COMPARATIVE STUDIES OF OCHRATOXIN A (OTA) PRODUCTION BY *Aspergillus niger*, *Aspergillus ochraceus* AND *Penicillium nordicum* IN COCOA, COFFEE AND YEAST EXTRACT SUCROSE AGAR

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ABSTRACT

The study aims to investigate the concentration of ochratoxin A (OTA) produced in dry cocoa beans by *Aspergillus ochraceus, A. niger* and *Penicillium nordicum* over a period of twenty-four days on three growth substrates of Yeast Extract Sucrose Agar (YES), Coffee and Cocoa agar. Quantitative analysis of OTA contamination in the samples was determined with a validated High Performance Thin Layer Chromatography (HPTLC) method with fluorescence detection by extraction with a mixture of 1.0 ml Dichloromethane and 1.0 ml of 1 % Acetic acid solution. OTA production by *Aspergillus ochraceus* in YES and Coffee media was high and more than observed in the Cocoa media. Highest levels of OTA produced during the growth of *A. ochraceus* in YES, Coffee and Cocoa media were 6.23, 8.09 and 2.29 ppm and occurred on day 23, 19 and 11 respectively. Although *A. niger* produced low levels of OTA in all the three media, YES and Coffee media were observed to support higher OTA production than Cocoa media. Maximum OTA concentration observed in YES media was 3.12 ppm on day 22. For Coffee media, 3.60 ppm occurred on day 15, while in Cocoa media 3.10 ppm was recorded on day 23. The production of OTA by *P. nordicum* showed the same trend with higher levels observed using YES and Coffee media, as compared with Cocoa media. Maximum levels of OTA produced during the growth of P. nordicum in YES, Coffee and Cocoa media were 8.16, 8.96 and 0.84 ppm and occurred on day 7, 19 and 12 respectively. Cocoa substrate is not suitable to OTA production by *A. ochreaceus* as compared to YES and Coffee media which are more suitable to OTA production by *A. niger* and *P. nordicum*.

Keywords : Ochratoxin A (OTA), HPTLC chromatography, *Aspergillus ochraceus, Aspergillus niger, Penicillium nordicum*, cocoa, coffee.

RESUME

SUBSTRATS ET CONTAMINATION EN OTA

La production d'Ochratoxine A (OTA) par Aspergillus ochraceus, Aspergillus niger *et* Penicillium nordicum *a été testée sur trois substrats, à base d'extrait de levure (YES), de café et de cacao pendant une période de 24 jours. L'analyse quantitative de l'OTA produite sur ces différents milieux par les moisissures étudiées a été effectuée par la méthode validée de la chromatographie en couche mince dénommée HPTLC avec détection par fluorescence. L'extraction de l'OTA s'est faite à l'aide d'une solution de 1 ml de dichlorométhane et 1,0 ml d'acide acétique 1 %.* Aspergillus ochraceus *est plus active sur les substrats YES et café que sur le substrat cacao. En effet, la production maximum d'OTA sur le substrat café est de 8,09 ppm après 19 jours d'incubation, 6,23 ppm après 23 jours d'incubation sur le substrat YES et, enfin, 2,29 ppm après 11 jours d'incubation sur le substrat cacao. Bien que les trois substrats sont faiblement contaminés par* Aspergillus niger*, les substrats YES et café présentent un plus grand niveau de contamination que le substrat cacao. Un maximum de 3,12 ppm d'OTA sont produits sur YES après 22 jours d'incubation et 3,60 ppm sur le substrat café après 15 jours d'incubation contre 3,10 ppm d'OTA produits sur le substrat cacao après 23 jours d'incubation. La production d'OTA par* Penicillium nordicum *présente les mêmes caractéristiques que celles*

observées avec Aspergillus ochraceus *sur ces trois substrats. La contamination maximum en OTA est de 8,16 ppm sur YES après 7 jours d'incubation, 8,96 ppm après 19 jours d'incubation sur café et enfin de 0,84 ppm après 12 jours d'incubation sur le substrat cacao. Bien que les trois souches soient productrices d'OTA, leur activité métabolique (toxinogénèse) est fortement influencée par les substrats dans lesquels elles se trouvent. Le milieu à base de cacao est moins favorable à la production d'OTA par* A. ochraceus *que* Aspergillus niger, Penicillium nordicum.

