



Research Journal of **Microbiology**

ISSN 1816-4935



Academic
Journals Inc.

www.academicjournals.com



Research Article

Cropping Practices and Fungal Contamination in Banana Plantations in Côte d'Ivoire

^{1,2}Toualy Serge Ouina, ¹Jean-Michel Panoff, ³Stephanie Gente, ³David Garon, ³Jean-Philippe Rioult, ⁴Tia Jean Gonnety and ²Marina Koussémon

¹Food, Bioprocess, Toxicology, Environments (Team 4651)-Food Matrix and Microbiota-Campus 1, Institute for Fundamental and Applied Biology, University of Caen Normandie, Esplanade de la Paix, 14032 Caen cedex 5, France

²Laboratory of Biotechnology and Food Microbiology, Faculty of Science and Food Technology, Nangui Abrogoua University, 02 BP 801 Abidjan 02, Côte d'Ivoire

³University of Caen Normandie, Food, Bioprocess, Toxicology, Environments (Team 4651)- Environmental Toxicology, Aerial Environments and Cancer, 14000 Caen, France

⁴Laboratory of Biocatalysis and Bioprocessing, Faculty of Science and Food Technology, Nangui Abrogoua University, 02 BP 801 Abidjan 02, Côte d'Ivoire

Abstract

Background and Objective: Bananas are very important crops for food and economic level in Côte d'Ivoire. For purposes of contributing to the development of this crop, this study aimed at determining the social and microbiological characteristics of some banana plantations in the region of Loh-Djiboua (Côte d'Ivoire). **Materials and Methods:** Cropping practices observed in banana plantations were determined through an investigation carrying out in August, 2014 and focusing on the identification of these plantations and human resources, maintenance of plantations, generally. Fungal species were isolated on culture media, from soils of investigated plantations. *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (Foc TR4) was researched through culture-dependent and culture-independent molecular approaches. **Results:** All investigated banana plantations are small farms. Banana plants were grown using the system of pure or associated crops. An aligned or random disposition was observed for these plants. Banana producers used pesticides in their plantations. Fungi found in the soil of these plantations belong to the genera *Aspergillus*, *Chrysonilia*, *Eupenicillium*, *Fusarium*, *Paecilomyces*, *Penicillium*, *Phoma*, *Rhizopus*, *Syncephalastrum* and *Trichoderma*. Genus and species found in more plantations are *Trichoderma* and *F. oxysporum*, respectively. From 6 plantations harboring *F. oxysporum*, Foc TR4 is found in 5 plantations. **Conclusion:** Soil mycoflora of investigated plantations include phytopathogenic, endophytic and toxinogenic fungi. These social and microbiological characteristics constitute important indicators for taking adequate measures to prevent the growth of phytopathogenic and toxinogenic fungi and mycotoxin production in post-harvest products.

Key words: *Fusarium oxysporum*, phytopathogenic, endophytic, toxinogenic, microbiological characteristics, banana plantations

Citation: Ouina, T.S., J.M. Panoff, S. Gente, D. Garon, J.P. Rioult, T.J. Gonnety, J.M. Koussémon, 2020. Cropping practices and fungal contamination in banana plantations in Côte d'Ivoire. Res. J. Microbiol., 15: 98-108.

Corresponding Author: Toualy Serge Ouina, Food, Bioprocess, Toxicology, Environments (Team 4651)-Food Matrix and Microbiota-Campus 1, Institute for Fundamental and Applied Biology, University of Caen Normandie, Esplanade de la Paix, 14032 Caen cedex 5, France
Tel: +22577849878/+22505674687

Copyright: © 2020 Toualy Serge Ouina *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Bananas (*Musa* spp.) constitute an essential basis in the diet of the population of the developing world^{1,2}. In Côte d'Ivoire, plantain banana occupies the 3rd place in terms of food crop production after yam and cassava³ and in terms of most consumed food after rice and yams⁴. With an average production per year of 1,600,000 and 300,000 T, for plantain and dessert bananas, respectively⁵, Côte d'Ivoire provides with bananas, various markets at the sub-regional level (Burkina Faso, Mali)⁶. The commercialization of plantain banana represents a stable source of income for producing populations. Dessert banana, mainly intended for export, plays a leading role in the Ivorian economy⁵.

Despite the potential of the banana sector in economic development and food security in Côte d'Ivoire, this sector has unfortunately periods of shortages. These shortages are related to abiotic stresses (reduction of soil fertility, water shortage, gales) and biotic stresses (pests and diseases) to which bananas are faced^{7,8}. These various stresses cause low yields and production losses of up to 80% according to El-DougDoug and El-Shamy⁷. But, the Ivorian population growth estimated in 2050 to 48,796,000 inhabitants⁹ requires an increase of food production.

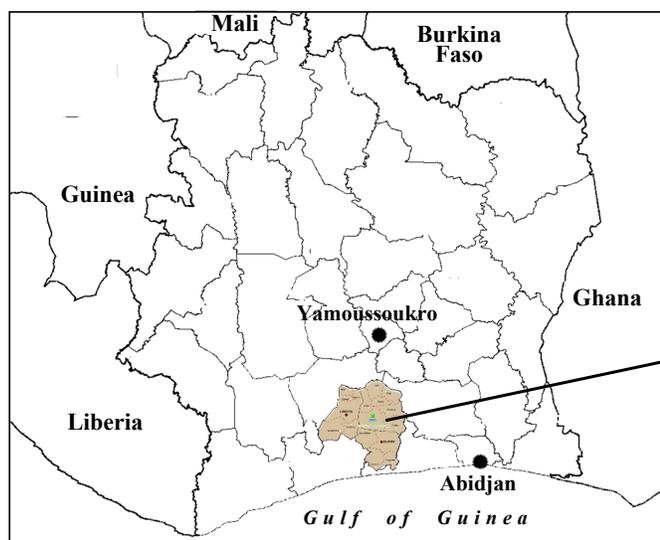
Bananas are mainly grown in the southern forest half of Côte d'Ivoire. The uses of good farming techniques and varietal improvement have to be observed for increasing the productivity of banana crops and the development of the

banana sector. Thus, the CNRA (Centre National de Recherche Agronomique) developed and recommended both Sigatoka resistant varieties of banana (Pita 3 and Fiha 21) and technical itinerary for the good cultivation of bananas in Côte d'Ivoire⁶. However, improved varieties are out of the reach of low-income producers in general and don't always meet the taste expectations of consumers⁶. The observation of good cultural practices would remain by default, the way of reducing the incidence of banana attacks and therefore, of increasing banana production.

