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Identification of Fungus Flora Associated with *Lagenaria siceraria* (Molina) Standl in Côte d'Ivoire

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Abstract

Lagenaria siceraria (Molina) Standl is a cucurbit which seeds are consumed by people in rural and urban Africa. This plant is subjected to a strong parasitic and diseases pressure that reduces seeds production. Efficient fight against plant parasite, particularly fungus is a prerequisite for an improved productivity. This study was undertaken in five localities (Alepe, Bondoukou, Bongouanou, Divo and Korhogo) belonging to three agroecological areas of Côte d'Ivoire. The aim was to identify fungal genera infecting *L. siceraria* in order to design an efficient control measure. Leaf samples with necrosis and discoloration symptoms were collected throughout the localities and subsequently, fungus were isolated and identified in laboratory. From a total of 750 samples collected, 7 types of symptoms were distinguished. Fungal genera found in all of the localities were *Aspergillus, Botryosphaeria, Cochliobolus, Colletotrichum, Fusarium, Lasiodiplodia and Phoma*. Only *Pestalotiopsis* was specific to the locality of Divo. An ANOVA test performed on the data showed a significant difference between fungal genera in terms of isolation frequency. Principal components analysis revealed that fungus distribution in each locality was correlated with climatic factors.

Keywords: Côte d'Ivoire, climatic factors, fungal genera, *Lagenaria siceraria*, symptom

Introduction

Seeds of African cucurbits commonly called Egusi in Benin and Nigeria are wrongly called "pistachio" in Côte d'Ivoire and are used in human food (Fondio *et al*., 2000; Zoro Bi *et al*., 2003). In Côte d'Ivoire, surveys made in various departments, have allowed to identify five species of "pistachio": *Citrillus lanatus*, *Cucubita moschata*, *Cucumeropsis mannii*, *Curcumis melo* and *Lagenaria siceraria* (Zoro Bi *et al.*, 2003). Of all these species, *Lagenaria siceraria* is the most strong and has a higher capacity of seeds production (Achigan *et al*., 2006; Zoro Bi *et al*., 2006). "Pistachios" are important in the diet and socio-cultural life of many people.

In Côte d'Ivoire, they represent a significant source of income especially for women that occur in association with major food crops (ANADER, 2004). *Lagenaria siceraria*, the most abundant species on the market of Abidjan after *Citrillus lanatus*, is the subject of this study. Seeds of *L. siceraria* are sold at an average price of 1500 CFA francs per kilogram; that is one and half times the price of cocoa and two and half times that of coffee (Anonymous, 2011).

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Cultivated for food, this cucurbit is prized for its oilseeds and is consumed as soup thickener. Seeds are good source of lipids and proteins (Loukou *et al*., 2007; Augem *et al*., 2011; Enzoga-Yoca *et al.*, 2011). Soup of *Lagenaria siceraria* is valued in sub-saharan african traditional societies. In Côte d'Ivoire, this soup is highly valued by the Akan during rejoicing times such as new year, births and wedding ceremonies (Zoro Bi *et al*., 2003).

In spite of their nutritional and socio-economic importance, african oil cucurbits remains a minor culture. In plantation, they are subjected to a high parasitic pressure that reduces seeds production from 40 to 70% (Fondio *et al.*, 2000; Vodouhe *et al.*, 2000). Among the pathogens, fungi cause various symptoms on cucurbits in plantations. Several previous studies have allowed the identification of fungi on Cucurbitaceae.

Thus, the most devastating fungal diseases are downy mildew (*Pseudoperonospora cubensis*) in Korea (Choi and Shin, 2006), powdery mildew (*Podosphaera xanthii*) in the United States (Kousik *et al*., 2007) and *Fusarium* wilt (*Fusarium oxysporum*) in Italy (Trionfetti Nisini *et al*., 2002).

In Côte d'Ivoire, few works on fungal diseases of *L. siceraria* have been conducted. The preliminary study conducted in 2008 at the University Nangui Abrogoua on leaves and seeds of *L. siceraria* revealed the presence of plants severely attacked by fungi. Accurate knowledge on these fungi would allow better control methods that would contribute to the improvement of *L. siceraria* productivity.

The objectives of this study are on one hand to isolate and identify fungal genera associated with leaves symptoms of *L. siceraria* from three agroecological areas in Côte d'Ivoire and on other hand to make a correlation between fungal genera isolation frequencies and climatic factors.

Study site

One step in this study involved the completion of surveys to collect samples. Surveys were carried out from 2008 to 2009 in various locations in three areas of Côte d'Ivoire (East, North and South). These areas were chosen in three agroecological regions in the main producing areas of *L. siceraria*. Thirty plantations of *L. siceraria* distributed in localities of Alepe, Divo (South) Bondoukou, Bongouanou (East) and Korhogo (North) were selected to conduct the investigations. The climate data used in this study are air moisture, rainfall, temperature and wind speed.

These data provided by SODEXAM (Society Development and Operations Airports, Aviation and Meteorology) in Abidjan, have been supplemented by data collected on www.tutiempo.net site. The geographic coordinates and agroecological characteristics of localities are as follows (Avit *et al.*, 1999; Brou, 2005).

The eastern area takes into account regions of Moronou (Bongouanou) and Gontougo (Bondoukou). It is localized between latitudes 6°00 N-8°1 N and longitudes 3°00 W-5°00 W. The climate is equatorial, Baouléen or transitional regime mitigated with two seasons interspersed with two dry rains seasons. Rainfalls vary from 1100 to 1600 mm. Annual mean temperature is between 25 and 28°C. Air moisture varies between 71.48 and 76.54% and the wind speed from 1.5 to 2.73 km/h. This area is characterized by the transitional woodland savannas, with several blocks of semi- deciduous forests.