Mots clés : *Ochratoxine A (OTA), HPTLC chromatographie,* Aspergillus ochraceus, Aspergillus niger, Penicillium nordicum*, cocoa, coffee.*

INTRODUCTION

Cocoa beans are the principal raw material for the manufacture of chocolate and cocoa beverage. The beans are subject to infestation by filamentous fungi during drying and storage. In temperate climates, such as Canada, eastern and northwestern Europe and parts of South America, *Penicillium verrucosum* has been blamed, while *Aspergillus ochraceus* is the main source of OTA contamination in tropical and subtropical countries (IARC, 1976, 1993). When formed, Ochratoxin A may persist in a variety of finished foods and beverages, and in particular cereal grains. *Aspergillus* is commonly found in stored food commodities, such as beans, fruits, peanuts and cereals worldwide (Pitt and Hocking 1985) and coffee (Rao *et al.,* 1979 ; Truckess *et al.,* 1999). OTA is a potent toxin and most commonly occurring of a structurally related group of compounds that are nephrotoxic (IARC, 1993), immunosuppressive, teratogenic and carcinogenic agent (IARC 1983, Sax and Lewis 1987, IARC 1993). It consists of a polyketide-derived dihydroiso-coumarin linked through the 12-carboxy group to phenylalanine. It is a colourless crystalline compound that exhibits blue fluorescence under UV light, with melting point of 169 ºC and recrystallizes from xylene to form crystals that emit green (acid solution) and blue (alkaline solution) fluorescence in ultraviolet light. IARC 1983 and IARC 1993 reported the free acid of OTA to be soluble in organic solvents, and its sodium salt as soluble in water.

OTA is unstable to light and air, degrading and fading even after brief exposure to light, especially, under humid conditions. However, in ethanol solutions OTA is stable for longer than one year if kept refrigerated and in the dark. OTA is fairly stable to heat. In cereal products up to 35 % of the toxin survives autoclaving for up to 3 hours (IARC 1976).

OTA survives processing regimes like boiling, baking, roasting or fermentation and may occur in finished consumer products. The degree of OTA destruction may be dependent on parameters such as pH, temperature and the other ingredients present. It has been reported of OTA survival during malting and brewing (Boudia and Lebars, 1999 ; Baxter, 2001 ; Benismail *et al.,* 2005 ; Adegoke *et al.,* 2007), bread making, processing of cereals into breakfast cereals (Scott, 1996), green coffee beans production and derived coffee products, in animal feed production where it may be carried over into meat products and food (Fischbach and Rodricks, 1973). Its mode of operation in biological systems is to bind to serum albumin. Surveillance of food commodities has confirmed OTA to occur in a wide range of stored products. Analyses of foods have shown estimates of daily intakes ranging from 0.7 to 4.7 ng/kg bw, and blood samples have shown 0.2 to 2.4 ng/kg bw. Due to the undesirability of OTA in foodstuffs, the European Commission has proposed statutory maximum limits of 5µg/kg in raw cereal grains including rice and buckwheat, 3 µg/kg for cereal derived products or for cereal grains for direct human consumption, and 10 µg/kg in dried vine fruits.

The regulatory limit of OTA in foods has been set in many countries (Kuiper-Goodman, 1996). Although concentration of OTA in naturally occurring foods are usually below 50 µg/kg, much higher concentrations can develop when such products are poorly stored.

Human exposure to OTA through contaminated foodstuffs is undesirable and can be detected in blood and breast milk samples. Clark and Snedeker (2006) indicated that OTA has been found in animals and human tissues, blood and breast milk. Animal cancer bioassays showing that female rats exposed orally to OTA had significantly increased incidence of mammary tumors (fibroadenomas) and both male and

female rats developed hepatocellular tumors of the kidney (designated as well-differentiated trabecular adenomas), renal cell tumors (renal cystadenomas and solid renal-cell tumors), hepatomas (some exhibiting the trabecular structure), and hyperplastic hepatic nodules (IARC 1983, Sax and Lewis 1987). Gavage administration of OTA to male and female rats resulted in a dose-related increase in the incidence of renal-cell adenomas and adenocarcinoma ; while metastasis of the renal-cell tumors was also observed in male and female rats (NTP, 1989). When administered by Gavage, OTA increased the incidence and multiplicity of fibroadenomas of the mammary gland in female rats (IARC 1993, NTP 1989). In humans, studies of the relationship between exposure to OTA and human cancer show incidence of, and mortality from urothelial urinary tract tumors, which is correlated with the geographical distribution of Balkan endemic nephropathy in Bulgaria and Yugoslavia in an area where a relatively high frequency of contamination of cereals and bread with OTA had been reported (IARC 1976, 1983, 1987, 1993).

Several researchers have proposed different ways of screening fungal isolates for mycotoxins and other secondary metabolites, with most of them using a simple method to detect and another for confirmation. Freshly grated coconut as a medium for aflatoxin production has been used (Arseculeratne *et al.,* 1969) and recommended as a useful substrate. A coconut-agar medium for fast detection of aflatoxigenic fungi has also been developed (Lin and Dianese, 1976). A method based on ultraviolet detection of aflatoxin on agar medium (Sara *et al.,* 1974) has been reported while Bastos (1979) established a method for detection of ochratoxins and sterigmatocystin production by *Aspergillus* sp. Also developed was a rapid and sensitive method for the identification of mycotoxin produced (aflatoxins, sterigmatocystin, ochratoxin A, patulin and penicillic acid) from the liquid medium where the fungi were cultivated (Krivobok *et al.,* 1987). Filtenborg and Frisvad (1980) also proposed a simple screening-method for extracellular mycotoxins taking a small plug from the agar substrate that may be applied to the TLC plate. A similar method to the agar plug procedure designed to detect the intracellular mycotoxins has also been developed (Filtenborg *et al.,* 1983). Since these methods are fast and simple, their sensitivity may be sufficient to detect the most important toxigenic isolates.