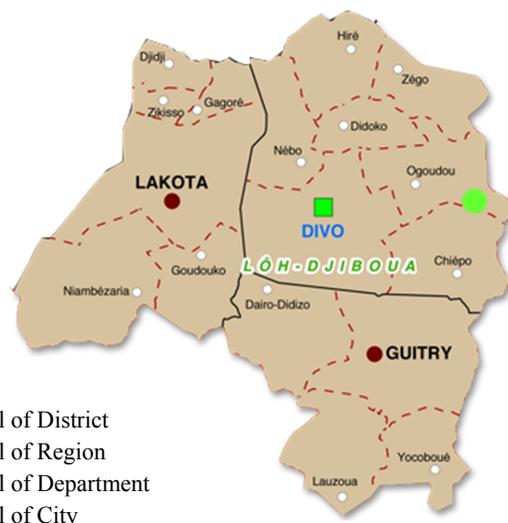
The region of Loh-Djiboua (Côte d'Ivoire) is a region that contributes alongside high banana production areas in Côte d'Ivoire, to supply markets across the country. Besides, one of the goals of the Ivorian authorities is to make this region an attractive future agricultural pole¹⁰. There is little information's about the characteristics of banana farms in this region. This study aimed at determining the social and microbiological characteristics of some banana farms in the region of Loh-Djiboua. Specifically, an inventory of observed cropping practices and soil mycoflora of banana plantations in this region was performed.

MATERIALS AND METHODS

Study site: This study was conducted for its field phase, in the region of Loh-Djiboua, located in the South-West of Côte d'Ivoire (Fig. 1). The localities investigated in this study were Divo and Lakota, with respective geographical coordinates



(a) Map of the regions of Côte d'Ivoire



(b) Map of the region of Loh-Djiboua

Fig. 1(a-b): Geographical representation of the study zone

Source: (a) <https://reliefweb.int/map/c-te-divoire/c-te-divoire-location-map-2013> and (b) https://favpng.com/png_view/map-view/map-1%C3%B4h-djiboua-zikisso-fromager-divo-bonoua-png/tWYYJ69U

(5° 49'59 "N and 5° 21'58" W) and (5° 50'49 "N and 5° 40'48" O), determined with the aid of Google Earth 7.1.5.1557 release (Google, Mountain View, California/USA). The distance between Abidjan (economic capital of Côte d'Ivoire) and each of the investigated localities is 189 km for Divo and 231 km for Lakota.

Investigation of cropping practices: An investigation was conducted during August, 2014, in eight banana plantations of which four were located in the locality of Divo (B₁, B₂, B₃ and B₄) and four in the locality of Lakota (B₅, B₆, B₇ and B₈). The minimum distance between two banana plantations were about ten (10) kilometers. Among these 8 banana plantations, 6 were of plantain banana type and two of dessert banana type. In each locality, 3 banana plantations of plantain type and one of the dessert type were chosen. The investigation consisted of determining the cropping practices observed in the chosen banana plantations in the locality of Divo and Lakota. It was implemented as follows: Each owner or manager of the banana plantation was submitted to a questionnaire about the identification of farmers and banana, objectives of production, used technical equipment and human resources. A guided tour was then performed in each banana plantation for a better assessment of the information collected after questioning, the type of planting and the system of cropping observed for banana plants. The state of banana plants and leaves was also observed.

Soil sampling of banana plantations: The sampling of soils of banana plantations were carried out during August, 2014 in all 8 banana plantations covered by the investigation. First, 30 banana plants approximately located 5 m from each other, were chosen by banana plantation. Two excavations have been performed at the dimensions 25×20×25 cm (length×width×depth) each, within a 1 m radius at the foot of each banana plant. These excavations were performed with the aid of hoe and machete, on opposed places, after getting rid of the soil surface of various plant and animal debris. The masses of land obtained (about 500 g from each excavation) were homogeneously mixed by hand (protected with gloves). A soil sample consists of approximately 2 g of soil collected from the mass of land mixed. In each banana plantation, 30 soil samples (codified 1-30 S for soil samples at the positions 1-30) were taken. The sampling was manually performed in cryotubes and conservation of soil samples was carried out in a cooler with ices. A total of 240 soil samples were collected and sent to the laboratory for analysis.

Research of fungal species in soil samples: The research of fungal species in the soil of investigated banana plantations was carried out on the culture media Potato Dextrose Agar (PDA), Cristomalt (CM) and Cristomalt+lithium (6 g L⁻¹) (CML), through the technique of soils plates¹¹. Briefly, amounts of 5 mg of each soil sample collected from banana plantations were weighed using a precision balance (Sartorius CP 225D, AG Germany) and placed in Petri dishes without ergot. Twenty-five milliliters of PDA, CM or CML medium, previously prepared and kept in super cooling at 45-50°C, were added thereto. Weighed soil was immediately dispersed in each culture medium with gentle stirring until solidification. Petri dishes were incubated at 25°C for 72 hrs to 7 days. Morphologically different colonies observed on each medium, have been picked individually on the PDA medium and incubated in the same conditions as previously. They have been identified based on their macroscopic and microscopic characters¹².

