal mouldness. The beans were washed twice with sterile distilled water by stirring. The cocoa beans were cut into two halves along the longitudinal axis with a sterile dissecting knife. Using a sterile dissecting forceps, mouldy cocoa beans were aseptically picked and placed on Malt Extract Agar and incubated at 28^{o}C for 7 days (**Amusa, 2001 and Arotupin, 2004**).

The fungi cultures were further subculture until pure colonies were obtained by successive hypha tip transfers. The cultures were examined under a dissecting microscope to determine the common fungi present.

Dilution Plate Method

This method was used to determine the type of fungi present in the mouldy cocoa beans. However, 1g of each bean sample was grounded and 10ml of sterile distilled water was added. This was shaken thoroughly and 1ml of the suspension was pipette into a sterile test tube and mixed again. This was repeated until dilutions 10^{-1} to 10^{-5} was obtained and 1ml from each of the dilution 10^{-3} to 10^{-5} was added to 18ml of molten Malt Extract agar. The plates were swirled gently to obtain a thorough mixing. The plates were incubated at 28^{o}C for 7days. Fungal colonies were subculture until pure cultures were obtained (**Atanda *et al.*, 1990**).

Washing Method

This method was carried out by weighing 1g of each sample into 10ml of sterile distilled water in a sterile test tube. This was shaken thoroughly and serial dilution of 10^{-1} to 10^{-5} was prepared from the resulting solution. However, 0.1ml of dilution of 10^{-4} and 10^{-5} were introduced into Malt Extract Agar plates. This was spread using a sterile glass spreader and incubated at 28^{o}C for 7 days.

Identification of microorganisms

The isolates were examined under day light for the colour of the culture and further examination was carried out using needle mount preparation method of **Tuite (1961), Crowley *et al*., (1969)** and **Burnett (1975)**.

Slide culture Method

The method of **Crowley *et al.*, (1969)** and **Dugan (2006)** was used whereby a 1cm^2 of malt extract agar was cut from a plate approximately 2mm deep and placed on a sterile glass slide. Fungus was maculated into the four vertical sides suing a sterile needle. A sterilized cover slip was applied so that it over lapped the medium on all sides and the preparation was placed on suitable support in a petri dish containing blotting paper soaked in 20% glycerol in water. The preparation is kept moist at 26^{o}C until adequate growth developed. After removing the medium the fungus adherent to both cover slip and slide was examined. A drop of alcohol added, followed by a drop of lactophenol blue and the preparation suitably covered and examined under the low power objectives microscope.

RESULTS AND DISCUSSION

In this study a total of nine fungi were isolated and identified based on their cultural and morphological characteristics. They are: *Aspergillus niger*, *Aspergillus flavus*, *Botryodiplodia theobromae*, *Fusarium* spp., *Mucor* spp., *Neurospora* spp., *Penicillium* spp., and *Phytophthora palmivora*.

The microorganisms isolated from mouldy cocoa beans from Ado, Ikere, Ise and Emure using three major methods (Direct plating, Dilution method and Washing method) are shown on Table 1.

This study showed that *Aspergillus* spp., *Penicillum* spp., *Mucor* spp., and *Phytophthora palmivora* were isolated from all the locations using the three methods of isolation, while those rarely isolated were *Fusarium* spp., *Neurospora* spp. and *Botryodiplodia theobromae*. These results is in agreement with the work of **ICCO (2004)** who worked on cocoa beans and reported the isolation of various species of fungi associated with internal mouldiness of cocoa beans in Ibadan..

The results of this study also showed that *Mucor* spp., *Rhizopus* spp., *Phythphora palmivora* and *Aspergillus* spp. were all isolated from cocoa beans from the three locations

(Ado, Ise, Emure). This is in agreement with the report of **ICCO (2006)** who found that *Mucor* spp., *Rhizopus* spp., *Phythophthora palmivora* and *Aspergillus* spp. were the principal fungi causing spoilage of stored cocoa beans.

The isolation of *Neurospora* spp. was common to only Ado and Emure while *Botryodiplodia theobromae* was common to Ado only. Isolation of *Penicillium* spp. and *Phytophthora* palmivora was common to Ado, Ise, Emure and Ikare. *Fusarium* spp. was common to Emure while *Mucor* spp. was common to Ikere. These results is in agreement with the previous findings by **Chatt (1953)** who reported that these moulds gained asses to cocoa bean at the various stages during the preparation of the crop from the market.