Routine plating methods employed by servicing laboratories for industries and exporters of these commodities is time-consuming and does not indicate previous fungal infestation (Gourama and Bullerman, 1995). But, it only provides a total mould population to indicate only the degree of sporulation instead of the actual fungal biomass with a subjective high degree of variations (Jarvis *et al.,* 1983). Other methods like immunoassays have been used to screen moulds and their toxins (Chu 1984 ; Li *et al.,* 2000) but limited in the application to estimate actual fungal biomass on solid substrate (Tsai and Yu, 1999). Milanez *et al.,* 2002 used coconut agar and moistened corn medium as growth substrates to evaluate mycotoxin production by *Aspergillus* strains.

This paper describes a qualitative and quantitative method of determining Ochratoxin A in cocoa, coffee and Yeast Extract Sucrose Agar in light of current and proposed regulatory limits, as well as appropriate management procedures to mitigate public health risk of OTA

MATERIALS AND METHODS

PREPARATION OF COCOA AND COFFEE BEAN POWDER

The beans were placed in liquid nitrogen to harden and then ground to fine powder. These were placed (100.0 g) and sealed in polyethylene bags which were then sterilized by irradiation.

PREPARATION OF SPORE SOLUTION

Spore solution was obtained by dissolving 4.5 g NaCl, 0.5 g Tween 80 and 0.5 g Agar agar in 500.0 ml distilled water.

STANDARD MOULD CULTURES

Ochratoxin A producers, *Penicillium nordicum* BFE 487, *Aspergillus ochraceus* BFE 635, *Aspergillus niger* BFE 632 isolated from cocoa beans and maintained in the culture collection at the Institute of Hygiene and Toxicology (BFEL), Karlsruhe (Germany) were used in the study.

PREPARATION OF AGAR MEDIA

YES agar was prepared with 20.0 g Yeast Extract, 150.0 g Saccharose and 15.0 g *Agar agar* dissolved in 1000 ml of distilled water. The final pH was 6.5. Cocoa agar was prepared by

using 400.0 g of sterile cocoa powder dissolved in sterile solution of 15.0 g Agar agar in 1000 ml of distilled water. Coffee agar was prepared as for Cocoa agar with sterile coffee powder. The media were sterilized at 121 °C for 15 minutes.

The mixture was dispensed in 40.0 ml quantity into sterile Petri dishes of 94 mm of diameter and left to solidify.

INOCULATION OF MOULD SPORES ON AGAR MEDIA

Spore solution (10.0 ml) was placed into a sterile conical flask out of which 200 µl was pipetted into the surface of each mould growth separately, on respective agar medium. A sterile loop was used to scrape the surface and pipetted back into the conical flask. This process was repeated until a uniform spore suspension was obtained. About 200 µl of the suspension was then placed into sterile agar media of YES, Cocoa, Coffee and spread evenly with a sterile spreader. Plates with *A. ochraceu*s were incubated at 30 °C, while those with *A. niger* and *P. nordicum* suspensions were at 25 °C.

SAMPLING

Cultured mould isolates on the respective agar media were sampled with a 10.0 mm cork borer and two plugs of growth transferred into each of duplicate Eppendorf tubes. These were placed in a freezer to harden. A sterile spatula was then used to break the plugs and macerate the samples.

OTA EXTRACTION

An extraction solution prepared from a mixture of 1.0 ml Dichloromethane and 1.0 ml of 1 % Acetic acid was added to the macerated plugs. It was disintegrated in a mixer (Omni, Model 17106) for 30 minutes, after which it was transferred into an ultrasonic water bath (Branson, type 5510, DTH 9,5 l) for 5 minutes. The samples were then centrifuged (Labofuge A, Heraeus Christ, West Germany) at 8,000 tours per minutes during 10 minutes. The solids were removed and the supernatant dried under nitrogen gas in an evaporating system at 30 °C. Methanol (500.0 µl) was added to each sample and dried with nitrogen gas. It was resuspended in 500.0 µl Methanol before placing in an ultrasonic water bath (Branson, type 5510, DTH, 9,5 l) for 5 minutes. The samples were then centrifuged at 7,000 tours per minute during 30 seconds. Pooled samples of 500.0 µl from each duplicate Eppendorf tubes were then aseptically pipetted into vials and stored in the freezer for High Performance Thin-Layer Chromatography (HPTLC) analysis.

