INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 18, No. 6, p. 25-36, 2021

RESEARCH PAPER OPEN ACCESS

Identification of *Aspergillus* section *Flavi* and *Fumigati* in maize grown in Burkina Faso

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Key words: *Aspergillus*, Identification, Aflatoxins.

http://dx.doi.org/10.12692/ijb/18.6.25-36 Article published on June 6, 2021

Abstract

Molds are frequent contaminants of foodstuffs. Their presence can alter sanitary and organoleptic qualities of food by secondary metabolites production such as mycotoxins. This study aims to isolate and identify *Aspergillus* section *Flavi* and *Fumigati* such as *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus fumigatus* strains from maize seeds grown in Burkina Faso. Three strains of *Aspergillus* M_1, M_2 and M_3 were isolated and purified on Potato Dextrose Agar (PDA) and Sabouraud chloramphenicol agar. The characterization focused on the morphological and microscopic criteria and the aflatoxins production capacity, achieved by blue-green fluorescence under ultraviolet light at 365 nm, of the colonies after three (3) days on coconut agar medium (CMA). Four reference strains served as a basis for comparison, such as *Aspergillus flavus* UBOCC-A-106031 France, *Aspergillus parasiticus var. globosus* UBOCC-A-111042 Japan and *Aspergillus fumigatus* S_1, *Aspergillus flavus* S_2 isolated and identified from previous work in Burkina Faso. According to morphological comparison results, the three strains M_1, M_2 and M_3 isolated from maize seeds belong to *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus parasiticus*. Both *Aspergillus flavus* and *Aspergillus parasiticus* produced aflatoxin.

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Introduction

Food crops and feed materials can be easily infected by fungal species which may produce mycotoxins during growth, harvest, and storage when weather conditions are favorable (Yizhi *et al.*, 2011). Maize is a food and feed crop that is commonly contaminated with mycotoxins, which are chemical products of molds, such as aflatoxins, fumagillin and zearalenone. The toxin-producing molds infest and cause many diseases in the maize plant (Nguyen *et al.*, 2015). Thus poor storage methods for agricultural products increase the risk of aflatoxin contamination (Cardwell, 2000).

A large number of mold species belonging mainly to the three very common genera, *Aspergillus*, *Penicillium* and *Fusarium* present in ambient air and soil, agricultural crops are able to develop on cereals to synthetize and excrete mycotoxins (Garba *et al.*, 2014; Glodjinon *et al.*, 2020). Certain strains of *Aspergillus* genus produce aflatoxins which are the most carcinogenic secondary metabolites, immunosuppressive and teratogens (Ouattara-Sourabie *et al.*, 2011). *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius* species have been the subject of several research works that have demonstrated their aflatoxins production capacity (Ito *et al.*, 2001; Johnsson *et al.*, 2008; Doster *et al.*, 2009). In addition to these three species, the aflatoxin production capacities were more recent discoveries in species of *Aspergillus*: *Aspergillus tamarii*, *Aspergillus ochraceoroseus* (Klich *et al.*, 2000) *Aspergillus pseudotamarii* (Ito *et al.*, 2001), *Aspergillus bombycis* (Peterson *et al.*, 2001) and *Aspergillus rambelli* (Frisvad *et al.*, 2005). In addition, fumagillin, produced by *Aspergillus fumigatus*, belongs to a sesquiterpenes family whose anti-angiogenic activity has aroused great interest since 1985. The cultural practices used could have a significant impact on the maize seed contamination in the field, especially in humid lands. Also, the improper storage or preservation methods used such as maize bad drying would also lead to attacks by micro-organisms including molds of the genus *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria*, etc. (Zinedine, 2004; Nguyen, 2007). These natural toxins threaten human and animal lives because their accumulation in foods and feeds causes serious health problems. Among the food crops, maize is the most important and used product by over 98% of rural households. Maize is the most important component in livestock feed and can be stored year-round in all households (Cardwell, 2000). Moreover, due to their negative impacts on performance, mycotoxins and the mold reduce the economic profits in agriculture.

According to Blanc (2001), the legislation European Community on Aflatoxins and the new requirements for health safety in trade international food products have a great impact on trade and developing countries economy. In effect, when cereals, oilseeds and animal products are contaminated with aflatoxins, their selling price drops, or these products are simply rejected at export (Ouattara-Sourabie *et al.*, 2011). FAO estimated that toxic fungi invade 25% of the world's food crops with many important foods, and in the world, around 1,000 million tons of foodstuffs per year are lost because of mycotoxins (Nguyen *et al.*, 2015). In Burkina Faso, during raining season, the conditions were favourable (high humidity and temperature) for the development of these fungi and mycotoxins production (Compaoré *et al.*, 2016a; Ouattara-Sourabié, 2018). Previously, the work carried out by Nikiéma *et al.* (1995), Ouattara-Sourabie *et al.* (2011) and (Sanou, 2000) have shown high levels of aflatoxins in maize, oilseeds in particular peanuts and their products derivatives in the western region of the country.

