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Performance of Maize Storage Technologies in Benin: Fungal Ecology and Mycotoxin Contamination

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Authors' contributions

This work was carried out in collaboration between all authors. Authors LBM and FG designed the study. Author MTDH performed the statistical analysis. Authors RB and UH wrote the protocol, wrote the first draft of the manuscript and managed literature searches. Authors RB, UH, BY, MTDH, NMFM, PA, PS, FG and LBM managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Objectives: The present work was to study the fungal ecology of maize according to the various storage technologies and evaluate the ability of fungal isolates to produce mycotoxin.

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Materials and Methods: Seven maize storage technologies (from A to G) were selected in seven agro-ecological zones and 198 samples of stored maize were collected based on storage technologies. The presence of mold was observed in all the areas prospected in all the type of technologies used. The identification of the mycotoxins produced by mildews isolated of the stored maize has been performed by Thin Layer Chromatography.
Results: Eleven (11) molds were isolated from the samples collected and three storage modes were observed. *Fusarium sp, Penicillium sp* and *Aspergillus niger* were the prevalent species with frequencies of 20.71%; 15.15% and 12.12%, respectively. Grain maize mode (55%) was the most used. Also the isolated molds have the ability to produce the toxins when the conditions are favorable. The identification of mycotoxins by Thin Layer Chromatography showed that the isolated and identified molds were producers of mycotoxins. *A. parasiticus* and *A. flavus* were not observed in technologies A and B in all the study areas. *A. flavus, A. parasiticus, A. ochraceus, F. graminearum, F. oxysporum* and *P. roqueforti* showed their ability to produce Aflatoxin B2, Aflatoxin G1, Ochratoxin A, Deoxynivalenol, moniliformin and Roquefortin C, respectively.
Conclusion: These results clearly show an correlation between the technologies of storage and the contamination by the mycotoxins.

Keywords: Cereals; method of preservation; mold; CCM.

1. INTRODUCTION

Cereals are the main source of food for humans and animals nutrition in the world. In Benin, maize is one of the most important cereal crops with high productivity and large food processing potential. Its cultivation occupies 54% of the field areas and has been the object of a renewed interest since the decline of cotton [1]. A most of cereals, maize is also much subjected to fungal contamination during storage due to the moisture. To avoid development of mold, various storage technologies were developed by producers across different agro-ecological zones where maize is cultivated. The diversity of technologies used does not always preserve maize against fungal infection during storage. Therefore, it is important to point out the problem related to the inefficiency of the observed technologies for maize preservation. Indeed, according to Neacşu and Madar [2], having good quality of corn during storage depends on the method of preservation. With maize, the postharvest losses varied from 30% to 40% [3]. These losses are caused by both physical and chemical damage. Attention is more and more concerned the problems of chemical contamination of maize caused by fungal pathogens. Fungal contamination of food is a chronic problem in developing countries and cause losses of the quality, quantity, nutritional value and monetary value of the concerned product [4]. Fungal species producing mycotoxin are the cause of bio-deterioration of a variety of food [5]. Approximately 25 to 40% of global cereals are contaminated by mycotoxin produced by different storage molds [6]. The presence of mold is very often accompanied by the production of mycotoxin in the farm produces [7]. The results of Prakash et al. [8] showed that mold alone cause nearly 20% reductions in yield and income from the sale of the stored foodstuffs. In Benin, the storage of maize is subjected to attack of fungi of the genus *Aspergillus, Penicillium* and *Fusarium* because of the hot and humid climate of this country. Moreover, these fungal species are a serious risk for human and animal consumption due to their production of secondary metabolites such as mycotoxin. The study conducted on 17.316 cereal samples (maize, wheat, barley and rice) and their derivative products from different regions (North and South America, Africa, Europe, Oceania and Asia) showed that the problem of mycotoxin is global [9]. These mycotoxin constitute a danger for food security. The ingestion of maize contaminated by mycotoxin can cause serious public health problem related to the liver, kidney, cancer, damage of the nervous system and immunosuppression for animals as well as humans. The danger associated with the presence of mold in maize leads us to ask questions about the effectiveness of various technologies used for its storage. It is then necessary to carry out investigations on the fungal ecology of storage technologies in each agro-ecological zone in order to identify the best storage technology which could showed low rates of fungal infection and even absence of mold during storage. Then, the objective of this study was to evaluate the performance of maize storage technologies on the fungal infection through agro-ecological zones of Benin. This will

contribute to prevent maize losses due to fungi infection and also reduce public health risks.

