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Detection of Genes involved in the Biosynthesis of Aflatoxin B1 in *Aspergillus* spp strains isolated from *Moringa oleifera* powder marketed in Abidjan (Côte d'Ivoire)

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A B S T R A C T

Moringa oleifera, traditionally consumed in some countries, is now widely distributed throughout the tropics, particularly in Africa. It is consumed in the form of food supplements in Côte d'Ivoire. However, the production method of Moringa leaf powder could be a source of contamination by potentially mycotoxigenic moulds. The objective of this work was to evaluate the level of contamination of Moringa powder by *Aspergillus Spp* strains and to detect the genes involved in the biosynthesis of aflatoxin B1. A total of 288 samples of Moringa powder were collected from vendors in six communes of Abidjan district. Isolation and purification of *Aspergillus* isolates were performed on Sabouraud medium with chloramphenicol. The identification of these strains was carried out by macroscopic and microscopic observations. The molecular aspect of the *Aspergillus* species and the detection of the *Nor*, *Ver*, *AvfA* and *AflR1* genes, involved in the biosynthesis process of aflatoxin B1, were carried out by PCR. The results show that the contamination rate of Moringa powder by *Aspergillus spp* was 62.46% with a predominance of *Aspergillus niger* (33.16%). Other species namely *Aspergillus flavus* (27.07%), *Aspergillus fumigatus* (24.87%) and *Aspergillus terreus* (13.98%) were also observed. The prevalences of strains harbouring the *AflR1*, *Nor*, *Ver*, and *AvfA* genes were 70.47%, 73.07%, 52.85% and 41.97% respectively. Strains harbouring *AflR*, *Nor*, *Ver*, and *AvfA* genes simultaneously were also detected in proportions of 39.37%. The presence of *Aspergillus* harbouring the genes coding for Aflatoxin B1 biosynthesis in Moringa powder could constitute a health risk for the consumer.

Introduction

Moringa oleifera, a tree native to India, is now widely distributed throughout the tropics, particularly in Africa where this species is attracting increasing interest from NGOs (Non Governmental Organization), scientists and even entrepreneurs (Adu-Gyamfi and Mahami, 2014). The nutritional quality of its leaves, which are very rich in vitamins, minerals and proteins, is currently giving rise to a significant number of initiatives in Africa, Europe and the United States in the fields of nutrition and dietetics (Gopalakrishnan *et al.,* 2016). Indeed, the leaves are usually dried and powdered, which facilitates preservation and consumption. However, the method of production of Moringa leaf powder does not always guarantee the safety of this food supplement (Houndji *et al.,* 2013). Indeed, the work of Houndji *et al.,* (2013) on the sanitary quality of Moringa powder marketed for the benefit of people living with HIV in Cotonou (Benin) revealed fungal contaminants, particularly moulds, in this food supplement. In Ghana, a study of the microbiological quality of *Moringa oleifera* leaves sold in markets in Accra revealed the presence of a widespectrum of microorganisms such as moulds whose load did not meet national and international standards (Adu-Gyamfi and Mahami, 2014). The main moulds of concern are fungi that are potentially mycotoxin-producing. Moreover, mycotoxin contamination of food stuffs is one of the main factors responsible for food insecurity (Udomkun *et al.,* 2017). According to the Food and Agriculture Organization of the United Nations (FAO), a quarter of the world's crops are affected by mycotoxins (Pankaj *et al.,* 2018). Among the different types of mycotoxins, aflatoxins (AFs) are highly toxic and are known to contaminate a wide variety of foods such as maize, peanuts, dried fruits, meat and milk products (Perrone *et al.,* 2014 ; Iqbal *et al.,* 2015). Aflatoxin B1 is widely regarded as the most potent liver carcinogen, affecting a large number of human and animal species, resulting in hepatotoxicity, immunotoxicity, teratogenicity and even death (Mehrzad *et al.,* 2011). Aflatoxins are generally produced by certain species of Aspergillus. These fungi usually grow in hot and humid conditions in tropical and subtropical regions (Battilani *et al.,* 2016). Ingestion of Aflatoxin from contaminated food stuffs has resulted in serious health complications in humans (Omondi *et al.,* 2013). In 2005 in Kenya, aflatoxin contamination of 317 people after eating maize resulted in the death of 125 of them after hospitalisation (Dieme *et al.,* 2016). In many African countries, aflatoxin contamination of food has economic, food and especially health consequences (Dieme *et al.,* 2016). In Côte d'Ivoire, studies have highlighted the contamination of food stuffs by aflatoxin-producing moulds (Boli *et al.,* 2014; Dedi *et al.,* 2017). However, little data exists on the molecular detection of potential aflatoxin-producing moulds. To strengthen the data on fungal contamination and molecular detection of aflatoxigenic moulds in food stuffs in Côte d'Ivoire, this study aimed to determine the aflatoxigenic potential of moulds of the genus *Aspergillus* contaminating samples of Moringa powder marketed in Côte d'Ivoire. In order to ensure the food safety of the consumer for a healthy life.

Materials and Methods

Sample Collection

During the period from July to September 2019, a total of 288 samples of Moringa powder (Figure 1) were purchased from retailers in different supply markets located in 6 communes of Abidjan district namely Abobo, Adjamé, Cocody, Koumassi, Marcory and Yopougon (Figure 2). The samples were collected in their original packaging under aseptic conditions and transported to the laboratory for mycological analysis.

Macroscopic and Microscopic Identification of the Different *Aspergillus* Species Contaminating the Moringa Powder

The method described by Giraud, (2011) was used for the isolation of the fungal flora. A stock suspension was made by homogenizing 10 g of each sample in 90 ml of Buffered Peptone Water (BPW).