Research of *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (Foc TR4): Sixteen soil samples (at the rate of two soil samples (S 2 and S 29) in each investigated plantation) were chosen for the research of Foc TR4. This research was performed both by culture-dependent molecular approach and culture-independent molecular approach. For the culture-dependent approach, after fungi isolation from the soil samples as described previously, molecular identification of Foc TR4 was performed from colonies morphologically identified as *F. oxysporum*. DNA was extracted from the mycelium of *F. oxysporum* by using the "NucleoSpin Plant II" kit (Macherey-Nagel, Hoerdt, France). The protocol of this kit was observed with some modifications as described by Seguin *et al.*¹³. For the culture-independent approach, DNA was extracted directly from soil samples by using the "NucleoSpin Soil" kit (Macherey-Nagel, Hoerdt, France). Extracted DNA through these two approaches, were quantified at the spectrophotometer (Nanodrop 2000) and their purity was checked by the ratio A_{260/280} (about 1.8 for non-contaminated DNA) and by assessing their ability to migrate on a 1% (w/v) agarose gel^{14,15}. Extracted DNA (from *F. oxysporum* and soil samples) were amplified by PCR (Polymerase Chain Reaction) by using universal primers ITS 1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS 4 (5'-TCCTCCGCTTATTGATATG-3') and specific primers Foc TR4-F (5'-CACGTTTAAGGTGCCATGAGAG-3') and Foc TR4-R (5'-CGCACGCCAGGACTGCCTCGTGA-3'). The primers ITS 1/ITS 4 and Foc TR4-F/Foc TR4-R, stood respectively for the confirmation of species *F. oxysporum* and the identification

of sub-species Foc TR4. Each PCR reaction mixture contained 100-500 ng of genomic DNA, 100 pmol of each of primers ITS 1 and ITS 4 or Foc TR4-F and Foc TR4-R (Eurogentec, Seraing, Belgique), 0.25 mM of dNTP, reaction buffer Taq DNA Polymerase 10X with MgCl₂ 25 mM and 2.5 U of AmpliTaq Gold DNA Polymerase (Applied Biosystems, Branchburg, NJ, USA) in a final volume of 50 µL. PCR was carried out using the following protocol: (i) 95°C for 4 min and 35 cycles of 95°C for 30 sec, 52°C for 30 sec and 72°C for 1 min, followed by an additional extension time for 7 min at 72°C for primers ITS 1/ITS 4¹² and (ii) 95°C for 10 min and 30 cycles of 95°C for 1 min, 62°C for 1 min and 72°C for 1 min, followed by an additional extension time for 10 min at 72°C for primers Foc TR4-F/Foc TR4-R¹⁵. The migration of PCR products was realized in a 1% (w/v) agarose gel, which was stained with Midori green and visualized under UV light. Amplicons obtained with the primers set ITS 1/ITS 4 were sequenced and sequences were aligned using BLASTN for the detection of *F. oxysporum*. For specific detection of Foc TR4, amplicons obtained with the primers set Foc TR4-F/Foc TR4-R had to present a 463 base pairs (bp) size¹⁶.

RESULTS

Characteristics of investigated banana plantations

Identification of the exploitations and their owners: The investigation carried out in the banana plantations, indicates that 75% of these plantations belong to private individuals (B₁, B₂, B₃, B₄, B₆ and B₈) and 25% are the propriety of agricultural cooperatives (B₅ and B₇). The exploitation's owners aged between 18 and 50 years consist of 25% of non-educated persons and 75% of educated persons (primary education, secondary education and high school). All investigated plantations have their surface area ranged between 1 and 5 ha, with flat reliefs (75% of plantations) and moderately sloping reliefs (25% of plantations). The different productions of banana are intended either for self-consumption or sales (Table 1).

Human resources and work equipment: The workforce in all investigated banana plantations is composed of the couple owners of banana plantations and contractors. Different modes of payment are observed for the employment contracts. In return to the work performed in a given banana plantation, the contractor either receives a pay or an equivalent work is performed conversely in its plantation. The workforce aged between 18 and 50 years, is partially educated and had 5-8 years of

experience in banana plantation work. Artisanal material (machete, hoe) is used as work equipment in all banana plantations. This equipment belonging to the plantations owners or contractors is not reserved for a specific plantation.

Planting and maintenance of plantations: In the investigated banana plantations, two banana cropping systems such as pure crops (25% of plantations) and associated crops (75% of plantations) are been observed (Table 1). In the system of associated crops, bananas are associated with industrial crops (coffee, cocoa) and subsistence crops (taro, yam, pepper, tomato). The plant material for dessert banana belongs to the group Cavendish. Plantain varieties grown, are Corne 1, Batard, Pita 3 and Fiha 23. In the banana plantations managed by agricultural cooperatives, plant material comes from CNRA or ANADER (Agence Nationale d'Appui au Developpement Rural). Concerning private individuals, plant material comes from old plantations or is obtained from a friend or a parent. Two modes of disposition (in-line and random) of banana plants have been observed in investigated banana plantations (Table 1). The random disposition which is more frequent is observed in 75% of all plantations and 83.33% of plantations with the system of associated crops. Only 25% of banana producers practice the disposition in-line. This latter disposition is observed in all plantations with the system of pure crops (Fig. 2). All the interviewed banana producers practice chemical fertilization and an application of pesticides in their plantation. The fertilization program is Nitrogen/Phosphorus/Potassium in proportion 20/10/10 and urea.

Concerning the applied pesticides, banana producers use the herbicides Detru-Herb 360 SL (glyphosate 360 g L⁻¹), Bin-Takara (glyphosate 360 g L⁻¹) and Glyphader 75 SG (glyphosate 75%), the insecticide Lambda 2.5% EC (cyhalothrin) and nematicide Furadan (carbofuran). Their knowledge level of banana diseases is variable. Concerning fungal diseases, 75% of the farmers do not know about

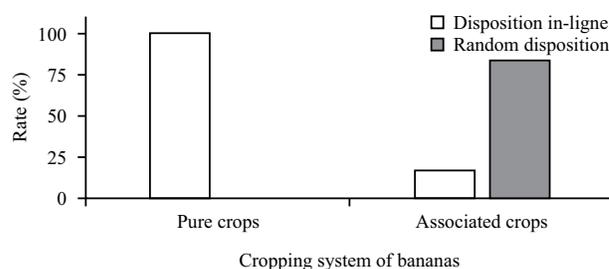


Fig. 2: Disposition of banana plants as a function of cropping system in banana plantations

