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Research Article

Analysis of factors influencing the occurrence of fungal contamination of coffee cherries during post-harvest treatment in CÔTE D'IVOIRE

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Abstract: OTA is a toxin produced by fungal strains and responsible for toxic and carcinogenic effect. The objective of this study was to evaluate the impact of several factors such as drying surface, load of cherries and mixing frequency on fungal contamination during drying process. The fungal strains found in coffee before drying were Fusarium and Yeasts but mainly Aspergillus and Penicillium. Moreover, the level of fungal contamination in cherries rose sharply from (28.42 - 51.18 %) to reach a high rate at the end of drying (70.59 – 80.37 %). A statistical analysis of data, indicate a uniform distribution of initial microbial contamination in fresh cherries regardless the provenance. However, the provenance was found to influence the level of fungal contamination in dried coffee. Hence, the contamination of coffee cherries with Penicillium at the end of drying was 3-folds higher in coffee originating from traditional plantation in Bingerville than in coffee originating from CNRA experimental plantation, and 2-folds higher in coffee originating from traditional plantation in Divo. Additionally, the drying surface interacting with the mixing frequency was also found to impact on microbial contamination in drying cherries; mixing being more susceptible to propagating fungal strains in cherries. In contrast, the load of cherries and the drying surface separately was not found to influence the development of fungal strains in drying coffee.
Keywords: Ochratoxin, coffee, contamination level, influencing factors, *Aspergillus*, *Penicillium.*

INTRODUCTION

OchratoxinA (OTA) is a mycotoxin produced as a secondary metabolite mainly in two ubiquitous fungal genera, *Aspergillus* and *Penicillium* [1, 2]. In animal models, OTA produce a wide array of toxicological effects, including nephrotoxicity and nephrocarcinogenicity, neurotoxicity and immunotoxicity [3, 4].OTA accumulates in several tissues in the body, with the kidney being its main target, where it exerts toxic and carcinogenic effects [5, 6].In the kidney, OTA mainly impairs proximal tubular functions and causes glucosuria, enzymuria, and a decrease in the transport of para-aminohippuric acid (PAH), a prototypical renal organic anion [7, 8]. Therefore, this carcinogen mycotoxin has been classified as a class 2B, possible human carcinogen by the International Agency for Research on Cancer [9]. OTA is distributed extensively in agricultural commodities and in the natural environment [10]. Foods associated with OTA contamination, are particularly cereals, nuts, and coffee beans [2].

Coffee standing among the most consumed food worldwide is reported to be a potential source of OTA contamination. Moreover, the presence of OTA in torrefied coffee sample available on the market has been reported [11, 12, 13]. Coffee cherries are subjected to fungal contamination during post-harvest processing which include drying of the cherries and dehusking of dried fruit. Specifically, fungal contamination of coffee cherries with ochratoxinogen fungal strains notably *Aspergillus*, *Penicillium* and *Fusarium*, occurs during the drying stage. No study has been undertaken on post-harvest processing of coffee, related to OTA contamination in Cote d'Ivoire. However, the agricultural practices of drying used by farmers are thought to be susceptible to influence the risk of OTA contamination according to the treatments of the coffee.

The objective of this study is to evaluate the impact of the drying surface, the load of coffee cherries, and of the number of mixings during drying, on the development of the potentially toxinogen mushrooms.

MATHERIALS AND METHODS

Biological material

Ripen coffee cherries where harvested from three areas in Côte d'Ivoire: the CNRA coffee experimental plot in Bingerville (geographic coordinates 5°21'21" North 3°53'24" West) located at 18 km from Abidjan, a traditional plantation in a village of Bingerville (geographic coordinates5°19'29" North 3°55'0" West) and a traditional plantation in a village of Divo(geographic coordinates 5°30' North 5°15' West), located at 184 km from Abidjan. Coffee cherries were collected and immediately subjected to drying process.

Drying process

The coffee cherries are put to dry each day in the blocks from 9 h to 17 h, and regularly mixed at 9h, 12h, 14h, and 16h. When experiment requires only one mixing, this was done at 9 h. Drying cherries where protected against moisture by using black plastic cover after 17 h until 9 h of the next day when the cover is retired to re-allow cherries to dry.