A series of decimal dilutions was made from this stock suspension and the dilutions 10^{-1}, 10^{-2} and 10^{-3} were retained. Then 0.1 millilitre of these retained dilutions was spread on Sabouraud agar supplemented with 10 µg/mL of chloramphenicol previously prepared and poured into Petri dishes. The plates were incubated at 30°C for 3 to 7 days. Daily observations were made as soon as the mycelium appeared. Each developed mycelium under went several successive inoculations until pure strains were obtained. The identification of the pure strains obtained was mainly based on cultural (macroscopic identification) and morphological (microscopic identification) characteristics. Morphological identifications were performed according to the taxonomic keys of Samson *et al.*, (2014) available for species of the genus *Aspergillus*.

Molecular Identification of Moulds of the Genus *Aspergillus*

DNA Extraction

The DNA of each fungal isolate was extracted according to the method described by Zhang *et al.*, (2010) with some modifications. DNA was extracted with 0.5 g of fungal mycelia harvested from freshly grown pure cultures on Sabouraud chloramphenicol medium. The mycelium was ground properly in a mortar and then transferred to phosphate buffered saline (PBS) for a series of three cycle of freezing/thawing in liquid nitrogen. Subsequently 500 µL of the fungal suspension was added to 500 µL of lysis buffer (1.4 M NaCl, 20 mM EDTA, 100 mM Tris-HCL) and incubated at 55°C for 3 hours in a dry bath. To the lysate, 500 µL of phenol-chloroform-alcohol-isoamyl (25-24-1) was added. The tubes were centrifuged at 13000 rpm for 10 minutes at 4°C. The upper aqueous phase was transferred to in a new sterile 2 ml Eppendorf tube. To the upper aqueous phase, 1/10th of 3M sodium acetate and 500 µl of ice-cold absolute ethanol were added and stored at -80°C for 2 hours. The tubes were centrifuged at 13000 rpm for 20 min at 4°C. Subsequently, 1 ml of ice-cold 70% ethanol was added to the pellet. The tubes were centrifuged at 13000 rpm for 10 min at 4°C. The pellet was dried in a dry water bath at 56°C for 2 h. The resulting DNA was dissolved in 50 µL of nuclease free water and stored at -20°C.

Molecular Identification of *Aspergillus* Strains

Verification that the Strains Belong to the Fungal Kingdom

Molecular identification by polymerase chain reaction (PCR) targeting the Internal Transcribed Spacer (ITS) region described in various works (Sudharsan *et al.*, 2017) was chosen as the target region for confirmation of the affiliation of the isolates to the fungal kingdom. The primer pair used ITS1 and ITS4 amplifies a region of size 590 bp (Table 1).

The PCR was performed in a final volume of 25 µL. The reaction medium consisted of 10.25 µL of Nuclease free water (Ambion), 5 µL of 5X buffer, 1.5 µL of 25 mM MgCl2 (Promega Corporation, Madison, USA), 0, 5 µL of 10 mM dNTPs, 1 µL of each 10 mM primer, 0.25 µL of Go Tag® G2 Flexi DNA polymerase 5 U/µL (Promega Corporation, Madison, USA) and 5 µL of DNA. Amplification was performed in a Gene Amp PCR-system 9700 thermal cycler (Applied Biosystems). The amplification programme included an initial denaturation phase at 95°C for 5 min, followed by a 35-cycle phase involving denaturation at 95°C for 30 s, hybridisation at 50°C for 30 s and elongation at 72°C for 1 min. This programme ends with a final elongation phase at 72°C for 3 min. All PCR amplification products were revealed on a Doc EZ® imager gel (Bio-Rad) after electrophoresis in 2% agarose gel containing Syber safe (Invitrogen).

Molecular Identification of *Aspergillus* Species

Monosporic cultures of *Aspergillus* isolates were specifically identified. This identification was done using primers specific to the species of *Aspergillus* strains. Four primer pairs (PEX1-PEX 2, PEPO 1-

PEPO 2, PEPI 1-PEPI 2 and ATE 1-ATE 2) specific to four (4) species of the genus *Aspergillus* were used for the species specific identification of *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus* and *Aspergillus terreus* (Table 2).

The 25 µL reaction medium consisted of 10.25 µL of Nuclease free water (Ambion), 5 µL of 5X buffer, 1.5 µL of 25 mM MgCl2, 0.5 µL of 10 mM dNTPs, 1 µL of each 10mM primer, 0.25 µL of Go Tag® G2 Flexi DNA polymerase 5 U/µL (Promega Corporation, Madison, USA) and 5 µL of DNA template. Amplification was performed in a Gene Amp PCR-system 9700 thermal cycler (Applied Biosystems). The amplification programme included an initial denaturation phase at 95°C for 10 min, followed by a 40-cycle phase involving denaturation at 94°C for 1 min, hybridisation at 59°C for 1 min and elongation at 72°C for 1 min. This programme ends with a final elongation phase at 72°C for 5 min. Revelation of the different amplification products was carried out by electrophoresis on an agarose gel prepared at 2% in a Tris-borate-EDTA solution (TBE, 1x). To the supercooled agarose, a SyBer® Safe DNA gel solution (Invitrogen) (8 µL per 100 mL) was added. The mixture was thoroughly homogenised and the gel poured into a holder. Migration was performed at 120 Volts for 25 min to 30 min in 1x Tris-borate-EDTA (TBE) buffer. After migration, the gel was placed in a computerized system with Gel Doc EZ TM Imager (Bio-Rad, USA) for visualization. The presence of bands corresponding to the amplified fragment was compared with the size of the 100 bp DNA Ladder (Promega) and the positive control. The absence of a band was considered a negative result.