The northern area takes into account the region of Poro (Korhogo). It is localized between latitude 9°26'N and longitude 6°38 W. The climate is tropical Sudanese or transitional regime with two seasons (dry, wet). The dry season, from November to March comes before the rainy season. Rainy season is marked by two maxima rainfall, one in June and other in September. Annual mean rainfall varies from 1000 to 1400 mm. Temperatures are high and vary between 28 and 32°C. Air moisture is 67.02% and the wind speed of 7.1 km/h. The vegetation consists of woodland savannas with extended ranges of herbaceous areas.

The southern area covers parts of Lôh-djiboua (Divo) and Massan (Alepe). It is localized between latitudes 5°00 N-6°00 N and longitudes 4°00 W-7°00 W. The climate is sub-equatorial, Guinea with two dry seasons and two rainy seasons. In this area, rainfalls are abundant (annual mean reach of 1500 to 2400 mm) and annual temperature is almost constant around 25.5 at 27°C. Air moisture varies between 78.11 and 89.48% and the wind speed from 2.8 to 10.14 km/h. Vegetation is mainly represented by the tropical rain forest, with mangrove on the coastal side.

Materials and methods

Plant material

The experiment was conducted using infected leaves of *L. siceraria* of 2-3 months-old. These infected leaves were come from five surveyed localities and have presented different fungal diseases symptoms.

Methods

Sampling and data collection

Symptoms of fungi infections were observed and described for the form, size and the color. Infected leaves of *L. siceraria* with symptoms were collected on thirty (30) randomly selected plantations: ten in Korhogo and five in each of the four localities. In each plantation, five leaves with the same type of symptoms were collected and placed on blotting paper, then in polyethylene plastic bag and brought to the laboratory. A total of seven hundred and fifty (750) leaves were collected to conduct the fungal identification study.

Disinfection and purification

Fragments of 2 cm long of infected leaves of *L. siceraria* were cut off the margin of lesions. These leaves explants were disinfected in 10 % sodium hypochlorite for 3 min, twice rinsed with sterile distilled water and air-dried. Dried leaves fragments were placed on Potato Dextrose Agar (PDA) then incubated at room temperature (25 ± 2^{o}C) for three days. The cultures were observed daily to prevent any mycelial colonies are confluent in the culture dishes. Growing mycelial tips of fungal strains were transferred to new PDA plates to obtain pure cultures. Different fungal colonies grown on different samples were systematically planted separately on PDA plate in three Petrie dish of sterile cultures strain to obtain individualized cultures. The experiment was twice repeated.

Characterization and identification of fungal isolates

Macroscopic descriptions of obtained fungal isolates were observed under a phase-contrast photonic microscope (ZEISS) to 40 X magnification. Identification of fungi was done using identification keys of Barnett and Hunter (1972) and Botton *et al.* (1990) after two weeks of culture on PDA plate. The identified fungi have been described. The observations focused on characteristics such as color and form of the spores, the partitioning and branching or not of mycelium. Isolates were then grouped into different fungal genera.

Isolation frequency of fungal isolates

The number of fungal genera isolation on different samples and the total number of isolates were evaluated. The isolation frequency of identified fungi was determined by genera according to Walder (1996):

$$FI\ (\%) = NI/NTI \times 100$$

FI (%): Isolation frequency
NI: Number of isolates belonging to a fungal genus
NTI: Total number of isolates of all strains belonging to a fungal genus

Relations between isolation frequencies of fungal genera and climatic factors

The occurrence of fungal genera in each locality has been linked with air moisture, rainfall, temperature and wind speed in order to clarify the existing correlations between fungal populations and climatic factors.

Statistical analyzes of data

Averages of fungi isolation frequency were compared. The results were statistically analyzed using the software Statistica 7.1 through analysis of variance (ANOVA). Whenever a significant difference was found, ANOVA is complemented by Fisher's LSD test, which allows identify the variables significantly different of others. The averages of the variables were separated at the probability threshold $P < 0.05$.

Principal Components Analysis (PCA) with Statistica 7.1 software package was applied to the frequencies of fungi and data of climatic factors. From the matrices of the original variables, we extracted a limited number of correlated combinations. The projection of individuals on the planes defined by the axes of the Principal Components was used to assess their dispersion and variability.

To admit that the variability is sufficiently expressed, the cumulative sum of the contributions of the main areas identified should be around 70% (Thomassone *et al*., 1993). Correlations test between variables were performed using the software Tanagra. Correlation measures the relationship between two or more variables. The correlation coefficient used is the Pearson r, also called linear correlation coefficient. The correlation coefficients were in the range from -1.00 to 1.00. Values -1.00 and +1.00 respectively represent a perfect negative or positive correlation and value of 0.00 represents a lack of correlation or independence between variables.

Results

Foliar symptoms observed on *Lagenaria siceraria*

Fungus attack symptoms observed on *L siceraria* leaves are of various forms, sizes and coloration. Seven types of symptom were distinguished. Three out of the seven were observed regardless the localities. These types are described as follows: small circular brown spots with yellow halation of approximately 1 cm in diameter (**Figure 1A**), brown coalescent spots of approximately 2 cm in diameter of which some are detached (**Figure 1B**) and large brown spots (**Figure 1C)**.

Two types of symptom were observed in Korhogo (North), Bondoukou and Bongouanou (East): Brown spots extended symmetrically to the main vein to the petiole on the upper surface of leaves (**Figure 1D**) and more or less circular brown spots that scatter thereafter (**Figure 1E**). The sixth type of symptom was observed in Alepe and Divo, and in Bondoukou and Korhogo; it is consisted of black flocculants spots located at the two sides of leaves (**Figure 1F**). The seventh type in Alepe, Bondoukou and Korhogo and consisted of numerous small white and powdery spots on the two sides of leaves (**Figure 1G**).\