2. MATERIALS AND METHODS

2.1 Study Sites

The study was conducted in seven agro-ecological zones of Benin namely zones II, III, IV, V, VI, VII and VIII (Fig. 1). These zones were selected based on the importance of maize production over other crops (Table 1). For each zone, two townships were selected. The choice of the townships was made together with the technicians of the Ministry of Agriculture, according to the availability and the diversity of stocks at the maize producers.

2.2 Sample Collection

At first, a survey was conducted among maize producers on the storage technologies using a questionary. Maize samples were collected according to the technologies of each township. A total of 198 maize samples were collected. A kilogram of maize was sampled, labeled and placed in a white cloth bag. In the same township and for the same technology used, three maize

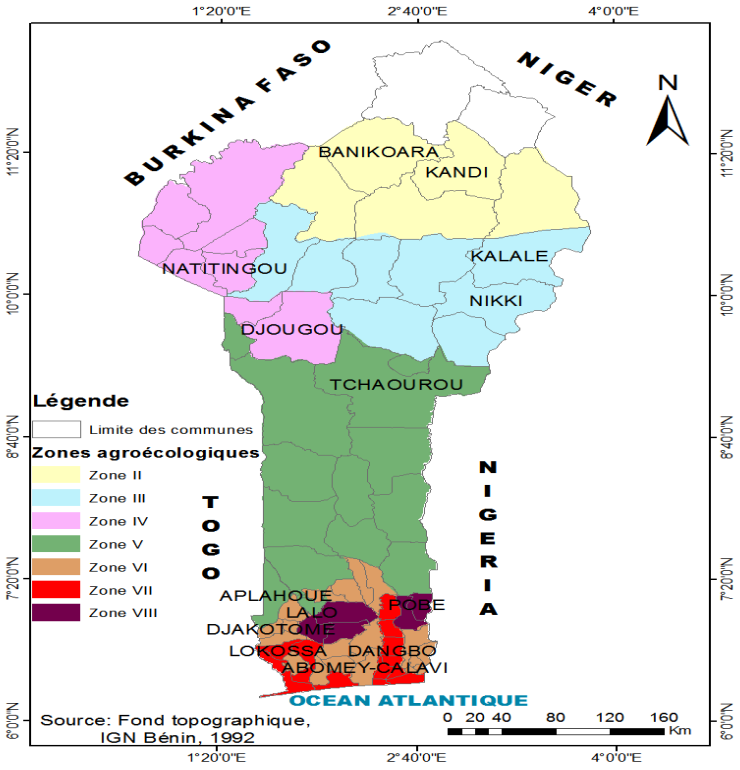

Fig. 1. Distribution of agro-ecological zones and township prospected

Table 1. Characteristics of agro-ecological zones

Zones	Designation	Townships	Different crops
II	Cotton zone of northern Benin	Banikoara, Kandi	Maize; Sorghum; Yam; Cotton
III	Food-producing zone of South Borgou	Kalalé; Nikki	Maize; Yam; Cotton; Cashew
IV	West-Atacora Zone	Djougou; Natittingou	Maize; Yam
V	Cotton zone of central Benin	Aplahoue; Tchaourou	Maize; Tubers; legume; Cotton
VI	Zone of bar lands	Abomey-calavi; Djakotomey	Maize; Cassava; Beans; Groundnut
VII	Zone of depression	Pobè; Lalo	Maize; Cassava; Beans; Tomato; Pepper
VIII	Zone of fisheries	Lokossa; Dangbo	Fishery; Maize; Cassava; Beans

samples were collected from different producers. For maize stored in bulk, maize samples were obtained direct taking from the storage structures, and for maize stored in polyethylene bags, collection was done with the aid of a conically shaped control instrument which was laterally open. In the storage structure, the final sample (1 kg) was obtained by mixing 3 kg of maize taken from the upper, middle and lower parts of the stock in order to obtain a statistically representative level of fungal infestation.