Experimental device

The drying of coffee cherries was performed on experimental units (block) designed as follow: 2m in length, 1m in width and 6 cm in height. Two drying surface were used notably cemented surface and plastic surface. Then we varied the load of coffee cherries on the drying surface from 25 kg/m^2 to 50 kg/m^2 and the daily number of mixing from 1 to 4 times. Hence, the drying of the coffee was led according to a device in completely randomized.

Block with 3 blocks in combination with three different factors. The blocks were constituted of block 1 (coffee from experimental plot in Bingerville), block 2 (coffee from a traditional plantation in a village of Bingerville) and block 3 (coffee from a traditional plantation in a village of Divo). The factors were constituted of the drying surface, the load of coffee cherries and the mixing frequency (Table 1).

Table-1: Experimental device

		Cemented surface (C)		Plastic surface (T)	
		25 kg/m^2 (L25)	50 kg/m^2 (L50)	25 kg/m^2 (L25)	50 kg/m^2 (L50)
Block (or origin of coffee cherries)	1 daily mixing (M1)	$CL25M_1$	$CL50M_1$	$TL25M_1$	$TL50M_1$
	4 daily mixing (M4)	$CL25M_4$	$CL50M_4$	$TL25M_4$	$TL50M_4$

C cemented surface (2m^2); T Plastic surface (2m^2); L25 load at 25 kg/m^2; L50 load at 50 kg/m^2; M_1 one mixing/day; M_4 four mixing/day.

ANALYSIS METHODS

During drying, the water content and the fungal load of coffee cherries were monitored by regularly withdrawing 200 g of sample at five different points on the experimental unit (block) at 16;00 each day.

Water content

To measure the water content, 10 g of sample contained in aluminum basin, were weighed (P_1) and put to dry at 105 °C in an oven (MEMMERT) during 24 h. Then the dried sample was allowed to cold in a desiccator for 4 h and weighed again (P_2). The water content was calculated as follows:

$$T(\%) = \frac{P_1 - P_2}{P_1} \times 100$$

T (%): water content
P_1: Weight of the fresh coffee
P_2: Weight of the dried and cooled coffee

Microbiological analysis

The inner load of fungal flora (yeast and mold) present in the cherries was analyzed at both the beginning and the end of the drying process. At the beginning of drying step, 100 fresh cherries randomly chosen from the 200 g of each sample are first subjected to surface sterilization with 70% alcohol and 1% sodium hypochlorite then the de pulped seed was plated on DG18 agar [14] and incubated at 30°C for 5-7 days. The overall percent contamination was expressed as the percentage of particles yielding visible growth of fungi. At the end of drying, cherries are dehulled and the seed are removed and plated as described above.

Isolation and identification of fungi

The isolated fungi were purified and identified according to Klich [15], Frisvad& *al.* [16]) and P. Simon [17] with slight modification. The DG18 medium was used instead of CYA. medium–Czapek yeast Agar. The isolates were incubated at 30°C for 7 days, the microscopic and macroscopic characteristics described by Klich [15] were observed.

Statistical analysis

The statistical analyses were carried out on software SPSS (Statistical Package for Social Sciences) version 20.0.An analysis of variance (ANOVA) was performed using the test of Duncan or Fisher at 95 % confidence [18]. The model of ANOVA used is designed as follow.

$X_{ijkln} - m\ldots.. = a_i + b_j + c_k + d_l + E_n + (ab)_{ij} + (ac)_{ik} + (bc)_{jk} + (ad)_{il} + (bd)_{jl} + (cd)_{kl} + (aE)_{in} + (bE)_{jn} + (cE)_{kn} + (dE)_{ln} + (abc)_{ijk} + (abd)_{ijl} + (acd)_{ikl} + (bcd)_{jkl} + (abE)_{ijn} + (acE)_{ikn} + (bcE)_{jkn} + (adE)_{iln} + (bdE)_{jln} + (cdE)_{kln} + (abcd)_{ijkl} + (abcE)_{ijkn} + (abdE)_{ijln} + (acdE)_{ikln} + (bcdE)_{jkln} + (abcdE)_{ijkln}$

a, b, c and d, are the main factors affecting fungal contamination and the letters in index represent the levels of these factors.
a = surface of drying (Surface) with i= 1 or 2;
b = coffee load (Load) with j = 1 or 2;
c = number of mixings (Mixing) with k = 1 or 2;
d = standard of micro-organism (Micro) with l= 1; 2 …… or 7 representing the 7 groups of micro-organisms identified on medium DG 18.