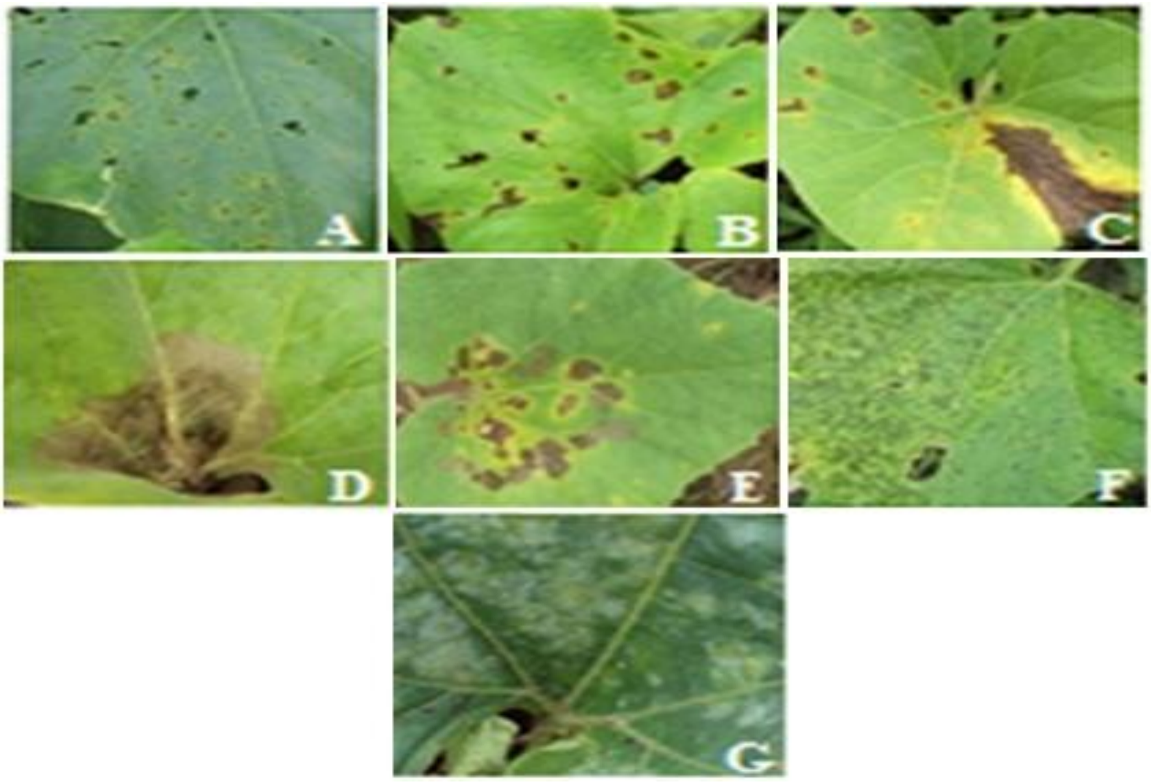

Figure 1: Fungus attacks **s**ymptoms on *L. siceraria* leaves: **A**: small circular brown spots with yellow halation of approximately 1 cm in diameter; **B**: brown coalescent spots of approximately 2 cm in diameter of which some are detached; **C**: large brown spots; **D**: Brown spots extended symmetrically to the main vein to the petiole on the upper surface of leaves; **E** : more or less circular brown spots that scatter thereafter; **F**: black flocculants spots located at the two sides of leaves; **G**: small white and powdery spots on the two sides of leaves.

Fungal genera isolated from leaves of *Lagenaria siceraria*

The fungus identification was done based on their macro and microscopic characteristics and revealed 17 genera associated with *L. siceraria* leaves. These are the following: *Aspergillus*, *Botryosphaeria*, *Curvularia*, *Cochliobolus*, *Colletotrichum*, *Diaporthe*, *Fusarium*, *Lasiodiplodia*, *Penicillium*, *Peronospora*, *Pestalotiopsis*, *Phoma*, *Phomopsis*, *Podosphaera*, *Pythium*, *Rhizoctonia* et *Trichoderma* (**Figure 2**).

Seven of theses genuses were encountered in all of the localities of this study. These are *Colletotrichum*, *Fusarium* and *Lasiodiplodia* isolated from the symptom types A, B, C, D and E; *Aspergillus* and *Botryosphaeria* isolated A, B, C and D, *Phoma* isolated from the symptom types A, C et D and *Cochliobolus* isolated from the symptom types B, C and D. *Curvularia* was isolated from the symptom types A, B and E, *Diaporthe* from the types A, B and D, *Podosphaera* from the type G, *Peronospora* from the type F, *Penicillium* from the types A, B and C, *Pestalotiopsis* from the types A and C, *Phomopsis* from the types A and B, *Pythium* from the types A, D and E, *Rhizoctonia* from the types A, C and E, and *Trichoderma* from the symptom types B and F (**Table 1**).

Thus, a total of 14, 13, 12, 10, and 8 fungal genera were observed in Alepe, Korhogo, Divo, Bondoukou and Bongouanou, respectively.