In this study three strains belong to *Aspergillus* section *Flavi* and *Fumigati* collected from maize seeds were identified based on their morphological characteristic comparison with reference strains.

Materials and methods

Sampling and fungi isolation from maize

Five samples of maize were collected from Ouagadougou markets, wet with sterile distilled water and stored at ambient temperature (27 °C to 32 °C) in Petri plates during 7 days. Fungi were isolated

according to Ulster or the direct method. It involved depositing directly ten maize seed samples on Potato Dextrose Agar (PDA). Mycelia of each isolate were cultured in this medium and incubated at 30 °C until seven (7) days. Purification of the isolates was performed by successive subcultures to obtain pure isolates. A total of twenty-three fungi were collected from local maize seed samples. *Aspergillus spp* strains research among these isolates has concern powdery, filamentous appearance, yellowish, greenish, or blackish colonies which may be the interest strains. Also, a microscopic view of isolates conidiophores of strains was performed on an optical microscope to put away strains having a septate and non-hyaline conidiophore. Thus, among these isolates, three *Aspergillus spp*. were retained for morphological characterization.

The identification medium "*Aspergillus flavus parasiticus* agar (AFPA)" was used to identify *Aspergillus flavus* and *Aspergillus parasiticus* according to Pitt *et al.* (1983) and Cotty (1994) protocols. Systematic determination and the identification of the strain were made on the Potato Dextrose Agar (PDA) at 25 °C and 37 °C depending on the methods used by Christensen (1981), Hocking (1982) and Cooney and Emerson (1964). Inoculation was done in three points equidistant.

Reference strains

Four reference strains served as a basis for comparison. It was UBOCC-A-106031 (*Aspergillus flavus* aflatoxinogenic) of French origin, UBOCC-A-111042 (*Aspergillus parasiticus var. globosus* aflatoxinogenic) of Japanese origin and those previously isolated and identified in Burkina Faso (Compaoré *et al.*, 2016b), such as S_1 (*Aspergillus fumigatus*), S_2 (*Aspergillus flavus* aflatoxinogenic). These reference strains stored at - 4 °C were revived in sabouraud broth at 30 °C for 4 days.

They were then transplanted onto the Potato Dextrose Agar (PDA) medium and incubated at 30 °C for seven days, for a comparison of cultural and microscopic characters with strains isolated from maize seeds.

Isolation and identification of strains isolated from maize seeds

A sample of ten maize seeds was wetted with sterile distilled water and left at laboratory temperature (30 °C to 34 °C) in Petri plates for 7 days. Isolation and purification of strains of interest (*Aspergillus spp*.) were made. For successive transplantations in points by exhaustion of mold, growths have been carried out on Sabouraud medium with chloramphenicol.

The last colonies pushed on the transplanting points successive by exhaustion constituted the pure strains. A microscopic view of conidiophores of strains newly isolated allowed to retain them in the function of a certain number of characters, for the rest of the study. Thus, the strains having a septate and non-hyaline conidiophore were ruled out. The isolated strains were purified on the Potato Dextrose Agar (PDA) slant was used for further purification and identification using the systematic classification of the *Aspergillus* strains based on morphological characters described by Christensen (1981), Raper and Fennell (1965), Hocking (1982), Cotty (1994), and Klich and Samson (2009). The identification medium "*Aspergillus flavus parasiticus* Agar (AFPA)" was used to identify *Aspergillus flavus* and *Aspergillus parasiticus* according to the protocols of Pitt *et al.* (1983) and Cotty (1994) and *Aspergillus fumigatus* was identified according to Raper and Fennell (1965) conventional key based on his thermophilic character. Thus, the main criteria used are growth time, colonies colors during incubation time, diameter and texture of colonies. This identification was completed with microscopic characteristics by equipping the optical microscope of an eyepiece micrometric and using a slide specimen micrometer. The sighting was carried out on cultures of 48 to 120 h and carried on the following characteristics of the aspergillary head: The hyphae texture, the number of divergent metulae (seriation) in a whorl. Thus, the number of phialides which bearing conidia, color and length of the conidiophore were also described for tentative identification (Compaoré *et al.*, 2016b). The media poured into 90 mm Petri dishes diameter were seeded at three points equidistant.