Detection of Genes Involved in Aflatoxin B1 Biosynthesis

The detection of genes involved in the Aflatoxin B1 biosynthetic pathway was performed using multiplex and monoplex PCR according to the method described by Sudharsan *et al.,* (2017) with some modifications. Four (4) primer pairs were selected for the detection of the presence of the four important genes (*AflR*, *AvfA*, *Ver-1* and *Nor-1*) involved in the aflatoxin B1 biosynthetic process (Table 3). Multiplex PCR was used to target the 3 structural genes (*Nor-1*, *Ver-1* and *AvfA*) that code for key enzymes in aflatoxin B1 production. Monoplex PCR was used to target the *AflR-1*gene which encodes a regulatory factor in the activation of transcription of genes involved in the aflatoxin B1 biosynthetic pathway.

The reaction was performed in a 25 µL volume containing 10.25 µL of Nuclease free water (Ambion), 5 µL of 5X buffer, 1.5 µL of 25 mM MgCl2, 0.5 µL of 10 mM dNTPs, 1 µL of each 10mM primer, 0.25 µL of Go Tag® G2 Flexi DNA polymerase 5 U/µL (Promega Corporation, Madison, USA) and 5 µL of DNA template. Amplification wasperformed in a Gene Amp PCR-system 9700 thermal cycler (Applied Biosystems). The amplification programme consisted of an initial denaturation phase at 95°C for 10 min, followed by a 40-cycle phase involving denaturation at 94°C for 30 s, hybridisation at 58°C for 1 min, elongation at 72°C for 1 min and final elongation at 72°C for 10 min.

The simplex PCR involved the detection of the AflR-1 gene and was performed under the same conditions as above, the difference being the amplification program consisting of an initial denaturation phase at 95°C for 15 min, followed by 40 cycles each consisting of a denaturation phase at 94°C for 1 min, a primer hybridization step at 60°C for 1 min, an extension phase at 72°C for 90 s and a final extension phase at 72°C for 10 min.

All PCR amplification products were revealed by agarose gel electrophoresis prepared at 2% in Tris-borate-EDTA (TBE, 1x) solution. To the supercooled agarose, a SyBer® Safe DNA gel solution (Invitrogen) (8 µL per 100 mL) was added. The mixture was thoroughly homogenised and the gel poured into a holder. Migration was performed at 100 Volts for 45 min in 1x Tris-borate-EDTA buffer (Sigma Aldrich, USA). After migration, the gel was placed in a computerized system with Gel

Doc EZ TM Imager (Bio-Rad, USA) for visualization. The presence of bands corresponding to the amplified fragment was compared with the size of the 100 bp DNA Ladder (Promega) and the positive control. The absence of a band was considered a negative result.

Results and Discussion

Level of Contamination of Moringa Powder Samples by *Aspergillus* Moulds

Of the 288 Moringa powder samples analysed, 244 (84.72%) samples were contaminated with moulds. Identification to species level was done according to the taxonomic characteristics of *Aspergillus* species. Macroscopic and microscopic observations of these isolates at the ×40 objective allowed us to identify a total of 309 strains, with 193 (62.46%) *Aspergillus* strains among which 4 species were identified, namely *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus terreus*. The predominant isolate was *Aspergillus niger* (64 strains or 33.16%), followed by *Aspergillus flavus* (54 strains or 27.98%), *Aspergillus fumigatus* (48 strains or 24.87%) and *Aspergillus terreus* (27 strains or 13.98%) (Figure 3).

Molecular Identification of *Aspergillus* Strains Isolated from Moringa Powder

Membership of the Isolated Strains to the Fungal Kingdom

The analysis of the results of the search for the fungal affiliation of the strains by the detection of the Its gene revealed that all 193 *Aspergillus* strains harboured the desired gene. The electrophoretic profile of the Its gene amplification product is shown in Figure 4. The size of the gene is 590 bp.

Molecular Identification of *Aspergillus* Species

The different *Aspergillus* species identified culturally on the basis of their morphological characteristics were confirmed by PCR analysis. All strains of *Aspergillus fumigatus* were successfully amplified by the PEX1-PEX2 primer pair producing 250 bp amplicons, the primer pair specific to *Aspergillus terreus* (ATE1-ATE2), *Aspergillus flavus* (PEPO 1-PEPO 2) and *Aspergillus niger* (PEPI1-PEPI2) amplified a segment of 450 bp, 150 bp and 200 bp respectively. The electrophoretic profiles of the amplification product of the *Pex*, *Ate*, *Pepi* and *Pepo* genes specific to *Aspergillus fumigatus*, *Aspergillus terreus*, *Aspergillus niger* and *Aspergillus flavus* species respectively are shown in Figure 5A-D.

Genes Involved in the Aflatoxin B1 Biosynthetic Pathway in *Aspergillus* Species Isolated from Moringa Powder

The multiplex and monoplex PCR used to detect the presence of the genes (*Nor*, *Ver*, *AvfA*) and the *AflR* gene coding for proteins involved in the aflatoxin B1 biosynthetic pathway respectively showed that all the targeted genes were successfully amplified and matched the size of their PCR products (Figure 6A-B). Among the 193 *Aspergillus* strains isolated from Moringa powder 141 isolates or 73.07% possessed the *Nor*gene, the *Ver* gene was detected in 102 (52.85%) isolates, the presence of the *AvfA* gene was detected in 81 (41.97%) isolates and the *AflR* gene was found in 136 (70.47%) isolates. The detection of *Nor* gene was observed mostly in *Aspergillus flavus* (85.16%) and *Aspergillus niger* (76.56%) followed by *Aspergillus fumigatus* (64.58%) and *Aspergillus terreus* (55.55%). The presence of the genes *Ver*, *AvfA* and *AflR* was predominantly found in *Aspergillus flavus* and *Aspergillus fumigatus* strains. The frequency of detection of the different genes according to the strains is shown in Table 4.