RESULTS AND DISCUSSION

Fungal contamination in drying coffee

At the beginning of the drying process, the microbiological analysis showed that the coffee cherries were contaminated with *Aspergillus*, *Penicillium*, *Fusarium* and yeasts as fungal strains **(Table 2).**

Table-2: The different fungal strains isolated from coffee cherries during drying process

Description of fungi strain	Identification
white colony and milky aspect, oval shaped at optic microscope	Yeasts
Powdery and fluffy aspect, black color	Black *Aspergillus*
Powdery and fluffy aspect, yellow color	*Aspergillusochraceus*
Powdery and fluffy aspect, green color	Green *Aspergillus(Aspergillusflavus)*
Powdery and fluffy aspect, brown color	Other *Aspergillus*
Creamy aspect, green color	*Penicillium*
Creamy aspect, light pink color	*Fusarium*

Strains were isolated on DG18 agar medium after 7 day incubation at 30°C.

These species mainly *Aspergillus* present in coffee cherries in Côte d'Ivoire, were also found in coffee from Brazil [19, 20], from Thailand [21], from Saudi Arabia [28] and from Vietnam [22] and seems to be closely linked to coffee beans as natural contaminant regardless the geographic area.

A statistical analysis was then performed in order to find whether the level of contamination is subjected to variation with the different factors notably drying surface, coffee load and number of mixings. Hence the level of contamination expressed in percentage, was transformed into a logarithmic variable Log (X+1) to normalize the distribution of this variable and to allow variance analysis.

The Table 3 from ANOVA shows that at the beginning of the drying process, there was not a significant difference between the levels of the factors, even with interactions of 2; 3; 4 or 5 factors, with p-value superior to 0.05. Only, the levels of contamination of the microorganisms with p-value lower than 0.001 proved to be very highly different. These observations indicate that no bias susceptible to affect artificially the results of this study was introduced in the experimental device designed at the beginning.

Furthermore, the analysis of variance (Table 3) also shows that there is not a difference in the contamination of coffee cherries from different origin (block) before the drying process. This suggests a uniform distribution of the microbial strains in coffee bean in these three areas of Ivory Coast [27] have also reported a uniform distribution of the filamentous fungi in coffee samples from different regions of Brazil.

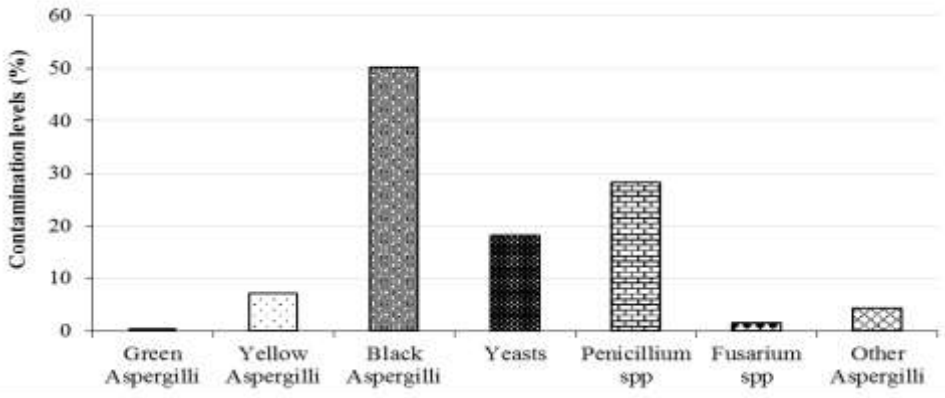
Microorganisms were already present in the fresh cherries at the beginning with relatively high level of contamination (Fig. 1). This contamination undoubtedly occurred on tree before post-harvest processing. Likewise, [19] reported a high contamination of coffee beans on tree by certain fungal species such as *Penicillium* and *Fusarium.*

The main groups of fungal strains isolated were black *Aspergillus* responsible for 51.18 % of coffee cherries contamination, followed by the group of *Penicillum* with 28.27 % of contamination and yeasts 18.21 %. *Aspergillus* and *Penicilium* were also found to be dominant in coffee in Brazil and many countries [19, 20, 21, 22]. However, among the remaining groups of fungal strains showing low level of contamination the presence of yellow type of *Aspergillus*, characteristic of *Aspergillus Ochraceus* at the rate of 7.20 % has been observed. This species is known to have a high potential of ochratoxin A production.