HPTLC ANALYSIS

Samples were analyzed for ochratoxin production using HPTLC machine (CAMAG TLC Scanner 3 and CAMAG Sprayer system, Germany) with computer programmed software. The HPTLC plates were pre-heated at 70 °C 10 to 30 minutes, prior to use. A calibration curve was prepared for each plate. The mobile phase was prepared in the ratio of 1 part Formic acid : 30 parts Ethylacetate : 60 parts Toluene. The concentration of the standard OTA used was 4.74 ppm. The fungal extracts obtained were subjected to HPTLC and the toxins estimated according to the intensity of their fluorescence observed under UV light.

STATISTICAL ANALYSIS

The statistical analysis of the results was carried out using the standard software XL STAT-PRO 7.52 The significance of the differences between the results was calculated with the test of Duncan, $P < 0, 05$. A variance analysis (ANOVA) was carried out on the obtained different results.

RESULTS

The elementary statistics of different tests carry out on the capacity of OTA production by *Aspergillus ochraceus, Aspergillus Niger* and *Penicillium nordicum* according to the subtrate, the interactions between the micro-organisms and the substrates are recapitulated in figure 7, tables 1, 2 and 3. In the same time, Ochratoxin A producing capacity of mould isolates obtained from heap and tray fermented cocoa beans is shown in figures 1, 2 and 3.

The production of OTA by *Aspergillus ochraceus* (Figure 1) in YES and Coffee media were higher than observed in the Cocoa media. Maximum amount of OTA produced during the growth of *A. ochraceus* in YES, Coffee and Cocoa media were 6.23, 8.09 and 2.29 ppm and occurred on day 23, 19 and 11 respectively.

The production of OTA by *Aspergillus niger* in the three different media investigated is shown in Figure 2. *A. niger* produced low levels of OTA in all the three media. However, YES and Coffee media were observed to support higher OTA production than Cocoa media. Maximum OTA concentration observed in YES media was 3.12 ppm on day 22. For Coffee media, 3.60 ppm occurred on day 15 while in Cocoa media 3.10 ppm was observed on day 23.

OTA produced by *Penicillium nordicum* in the three different media investigated is illustrated by Figure 3. OTA production by *P. nordicum* showed the same trend with higher levels observed using YES and Coffee media, as compared with Cocoa media. Maximum amount of OTA produced during the growth of *P. nordicum* in YES, Coffee and Cocoa media were 8.16, 8.96 and 0.84 ppm and occurred on day 7, 19 and 12 respectively.

Table 1 : Descriptive statistics of OTA production on various culture media.

Statistiques descriptives de production d'OTA sur différents milieux de culture.

Source	ddl	∑ squares	Average squares	F of Fisher	Pr > F
Media	2	174,612	87,306	29,489	< 0,0001
Microorganisms	2	97,429	48,714	16,454	< 0,0001
Media*microorganisms	4	61,689	15,422	5,209	0,001

Table 2 : Average production of OTA according to the media and the micro-organisms.

Production moyenne d'OTA en fonction des milieux et des micro-organismes.

	Milieu de culture	Moyenne	Min	Max
	Yes	3,54±1,80[a]	0,14	6,23
A. Ochraceus	Coffee	2,97±2,06[a]	0,44	8,09
	Cocoa	0,84±0,60[b]	0,18	2,29
	Yes	1,57±0,77[a]	0,24	3,12
A. Niger	Coffee	1,41±0,82[a]	0,11	3,60
	Cocoa	0,70±0,63[b]	0,20	3,10
	Yes	3,62±2,61[a]	0,06	8,16
P. nordicum	Coffee	4,60±2,66[a]	0,19	8,96
	Cocoa	0,35±0,24[b]	0,31	0,84

Variables with different superscript are significantly different (P < 0.05).

Les variables affectées d'une même lettre ne sont pas significativement différentes au seuil de 5 %.

Table 3 : Interaction between micro-organisms and media (Duncan, SNK, PPDS).

Interaction entre microorganismes et milieux de culture (Duncan, SNK, PPDS).

Modalités	Moyenne	Regroupements		
Coffee**P. nordicum*	4,602	A		
Yes* *P. nordicum*	3,620	A	B	
Yes**A.ochraceus*	3,542	A	B	
Coffee**A.ochraceus*	2,970		B	
Yes**A. niger*	1,568			C
Coffee**A. niger*	1,415			C
Cocoa**A.ochraceus*	0,836			C
Cocoa**A. niger*	0,704			C
Cocoa**P. nordicum*	0,350			C

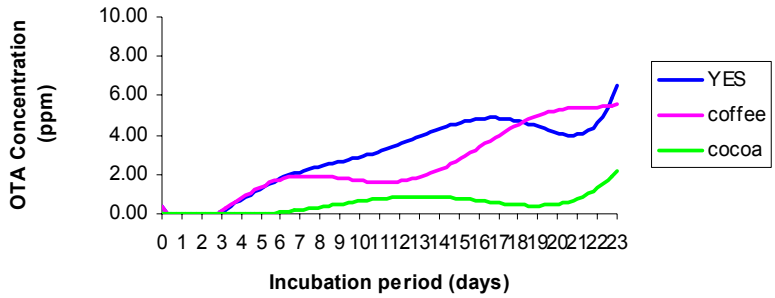

Figure 1 : Ochratoxin A (OTA) Production by *Aspergillus ochraceus* in YES, Coffe and Cocoa media.