The analysis of moringa powder sold in the markets revealed that 62.46% of the samples were contaminated with moulds of the genus *Aspergillus* with the presence of four species of *Aspergillus*. These were *Aspergillus flavus* (27.98%), *Apergillus niger* (33.16%), *Aspergillus fumigatus* (24.87%), and *Aspergillus terreus* (13.98%). The present study

results corroborate with those of (Priyanka *et al.*, 2015) who also isolated the same *Aspergillus* species from food grain samples in India. Among the *Aspergillus* isolated from Moringa powder samples, *Aspergillus niger* aggregates were the predominant species, followed by *Aspergillus flavus*. These results corroborate with those of Muhammad *et al.*, (2010) who identified among the predominant species of *Aspergillus* contaminating food for human consumption *Aspergillus niger* a (3.74%) and *Aspergillus flavus* (22.64%) Studies conducted by (Riba *et al.*, 2008) in Algerian wheat samples, also revealed that 66% of the wheat sampleswere contaminated by moulds of the genus *Aspergillus*. These fungal species are mostly present in poorly preserved or dried foods (Hissein *et al.*, 2019). Thus, similar studies were conducted by (Dedi *et al.*, 2017) on the characterisation of maize flour sold in the markets of Adjamé, Yopougon and Abobo in Côte d'Ivoire. These authors reported in their work the presence of moulds belonging to the genera *Aspergillus* such as *Aspergillus flavus* and *Aspergillus niger*. The high frequency of *Aspergillus* contamination in Moringa powder samples could be explained by the fact that *Aspergillus*is a very common fungus, in the soil and air through its spores (Hissein *et al.*, 2019). As Moringa powder is obtained after a process of grinding and drying the biomass, contamination could therefore occur during handling (harvesting, washing, drying, storage, packaging). This point was also made by Hocking (2006) who pointed out that fungi of the genus *Aspergillus* belonging to the sub-branch Ascomycotina and having a sexual reproduction mode, easily colonise food products when storage conditions are not suitable. In addition, *Aspergillus* contaminate many food stuffs such as cocoa beans, coffee beans, cassavaflour, cereals, fish, peanuts, dried fruits, wine, poultry eggs and milk (Micheline *et al.*, 2020). In addition, local weather conditions as well as environmental conditions in storage facilities, including temperature and relative humidity, contribute to the growth of certain *Aspergillus* species and are therefore potential risks for aflatoxin production (Davari *et al.*, 2014). Studies have shown that commonly dried and processed food stuffs are significant targets for toxigenic moulds including aflatoxin B1 which is the most abundant and toxicaflatoxin (Lahouar, 2016). In this study the aflatoxigenicity of the identified *Aspergillus* strains was examined using PCR, a molecular detection technique for the identification of genes involved in the aflatoxin B1 biosynthesis pathway. The results revealed the simultaneous presence of all 4 genes (*Nor*, *Ver*, *AvfA* and *AflR*) in 39.38% of *Aspergillus* isolates. These results are in agreement with the results of Rashid *et al.*, (2008), who detected the same genes in aflatoxigenic isolates of some *Aspergillus* strains in their work. This study showed that among the genes involved in the aflatoxin bisonythesis pathway, the *Nor* gene (73.07%) and the *AflR* gene (70.47%) were the most detected in the four (4) *Aspergillus* species. However a predominance of detection of these 4 genes was most observed in *Aspergillus flavus* isolates.

The high detection rate of *Nor* gene in all 4 *Aspergillus* species, was also reported by Priyanka *et al.*, (2015), who observed the presence of *Nor* gene in several *Aspergillus* isolates while working on molecular detection of toxigenic *Aspergillus* species in food cereal samples in India. The presence of these aflatoxin biosynthetic genes shows the potential capacity of *Aspergillus* species isolated from Moringa powder to produce Aflatoxin. Indeed the study conducted by Priyanka *et al.*, (2015) showed that all the *Aspergillus* strains that tested positive to HPLC produced aflatoxin equally tested positive to molecular detection genes involved in Aflatoxin biosynthesis. Rodrigues *et al.*, (2009) presented details of a multiplex PCR assay for the detection of aflatoxigenic fungi. Three structural genes (*Nor*, *Ver* and *AvfA*) involved in aflatoxin biosynthesis were targeted as markers to distinguish aflatoxinogenic from non-aflatoxinogenic fungi. The presence of these genes could be due to the way Moringa powder is dried, stored and processed. Indeed, Moringa leaves are often stored in conditions that do not respect hygienic rules. Then, for processing, the leaves are first soaked in water and then dried before milling.

Table.1 Primer pair used for fungal strain identification

Targets	Primers	Sequence (5´-3´)	Size (bp)	Reference
Fungalstrains	ITS1	TCC GTA GGT GAA CCT GCG G	590 pb	Sudharsan *et al.*, (2017)
	ITS4	TCCTCCGCTTATTGATATGC		

Table.2 Specific primers used for the identification of *Aspergillus* species

Targets	Primers	Sequence (5´-3´)	Size (pb)	References
Aspergillus fumigatus	PEX1	TATGTCTTCCCCTGCTCC	250 pb	Priyanka *et al.*, (2015)
	PEX 2	CTATGCCTGAGGGGCGAA		
Aspergillus flavus	PEPO 1	CGACGTCTACAAGCCTTCTGGAAA	200 pb	Priyanka *et al.*, (2015)
	PEPO 2	CAGCAGACCGTCATTGTTCTTGTC		
Aspergillus niger	PEPI 1	CCAGTACGTGGTCTTCAACTC	150 pb	Logotheti *et al.*, (2012)
	PEPI 2	CATCACCATGACCATCGTTTGCT		
Aspergillus terreus	ATE 1	CTATTGTACCTTGTTGCTTCGGCG	450 pb	Logotheti *et al.*, (2012)
	ATE 2	AGTTGCAAATAAATGCGTCGGCGG		