Table-3: Analysis of variance of the level of contamination of the micro-organisms at the beginning of the drying process

Variation Source (VS)	FD	SS	MS	F	p-value
Surface	1	0,133	0.133	1.615	0.332
Load	1	1.3710^{-4}	1.3710^{-4}	0.005	0.951
Mixing	1	5.8010^{-2}	5.8010^{-2}	0.120	0.762
Microorganism	6	41.027	6.838	11.627	**0.000**
Block	2	0.519	0.260	0.578	0,690
Surface - Load	1	0.158	0.158	1.290	0.374
Surface - Mixing	1	7.1110^{-2}	7.1110^{-2}	0.231	0.678
Load - Mixing	1	$3,8810^{-2}$	3.8810^{-2}	0.690	0.494
Surface - Load - Mixing	1	4.0310^{-2}	4.0310^{-2}	1.051	0.413
Microorganism - Surface	6	0.442	$7.3\ 10^{-2}$	0.472	0.816
Load - Microorganism	6	0.770	0.128	0.573	0.745
Microorganism - Surface - Load	6	1.015	0.169	1.611	0.227
Microorganism - Mixing	6	0.267	$4.4\ 10^{-2}$	0.238	0.955
Microorganism - Surface - Mixing	6	0.728	0.121	1,090	0,421
Load - Microorganism - Mixing	6	1.789	0.298	1,339	0,313
Surface - Microorganism - Load - Mixing	6	0.949	0.158	1.001	0.468
Surface - Block	2	0.165	$8.2\ 10^{-2}$	0.168	0.851
Load - Block	2	$5.6\ 10^{-2}$	$2.8\ 10^{-2}$	0.145	0.874
Surface - Load - Block	2	0.245	0.251	0.718	0.946
Mixing - Block	2	0.969	0.485	1.441	0.417
Surface - Mixing - Block	2	0.615	0.109	1.019	0.573
Load - Mixing - Block	2	0.113	$6.1\ 10^{-2}$	0.124	0.531
Surface - Load - Mixing - Block	2	$7.6\ 10^{-2}$	$3.8\ 10^{-2}$	0.243	0.788
Microorganism - Block	12	7.057	0.588	2.057	0.228
Surface - Microorganism - Block	12	1.873	0.156	2.677	0.483
Load - Microorganism - Block	12	2.688	0.224	1.319	0.427
Surface - Load - Microorganism - Block	12	1.261	0.105	0.665	0.755
Mixing - Microorganism - Block	12	2.242	0.187	1.062	0.522
Surface - Mixing - Microorganism - Block	12	1.335	0.111	0.704	0.724
Load - Mixing - Microorganism - Block	12	2.673	0.223	1.409	0.281
Surface - Load - Mixing - Microorganism - Block	12	1.897	0.158	0.832	0.316

FD Freedom degree; SS Sum of squares; MS Mean square; F Test of Fisher; p-value level of significance. When p-value ≤0.05 the difference is significant - When p-value ≤0.01 the difference is highly significant - When p-value ≤0.001 the difference is very highly significant.



[23].

Fig-1: The different groups of fungal strains and their levels of contamination in coffee cherries at the beginning of the drying process.

The presence of fungal contamination of coffee cherries before drying suggests that, the final quality of dried coffee should rely on the efficiency of the drying process to lower the load of microbial strains, notably those which are highly toxigenic. We observed that, the groups of fungal microflora present initially was also the same found at the end of drying process but with different level of contamination depending on the surface drying and the origin of beans (Fig. 2 and 3). From the beginning to the end of drying, several factors in combination may contribute to impact differently the microbial growth in contaminated cherries. This is strongly supported by the fact that the ANOVA performed before and at the end of drying shows a distribution of contamination levels of cherries initially uniform in samples from different area, but this distribution is no longer uniform at the end of drying. Moreover, an interaction of different factors has been evidenced (Table 4).