Visual determination of the aflatoxin capacity production

The strains isolated and those of references were all cultured on diluted coconut medium (1/5th) at 30 °C for 4 days. Thus, 20 g of shredded coconuts have been homogenized for 5 minutes with 300 ml distilled water heated to about 70 °C. The solution was filtered through tissue. The agar has been added to coconut broth at the rate of 20 g/L then the mixture was sterilized in the autoclave at 121 °C for 15 min. The final pH was adjusted to 7.0. UV plate is used to view colonies under ultra-light violet at 254 and 312 nm. This visualization has allowed us to qualitatively highlight the aflatoxin production capacity of strains through blue fluorescence (Davis *et al.*, 1987; Ouattara-Sourabie *et al.*, 2011). When the test is negative there is no blue fluorescence around the colonies observed.

Results

Morphological identification

Macroscopic characteristics

The strain M_1 isolated from maize seeds showed the same visual characteristics as those of S_1 reference strains on the PDA medium however the edges of reference colonies are rough whereas M_1 are smooth. Both strains present rapid growth after 24 h incubation at 30 °C; colonies are white and turn blue-green then dark green to blackish gray; the surface texture is smooth and powdery.

Colonies diameter is between 30 and 35 mm and the reverse was greyish ash or olivaceous grey. Both strains can grow at 50 °C (Fig. 1 and Table 1).

Table 1. Macroscopic characters on the 7th day incubation at 30 °C of *Aspergillus* strains on PDA medium.

Strain	Macroscopic characteristics
M_1	Colonies were white and turn blue-green then light green to blackish gray, with smooth edge, the surface texture is smooth and powdery, colonies diameter is between 30-35 mm with a yellowish reverse. The isolate can grow at 50 °C.
S_1 *Aspergillus fumigatus*	Colonies were white and turn blue-green then dark green to blackish gray, with rough edge, the surface texture is smooth and powdery, colonies diameter is between 30-35 mm with a yellowish reverse. The isolate can grow at 50 °C.
M_2	Colonies were white, becoming yellowish green then dark green, downy, raised; the texture is velvety radiant at the edge, fluffy, cottony and denser and dark green in the center, colonies smaller than those of S_2 whose diameter between 25-30 mm.
UBOCC-A-106031 *A. flavus* aflatoxinogenic	Colonies were white, becoming yellowish green then dark green, downy, raised; the texture is velvety radiant at the edge, fluffy, cottony and denser and dark green in the center, colonies smaller than those of S_2 whose diameter between 25-30 mm.
S_2 *A. flavus* aflatoxinogenic	Colonies were white, becoming yellowish green then dark green, downy, raised; the texture is velvety radiant at the edge, fluffy, cottony and denser and dark green in the center with white sclerotia, colonies diameter is between 40-55 mm with a yellowish reverse.
M_3	Colonies were deep yellow green, velutinous in its center compared to that of S_2-*A. flavus* and UBOCC-A-106031 *A. flavus*, yellow-brown reverse with increased pigmentation, some white sclerotia were in the colonies center whose diameter was about 30–35 mm.
UBOCC-A-111042 *A. parasiticus var. globosus* aflatoxinogenic	Colonies were deep yellow green, velutinous in its center compared to that of S_2-*A. flavus* and UBOCC-A-106031 *A. flavus*, yellow-brown reverse with increased pigmentation, some white sclerotia were in the colonies center whose diameter was about 30–35 mm.

The strain M_2 also exhibits the same visual characteristics as both reference strains UBOCC-A-106031 and S_2 on the PDA medium and with, however, some white sclerotia are present in the colonies center of strain S_2. In addition, the strain S_2 forms large colonies attained 40 to 55 mm, as diameter in 7 days of incubation, while both M_2 and UBOCC-A-106031 strains have small colonies whose diameter between 25 to 30 mm. All colonies were yellow-green with white mycelia at the edges, a yellow

reverse was noted, even if those of UBOCC-A-106031 are darker than two strains. They easily produce superficial and submerged hyphae with fruiting bodies (Fig. 1 and Table 1).

The strain M_3 presents a lot of similar characteristics on PDA medium with reference strain UBOCC-A-111042. The colonies were yellow-green with white to cream mycelia and yellow-green edges. Sporulation rings formed in the colonies whose diameter between 30 to 35 mm. However, strain UBOCC-A-111042 color was darker and produced white sclerotia, strain M_3 produced uncolored exudates from 96 hours of incubation (Fig. 1 and Table 1).

Table 2. Summary table of the culture on AFPA, and CAM diluted to 1/5th results.