Table.3 List of primers used for the detection of genes involved in the aflatoxin B1 biosynthetic pathway

Targets	Primers	Sequence (5´-3´)	Size (pb)	Reference
Nor-1	NorF	ACCGCTACGCCGGCACTCTCGGCAC	400 pb	Davari *et al.*, (2014)
	NorR	GTTGGCCGCCAGCTTCGACACTCCG		
Ver-1	VerF	GCCGCAGGCCGCGGAGAAAGTGGT	537 pb	Davari *et al.*, (2014)
	VerR	GGGGATATACTCCCGCGACACAGCC		
AvfA	AvfA-F	GGTCACATACGCTCTTCTCG	811 pb	Yu *et al.*, (2000)
	AvfA-R	CACAACGCCGTCAACTACTG		
AflR-1	AflR1-F	AACCGCATCCACAATCTCAT	798 pb	Manonmani *et al.*, (2005)
	AflR1-R	AGTGCAGTTCGCTCAGAACA		

Table.4 Detection frequencies of genes involved in aflatoxin B1 biosynthesis in *Aspergillus* strains isolated from Moringa powder

Strains	Number of isolates	Number (Gene detectionfrequency(%))			
		Nor	*Ver*	*AvfA*	*AflR1*
Aspergillus flavus	54	46 (85,2)	31 (57,4)	27 (44,1)	43 (79,6)
Aspergillus niger	64	49 (76,6)	33 (51,6)	23 (35,9)	39 (60,9)
Aspergillus fumigatus	48	31 (64,6)	27 (56,3)	23 (47,9)	35 (72,9)
Aspergillus terreus	27	15 (55,6)	11 (40,7)	8 (64,7)	19 (70,4)
Total	193	141 (73,1)	102 (52,8)	81 (41,9)	136 (70,5)