Table 1: Characteristics of the investigated banana plantations

Locality	Banana plantations							
	Lakota				Divo			
	B ₁ ^(P)	B ₂ ^(P)	B ₃ ^(P)	B ₄ ^(D)	B ₅ ^(P)	B ₆ ^(P)	B ₇ ^(P)	B ₈ ^(D)
Ownership								
Private individuals	+	+	+	+	-	+	-	+
agricultural cooperatives	-	-	-	-	+	-	+	-
Surface area (ha)								
<1	-	-	-	-	-	-	-	-
1-5	+	+	+	+	+	+	+	+
5-10	-	-	-	-	-	-	-	-
>10	-	-	-	-	-	-	-	-
Relief of the terrain								
Flat	+	-	+	+	+	-	+	+
Moderately sloping	-	+	-	-	-	+	-	-
Sloping	-	-	-	-	-	-	-	-
Planting and maintenance								
Banana	-	-	-	+	-	-	-	+
Plantain	+	+	+	-	+	+	+	-
Pure crops	-	-	-	-	+	-	+	-
Associated crops	+	+	+	+	-	+	-	+
Disposition in-line	-	-	-	-	+	+	+	-
Random disposition	+	+	+	+	-	-	-	+
Chemical fertilization	+	+	+	+	+	+	+	+
Application of pesticides	+	+	+	+	+	+	+	+
Managers age (years)								
<18	-	-	-	-	-	-	-	-
18-30	-	-	+	-	-	-	-	-
30-50	-	+	-	+	+	+	+	+
>50	+	-	-	-	-	-	-	-
Managers schooling	+	+	+	-	+	-	-	-
Primary school	-	+	-	-	-	-	-	-
Secondary education	-	-	+	-	-	-	-	-
High education	+	-	-	-	+	-	-	-
Objective of production								
Self-consumption	+	+	+	+	-	+	-	+
Sale	-	-	-	+	+	+	+	-
Managers' knowledge level of banana and plantain diseases								
<i>Fusarium</i> wilt	-	-	-	-	-	-	-	-
Diseases other than <i>Fusarium</i> wilt	-	-	-	+	+	-	-	-

B₁-B₈: Banana plantation 1-8, (P)/(D): Plantain/banana, + or -: Observed or unobserved characteristic for a banana plantation

banana diseases and 25% know black sigatoka. In all investigated banana plantations, *Fusarium* wilt is unknown by the producers. In the case of diseases suspicion, banana plants are suppressed (Table 1).

Fungal diversity of soil of investigated banana plantations

Global distribution of fungi in all investigated banana plantations: Microbiological analysis of different soil samples collected from banana plantations investigated in Loh-Djiboua region (Côte d'Ivoire), has permitted to assess soil mycoflora of these plantations (Table 2). Fungal species isolated from soil samples belong to ten genera which are *Fusarium*, *Aspergillus*,

Trichoderma, *Penicillium*, *Eupenicillium*, *Rhizopus*, *Paecilomyces*, *Chrysonilia*, *Syncephalastrum* and *Phoma*. A predominance has been observed for the genus *Trichoderma* (100% of banana plantations) followed by *Fusarium* (75%) and *Aspergillus* (62.5%). Concerning the species, the presence of *Fusarium oxysporum* has been recorded in more plantations (75%). It's followed by *Trichoderma hamatum* (62.5%), *T. viride* (50%), *T. pseudokoningii* (50%) and *Aspergillus niger* (50%) which were found in more than half of banana plantations (Fig. 3). The phytopathogenic fungus Foc TR4 is found in 5 banana plantations (Table 3) among these 6 harboring *F. oxysporum* (Table 2).

Table 2: Diversity of fungi species of the investigated banana plantations

Fungi (species)	Banana plantations							
	Lakota				Divo			
	B ₁ ^(P)	B ₂ ^(P)	B ₃ ^(P)	B ₄ ^(D)	B ₅ ^(P)	B ₆ ^(P)	B ₇ ^(P)	B ₈ ^(D)
<i>Fusarium oxysporum</i>	+	+	-	+	-	+	+	+
<i>Aspergillus parasiticus</i>	+	-	-	-	+	-	-	-
<i>Aspergillus niger</i>	+	+	-	-	+	-	+	-
<i>Aspergillus versicolor</i>	-	+	-	-	-	-	-	-
<i>Aspergillus terreus</i>	-	+	-	-	-	-	+	-
<i>Aspergillus niveus</i>	-	-	-	-	+	-	-	-
<i>Trichoderma hamatum</i>	+	+	+	+	+	-	-	-
<i>Trichoderma viride</i>	+	-	+	-	-	-	+	+
<i>Trichoderma pseudokoningii</i>	-	+	-	-	-	+	+	+
<i>Rhizopus oryzae</i>	-	-	-	+	+	-	-	-
<i>Eupenicillium shearii</i>	-	-	-	+	-	-	-	-
<i>Penicillium</i> sp.	-	-	+	+	-	-	+	-
<i>Paecilomyces</i> sp.	-	-	+	-	-	-	-	-
<i>Chrysonilia</i> sp.	-	-	-	+	-	-	-	-
<i>Syncephalastrum</i> sp.	+	-	-	-	-	-	-	-
<i>Phoma</i> sp.	-	-	-	-	-	-	-	+
Number of contaminating species	6	6	4	6	5	2	6	4
Occurrence rate of categories of fungi (%)								
Molds at the fields	66.66	50	25	57.14	28.57	100	50	100
Conservation molds	33.33	50	75	42.87	71.43	0	50	0
Rate of toxinogenic molds (%)	63.33	52.5	41.66	36.66	55	50	75	
41.66								

B₁-B₈: Banana plantation 1-8, (P)/(D): Plantain/dessert, + or -: Presence or absence of the fungus in a given banana plantation, respectively

Table 3: Contamination by Foc TR4 of the soil of investigated banana plantations

Soil samples	Banana plantations							
	B ₁ ^(P)	B ₂ ^(P)	B ₃ ^(P)	B ₄ ^(D)	B ₅ ^(P)	B ₆ ^(P)	B ₇ ^(P)	B ₈ ^(D)
S 2	+	+	-	-	-	+	-	+
S 29	+	-	-	+	-	+	-	+

B₁-B₈: Banana plantation 1-8, (P)/(D): Type plantain/type dessert, +/-: Presence/absence of Foc TR4 in a given banana plantation

Table 4: Categorization of fungi isolated from the investigated banana plantations