2.3 Identification and Characterization of Mycoflora

2.3.1 Isolation and identification of mold

The Culture of molds from collected samples was done according to the modified method of Pitt and Hocking, [10]. 5 g of maize were placed in each flask containing 45 ml of sterile peptone water. One hour (1 h) after homogenization, 0.1 ml of the supernatant was plated in each Petri dish containing culture medium OGYEA Agar (Oxytetracyclin-Glucose-Yeast Extract Agar). The prepared Petri dishes were incubated at 25℃ for 5 days. The colonies obtained were observed under an optical microscope and those belonging to the genus Aspergillus, Penicillium or Fusarium were resown on the selective medium MEA for 14 days in order to obtain pure strains.

2.3.2 Evaluation of mycotoxin production by isolated mold

The ability of fungal isolates (isolated mold) to produce mycotoxins was assessed by thin-layer chromatography according to the method reported by Sessou et al. [4]. A fungal disc of 5 mm in diameter was introduced into flasks of 100 ml containing 25 ml of sterile SMKY broth medium (sucrose, 200 g; $MgSO_4$ $7H_2O$, 0.5 g;. KNO_3, 0.3 g; and yeast extract, 7 g; 1 L of distilled water). The flasks were incubated at 28±2℃ for 10 days and then filtered through Whatman paper N°1. Twenty (20) ml of chloroform were added to each filtrate for decanting with the aid of a funnel. The obtained extracts were evaporated and dried in a water bath. Each extract was recovered with 1 ml chloroform. 50 µl of each recovery extract were spotted on TLC plates of silica gel (TLC Silica gel 60 F_{254}, Merck, Germany) with Griseofulvin as standard (Sigma, G4753-5G; 010M0537 Product of China, MSDS available SL 10243, EC. 204-767-4, WGK. 3). The plates were developed in TEF (Toluene / Ethyl acetate / Formic acid, 5: 4: 1 v / v / v) and CAP (Chloroform / acetone / 2-propanol, 85:15:20, v / v / v) mobile phases and subsequently dried at room temperature and observed under UV light at 365 nm. After spraying with sulfuric anisaldehyde (ANIS: 0.5% p-anisaldehyde in methanol / acetic acid / concentrate sulfuric acid (17: 2: 1v / v / v), the plates were dried and observed at 365 nm in the dark. The retention factors RF CAP and RF TEF of each spot were calculated and compared to the standard Griseofulvin having RF TEF = 1.0 and RF CAP = 1.0

2.4 Statistical Analysis

Data were analyzed using the statistical software R.3.2.3 (R Core Team, 2015). The software was used to categorize the technologies per agro-ecological zones and fungal strain. This categorization was made by using a multidimensional positioning on the matrix of frequencies occurrence of fungal strains in relation with each storage technology. The Excel software was used to draw graphs.

3. RESULTS

3.1 Storage Mode Through Agro-ecological Zones

Three storage modes and two methods of corn shelling were observed. Among the modes of storage, there is the storage of grains, the storage of dry and fresh maize cobs., cobs. Among all prospected areae, 53% of producers stored maize grain, 24% for dry maize cobs and 15% of producers stored fresh maize cobs (Fig. 2). Nevertheless, some producers combine both storage modes (dry maize cobs and maize grain or fresh maize cob and maize grain). To pick off maize grains, an average of 76% of producers used sheller (machine) for 13% who did it manually (with a pestle and mortar) (Fig. 3). Depending on the storage mode the duration of storage can extend over a whole year.