Fig.1 Moringa leaf powders old in markets

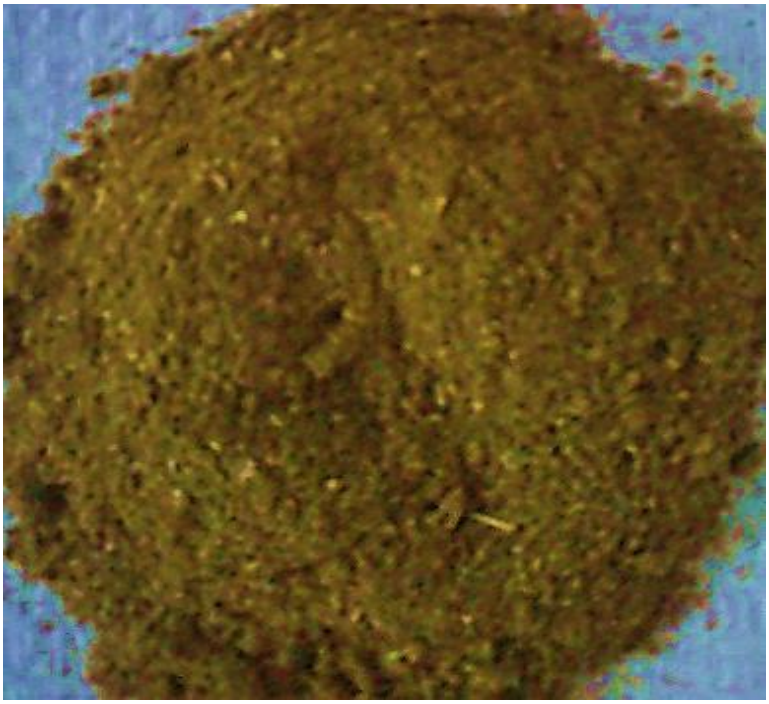


Fig.2 Map of the city of Abidjan showing the sites used for the study

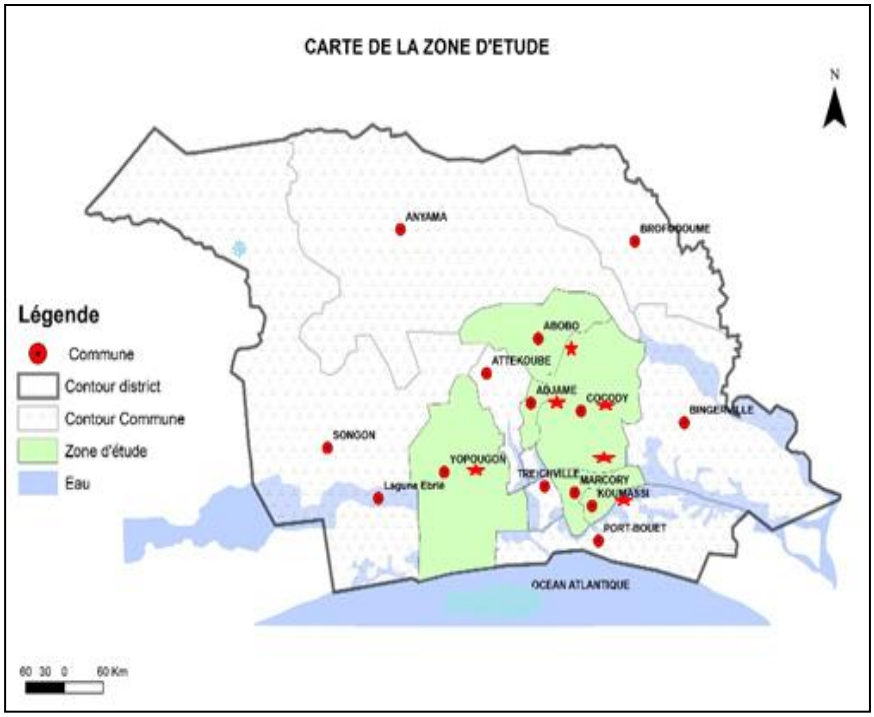


Fig.3 Level of contamination of Moringa powder samples by *Aspergillus* moulds

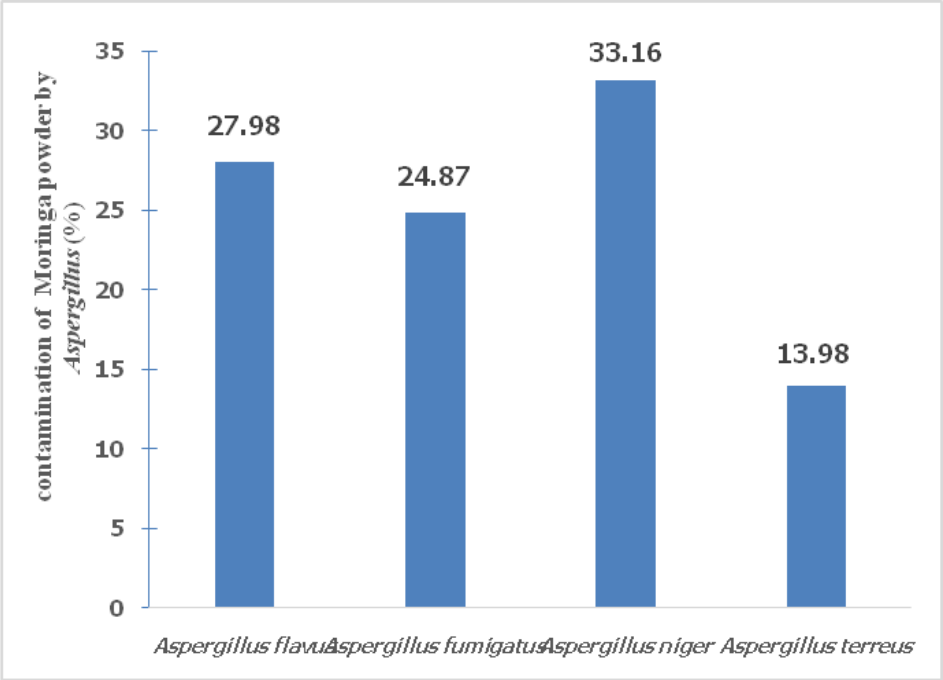


Fig.4 Electrophoretic profile of *Its* gene amplification product on 2% agarose gel performed with genomic DNA of *Aspergillus* strains (M : 100 bp molecular marker; Lanes 1, 2, 3, 4, and 5 : positive test strains; T+ positive control; T-, negative control)

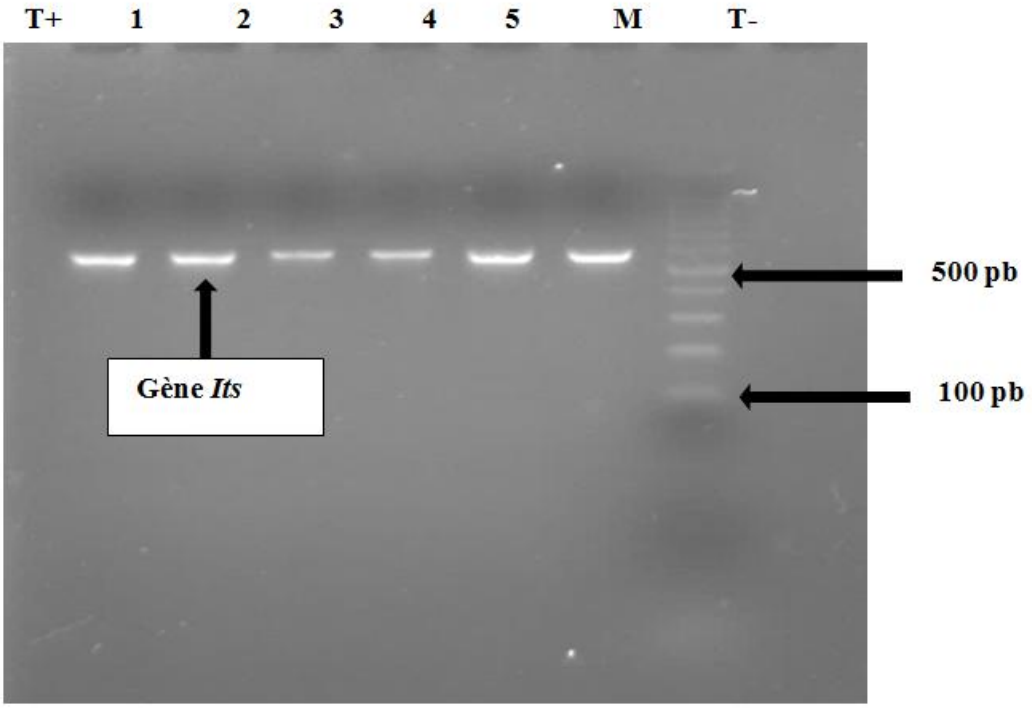


Fig.5A Electrophoretic profile of the *Pex*gene amplification product on a 2% agarose gel made with *Aspergillus fumigatus* genomic DNA (M : 100 bp molecular marker; Lanes 1, 2, 3, 4, and 5 : positive test strains; T+ positive control; T-, negative control)