Table-4: Analysis of variance of the level of contamination of the micro-organisms at the end of the drying process

Variation Source (VS)	FD	SS	MS	F	p-value
Surface	1	2×10^{-6}	2×10^{-6}	0.000	0.998
Charge	1	0.396	0.396	10.085	0.086
Mixing	1	9.3×10^{-2}	9.3×10^{-2}	5.958	0.135
Microorganism	6	55.163	9.194	6.581	**0.003**
Block	2	2.401	1.200	1.009	0.426
Surface - Load	1	0.221	0.221	3.506	0.202
Surface - Mixing	1	1.2×10^{-3}	1.2×10^{-3}	0.012	0.923
Load - Mixing	1	0.110	0.110	1.233	0.382
Surface - Load - Mixing	1	5.7×10^{-2}	5.7×10^{-2}	0.827	0.459
Micro - Surface	6	1.420	0.237	1.040	0.447
Load - Microorganism	6	1.675	0.279	1.800	0.182
Microorganism - Surface - Load	6	0.875	0.146	1.989	0.146
Microorganism - Mixing	6	0.676	0.113	0.398	0.866
Microorganism- Surface - Mixing	6	1.931	0.322	3.386	**0.034**
Load - Microorganism - Mixing	6	0.755	0.126	0.774	0.605
Surface - Microorganism- Load - Mixing	6	1.467	0.245	1.813	0.179
Surface - Block	2	0.783	0.391	1.352	0.339
Charge - Block	2	7.8×10^{-2}	3.9×10^{-2}	0.286	0.789
Surface - Load - Block	2	0.126	6.3×10^{-2}	8.238	0.953
Mixing - Block	2	3.1×10^{-2}	1.5×10^{-2}	0.056	0.947
Surface - Mixing - Block	2	0.203	0.102	3.446	0.713
Load - Mixing - Block	2	0.178	0.309	1.128	0.601
Surface - Load - Mixing - Block	2	0.138	6.9×10^{-2}	0.513	0.611
Microorganism - Block	12	16.765	1.397	2.975	**0.034**
Surface - Microorganism - Block	12	2.731	0.228	6.790	0.520
Load - Microorganism - Block	12	1.860	0.155	1.536	0.432
Surface - Load - Microorganism -Block	12	0.879	7.3×10^{-2}	0.543	0.848
Mixing - Microorganism - Block	12	3.397	0.283	2.306	0.247
Surface - Mixing - Microorganism - Block	12	1.141	9.5×10^{-2}	0.705	0.723
Load - Mixing - Microorganism - Block	12	1.950	0.163	1.205	0.376
Surface - Load - Mixing - Microorganism -Block	12	1.618	0.135	0.915	0.447

FD Freedom degree; SS Sum of squares; MS Mean square; F Test of Fisher; p-value level of significance. When p-value ≤0.05 the difference is significant - When p-value ≤0.01 the difference is highly significant - When p-value ≤0.001 the difference is very highly significant.

Origin of coffee as factor influencing the fungal contamination

The interaction Block-Microorganism was found to be very significant. This indicates that the level of contamination of microorganisms is impacted by the origin (Block) of coffee sample at the end of the drying process. Accordingly, the level of contamination of coffee cherries with *Penicillium* at the end of drying, is three folds higher in block 2 (coffee originating from a traditional plantation in Bingerville) than in block 1

(coffee originating from CNRA experimental plot in Bingerville), and 2-folds higher in block 3 (coffee originating from a traditional plantation in Divo) than in block 2 (Fig. 2). Yellow *Aspergilli* characteristics of *Aspergillus ochraceus* were more present in dried cherries in the block 2 corresponding to a levels of contamination 3-folds higher than in block 1 and 18-folds higher than in block 3 (Fig. 2). Intrinsic biochemical characteristic of beans are sometime related to the type of soil and geographic origin. Some of these characteristics are susceptible to influence microbial growth. For instance, *Penicillium* is known to require high water content for growth [24], while the presence of tannin and lignin in cherries is believed to limit fungal growth [25]. Hence, a difference in biochemical properties of beans linked to the soil may explain the impact of coffee origin on fungal contamination. Furthermore, the stage of maturation was also reported to influence the presence of fungi in the fruit [26].