Categories	Isolated fungi	Indicatives references
Molds at the field		
Phytopathogens	<i>Fusarium oxyporum</i> *	Ellis <i>et al.</i> ¹⁷
Endophytes	<i>Trichoderma viride</i>	Bezerra <i>et al.</i> ¹⁸
	<i>T. hamatum</i>	Mulaw <i>et al.</i> ¹⁹
	<i>T. pseudokoningii</i>	Mulaw <i>et al.</i> ¹⁹
	<i>Syncephalastrum</i> sp.	Huang <i>et al.</i> ²⁰
	<i>Fusarium oxyporum</i> *	Bezerra <i>et al.</i> ¹⁸
	<i>Paecilomyces</i> sp.	Esfahani and Pour ²¹
	<i>Eupenicillium shearii</i>	Hoff <i>et al.</i> ²²
	<i>Chrysonilia</i> sp.	Bezerra <i>et al.</i> ¹⁸
	<i>Phoma</i> sp.	Kedar <i>et al.</i> ²³
Conservation molds	<i>Aspergillus parasitus</i> *	Njobeh <i>et al.</i> ²⁴
	<i>A. niger</i>	Misra <i>et al.</i> ²⁵
	<i>A. versicolor</i> *	Misra <i>et al.</i> ²⁵
	<i>A. terreus</i> *	Misra <i>et al.</i> ²⁵
	<i>Aspergillus</i> sp.*	Njobeh <i>et al.</i> ²⁴
	<i>Rhizopus oryzae</i>	Ghosh and Ray ²⁶
	<i>Penicillium</i> sp.*	Njobeh <i>et al.</i> ²⁴

*Toxinogenic molds

Specific distribution of fungi in each banana plantations:

The specific distribution of fungi in each banana plantations is presented in Table 2. Mycoflora identified in soil samples of the investigated banana plantation is heterogeneous from a plantation to another. No species of the genus *Aspergillus*, *Paecilomyces* and *Syncephalastrum* have been isolated in banana plantations (dessert type). The diverse fungal species found in each plantation may be grouped in different categories of fungi such as molds at the field and conservation molds (Table 4). The same occurrence level of molds at the field and conservation molds is observed in plantations B₂, B₄ and B₇. Likewise, in the locality of Lakota, the number of fungi belonging to each category is identical (49.70% for molds at the field and 50.30% for conservation molds). A difference is however observed in the locality of Divo with 69.64% for molds at the field and 30.36% for conservation molds (Fig. 4). Furthermore, this mycoflora isolated from soil of investigated banana plantations contains toxinogenic species belonging to

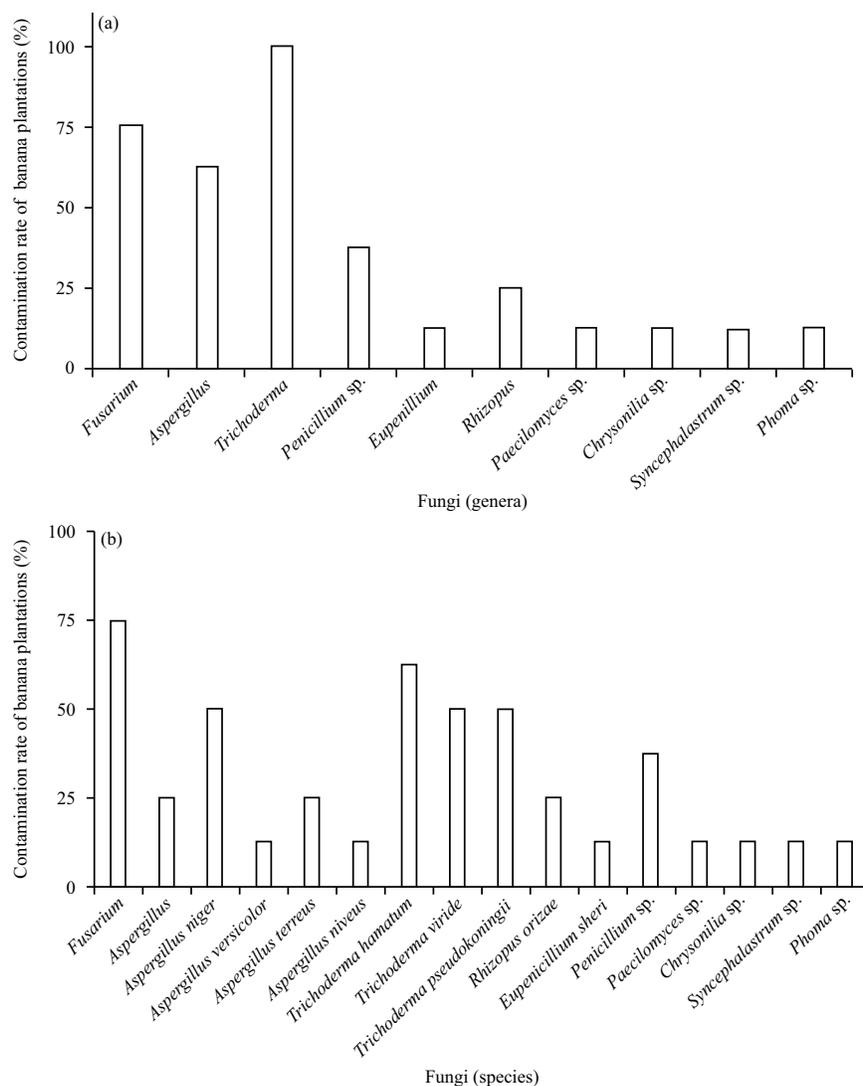


Fig. 3(a-b): Contamination rate of banana plantations as a function of fungi, (a) Genera and (b) Species

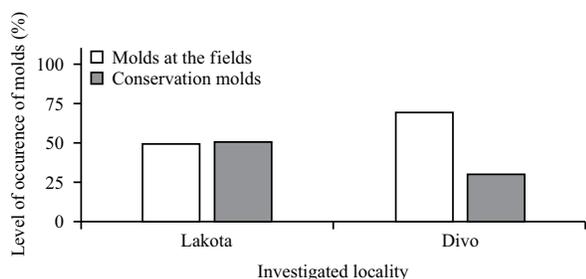


Fig. 4: Categories of isolated molds as a function of locality

the genera *Fusarium*, *Aspergillus* and *Penicillium*. Among these toxinogenic species, a predominance of *Aspergillus* sp. (section *Flavi*, *Terrei* and *Versicolores*) has been observed in all plantations.

DISCUSSION

Banana production in the Côte d'Ivoire has a very important economic and nutritional character, its development requires a better knowledge of the characteristics of banana farms. In this context, an inventory of cultural practices and fungal diversity was carried out in banana plantations in the localities of Divo and Lakota (Côte d'Ivoire).