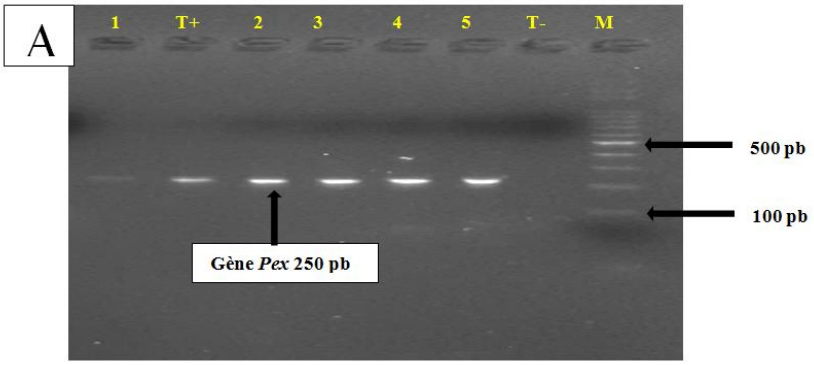


Fig.5B Electrophoretic profile of the Ate*gene* amplification product on a 2% agarose gel made with *Aspergillus terreus* genomic DNA (M: 100 bp molecular marker; Lanes 1, 2, 3, 4, 5, 6, 7, 8, 9: positive test strains; T+ positive control; T-, negative control)

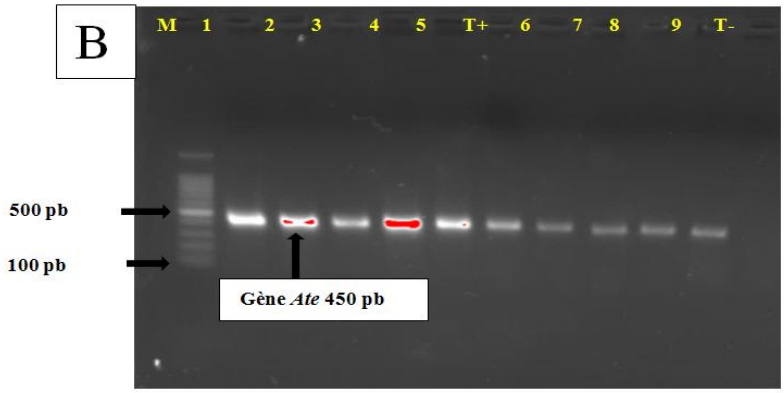


Fig.5C Electrophoretic profile of the *Pepi*gene amplification product on a 2% agarose gel made with *Aspergillus niger* genomic DNA (M : 100 bp molecular marker ;Lanes 1, 2, 3, 4 : positive test strains; T+ positive control; T-, negative control)

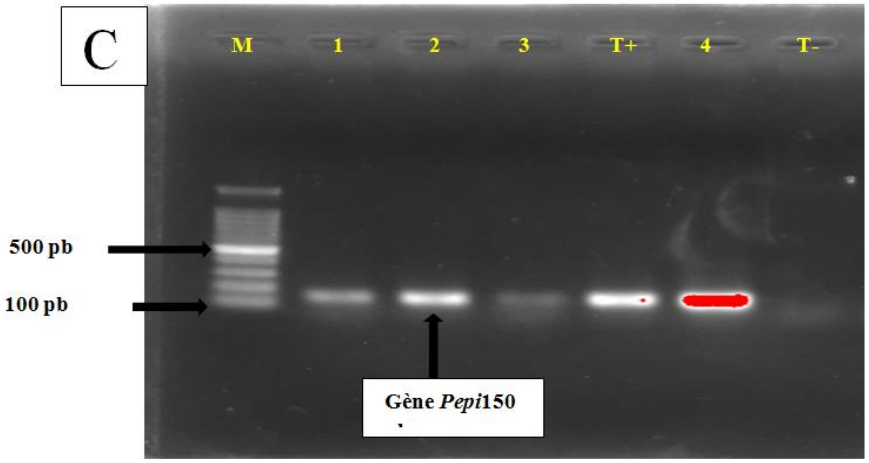


Fig.5D Electrophoretic profile of the *Pepo*gene amplification product on a 2% agarose gel made with *Aspergillus flavus* genomic DNA (M : 100 bp molecular marker; Lanes 1, 2, 3, 4: positive test strains; T+ positive control; T-, negative control)

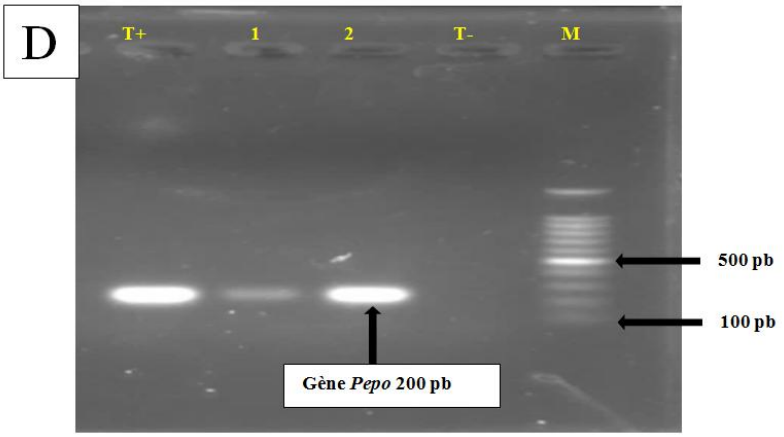


Fig.6A Electrophoretic profile of the *Nor*, *AvfA* and *Ver*gene amplification product on a 2% agarose gel using genomic DNA from *Aspergillus* strains (M: 100 bp molecular marker; Lanes 4, 5, 6, 7, 12, 14 and 15: positive test strains; T+ positive control; T-, negative control)