Strain	Orange yellow color in the colonies reverse on AFPA as shown Fig. 2	Aflatoxin's production capacity by fluorescence emission by colonies under UV on Coconut Agar medium
M_1	+	+
S_1 *Aspergillus fumigatus*	+	+
M_2	+	+
UBOCC-A-106031 *A. flavus* aflatoxinogenic	+	+
S_2 *A. flavus* aflatoxinogenic	+	+
M_3	+	+
UBOCC-A-111042 *A. parasiticus var. globosus* aflatoxinogenic	+	+

It should be noted, however, that the green and yellow pigmentations are more intense at UBOCC-A-111042 and M_3 than at M_2, UBOCC-A-106031 and S_2 strain. So, these five strains although very relatives are different in terms of the shade of pigments. This proximity to stump colors could be correlated with their ability production of aflatoxins confirmed by fluorescence of the colonies under UV light as well as the yellow-orange color observed on the reverse of the Petri dishes on AFPA medium as shown in Fig. 2 and Table 2.

To this end, all five strains showed blue fluorescence under UV with however a low intensity for the M3 strain, which corresponds to a positive response on the coconut agar medium (Table 2). The cultural characteristics of all strains on the PDA medium are shown in Table 1.

Table 3. Microscopic characters of *Aspergillus* strains.

Strain	Stipe texture	Seriation	Length Conidiophore (µm)	Vesicle diameter (µm)	Conidia size (µm)	Conidia Head/Shape
M_1	smooth	uniseriate	250-430	20-35	2-3	short columnar
S_1	smooth	uniseriate	80-300	20-35	2-3	short columnar
M_2	rough	biseriate	450–930	14-43	3-5	Radiate
UBOCC-A-106031	rough	biseriate	450–930	14-43	3-5	Radiate
S_2	rough	biseriate	400-800	14-43	3-5	Radiate
M_3	rough	uniseriate	200–430	28-32	4-7	Radiate
UBOCC-A-11042	smooth	uniseriate	200–430	28-32	4-7	Radiate

Culture on AFPA medium allowed to identify of M_2 and M_3 strains as *Aspergillus flavus* or *Aspergillus parasiticus* because they gave a positive response on AFPA medium, illustrated by orange-yellow color from the colonies reverse (Figs 2 and 3). The reference strains S_2, UBOCC-A-111042 and UBOCC-A-106031 have also given a positive answer on AFPA (Table 2).

Table 4. *Aspergillus* strains microscopic characters results by other authors.

References	Strain	Stipe texture	Seriation	Length Conidiophore (µm)	Vesicle diameter (µm)	Conidia size (µm)	Conidia Head/Shape
Nyongesa *et al.* (2015)	*A. fumigatus*	smooth	Uniseriate	-	19-31	2-3	short columnar
	A. flavus	rough	Uniseriate and biseriate	-	18-36	3.5-5 4-5	Radiate
	A. parasiticus	rough	Uniseriate and biseriate	-	19-35	3-7	Radiate
	A. parasiticus	smooth	Uniseriate or biseriate	-	24-30	4-5.8	Radiate
Christensen (1981)	*A. Flavus Var. fla*	smooth	Uniseriate and biseriate	400-850 2000-2500	10-85	3-7.5 4-5	Radiate
	A. parasiticus	smooth	Uniseriate Rarely biseriate	300-700	20-35	3.5-5.5	Radiate
Gao *et al.* (2009)	*A. flavus S*	rough	uniseriate	150-1130	17-22 x 16-21	2-7 x 2.5-6	Radiate
	A. flavus L	rough	uniseriate and biseriate	450-930	16-50 x 15-47	3-5 x 3-4.5	Radiate
	A. parasiticus	smooth	-	350-387.5	-	4-8 x 3-8	Radiate
Gao *et al.* (2007)	*A. flavus S*	smooth	uniseriate and biseriate	150-1130	17-22 x 16-21	2-7 x 2.5-6	Radiate
	A. flavus L	smooth	uniseriate and biseriate	210-700	28-32 x 15-47	2-5 x 3-4.5	Radiate
	A. parasiticus	smooth	-	350-387.5	-	4-8 x 3-8	Radiate

Microscopic characteristics

In optical microscopy, all strains have a conidiophore, not septate, hyaline with a radiant conidial head and variations at the seriation level, form and dimensions of the vesicle, hyphae and conidia (Fig. 3, 4, 5 and Table 3). Thus, reference strain S_1 and local strain M_1 showed numerous round and small conidia sizes; 2-3 µm, globose finely rough. Conidiophores were upright, simple, uncolored and smooth terminating in a globose swelling. We noted that the conidiophore is shorter in the reference strain S_1 (80-300 µm) than in the maize strain M_1 (250-430 µm). The terminal vesicle has a compact columnar, measured 20-35 µm long, it is uniseriate and directly carries numerous phialides arranged parallel to the axis of the conidiophore. Reference strains M_2 and UBOCC-A-106031 showed a longer rough conidiophore (450-930 µm) than reference strain UBOCC-A-111042 and local strains M_3 (200-430 µm). Strains S_2, M_2 and UBOCC-A-106031 showed several similitudes, they have biseriate vesicles which were spherical to globose with a diameter between 14-43 µm. The conidia size range was between 3-5 µm; globose; smooth to finely rough and yellow-green colour. Strain M_3 was comparable to the reference strain S_3. In addition to the shorter conidiophores, the vesicles were uniseriate, pyriform to globose and measured between 28-32 µm in diameter. The stems have thick, smooth walls that are not stained.