Production d'Ochratoxine A (OTA) par Aspergillus ochraceus *sur milieux YES, Café et Cacao.*

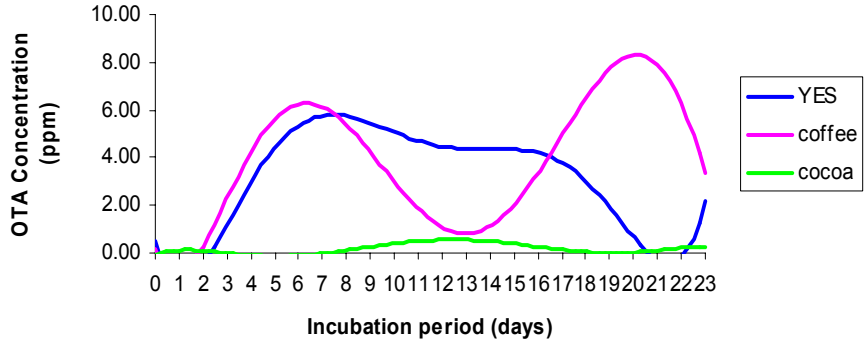

Figure 2 : Ochratoxin A (OTA) Production by A. niger in YES, Coffee and Cocoa media.

Production d'Ochratoxine A (OTA) par A. niger sur milieux YES, Café et Cacao

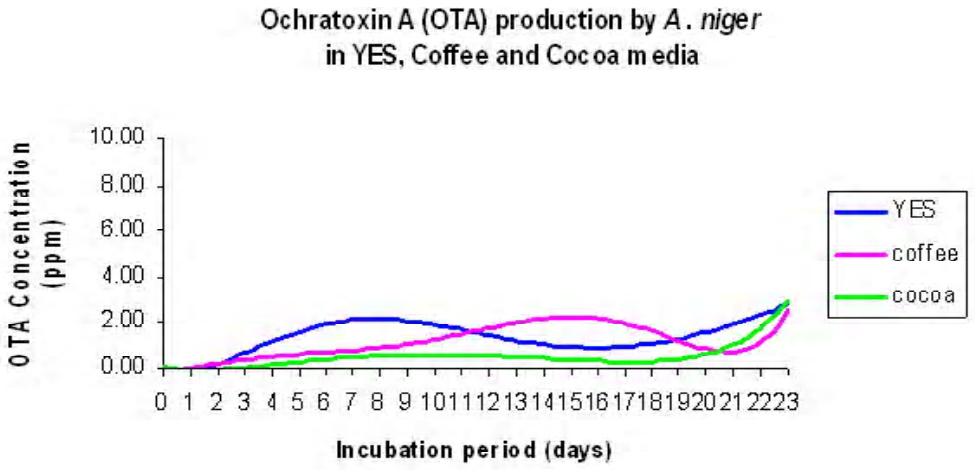

Figure 3 : Ochratoxin A (OTA) Production by *P. nordicum* in YES, Coffe and Cocoa media.

Production d'Ochratoxine A (OTA) par P. nordicum *sur milieux YES, Café et Cacao.*

Screening fungal isolates for OTA production, using Cocoa, coffee and YES agar, showed different abilities and time of elaboration. Concentrations of OTA produced by *A. ochraceus, A. niger* and *P. nordicum* on YES agar, Coffee agar and on Cocoa agar are illustrated by figures 4, 5 and 6.

The production of OTA by *A. ochraceus, A. niger* and *P. nordicum* in the three investigated different media is shown in figure 4. At the initial stages of growth of the three mould isolates on YES agar, *P. nordicum* produced maximum levels of OTA and declined to the lowest level of production during the last days. *A. ochraceus* however exhibited quite the opposite trend to *P. nordicum* in that it produced the least levels at the beginning of growth and maintained maximum levels at the end of the trials. *A. niger* exhibited medium levels of OTA concentration throughout the growth period.

Figure 5 shows the concentrations of OTA produced by *A. ochraceus, A. niger* and *P. nordicum* on Coffee agar. *P. nordicum* produced maximum levels of OTA throughout its growth during the 30 days as compared to the other two moulds. Although *A. ochraceus* recorded the least amount of OTA at the initial stages of growth, it produced steady increase in concentration almost throughout the 30 days of incubation. *A. niger* produced the least amount of OTA among the fungi on Coffee agar. Production of OTA on Cocoa agar (Figure 6) by *A. ochraceus, A. niger* and *P. nordicum* showed *A. niger* to have the highest concentration throughout the days of growth, with *P. nordicum* having the least levels. While *A. niger* and *A. ochraceus* produced large amounts of OTA over time, that produced by *P. nordicum* was, comparatively, low.