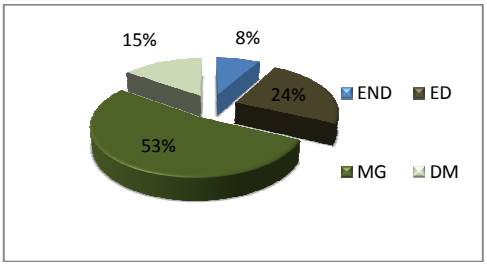

Fig. 2. Mode of storage
END: Fresh cob; ED: dry cob; MG: grain maize; DM: both modes

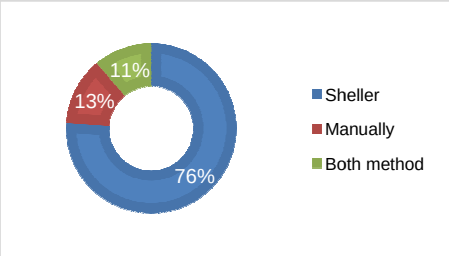

Fig. 3. Shelling method

3.2 Storage Structures

The main structures used by producers for preservation of maize are polyethylene bags, granary, store, room and roof of houses. The most common storage structures were polyethylene bags and granary. Moreover, it has been noticed that, 82% of maize producers usually use at the same time two storage structures. Seven maize storage technologies were recorded in the 14 townships prospected (from A to G) (Fig. 4). An average of five different maize storage technologies was identified in each township (Table 2).

3.3 Fungal Flora Isolated From Maize Based on Storage Technologies

Eleven (11) molds were isolated from maize samples collected among which *Fusarium sp, Penicillium. sp* and *Aspergillus. niger* were the prevalent species with frequencies of 20.71%; 15.15% and 12.12% (Table 3), respectively. Figs. 5 and 6 show the mold isolated from maize samples according to the areas of study and storage technologies. Toxigenic molds were recorded in all the maize samples. In general, the main mold producing aflatoxins (*A. flavus* and *A. parasiticus*) were not recorded in the A and B technologies for all study areas.

Table 2. Samples of maize collected per township and per technology

Agro-ecological zones	Townships	Technologies	Number of technologies	Number of replicates	Number of samples
II	Banikoara	ABCDE	5	3	15
	Kandi		5	3	15
III	Kalalé	ABCDE	5	3	15
	Nikki		5	3	15
IV	Djougou	ABDE	4	3	12
	Natittingou		4	3	12
V	Aplahoué	ABDEF	5	3	15
	Tchaourou	ABDEG	5	3	15
VI	Abomey-Calavi	ABDEFG	6	3	18
	Djakotomey		6	3	18
VII	Lalo	ABDE	4	3	12
	Pobè		4	3	12
VIII	Lokossa	ABDE	4	3	12
	Dangbo	BDEF	4	3	12
Total					198

Table 3. Mold isolated from samples of stored maize (N=198)

Molds	Samples nomber	Frequency (%)
A. niger	24	12,12
A. flavus	18	9,09
A. parasiticus	15	7,58
A. ochraceus	7	3,53
F. sp	41	20,71
F. graminearum	9	4,55
F. verticillioïdes	31	15,65
F. oxysporum	9	4,55
P. sp	30	15,15
P. roqueforti	6	3,03
P. griseofulvum	8	4,04
Total	198	100