The size of the conidia varied between 4-7 µm; smooth; globose and yellow as shown in Table 3. This result was compared with those of any authors (Table 4), was Raper and Fennell (1965), Christensen (1981), Ito *et al.* (2001), Chabasse *et al.* (2002), Ouattara-Sourabie *et al.* (2011) and Nyongesa *et al.* (2015).

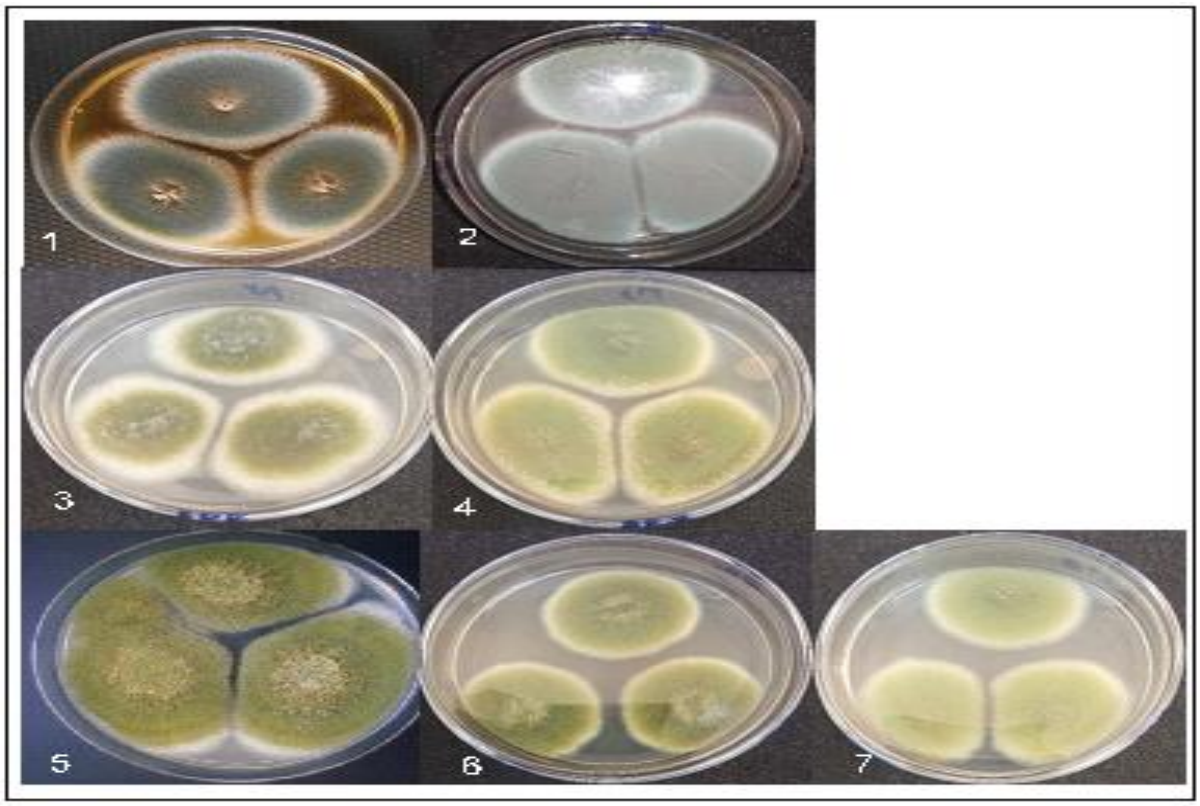

Fig. 1. Strains macroscopic aspects S_1 (1), M_1 (2), UBOCC-A-111042 (3), M_3 (4), S_2 (5), UBOCC-A-106031 (6) and M_2 (7) in PDA medium at 7 days of incubation at 30 °C.

Aflatoxin production capacity

Analysis of strains' ability to produce aflatoxins through blue fluorescence emission from colonies in coconut agar medium (CAM) showed that reference strains UBOCC-A-106031, UBOCC-A-111042, S_1 and S_2, as well as local strain M_1 and M_2 are aflatoxins producer (Fig. 2 and Table 2). These results were corroborated with their positive response to AFPA medium. A confirmation of aflatoxin production by high-performance liquid chromatographic (HPLC) was made and the results presented in another unpublished article and are in perfect adequacy with the fluorescence under UV results and those of CAM.