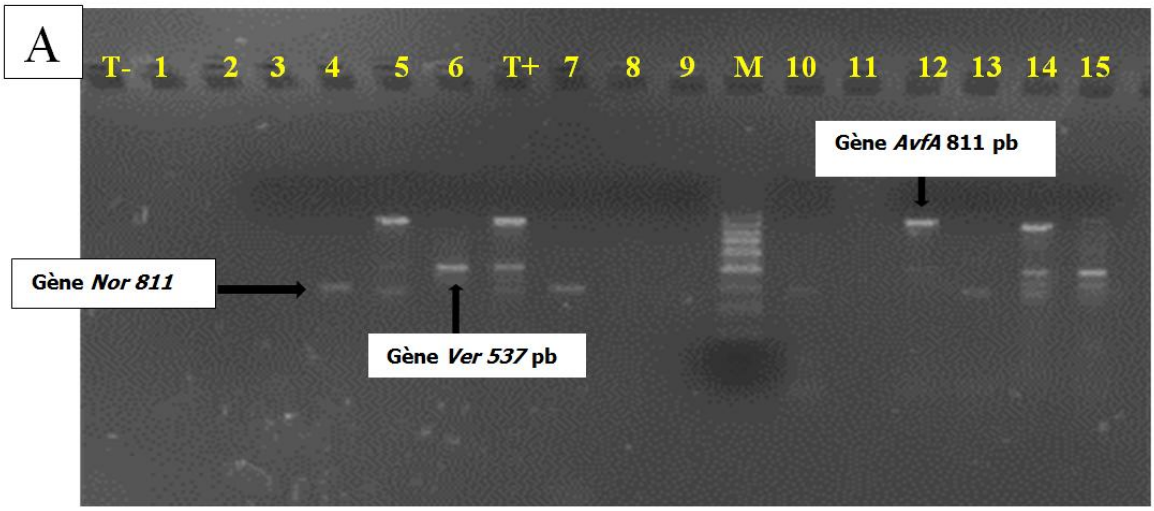
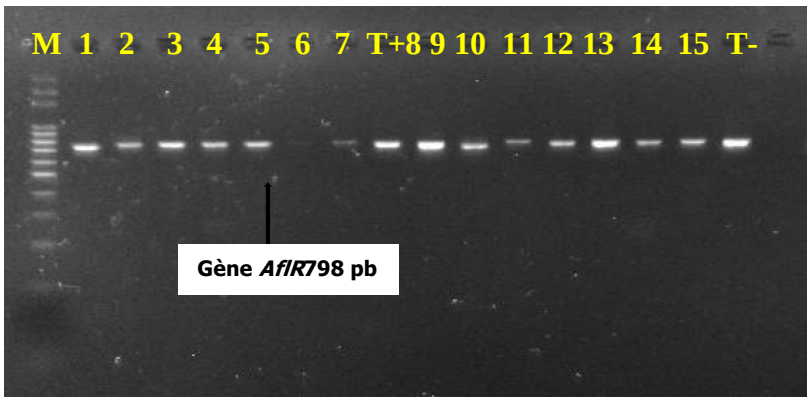


Fig.6B Electrophoretic profile of the *AflR*gene amplification product on a 2% agarose gel using genomic DNA from *Aspergillus* strains (M : 100 bp molecular marker; Lanes 1, 2, 3, 4, 5, 6, 8 and 9-23: strains tested positive; Lane 7 : strain tested negative; T+ positive control; T-, negative control)



This soaking and drying step could favour the development of moulds and the production of aflatoxins due to the moisture content. According to Nguyen (2007), the moisture content of the food stuffs would increase the susceptibility to fungal contamination and aflatoxin production. The high aflatoxin levels obtained in the Moringa powder samples could be explained by a fungal infestation of the dried leaf samples. Indeed, during phenomena such as drying and storage, the quality of the Moringa powder and the production of aflatoxins may be affected by poor handling associated with poor hygiene. This point was also made by (Okigbo, 2017) in their work on the Prevalence of aflatoxins in okra and tomatoes marketed in Ibadan metropolis. Moreover these fungi usually thrive in the hot and humid conditions of tropical and subtropical regions (Battilani *et al.*, 2016). Food processing techniques are not sufficient to remove aflatoxins from foodstuffs contaminated with aflatoxigenic moulds due to their heat resistant nature (Medina *et al.*, 2017b).

From the data collected in this study, it appears that Moringa powder sold in Abidjan markets is contaminated by *Aspergillus* moulds. The rate of contamination by *Aspergillus* isolates was 62.46%. The isolates consisted of species such as *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus* and *Aspergillus fumigatus*. The genes coding for the biosynthesis of aflatoxin B1 were found in the majority of the *Aspergillus* isolates. A predominance of *Nor* and *AflR1*gene detection was observed in Aspergillus *flavus* strains. A rate of 39.37% of the *Aspergillus* strains isolated from Moringa powder successfully amplified the 4 genes tested indicating a potential aflatoxigenic activity of these isolates. Several researches are focused on the use of PCR as a method of choice for the diagnosis of aflatoxigenic moulds. This is because it could be a complementary strategy to current conventional mycotoxin analysis techniques such as Thin Layer chromatography (TLC), High Performance Liquid Chromatography (HPLC). The results of this study therefore reveal the importance of ensuring adequate measures to prevent fungal contamination of food stuffs in order to limit the presence of mycotoxins and the eminent hazards. In addition, mycotoxin regulations should be adopted in our country to help regulate the level of mycotoxin such as aflatoxin B1 in locally consumed foods to ensure food safety, which is fundamental for good health.

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