The banana plantations investigated in the localities of Divo and Lakota generally belonging to the autochthon private individuals are created on flat or moderately sloping terrains. These terrains are acquired by inheritance (homesteads) and therefore, represent most often the unique agricultural plots to exploit for the farmers. Banana plantations

are small to medium size, with areas ranging between 1 and 5 ha. These observations comply with those of Traore *et al.*²⁷, focusing on the cropping system of plantain in farmer fields in the Departments of Aboisso, Agboville, Bouafle, Gagnoa and San-Pedro (Côte d'Ivoire). The cropping system of banana in an association and in random arrangement of banana plants, observed in 75% of farms shows the secondary character of this crop. Under this system (association crops or intercropping), banana plants represent shade plants for perennial crops (cocoa, coffee). The disposition in-line of banana plants otherwise applied in 25% of the banana plantations and also in all the banana plantations with the system of pure crops, is part of a banana plantation whose the objective of production is mainly the sale. The work material is not reserved only for a specific banana farm, would be an artificial means of dissemination of phytopathogenic microorganisms between plantations. This is the case of the fungi *Fusarium oxysporum* f. sp. *cubense* (responsible for *Fusarium* wilt of banana), which is scattered through the soil adhering to farm implements²⁸. The knowledge level of the farmers about the banana disease is variable. Seventy-five percent (75%) of the farmers don't know banana pathologies, because of their low literacy or lack of support by the specialized structures in banana cropping. Farmers (25%) having a partial knowledge of banana diseases don't know *Fusarium* wilt of banana due to plantain type which is widely grown in the surveyed area. Indeed, plantain would be more prone to threats other than *Fusarium* wilt. Thus, these farmers are educated about common threats such as leaf spot diseases, nematodes (*Radopholus similis*) and weevils (*Cosmopolites sordidus*).

Mycological analysis of soil samples from investigated banana plantations, reveals a diversity of fungal agents in the soil of these plantations. The isolated fungi belong to ten genera (*Aspergillus*, *Chrysonilia*, *Eupenicillium*, *Fusarium*, *Paecilomyces*, *Penicillium*, *Phoma*, *Rhizopus*, *Syncephalastrum* and *Trichoderma*) which constitute common soil saprophytes^{12,29,30}. Among these ten genera, the predominance observed for *Trichoderma* (100% of banana plantations), *Fusarium* (75%) and *Aspergillus* (62.5%) is related to their ubiquitous nature, their antagonist and dominating activity on others fungi¹⁹. The fungi *Trichoderma* can colonize different ecological niches because of various metabolites (cellulase, endo-polygalacturonase, chitinases, glucanases) they secrete^{31,32}. Its presence in banana plantations has advantages owing to its ability to induce in plant systemic resistance to the phytopathogenic microorganisms and abiotic stress³³. *Fusarium oxysporum* has been isolated from the soil of most farms (75%). Crops associated with banana plants in the plantations investigated are hosts and secondary

reservoirs for this fungus. Indeed, it is a fungal subspecies complex, with many of which are capable of infecting a hundred plants very important at the economic and dietary level^{16,17,34}. The phytopathogenic fungus Foc TR4 is found in 5 farms (Table 3) among these 6 harboring *F. oxysporum* (Table 2). In these plantations with Foc TR4 (62.5%), the terrain is often moderately sloping and the used plant material is obtained from a parent, a friend or previous plantations. The presence of Foc TR4 in the soil of banana plantations would be linked to cropping as reported by Perez-Vicente *et al.*²⁸. This occurrence of Foc TR4 is a concern for the maintenance of food security in Côte d'Ivoire. Measures must be taken to prevent the propagation of Foc TR4 in other plantations not infected by this fungus. Representatives of the genera *Aspergillus*, *Penicillium* and *Rhizopus* are most often found in terrestrial habitats, where they are commonly isolated from the soil and plant products³⁵⁻³⁷. The presence of these fungi in investigated farms constitute a threat for fruits and vegetables. They are responsible of post-harvest rotting.^{38,39}. These micromycetes are also opportunistic pathogens and toxins (aflatoxins, citreoviridine, citrinin and palutin) producers^{40,41}. Some *Penicillium* sp. are capable to produce antibiotic substances and to act therefore as bio-control agents (case of *Penicillium restrictum*)⁴² and as bio-remediation agents^{43,44}. The genera *Eupenicillium*, *Paecilomyces*, *Chrysonilia*, *Syncephalastrum* and *Phoma* are part of the fungi less isolated from the soil of investigated banana plantations. This could be explained by potentially important uses of pesticides in banana plantations. Their presence in banana farms could be an advantage for crops practiced there. Indeed, these fungi are identified as biological control agents and endophytic fungi (Table 4)^{21,20,45,46}.

Mycoflora identified in soil samples is heterogeneous from a plantation to another. This could be due to the possible variation of the physicochemical characteristics of the soil (structure, aeration, temperature, water content, pH) between these banana plantations⁴⁷. This heterogeneous distribution of fungi indicates that the presence of these fungi in banana plantations might be correlated with cropping practices that are observed there. These isolated fungi can be categorized into molds at the field and conservation molds. Molds at the field carrying out their main activities in the fields contain endophytes and plant pathogens species. The conservation molds have their activity mainly focusing on the alteration of post-harvest products. They contaminate food products from agricultural plots during harvesting and other post-harvest processes (transport, drying, handling, storage) but their spores or possibly established hyphae come into the vegetative phase during storage^{48,49}.

The presence at identical rates of molds at the field and conservation molds, observed in the plantations B₂, B₄ and B₇ and all the banana plantations of Lakota could be explained by the existence of growth conditions (structure, aeration, temperature, Aw, pH) favorable to each category of fungi, in the soil of these farms. Farmers in Lakota observe in their plantations, a system of associated crops and a random disposition of banana plants. This cropping system creates a density of banana plants and relatively high humidity conditions. In contrast in Divo, the disposition in-line creates a less humid climate by comparison to that of Lakota. However, molds at the field, more adapted to colonize the plant tissues, would find these conditions acceptable for growth and are, therefore, more present than the conservation molds.