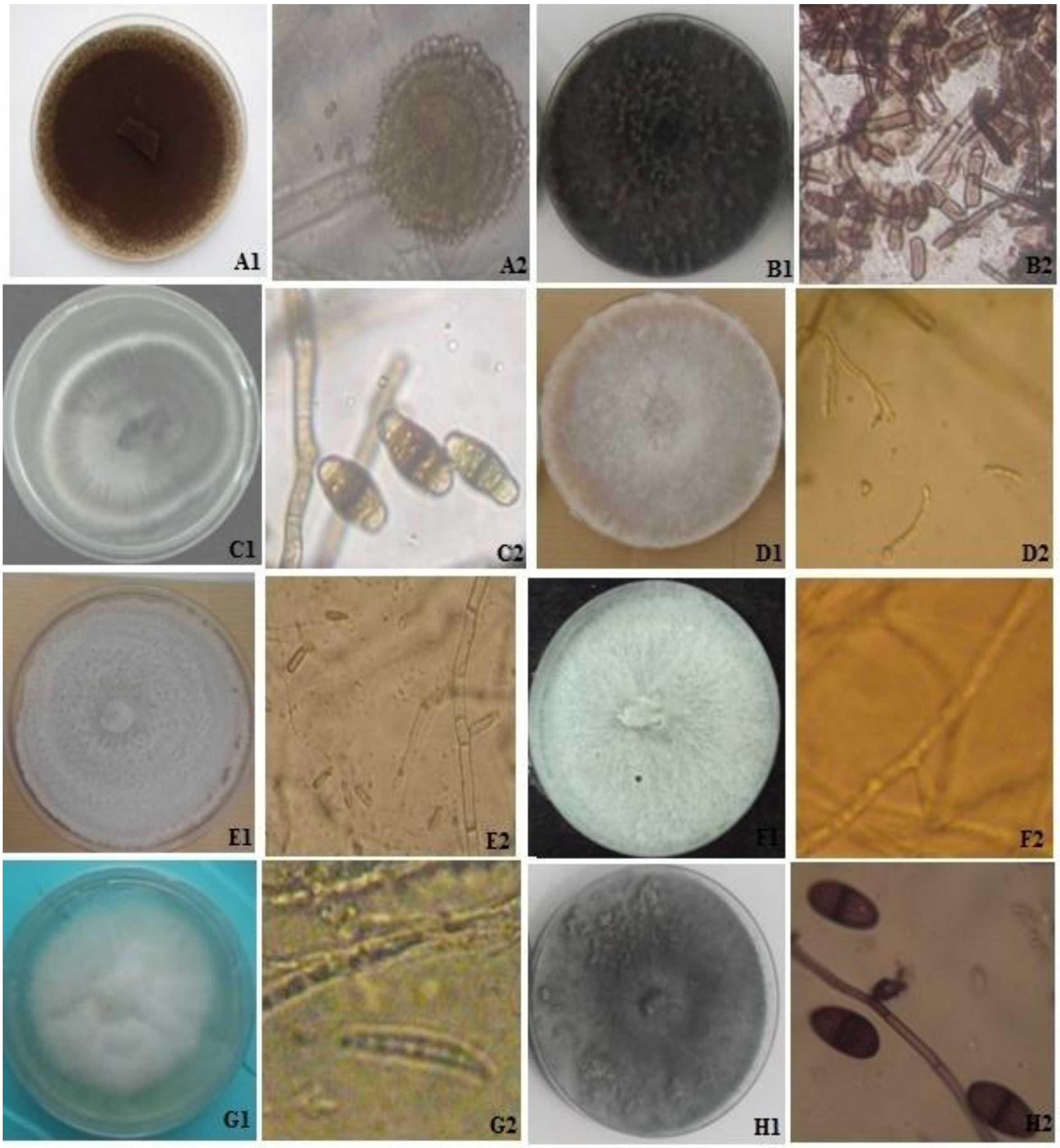

Figure 2: Macroscopic (A1, B1, C1, D1, E1, F1, G1 et H1) and microscopic appearance (A2, B2, C2, D2, E2, F2, G2 et H2) appearance of fungi isolated on Potato Dextrose Agar medium from disease symptoms of *Lagenaria siceraria* leaves.

A1. Brown mycelia colonies of *Aspergillus* sp., **A2.** Biseriate head spore of *Aspergillus* sp. with rounded conidia; **B1**. Mycelia colonies of *Botryosphaeria* sp., **B2**. Conidia of *Botryosphaeria*.; **C1**. Mycelia colonies of *Curvularia* sp., **C2**. Solitary conidia of *Curvularia* sp.; **D1**. Mycelia colonies of *Cochliobolus* sp., **D2.** Conidia of *Cochliobolus* sp.; **E1.** Mycelia colonies of *Colletotrichum* sp., **E2.** Conidia of *Colletotrichum* sp.; **F1.** Mycelia colonies of *Diaporthe* sp., **F2.** Conidia of *Diaporthe* sp.; **G1**. Mycelia colonies of *Fusarium* sp., **G2.** Conidia of *Fusarium* sp.; **H1.** Mycelia colonies of *Lasiodiplodia* sp., **H2.** Conidia of *Lasiodiplodia* sp.