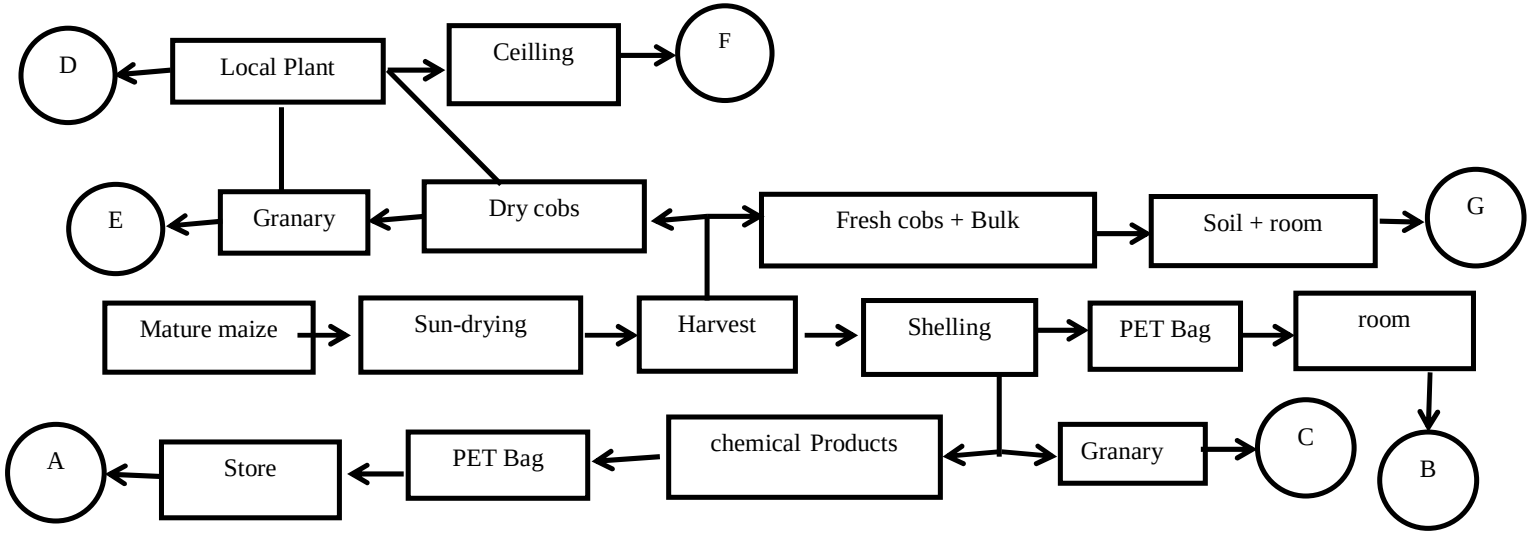

Fig. 4. Maize storage technologies identified

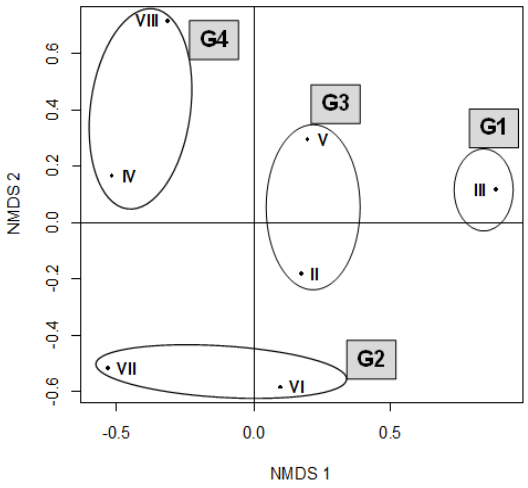

Fig. 5. Multi-dimensional positioning of groups according to agro-ecological zones

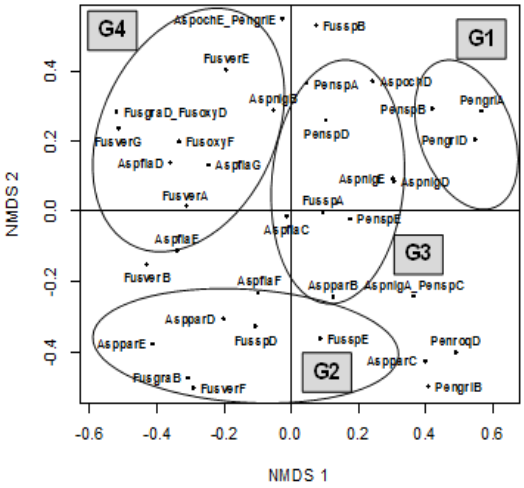

Fig. 6. Multi-dimensional mold isolated positioning according to technologies

Final stress to the convergence for agro-ecological zones: 12.27.

Final stress to the convergence for agro-ecological zones, the fungal species by technologies: 25.79.

3.4 Characterization of Mycotoxins in Stored Maize

The molds and their ability to produce mycotoxins are presented in Table 4. The analyses of the results of Table 4 show that all isolated mold produced mycotoxins. *A. flavus, A. parasiticus, A. ochraceus, F. graminearum, F. oxysporum* and *P. roqueforti* showed their ability to produce Aflatoxin B2, Aflatoxin G1, Ochratoxin A, Deoxynivalenol, moniliformin and Roquefortin C, respectively. The metabolites of other species could not be determined due to lack of reference data. Using Griseofulvin as a reference, the retention factors of secondary metabolites were determined by Thin Layer Chromatography and the spots were observed in the UV at 365 nm. The observation of these spots showed blue fluorescence except the metabolite 8 which showed red fluorescence (Fig. 7).