CONCLUSION

The examination of the cropping practices observed in the banana plantations investigated in Lakota and Divo showed that farmers grow generally banana plants in association with various industrial and/or subsistence crops. They observe the random disposition of banana plants and all use pesticides in their plantations. Fungi found in the soil of these banana plantations are abundant, 10 genera and 16 species. Fungal genus and species frequently found in banana farms are, respectively, *Trichoderma* (100% of plantations) and *Fusarium oxysporum* (75%). Foc TR4 is found in 50% of plantations. The cropping practices of farmers contribute to the appearance and spreading of banana diseases.

SIGNIFICANCE STATEMENTS

This study discovers some social and microbiological characteristics of some banana plantations located in the region of Loh-Djiboua (Côte d'Ivoire), which can be beneficial for the development of the banana sector in this region. This study will help the researchers to uncover the critical areas of banana diseases that many researchers were not able to explore. Thus, a new theory on the link of cropping practices in banana plantations on the appearance and propagation of banana diseases may contribute to reducing diseases in this crop.

ACKNOWLEDGMENT

This work was supported by the Ministère des Affaires Étrangères (France) and by the Ministry of Higher Education and Scientific Research (Côte d'Ivoire) through doctoral training fellowships.

REFERENCES

1. Dotto, J., A.O. Matemú and P.O. Ndakidemi, 2018. Potential of cooking bananas in addressing food security in East Africa. *Int. J. Biosci.*, 13: 278-294.
2. Lassoudière, A., 2007. *Banana Tree and its Cultivation*. Editions Quae, Paris, ISBN: 2759209571. Pages: 383.
3. Yao, A.K., D.M. Koffi, Z.B. Irié and S.L. Niamké, 2014. Conservation of unripe plantain banana (*Musa AAB*) by using polyethylene films with different thicknesses. *J. Anim. Plant Sci.*, 23: 3677-3690.
4. Kouakou, K.A.K., S. Coulibaly, L.O.A. Atchibri, G. Kouamé, and A. Meité, 2012. Comparative nutritional assessment of the fruits of three hybrid bananas (CRBP 39, FHIA 17 and FHIA 21) with those of *Orishéle* variety. *Tropicultura*, 30: 49-54.
5. Sangaré, A., E. Koffi, F. Akamou and C.A. Fall, 2009. National report on the state of phylogenetic resources for food and agriculture. FAO, Rome, pp: 65.
6. Perrin, A., 2015. Study of plantain sector in Côte d'Ivoire. RONGEAD, Lyon, France, pp: 66.
7. EL-DougDoug, K.A. and M.M. El-Shamy, 2011. Management of viral disease in banana using certified and virus tested plant material. *Afr. J. Microbiol. Res.*, 5: 5923-5932.
8. Kra, K.D., H.A. Diallo, K. Kobenan, D. Koné and Y.J. Kouadio, 2011. Diagnosis of *Fusarium* wilt on cultivars grand nain (*Musa AAA*) and corne 1 (*Musa AAB*) in the periphery of Abidjan District (Côte d'Ivoire). *Int. J. Bio. Chem. Sci.*, 5: 1501-1514.
9. UN (United Nations), 2015. World population prospects: The 2015 revision, key findings and advance tables. Department of Economic and Social Affairs, Population Division, Working Paper No. ESA/P/WP, 241, pp: 66.
10. Anonymous 1, 2016. Presentation of the region of Loh-Djiboua. General Direction of Decentralisation and local Development, pp: 8.
11. Warcup, J.H., 1950. The soil-plate method for isolation of fungi from soil. *Nature*, 166: 117-118.
12. Domsch, K.H., W. Gams and T.H. Andersen, 1980. *Compendium of Soil Fungi Volume 1*. Academic Press, London, Pages: 860.
13. Seguin, V., S. Gente, N. Heutte, P. Verite and V. Kientz-Bouchart *et al*, 2014. First report of mycophenolic acid production by *Eurotium repens* isolated from agricultural and indoor environments. *World Mycotoxin J.*, 7: 321-328.
14. Zhang, M.Z. and S. Zhang, 2011. An efficient DNA extraction method for polymerase chain reaction-based detection of *Mycobacterium avium* subspecies *paratuberculosis* in bovine fecal samples. *J. Vet. Diagn. Invest.*, 23: 41-48.
15. Del-Toro-Sánchez, C.L., S. Villaseñor-Alvarado, F. Zurita-Martínez, O. Castellanos-Hernández and A. Rodríguez-Sahagún *et al*, 2013. Optimization of DNA isolation and PCR protocol for analysis and evaluation of genetic diversity of the medicinal plant, *Anemopsis californica* using RAPD. *Acta. Biol. Hung.*, 64: 184-195.