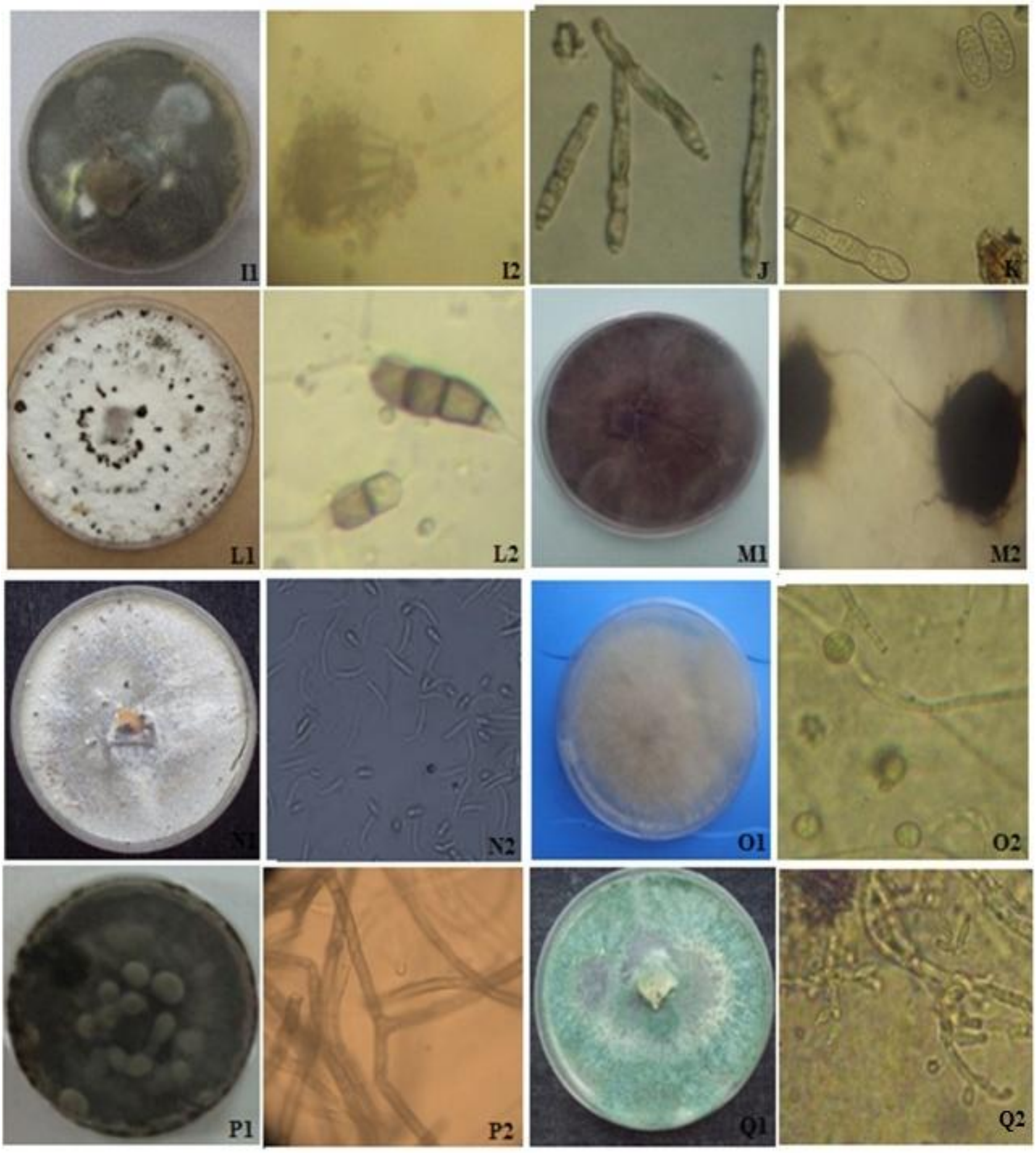

Figure 2: (continuation): Macroscopic (I1, L1, M1, N1, O1, P1 et Q1) and microscopic appearance (I2, J, K, L2, M2, N2, O2, P2 et Q2) appearance of fungi isolated on Potato Dextrose Agar medium from disease symptoms of *Lagenaria siceraria* leaves.

I1. Mycelia colonies of *Penicillium* sp., **I2**. Conidia of *Penicillium* sp ; **J.** Conidia of *Peronospora* sp., **K** Conidia of *Podosphaera* sp. **L1**. Mycelia colonies of *Pestalotiopsis* sp., **L2.** partitioned conidia of *Pestalotiopsis* sp; **M1** Mycelia colonies of *Phoma* sp., **M2.** Pycnidia of *Phoma* sp., **N1.** Mycelia colonies of *Phomopsis* sp., **N2**. Conidia of *Phomopsis* sp., **O1.** Mycelia colonies of *Pythium* sp., **O2**. Oospore of *Pythium* sp**.**, **P1**. Mycelia colonies of *Rhizoctonia* sp., **P2**. Brown and septate mycelia with a right angle of *Rhizoctonia* sp**.**, **Q1**. Mycelia colonies of *Trichoderma* sp., **Q2**. Conidia de *Trichoderma* sp.

Table 1: List of fungal genera encountered on *Lagenaria siceraria* leaves collected in the five localities. SA: Type A symptom; SB: Type B symptom; SC: Type C symptom (to be continued)

		Surveyed localities				
		East		North	South	
	Symptom types	**Bondoukou**	**Bongouanou**	**Korhogo**	**Alepe**	**Divo**
Fungal genera	SA	*Colletotrichum* *Fusarium* *Pythium* *Phoma*	*Botryosphaeria* *Diaporthe* *Fusarium* *Phoma*	*Aspergillus* *Botryosphaeria* *Colletotrichum* *Lasiodiplodia* *Phoma* *Phomopsis*	*Aspergillus* *Botryosphaeria* *Colletotrichum* *Curvularia* *Fusarium* *Penicillium* *Phomopsis*	*Botryosphaeria* *Colletotrichum* *Diaporthe* *Fusarium* *Pestalotiopsis* *Lasiodiplodia*
	SB	*Botryosphaeria* *Colletotrichum* *Fusarium*	*Aspergillus* *Cochliobolus* *Colletotrichum* *Diaporthe*	*Aspergillus* *Colletotrichum* *Curvularia* *Fusarium* *Phomopsis*	*Botryosphaeria* *Cochliobolus* *Colletotrichum* *Lasiodiplodia* *Phomopsis* *Rhizoctonia*	*Cochliobolus* *Colletotrichum* *Diaporthe* *Penicillium* *Lasiodiplodia* *Trichoderma*
	SC	*Aspergillus* *Colletotrichum* *Lasiodiplodia* *Phoma*	*Botryosphaeria* *Colletotrichum* *Fusarium* *Lasiodiplodia*	*Cochliobolus* *Colletotrichum* *Fusarium* *Phoma* *Rhizoctonia*	*Botryosphaeria* *Colletotrichum* *Fusarium* *Lasiodiplodia* *Phoma*	*Botryosphaeria* *Colletotrichum* *Penicillium* *Pestalotiopsis* *Phoma*
	SD	*Cochliobolus* *Colletotrichum* *Fusarium*	*Aspergillus* *Diaporthe* *Pythium*	*Botryosphaeria* *Colletotrichum* *Fusarium* *Lasiodiplodia* *Phoma*		
	SE	*Colletotrichum* *Fusarium* *Lasiodiplodia*	*Colletotrichum* *Fusarium*	*Colletotrichum* *Curvularia* *Fusarium* *Pythium* *Rhizoctonia*		
	SF	*Peronospora*		*Peronospora*	*Peronospora*	*Peronospora*
	SG	*Podosphaera*		*Podosphaera*	*Podosphaera*	

Occurrence of the fungal genera isolated from *Lagenaria siceraria* leaves

Significant difference was observed between the occurrences of the 17 fungus genera (P ≤ 0.001) on *L. siceraria* leaves. The occurrence showed significant variation between localities and within localities as well. Between locality variation was observed for 14 genera: *Botryosphaeria*, *Curvularia*, *Cochliobolus*, *Diaporthe*, *Lasiodiplodia*, *Penicillium*, *Peronospora*, *Pestalotiopsis*, *Phoma*, *Phomopsis*, *Podosphaera*, *Pythium*, *Rhizoctonia* et *Trichoderma* and not for *Aspergillus*, *Colletotrichum* and *Fusarium* (**Table 2**). However, the occurrence of all the 17 genera varied within the localities. They varied from 3.23 % to 6.45 % in Alepe, 3.33 % to 9.33 % in Bondoukou, 3.85 % to 7.69 % in Bongouanou, 3.85 % to 5.38 % in Divo and 1.89 % to 7.92 % in Korhogo. Ranking the occurrences revealed 3 groups of genera in Bondoukou and Divo, 4 groups in Alepe, five groups in Bongouanou and 6 groups in Korhogo (**Table 2**).