Discussion

Dangers aflatoxins pose to human health, livestock productivity and trade are widely recognized (Johnsson *et al.*, 2008). Molds proliferation in cereals seeds is accentuated by environmental conditions such as temperature and humidity that prevail in Burkina Faso during the rainy season. Also, poor farming practices are an undeniable cause of contamination of the maize in the open field by molds and, therefore, later by mycotoxins once the favorable conditions are met for their production by the latter. These results are in good agreement with those of several authors showing that humidity and temperature are determining factors for the proliferation of molds, with possible production of mycotoxins of all kinds (Zinedine, 2004; Waré *et al.*, 2017). Fungi of the genus *Aspergillus* contaminating peanuts in Burkina Faso are various microscopic characteristics and metabolic. So, through this present work whose objective is *Aspergillus* section *Flavi* and *Fumigati* identification from maize seeds grown in Burkina Faso and their aflatoxins ability, it appears that the M_1 strain isolated from maize has common characteristics to *Aspergillus fumigatus*. This classification was based on cultural characteristics observed on PDA medium and microscopic characters that correspond to those of *Aspergillus fumigatus*. This classification was confirmed by the Cooney and Emerson (1964) key for thermophilic filamentous fungi identification. Indeed, *Aspergillus fumigatus* is the only species of the genus *Aspergillus* capable to grow at 50 °C.

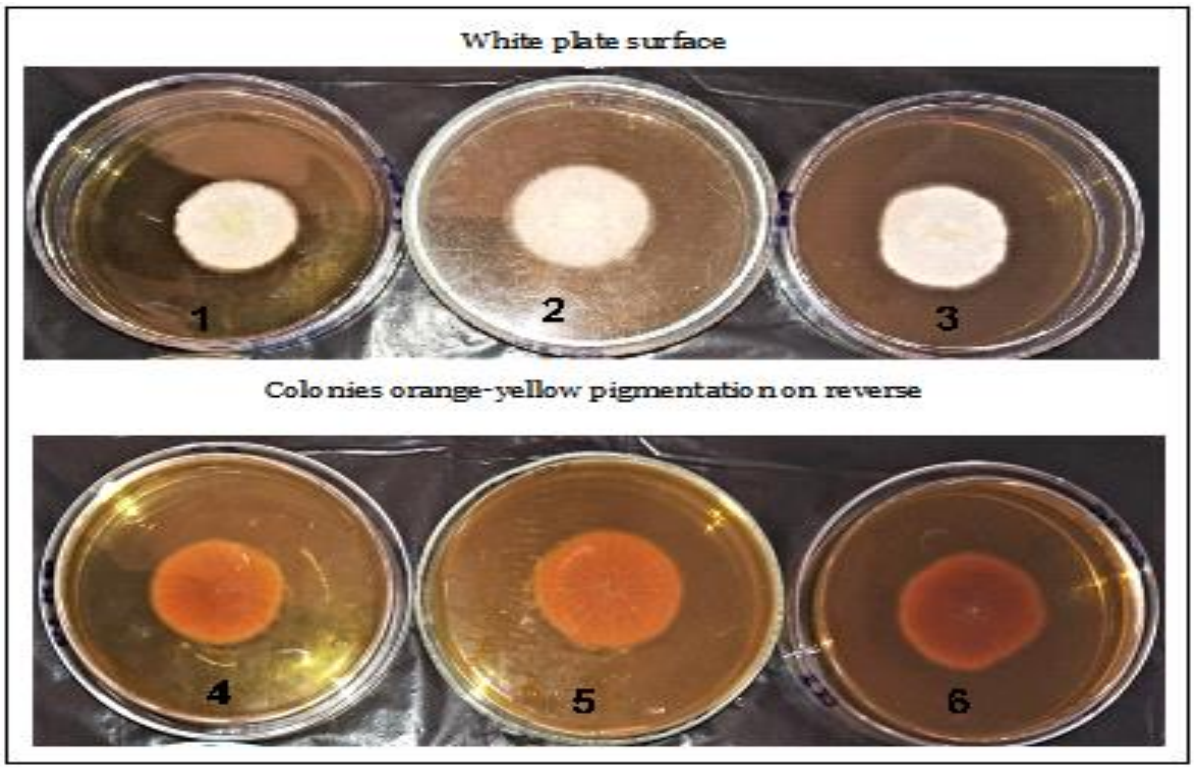

Fig. 2. Positives strains S_2 (1,4), UBOCC-A-106031 (2,5) and M_2 (3,6) as (yellow-orange reverse) on AFPA medium after 72 h of incubation at 30 °C.

These results are in agreement with those of Lamrani *et al.* (2008) who isolated *Aspergillus fumigatus* strain from traditional trituration units in Marocco, that grow at 50 °C.