4. DISCUSSION

Among all agro-ecological zones prospected three maize storage modes were adopted by producers. The most common method is the storage of grains. This observation was also made by Gueye et al. [11]. The significant use of this mode could be explained by the fact that this cereal is produced to be sold in the major

Table 4. Characteristics of stored maize mycotoxins

Number	Molds	RFg TEF	RFg CAP	Colors	Metabolites identified
1	*A. niger*	ND	0.94	Blue fluorescence	ND
2	*A. flavus*	0.39	0.85	Blue fluorescence	Aflatoxin B2
3	*A. parasiticus*	0.39	0.96	Blue fluorescence	Aflatoxin G1
4	*A. ochraceus*	1.39	0.31	Blue fluorescence	Ochratoxin A
5	*F. sp*	ND	0.81	Blue fluorescence	ND
6	*F. graminearum*	0.48	0.62	Blue fluorescence	Deoxynivalenol
7	*F. verticillioïdes*	ND	0.95	Blue fluorescence	ND
8	*F. oxysporum*	0.1	0.00	Red	moniliformin
9	*P. sp*	ND	0.92	Blue fluorescence	ND
10	*P. roqueforti*	0.19	0.46	Blue fluorescence	Roquefortine C
11	*P. griseofulvum*	1.16	0.85	Blue fluorescence	ND

ND: Non determined

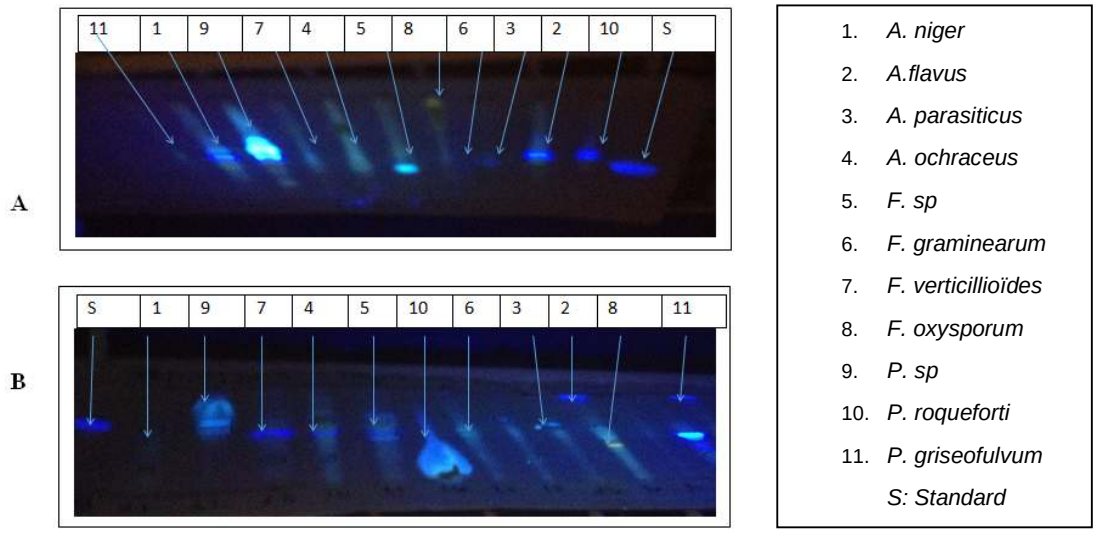

Fig. 7. Spots of secondary metabolites extracted from mold of stored maize observed at 365 nm after TLC in solvent CAP (A) and TEF (B)

markets even exported to some neighboring countries to meet their financial needs. However, the mode of storage depends on the use of the maize according that it will be sold or consumed by family. Then, some producers adopted both storage modes (dry maize cobs and maize grain or fresh maize cob and maize grain). The maize for consumption is often stored in granaries while the ones reserved for sale are stored in polyethylene bags. Thus, according to the storage method adopted, maize can be stored for a whole year. To obtain maize grain, 76% of producers shelled maize mechanically (by using sheller) against 13% who did it manually (by means of mortar and pestle). Indeed, the maize intended to be sale is shelled mechanically by men and women while that intended for consumption is mainly done manually by women. The use of the sheller could be explained by the fact that it is an easy technique, fast; less tedious and responding most to the market demand, and the manual shelling is slow and requires a lot of physical effort.