Colletotrichum was the most isolated in Bondoukou, Bongouanou and Korhogo with respective frequencies of 9.33 %, 7.69 % and 7.92 %. In Alepe, the most isolated fungi were *Cochliobolus* and *phoma* with respectively 5.81 % and 6.45 %. With both, 5.38 % of frequency *Botryosphaeria* and *Fusarium* were more isolated in Divo

Table 2: Mean frequency of the fungal genera isolation in each of the five localities

	Surveyed localities							
	East		North		South		Statistics	
Fungal genera	**Bondoukou**	**Bongouanou**	**Korhogo**	**Alepe**	**Divo**	**Averages**	**F**	**P**
Aspergillus	3.33±0 c	3.85±0 **d**	3.77±0 **bc**	3.23±0**c**	3.85±0 **b**	3.61±0	2.28	0.10
Botryosphaeria	6.67±0 **b**	5.38±2.11**bcd**	2.26±0.84 **cd**	3.87±1.44 **bc**	5.38±2.11 **a**	4.71±2.08	6.12	0.002
Curvularia	-	-	1.89±0 **d**	3.23±0**c**	-	1.02±0	185.86	<0.001
Cochliobolus	3.33±0 **c**	3.85±0 **d**	1.89±0 **d**	5.81±1.44 **a**	3.85±0 **b**	3.74±1.41	23.70	<0.001
Colletotrichum	9.33±3.65 **a**	7.69±3.85 **a**	7.92±3.63 **a**	4.52± 1.77 **b**	4.62±1.72 **ab**	6.82±3.43	2.44	0.08
Diaporthe	-	6.15±2.11 **abc**	-	-	3.85±0 **b**	2±0.42	129.41	<0.001
Fusarium	6±1.49 **b**	4.62±1.72 **cd**	4.15±3.10 **b**	4.52±1.77 **b**	5.38±2.11 **a**	4.93±2.05	0.62	0.65
Lasiodiplodia	5.33±1.83 **b**	3.85±0 **d**	1.89±0 **d**	3.23±0**c**	3.85±0 **b**	3.63±1.36	11.62	<0.001
Penicillium	-	-	-	3.23±0**c**	3.85±0 **b**	1.41±0	242.57	<0.001
Peronospora	5.33±1.83 **b**	-	1.89±0 **d**	3.23±0 **c**	3.85±0 **b**	2.86±0.36	38.60	<0.001
Pestalotiopsis	-	-	-	-	3.85±0 **b**	0.77±0	332.36	<0.001
Phoma	3.33±0 **c**	6.92±1.72 **ab**	3.02±1.03 **bcd**	6.45±0 **a**	3.85±0 **b**	4.71±1.86	20.84	<0.001
Phomopsis	-	-	3.77±0 **bc**	3.23 ± 0**c**	-	1.4±0	442.16	<0.001
Podosphaera	3.33±0 **c**	-	1.89±0 **d**	3.23±0 **c**	-	1.69±0	39.74	<0.001
Pythium	3.33±0 **c**	-	1.89±0 **d**	-	-	1.04±0	262.27	<0.001
Rhizoctonia	-	-	2.64±1.69 **bcd**	3.23±0 **c**	-	1.17±0.56	23.04	<0.001
Trichoderma	-	-	-	3.23±0 **c**	3.85±0 **b**	1.42±0	211.67	<0.001
F	32.71	24.13	12.06	26.55	29.34			
P	<0.001	<0.001	<0.001	<0.001	<0.001			

F: Fischer value, P: probability. In the same column, means with the same letter are not significantly different at the 0.05 level.

Relationships between occurrence of fungus genera and climatic factors

The occurrence of the fungus genera was correlated with climatic factors, namely air moisture, rainfall, temperature and the wind speed. Axes 1 and 2 explained 79.9 %, 81.5 % 87.5%, 88.2% and 78.1 % of the total variability in Alepe, Bondoukou, Bongouanou, Korhogo and Divo, respectively (**Table 3**).

For the locality of Alepe, 14 out of the 18 descriptors (variables) were significantly correlated with the two first axes. Therefore, these were the factors explaining variability in the distribution of fungus genera (**Table 3**). The axe 1 explained 59.5 % of variability and was linked to 11 traits as shown on the correlation circle (**figure 3A**).

These traits are isolation frequencies of *Aspergillus* (Asp), *Curvularia* (Curv) and *Colletotrichum* (Colle) which are positively correlated to the axe 1, *Cochliobolus* (Coch), *Lasiodiplodia* (Las), *Penicillium* (Pen), *Peronospora* (Per), *Phomopsis* (Pho), *Podosphaera* (Pod), *Rhizoctonia* (Rhi) and *Trichoderma* (Trich) which are negatively correlated to Axe 1 (|r|>70). *Fusarium* (Fus), rainfall (Pv) and temperature (Tm) are the parameters which correlated with Axe 2 (|r|>70) explaining 20.51 % of the total variability.

Isolation frequency of *Fusarium* (Fus) was positively correlated to Axe 2 while the rainfall and the temperature were negatively correlated to this axe. The correlation matrix (**Table 4**) revealed that *Fusarium* (r = -0.86%) is negatively correlated to temperature and *Phoma* (r = 0.88%) is positively to wind speed.