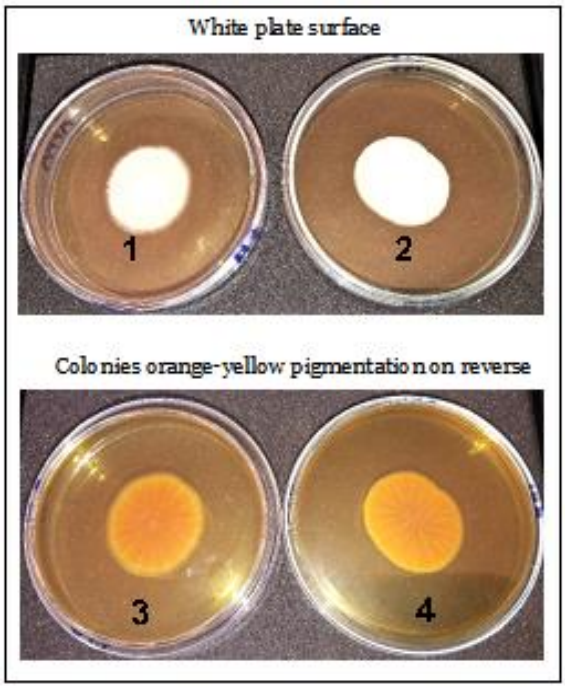

Fig. 3. Positives strains S_3 (1,3), UBOCC-A-111042 (2,4) as yellow-orange reverse on AFPA medium after 72 h of incubation at 30 °C**.**

According to the results, strain S_2 is morphologically close to the reference strains UBOCC-A-106031 and S_2. We, therefore, classified it as belonging to *Aspergillus flavus* species. This similarity was based on the determination of cultural characteristics observed on PDA and AFPA medium and microscopic characters. In addition to cultural characteristics and microscopic corresponding, strain S_2 is able to produce aflatoxins. The highlighting was done by the blue fluorescence on the coconut agar medium (CAM) and by HPLC. These results are therefore in agreement with those of Davis *et al.* (1987); Nguyen (2007); Ouattara-Sourabie *et al.* (2011) which was used the coconut agar medium to put in evidence by fluorescence the aflatoxins production. Our results are in concordance with those obtained by Ouattara-Sourabie *et al.* (2011) who showed the production capacity of aflatoxin by a local strain isolated from peanut grains in Burkina Faso using AFPA and CAM medium.

According to Cotty (1994), the AFPA medium would detect 100% of its strain targets but is not able to detect all strains that produce aflatoxins.