16. Dita, M.A., C. Waalwijk, I.W. Buddenhagen, J.M.T. Souza and G.H.J. Kema, 2010. A molecular diagnostic for tropical race 4 of the banana *Fusarium* wilt pathogen. *Plant Pathol.*, 59: 348-357.
17. Ellis, M.L., D.R.C. Jimenez, L.F. Leandro and G.P. Munkvold, 2014. Genotypic and phenotypic characterization of fungi in the *Fusarium oxysporum* species complex from soybean roots. *Phytopathology*, 104: 1329-1339.
18. Bezerra, J.D.P., M.G.S. Santos, R.N. Barbosa, V.M. Svedese and D.M.M. Lima *et al.*, 2013. Fungal endophytes from cactus *Cereus jamacaru* in Brazilian tropical dry forest: A first study. *Symbiosis*, 60: 53-63.
19. Mulaw, T.B., I.S. Druzhinina, C.P. Kubicek and L. Atanasova, 2013. Novel endophytic *Trichoderma* spp. isolated from healthy *Coffea arabica* roots are capable of controlling coffee tracheomyces. *Diversity*, 5: 750-766.
20. Huang, W.K., J.H. Sun, J.K. Cui, G.F. Wang and L.A. Kong *et al.*, 2014. Efficacy evaluation of funguvs *Syncephalastrum racemosum* and nematicide avermectin against the root-knot nematode *Meloidogyne incognita* on cucumber. *PLoS ONE*, Vol. 9. 10.1371/journal.pone.0089717.
21. Esfahani, M.N. and B.A. Pour, 2006. The effects of *Paecilomyces lilacinus* on the pathogenesis of *Meloidogyne javanica* and tomato plant growth parameters. *Iran Agric. Res.*, 24: 67-76.
22. Hoff, J.A., N.B. Klopfenstein, G.I. McDonald, J.R. Tonn and M.S. Kim *et al.*, 2004. Fungal endophytes in woody roots of Douglas-fir (*Pseudotsuga menziesii*) and ponderosa pine (*Pinus ponderosa*). *For. Pathol.*, 34: 255-271.
23. Kedar, A., D. Rathod, A. Yadav, G. Agarkar and M. Rai, 2014. Endophytic *Phoma* sp. isolated from medicinal plants promote the growth of *Zea mays*. *Nusantara Biosci.*, 6: 132-139.
24. Njobeh, P.B., M.F. Dutton, S.H. Koch, A. Chuturgoon, S. Stoev and K. Seifert, 2009. Contamination with storage fungi of human food from Cameroon. *Int. J. Food Microbiol.*, 135: 193-198.
25. Misra, J.K., E.B. Gergon and T.W. Mew, 1995. Storage fungi and seed health of rice: A study in the Philippines. *Mycopathologia*, 131: 13-24.
26. Ghosh, B. and R.R. Ray, 2011. Current commercial perspective of *Rhizopus oryzae*: A review. *J. Applied Sci.*, 11: 2470-2486.
27. Traoré, S., K. Kobénan, K.S. Kouassi and G. Gnonhour, 2009. Cropping systems of plantain banana and pests control methods in farm area in Côte d'Ivoire. *J. Appl. Biosci.*, 9: 1094-1101.
28. Perez-Vicente, L., M.A. Dita and E.M. De La Parte, 2014. Technical manual prevention and diagnostic of fusarium wilt (Panama disease) of banana caused by *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (TR4). *FAO*, Rome, pp: 74.
29. Lamovšek, J., G. Urek and S. Trdan, 2013. Biological control of root-knot nematodes (*Meloidogyne* spp.): Microbes against the pests. *Acta Agric. Slovenica*, 101: 263-275.
30. Mukherjee, P.K., B.A. Horwitz, A. Herrera-Estrella, M. Schmoll and C.M. Kenerley, 2013. *Trichoderma* research in the genome era. *Annu. Rev. Phytopathol.*, 51: 105-129.
31. Schuster, A. and M. Schmoll, 2010. Biology and biotechnology of *Trichoderma*. *Applied Microbiol. Biotechnol.*, 87: 787-799.
32. Rai, D. and P. Mehra, 2015. A review on interactions of *Trichoderma* with plant and pathogens. *Res. J. Agric. For. Sci.*, 3: 20-23.
33. Shores, M., G.E. Harman and F. Mastouri, 2010. Induced systemic resistance and plant responses to fungal biocontrol agents. *Annu. Rev. Phytopathol.*, 48: 21-43.
34. Smith, S.N., 2007. An overview of ecological and habitat aspects in the genus *Fusarium* with special emphasis on the soil-borne pathogenic forms. *Plant Pathol. Bull.*, 16: 97-120.
35. Horn, B.W., 2006. Relationship between soil densities of *Aspergillus* species and colonization of wounded peanut seeds. *Can. J. Microbiol.*, 52: 951-960.
36. Sharma, R., 2012. Pathogenicity of *Aspergillus niger* in plants. *CIBTech J. Microbiol.*, 1: 47-51.
37. Dedi, J.K.Y., K.R. Allou and A. Otchoumou, 2015. Inventory of fungal flora and identification of different horizons of the profile in banana plantation of TADMAIT. *Int. J. Bio. Chem. Sci.*, 9: 1419-1430.
38. Bhat, R., R.V. Rai and A.A. Karim, 2010. Mycotoxins in food and feed: Present status and future concerns. *Comprehen. Rev. Food Sci. Food Safety*, 9: 57-81.
39. Dijksterhuis, J., J. Houbraken and R.A. Samson, 2013. 2 Fungal Spoilage of Crops and Food. In: *Agricultural Applications*, Kempken, F. (Ed.). Springer Science & Business Media, London, ISBN: 978-3-642-36821-9, pp: 35-56.
40. Samson, R.A., S.W. Peterson, J.C. Frisvad and J. Varga, 2011. New species in *Aspergillus* section *Terrei*. *Stud. Mycol.*, 69: 39-55.
41. Howell, C.R., 2002. Cotton seedling preemergence damping-off incited by *Rhizoctonia oryzae* and *Pythium* spp. and its biological control with *Trichoderma* spp. *Phytopathology*, 92: 177-180.
42. Steinberg, C., J. Laurent, V. Edel-Hermann, M. Barbezant and N. Sixt *et al.*, 2015. Adaptation of *Fusarium oxysporum* and *Fusarium dimerum* to the specific aquatic environment provided by the water systems of hospitals. *Water Res.*, 76: 53-65.
43. Gharaei-Fathabad, E., M.A. Tajick-Ghanbary and N. Shahrokhi, 2009. Antimicrobial properties of *Penicillium* species isolated from agricultural soils of Northern Iran. *Res. J. Toxins*, Vol. 6. 10.3923/rjt.2014.1.7.
44. Leitao, A.L., 2009. Potential of *Penicillium* species in the bioremediation field. *Int. J. Environ. Res. Public Health*, 6: 1393-1417.

45. Hussain, H., M. John, A. Al-Harrasi, A. Shah and Z. Hassan *et al*, 2015. Phytochemical investigation and antimicrobial activity of an endophytic fungus *Phoma* sp. J. King Saud Univ. Sci., 27: 92-95.
46. Li, G., S. Kusari, M. Lamsho"ft, A. Schu"ffler, H. Laatsch and M. Spiteller, 2014. Antibacterial secondary metabolites from an endophytic fungus, *Eupenicillium* sp. LG41 J. Nat. Prod., 77: 2335-2341.
47. Rohilla, S.K. and R.K. Salar, 2012. Isolation and characterization of various fungal strains from agricultural soil contaminated with pesticides. Res. J. Recent. Sci., 1: 297-303.
48. Uma, V. and E.G. Wesely, 2013. Seed borne fungi of rice from South Tamil Nadu. J. Acad. Indus. Res., 1: 612-614.
49. del Palacio, A., L. Bettucci and D. Pan, 2016. *Fusarium* and *Aspergillus* mycotoxins contaminating wheat silage for dairy cattle feeding in Uruguay. Braz. J. Microbiol., 47: 1000-1005.