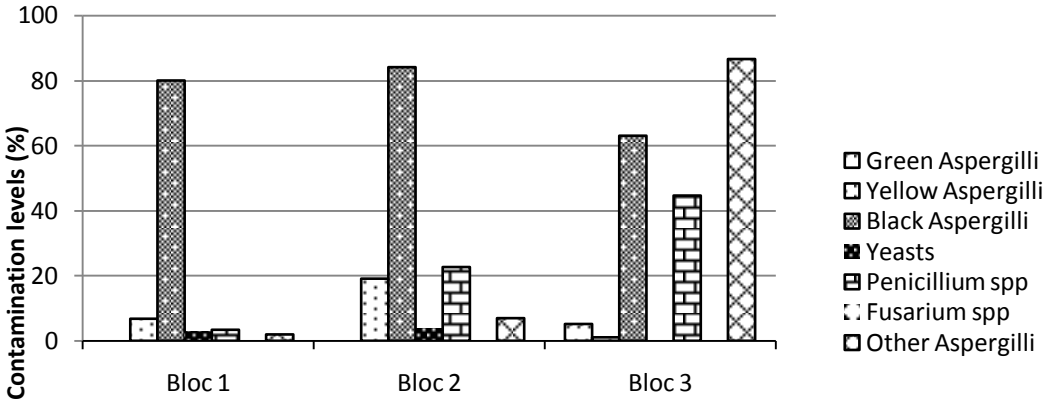


Fig-2: Levels of fungal contamination in the blocs at the end of drying.
Block 1 (coffee from experimental plot in Bingerville), Block 2 (coffee from a traditional plantation in a village of Bingerville) and block 3 (coffee from a traditional plantation in a village of Divo).

Interaction drying surface-mixing frequency as a factor influencing the level of fungal contamination

The other interaction found to be significant was the triple interaction Microorganism-Surface-stirring (Table 4) indicating that, the level of microbial contamination of coffee cherries depends on combined effects of drying surface and number of stirrings. Hence, on cemented surface, the contamination with the major fungal flora notably black *Aspergillus* and *Penicillium*, is more important in cherries mixed four times daily than cherries mixed once daily (Fig. 3). On plastic surface, although the major fungal flora seems to be stable at different mixing frequency, the contamination with yellow *Aspergillus* sharply raised to reach the level of *Penicilium* contamination and become a major flora in cherries mixed four times daily comparatively to cherries mixed once daily (Fig. 3). From these observations, one could assume that, stirring contribute to spread the microbial strains in the cherries and raise the contamination level. However, a contrast has been observed with some minor flora notably yeasts (on both drying surface) and yellow *Aspergillus*(on cemented surface) which were less important in cherries mixed four times daily than in in cherries mixed once daily (Fig.3). Although the mixing and type of dryers cannot be considered separately due to interaction between these factors, it is strongly believable that a high frequency of mixing should result in raising the level of fungal contamination in cherries during drying.

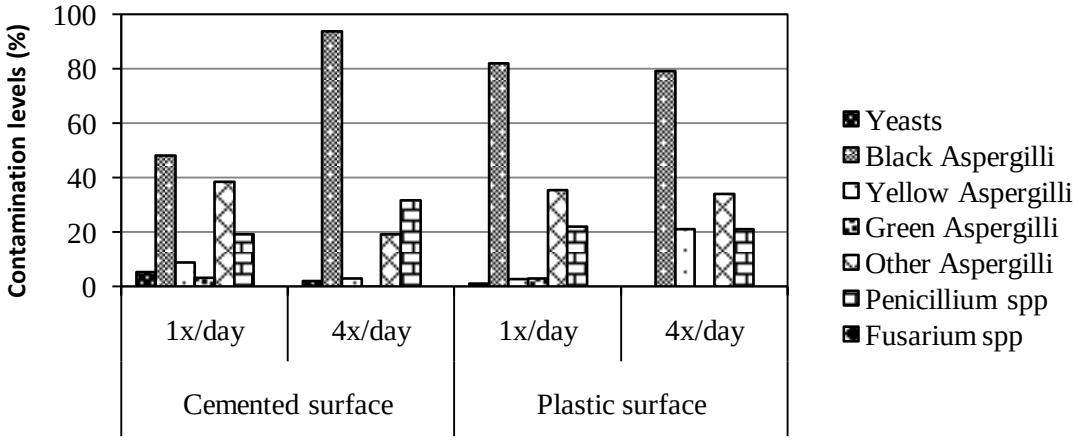


Fig-3: Level of fungal contamination according to the drying surface and mixing frequency at the end of drying

CONCLUSION

This study shows a high level of fungal contamination in coffee cherries in Côte d'Ivoire mainly with *Aspergillus* and *Penicillium*. The level of fungi contamination of coffee cherries during drying depends on origin of the fruits but also on the combined effect of mixing and drying surface. A high frequency of mixing may contribute to a high level of coffee contamination during drying. Among the drying surface tested notably cemented and plastic surface, no surface was found as better than other.

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