Amongst the 11 fungi isolated, *Fusarium sp* was the most frequent species with a frequency of 20.71%. Identification of the genus *Fusarium* among fungal confirmed the results reported by Fandohan et al. [12] who reported that Fusarium is a major fungal corn in Africa. Its presence in samples during storage could be explained by the contamination of cobs during the harvest in the field. The genera *Penicillium* and *Aspergillus* are fungi which proliferate mainly during storage. Their presence in maize samples with frequency of 15.15% for *Penicillium sp* and 12.12% for *Aspergillus niger* indicates the harmful impact that can cause bad storage conditions on the preservation of crops. Indeed, according to Hell et al. [13], maize presents qualitative post-harvest losses, related to the inadequacy of traditional storage structures. Amongst the genus *Aspergillusi* found in the samples collected, there were *Aspergillus flavus* and *Aspergillus parasiticus* with 9.09% and 7.58% respectively. The presence of this two species illustrates the risk of contamination by mycotoxin in particular aflatoxin. Fandohan et al. [14], reported the presence of aflatoxin and fumonisin in maize grown in Benin and Allogni et al. [15] who confirmed that over 30% maize kernels are contaminated by aflatoxin in Benin. Toxigenic molds produce different mycotoxins. The findings of Forget et al. [16] showed that among mycotoxins, some are hepatotoxic even carcinogenic, others are found to be nephrotoxic, neurotoxic or endocrine disruptor. Molds are useful in some industries such as cheese and pharmaceutical industries, but can also in some cases be harmful (toxigenic molds) by altering the physical and chemical properties of the substrate they colonize Alborch et al. [17]. In addition to these results, the presence of molds was noticed in all the prospected area and storage technologies. This result suggest that environmental conditions (temperature, moisture) are favourable for development of fungi and no recorded traditional storage technology was

efficiency for inhibition of mold development. This is because the molds are very stable to acid and heat. Moreover, for all technologies, there is no antifungal treatment popularized for the prevention of post-harvest losses due to attacks of molds. In all areas prospected, the presence of *Aspergillus flavus* and *Aspergillus parasiticus* was not observed in technology A and B. With the technology A where maize was sprayed with a chemical and stored in PET bags for a relatively long period of time, the absence of these two fungi producing aflatoxins may be related to the chemical application. This result confirms the studies of Hell et al. [13] who revealed that the use of insecticides reduce the risk of contamination by aflatoxins. Technology B is almost the technology A except that there is no use of chemicals and the storage duration is short. The absence of the two molds in this technology could be related to the short period of storage and the fact that it is sold earlier. On the other hand, technologies E, F and G were the most sensitive to the proliferation of aflatoxin producers. This sensitivity may be due to the exposure of the structures to moisture and conservation of maize over a long period. These results are in accordance with the studies of Fandohan et al. [14] and Neacşu and Madar, [2] who showed that maize stored directly on a concrete floor can be favorable to fungal infestation. Moreover, Hell et al. [13] also revealed that maize storage structures that had a higher risk of development of mold and production of aflatoxin were traditional granaries and roof of the house isolated species showed ability to produce aflatoxin. Joubranne, [18] also revealed the ability of fungal isolates from wheat to produce mycotoxins.

5. CONCLUSION

The present study allowed us to assess the effectiveness of maize storage technologies on the fungal infestation in agro-ecological zones of Benin. However, it will be useful to conduct more investigations in order to determine the real conditions of fungi development during maize storage. Moreover, an improvement of the existing storage methods and the use of news storage materials will contribute highly to the reduction of fungi contamination and extend the duration of preservation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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