The average production of OTA during 22 days, the minimum and the maximum of OTA production per day by *A. ochraceus, A. Niger* and *P. nordicum* on YES, Coffee and Cocoa media is indicated in table 2. The minimum daily of OTA production r by the micro-organisms on the three mediums vary between 0,06 and 0,44 ppm whereas the maximum daily of OTA production by the same micro-organisms on three media vary between 0,84 and 6,23 ppm. The production of OTA per *A. ochraceus* (0,84 ± 0,60), *A. Niger* (0,70 ± 0,63) and *P. nordicum* (0,35 ± 0,24) remains weak on Cocoa media with significant different values with the average productions of OTA by *A.niger* and *P. nordicum* observed on YES and Coffee media.

The interactions between the media and the micro-organisms on the production of OTA are represented in table 3 and figure 7. These interactions show three groups which are A, AB and C according to the OTA production by the micro-organisms according to the medias. The production of OTA remains weak on the cocoa media despite the type of micro-organisms (Group C). Always in the group C, *A. Niger* presents a weak production of OTA on YES and Coffee media. The groups A and B are consisted of micro-organisms *A. ochraceus* and *P. nordicum* which undertake a significant metabolic activity on Coffee media with however an OTA production in favour of *P. nordicum.* Group AB is consisted of *P. nordicum and A. ochraceus* presents the same metabololic activity on YES media.

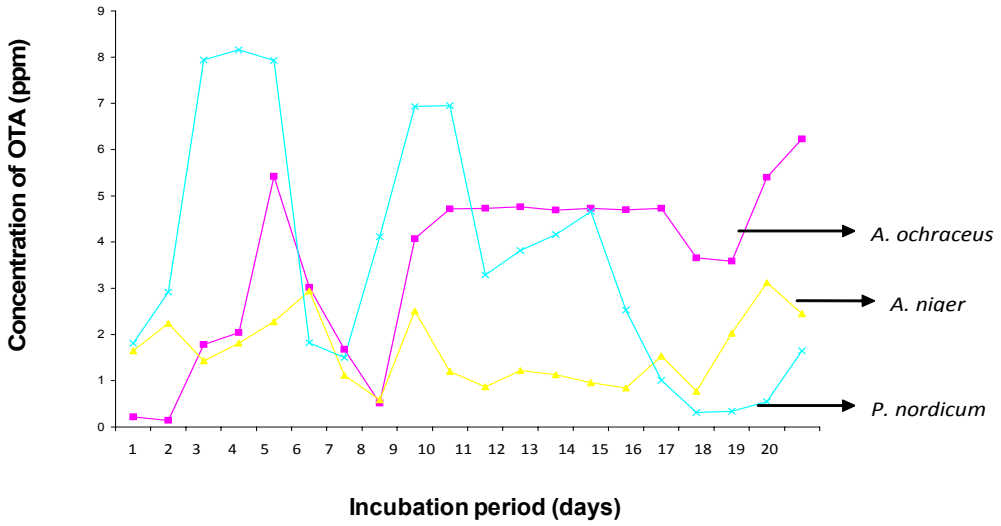

Figure 4 : Comparaison du niveau de production d'Ochratoxine A (OTA) par *A. ochraceus, A. niger* et *P. nordicum* sur milieu YES.

Comparative ochratoxin A (OTA) production of A. ochraceus, A. niger *and* P. nordicum *on YES agar.*

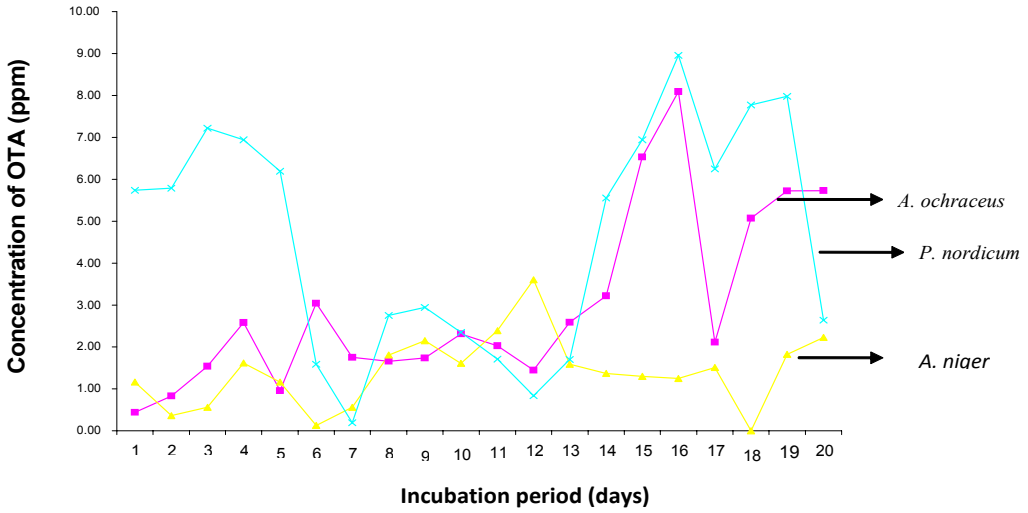

Figure 5 : Comparative ochratoxin A (OTA) production of *A. ochraceus, A. niger* and *P. nordicum* on Coffee agar.