At Ikere, the following fungi were isolated using direct plate method namely, *A. flavus, P. palmivora, Penicillium* spp., while *A. niger, B. theobromae*, *Fusarium* spp., and *Mucor* spp. were isolated using dilution plate method and *Rhizopus* spp. was additionally isolated using washing method. The isolated fungi are in agreement with the work of **Oyeniran (1976)** who isolated most of the fungi from a survey of internal mouldiness of cocoa. These moulds gained access when the pods have been damaged. Similarly **Oyeniran (1976)** recorded 66% of internally mouldy cocoa beans which could have resulted from pre- harvest infection. Previous findings by **Broadbent *et al.*, (1969)** showed that brown and black cocoa beans contained internally mould beans.

At Emure and Ise, the fungi isolated using direct plating and dilution plate method respectively are *A. niger, A.flavus, P. palmivora* and *Penicillium* spp., while *Rhizopus* spp. was isolated using washing method. These findings are in agreement with the work of **Oyeniran (1976)** who isolated some of these fungi from a survey of mouldy cocoa beans. The possible source of these moulds could have been during sun drying process or the handling process during storage of the product. **(ICCO (2004)** reported an internal mouldiness of 25% when drying was prolonged for 13-14 days as a result of dull weather. **Copetti *et al., (*2011)** and **Magalhães *et al*., (2011)** also reported the isolation .*Aspergillus flavus, Aspergillus niger, Asperillus carbonarius, Aspergillus nomius, Aspergillus ochraceus* and *Penicillium paneum Absidia corymbifera* from the dried and stored cocoa beans.

In Ado, *Aspergillus* spp., *B. theobromae P. palmivora*, *Mucor* spp. were consistently isolated using direct plating and washing methods while *Neurospora* spp. was isolated using dilution plate method. The presence of these storage fungi are in agreement with the findings of **Mounjouenpou *et al.,* (2007)** and **Sánchez-Hervás *et al*., (2008)** who found *A. niger, A.carbonarius* to be associated with the processing and storage of cocoa beans in Cameroon.

Some of these fungi according to **Fandohan *et al.*, (2003)** gain asses to cocoa beans during storage either by air or by contamination from handlers of the stored cocoa beans.

The control of mouldiness of cocoa beans during drying is difficult. The wet season prolong the drying process of cocoa bean thereby encouraging the growth of spoilage fungi. Moreover, during fermentation fungi gain asses to bean when temperatures are extremely high. A few species such as *Aspergillus* spp., and *Mucor* spp., has been frequently isolated **(Hamzat, 2005; Sanusi and Oluyole, 2005)**. Other factors that enhance penetration of beans during this stage are germination and mechanical damage through which storage fungi gain asses to the inside of the beans of the stored sundried cocoa.

Tab 1 A summary of fungi isolated from the cocoa stores during the survey using various methods of isolation.

ISOLATED FUNGI	TOWNS AND METHOD			
	ADO	ISE	EMURE	IKERE
	A, B, C	A, B, C	A, B, C	A, B, C
Aspergillus flavus	+ + +	+ - +	+ + -	+ + -
Aspergillus niger	+ - +	+ - +	+ + +	- + +
Botryodiplodia theobromae	+ + -	- - -	+ - -	- + -
Fusarium spp.	- - -	- - -	+ - +	- + +
Mucor spp.	+ - +	+ + -	+ - -	- + +
Neurospora spp.	- + +	- - -	+ - +	+ - +
Penicillium spp.	+ + +	+ + +	- + +	+ - +
Phytophthora palmivora	+ + +	+ + +	+ + +	+ + +
Rhizopus spp.	- - -	- - +	- + -	- - +

Legend:

Key		Method of isolation
+	Present	(A) = Direct plating method
-	Absent	(B) = Dilution plating method
		(C) = Washing method

CONCLUSION

Cocoa beans are of great economic importance and in order to maintain the quality, they should be stored under controlled environment that would not be favourable for the growth of fungal flora thereby preventing deterioration of the stored cocoa bean and reduction in the chemical composition. This present study has revealed the various fungi associated with stored cocoa bean at different towns in Ekiti State. However, apart from good hygiene, proper handling and processing practice should be employed to reduce the contamination of stored cocoa bean. The isolated fungi can degrade the cocoa bean as substrate thereby reducing the market value, also making consumers especially the immunocompromised individual vulnerable to microbial infection.

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