This suggests that the higher the temperature, the lower the spore dissemination by *Fusarium* and the higher the wind speed, the higher the spore dissemination by *Phoma*.

Table 3: Matrix of eigenvalues and correlations between variables and the two axes 1 and 2 after the PCA. The correlations used to interpret the factors are those above 0.7 (R2 ≥ 0.7)

	Surveyed localities									
	East				North		South			
	Bondoukou		Bongouanou		Korhogo		Alepe		Divo	
Parameters	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2
Eigenvalue	6,91	4,50	6,43	4,06	9,53	3,77	10,72	3,70	10,20	3,92
Percent of total variance	49,34	32,15	53,62	33,85	55,99	22,14	59,46	20,51	63,72	24,53
Percent of total cumulative variance	49,34	81,49	53,62	87,47	55,99	78,14	59,46	79,97	63,72	88,25
Aspergillus	-0,96	0,23	0,86	-0,50	-0,97	-0,23	0,99	-0,05	-0,99	-0,05
Botryosphaeria	0,43	0,62	0,86	-0,50	0,63	0,42	0,39	0,41	-0,15	-0,69
Curvularia	-	-	-	-	-0,31	0,30	0,99	-0,05	-	-
Cochliobolus	-0,96	0,23	0,86	-0,50	0,56	-0,64	-0,70	0,52	-0,99	-0,05
Colletotrichum	0,31	-0,73	-0,89	-0,18	-0,68	0,72	0,89	-0,09	0,81	-0,07
Diaporthe	-	-	0,98	-0,07	-	-	-	-	-0,99	-0,05
Fusarium	0,55	0,22	0,49	-0,86	-0,95	0,30	0,31	0,86	-0,51	0,74
Lasiodiplodia	-0,14	-0,91	0,86	-0,50	-0,97	-0,23	-0,99	0,05	-0,99	-0,05
Penicillium	-	-	-	-	-	-	-0,99	0,05	-0,99	-0,05
Peronospora	0,76	0,41	-	-	-0,97	-0,23	-0,99	0,05	-0,99	-0,05
Pestalotiopsis	-	-	-	-	-	-	-	-	-0,99	-0,05
Phoma	-0,96	0,23	0,72	-0,17	-0,84	-0,34	0,10	0,51	-0,99	-0,05
Phomopsis	-	-	-	-	-0,97	-0,24	-0,99	0,05	-	-
Podosphaera	-0,96	0,23	-	-	-0,97	-0,23	-0,99	0,05	-	-
Pythium	-0,96	0,23	-	-	-0,97	-0,23	-	-	-	-
Rhizoctonia	-	-	-	-	-0,61	-0,31	-0,99	0,05	-	-
Trichoderma	-	-	-	-	-	-	-0,99	0,05	-0,99	-0,05
Air moisture	-0,41	-0,79	-0,49	-0,86	0,55	-0,64	0,02	-0,64	0,15	-0,92
Rainfall	-0,41	-0,79	0,48	0,08	0,55	-0,64	-0,26	-0,80	0,15	-0,92
Temperature	-0,76	0,62	-0,49	-0,86	-0,13	0,75	0,06	-0,88	0,15	-0,92
Wind speed	-0,41	-0,79	-0,49	-0,86	-0,13	0,75	0,43	0,63	0,50	0,56

At Bondoukou, the two first axes explained 81.49% of the variability. The Axe 1 (F1) explained 49.34% while the Axe 2 explained (F2) 32.15% of variation (**Table 3**). The correlation circle (**Figure 3B**) showed that Axe 1 was negatively correlated with isolation frequencies of *Aspergillus* (Asp), *Cochliobolus* (Coch), *Peronospora* (Per), *Phoma* (Pha), *Podosphaera* (Pod), and *Pythium* (Pyth) and positively with the temperature (Tm). Axe 2 was negatively correlated to isolation frequencies of *Colletotrichum* (Colle), *Lasiodiplodia* (Las), air moisture (Hm), rainfall (Pv) and the wind speed (Vt). Five genera, *Aspergillus*, *Cochliobolus*, *Phoma*, *Podosphaera* and *Pythium* showed a great and positive (r= 0.87%) correlation with temperature (**Table 4**).

At Bongouanou, Axe 1 and Axe 2 contributed to 53.62% and 33.85% to the variability, i.e. a total of 87.47 % (**Table 3**). The correlation circle shows that Axe 1 is made up with *Aspergillus* (Asp), *Botryosphaeria* (Bot), *Cochliobolus* (Coch), *Lasiodiplodia* (Las), *Phoma* (Pha) *Diaporthe* (Dia) and *Colletotrichum* (Colle). *Colletotrichum* (Colle) is positively correlated to this axe while the other genera are negatively correlated. *Fusarium* (Fus), air moisture (Hm), temperature (Tm) and wind speed were those which correlated with Axe 2 (|r|>70) negatively (**Figure 3C**).

The correlation matrix (**Table 4**) shows that *Fusarium* was negatively correlated (-0.86 %) to air moisture, temperature and wind speed suggesting that *Fusarium* is fairly isolated when the factors are low. At Divo, 14 out the 16 descriptors were correlated to the two first axes. Axe 1 explained 63.72% of variability and was correlated to 10 genera (**Table 3**), negatively with isolation frequencies of *Aspergillus* (Asp), *Cochliobolus* (Coch), *Diaporthe* (Dia), *Lasiodiplodia* (Las), *Penicillium* (Pen), *Peronospora* (Per), *Pestalotiopsis* (Pes), *Phoma* (Pha), *Trichoderma* (Trich), and positively (|r|>70) with *Colletotrichum* (Colle). *Fusarium* (Fus), air moisture, rainfall (Pv) and temperature were the variables which correlated (|r|>70) with Axe 2 (24.53 % of variability). *Fusarium* (Fus) was positively correlated to this axe and the others, negatively. The Axe 2 can be defined as the axe of climate variability in Divo.