Comparaison du niveau de production d' ochratoxin A (OTA) par A. ochraceus, A. niger *et* P. nordicum *sur gélose à base de café.*

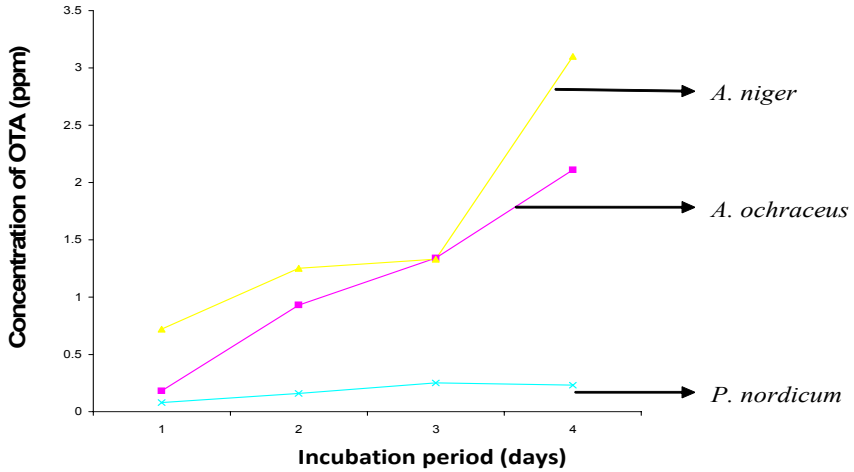

Figure 6 : Comparative ochratoxin A production of *A. ochraceus, A. niger* and *P. nordicum* on Cocoa agar.

Comparaison du niveau de production d'ochratoxine A (OTA) par A. ochraceus, A. niger *et* P. nordicum *sur gélose cacao.*

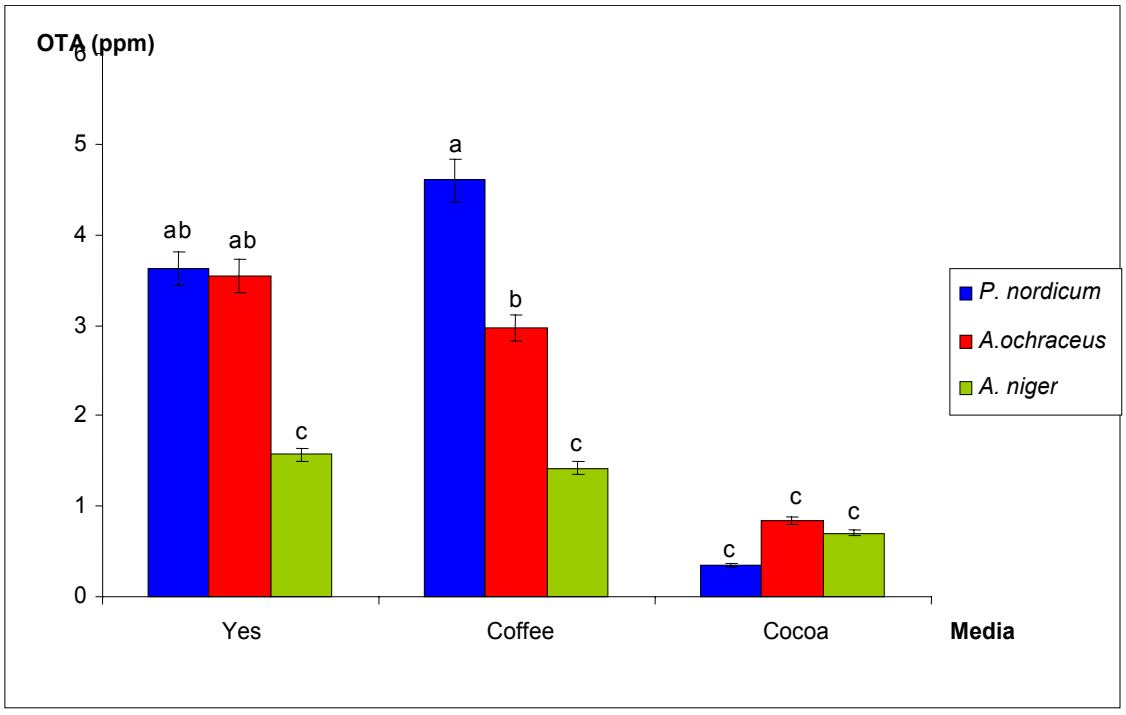

Figure 7 : Comparison of the level of produced OTA in various culture media by different microorganisms.