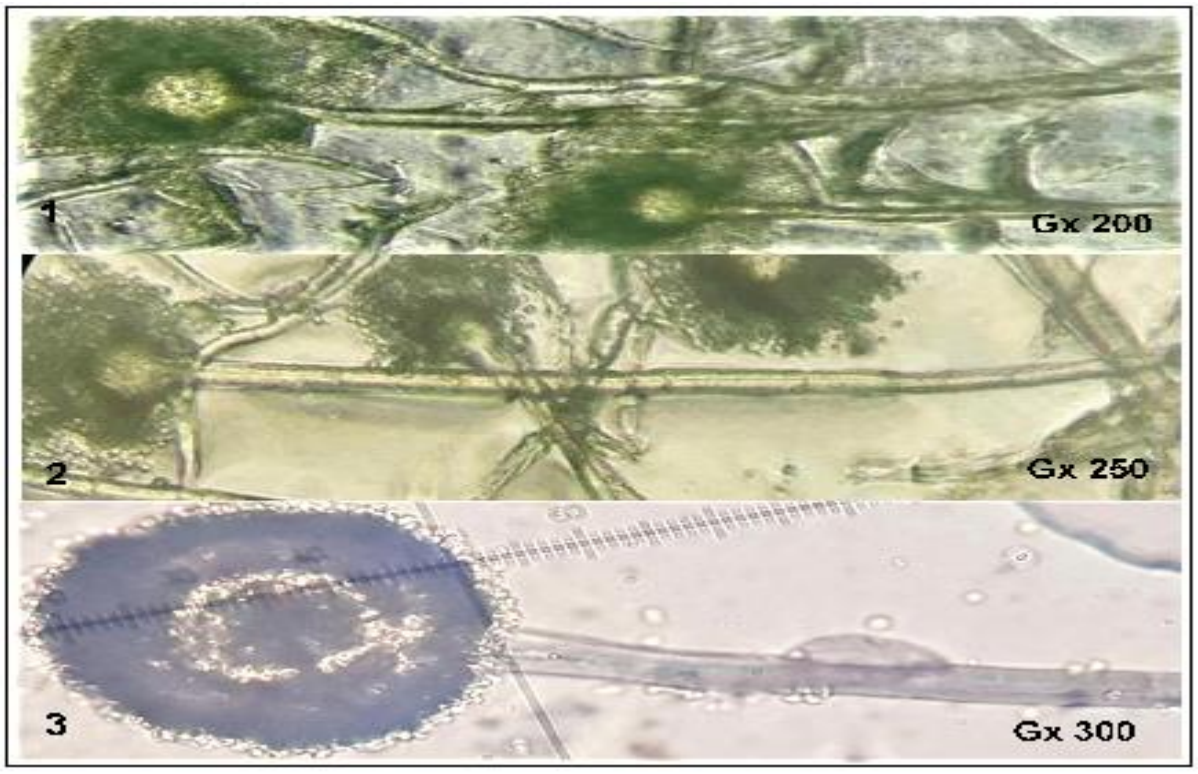

Fig. 4. Strains aspergillaires heads S_2 (1), UBOCC-A-106031 (2) and M_2 (3).

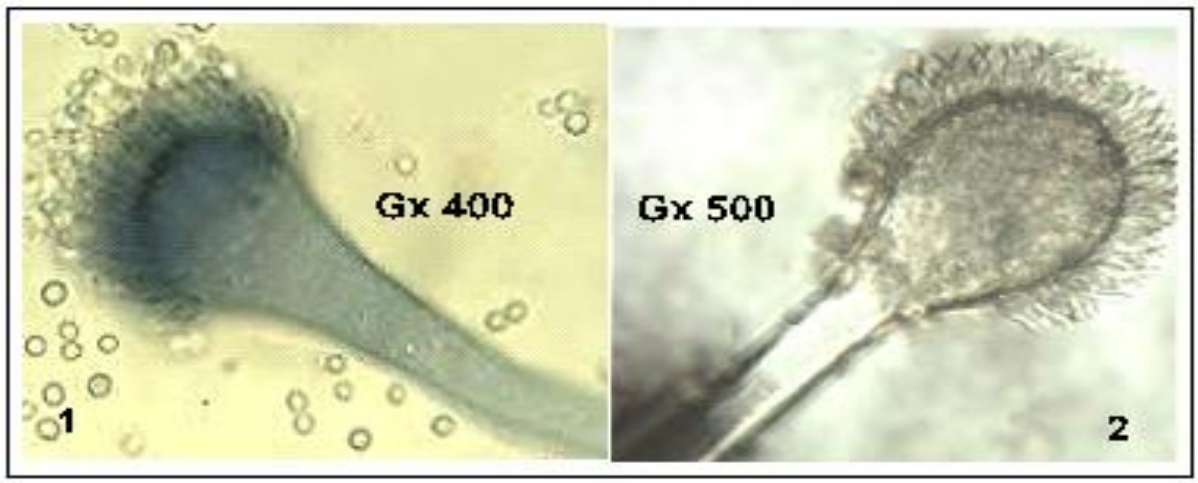

Fig. 5. Strains aspergillaires heads S_1 (1) and M_1 (2).

The local strains M_3 isolated from maize seeds grown in Burkina Faso have characteristics common to reference strain *Aspergillus parasiticus var. globosus* UBOCC-A-111042, with the exception of stipe texture which is rough for M_3 and smooth for reference strain. Both strains showed shorter conidiophore (200-430 µm) than that of *Aspergillus flavus* strains (450-930 µm). Several authors who isolated *Aspergillus parasiticus* strains, such as Gao *et al.* (2007); Nyongesa *et al.* (2015) and Christensen (1981) obtained similar characteristics to M_3 and UBOCC-A-111042 strains. However, small differences exist in the vesicle seriation and the size of the conidiophore. Thus, vesicles are uniseriate in our case while Nyongesa *et al.* (2015) and Gao *et al.* (2007) obtained *Aspergillus parasiticus* strains with the uniseriate and biseriate vesicle.

Christensen noticed longer conidiophores (300-700 µm) than those obtained in this present work (200-430 µm). The strains had a positive reaction to AFPA and CAM medium.

Conclusion

Three strains M_1, M_2 and M_3 isolated from maize seed grown in Burkina Faso were respectively identified as being *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus parasiticus* by comparison of their morphological characteristics with those of reference strains. In addition, any isolated strains produce aflatoxin, which can cause serious diseases in consumers at certain concentrations, it is, therefore, necessary to improve cultural practices, respecting good practices during harvest and storage.

It will therefore be necessary to make farmers and processors aware of the need to dry cereal grains well before placing them in the granary. Morphological characteristics are the primary tools in the identification of various *Aspergillus* species; before delineating them into species using the other rapid technologies. Thus, identification must be confirmed by more taxonomic modern methods technologies such as DNA sequencing using household genes and phylogenetic tree construction.

Acknowledgements

The authors thank Ms. Ouattara/Sourabié Pane B. for her advice, technical support and guidance which enabled us to carry out this work.

Conflict of interest

The authors have no conflict of interest to declare.

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