Comparaison du niveau de production d'OTA sur différents milieux par différents microorganismes.

The histograms affected with different superscript are significantly different ($P < 0.05$).

Les histogrammes affectés de lettres différentes sont significativement différents au seuil de 5%.

DISCUSSION

Cocoa substrate is not suitable to OTA-production by *Aspergillus ochraceus* to any significant level as to cause any health hazard, as the growth was minimal. Additionally, the initiation of growth of *Aspergillus ochraceus* in cocoa media was delayed until after day 5 ; whereas YES and coffee media encouraged growth as early as the third day of incubation. The implication therefore is that consignments of cocoa beans would ordinarily not support significant growth of *Aspergillus ochraceus* ; and if there was any, the onset would be delayed as compared to its growth in YES and coffee substrates. This is in line with the incubation of *A. niger* on cocoa media. Cocoa media supported less OTA production and also had a delayed onset during incubation of *A. niger*, beginning on the third day ; as compared to YES and coffee media which not only encouraged early growth, but also, produced comparatively higher levels of ochratoxin A. These statements are confirmed by the results obtained with *P. nordicum* and imply that a consignment of cocoa may not encourage the growth and elaboration of OTA ; and therefore cocoa powder is not a major source of OTA in the diet. In a study of 170 samples of cocoa products from different geographical origins which included cocoa bean, cocoa cake, cocoa mass, cocoa nib, cocoa powder, cocoa shell, cocoa butter, chocolate, and chocolate cream, it was observed that OTA contamination in cocoa beans was principally concentrated in the shell. Therefore, improvements of the industrial shelling process could prevent OTA occurrence in cocoa final products. This, implies that roasted cocoa powder is not a major source of OTA in the diet which is collaborated by this present study.

Regarding the screening fungal isolates for OTA production, it has been observed that during the growth of the three fungal isolates on YES agar, *P. nordicum* initially utilized the substrate and elaborated more copious amounts of OTA than *A. ochraceus* and *A. niger.* This observation is in line with Abarca *et al.* (1997) who indicated that *A. versicolor* produced OTA in corn culture but not in YES broth (yeast extract sucrose). The same observation corroborates with those of several authors. Indeed, Bastos (1979) tested 92 isolates of *Aspergillus* from seeds and observed that only one isolate of the *A. niger* was characterized as an OTA producer. When some *Aspergillus* isolates were analyzed, Ueno, (1991) observed that two *A. foetidus* isolates and one variety of *A. niger* used in the production of a local alcoholic beverage, produced OTA. Also among 19 isolates of *A. niger* var. *niger,* two OTA producers were observed not only in liquid medium (yeast and sucrose) but also in moistened corn (Abarca *et al.,* 1994). Ueno *et al.* (1991) also examined 100 fungal isolates and verified that 26 were OTA producers. Among the *Aspergillus* species producers were *A. sydowii, A. terreus, A. ustus* and *A. foetidus.* Since it is likely that some toxigenic fungi may be resident flora of the natural terrestrial environments in which the products were harvested, it would imply that the cocoa and coffee beans may have acquired these from the soil, wood or humus on the farms. The formation of OTA also depends upon the commodity on which the moulds grow and the prevailing climatic conditions. Some of these fungi may proliferate and produce OTA on such substrates as indicated by one strain of *P. nordicum* that was not associated with wood, producing OTA when cultured on wood chips (Land and Hult, 1987).

CONCLUSION

OTA production by *A. ochraceus, A. Niger* and *P. nordicum* according to the substrates on which these moulds were grown and the interaction between the substrates and the metabolic activity of these moulds were studied. Different culture media may support OTA produces varyingly, dependent on the composition of the media, the substrate and the fungus. From this study, Cocoa substrate is not suitable to OTA production by *Aspergillus ochraceus* to any significant level as compared to YES and Coffee media inoculated with *A. niger* and *P. nordicum.* Cocoa media was the substrate that least supported growth of *Aspergillus ochraceus, A. niger* and *P. nordicum* and, consequently, discouraged the production of Ochratoxin A. It knows that in some producers countries like Côte d'Ivoire, Ghana and Nigeria where large consignments of cocoa and coffee beans are produced and stored for export. The presence of toxigenic fungi is of great concern as the presence of OTA has been reported to persist worldwide in a variety of raw agricultural products and finished foods which may accumulate OTA in humans who consume them. Suitable monitoring strategies, control measures

and analytical methods are therefore necessary for tracking OTA levels in such foods. This study contributes to achieve this goal. Further investigations are needed to be carried out to apprehend the complexity of the substrates and OTA contamination.

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