At Korhogo, the Axes 1 and 2 concentrated 55.99% and 22.14% of variability, i.e. at total of 78.13% (**Table 3**). The correlation circle (Figure 3E) shows that the Axe 1 (F1) is (negatively) correlated to isolation frequencies of *Aspergillus* (Asp), *Fusarium* (Fus), *Lasiodiplodia* (Las), *Peronospora* (Per), *Phoma* (Pha), *Phomopsis* (Pha), *Podosphaera* (Pod), *Pythium* (Pyth) the Axe 2 is positively correlated to isolation frequencies of *Colletotrichum* (Colle), temperature (Tm) and wind speed (VT).

It appears from the correlation matrix that isolation frequency of *Cochliobolus* was positively correlated to air moisture and rainfall while *Colletotrichum* isolation frequency was negatively correlated to these two factors (**Table 4**). This suggests that the higher the rainfall and the air moisture, the higher the isolation frequency of *Colletotrichum* (-0.82), and the lower the rainfall and air moisture, the lower higher the isolation frequency of *Cochliobolus* (0.99).

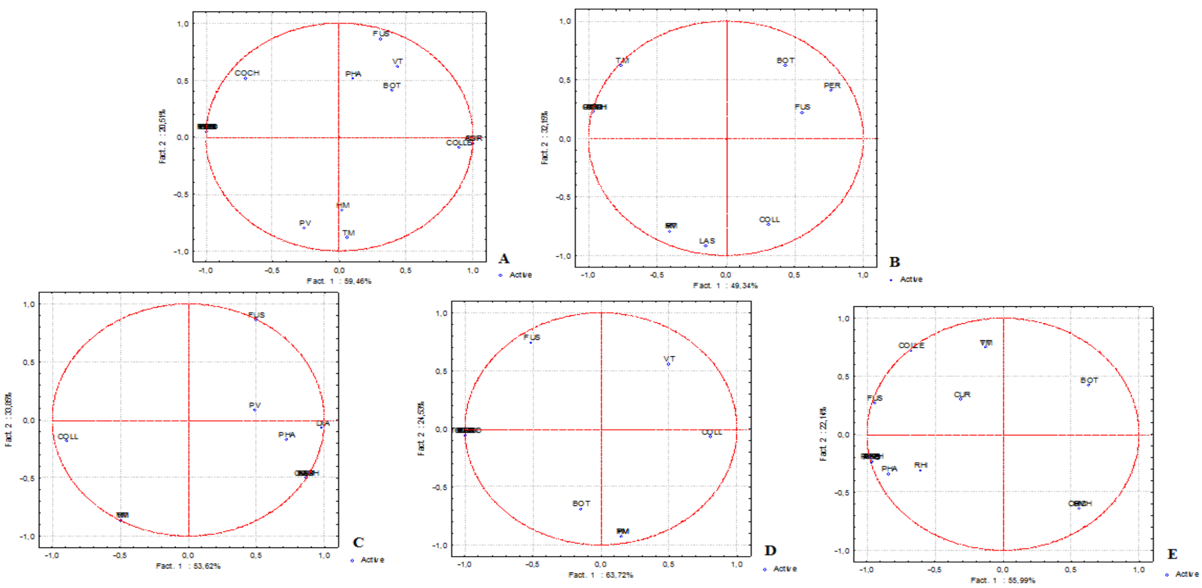

Figure 3: Distribution of variables in the factoriel plane (1x2) in each of the localities: A: Alepe; B: Bondoukou; C: Bongouanou; D: Divo; E: Korhogo

Table 4: Correlation matrix between fungus genera and climatic factors

Localities	Pair of variables		r	r²	t	Pr (>|t|)
Bondoukou	**Temperature**	*Aspergillus*	0,88	0,77	3,20	0,049*
	Temperature	*Cochliobolus*	0,88	0,77	3,20	0,049*
	Temperature	*Podosphaera*	0,88	0,77	3,20	0,049*
	Temperature	*Phoma*	0,88	0,77	3,20	0,049*
	Temperature	*Pythium*.	0,88	0,77	3,20	0,049*
Bongouanou	**Air moisture**	*Fusarium*	-0,86	0,74	-2,97	0,049*
	Temperature	*Fusarium*	-0,86	0,74	-2,97	0,049*
	Wind speed	*Fusarium*	-0,86	0,74	-2,97	0,049*
Korhogo	**Air moisture**	*Cochliobolus*	0,99	0,98	15,75	0,0006**
	Air moisture	*Colletotrichum*	-0,82	0,67	-2,50	0,087 ns
	Rainfall	*Cochliobolus*	0,99	0,98	15,75	0,0006**
	Rainfall	*Colletotrichum*	-0,82	0,67	-2,50	0,087 ns
Alepe	**Temperature**	*Botryosphaeria*	-0,70	0,49	-1,73	0,18ns
	Temperature	*Cochliobolus*	-0,70	0,49	-1,73	0,18ns
	Temperature	*Fusarium*	-0,86	0,74	-2,97	0,049*
	Wind speed	*Phoma*	0,89	0,79	3,37	0,043*

r = Corrélation; t = Student test; Pr = probability; ns = not significant; * = significant correlation at P < 0, 05; ** = significant correlation at P < 0,001

Discussion

This study led to the distinction of various fungus attack symptoms on *Lagenaria siceraria* leaves in field. Seven symptom types were observed in Korhogo and Bondoukou, five types in Alepe and Bongouanou, and four types in Divo. The difference among localities in terms of symptom types may be attributed to the difference in plant species composition of vegetations surrounding *L. siceraria* farms as well as to the association of *L. siceraria* with other crops on the same farm (Anahosur, 1992).

Indeed, fungus species hosted by the surrounding vegetation may vary according to the plant species composition. The presence of a fungus species in a given locality implies that it has host plant species or other sink in that locality and that environmental conditions are conducive to its growth and development (Anahosur, 1992; Banvart, 1998).

The fact that the localities have in common a great part of the fungus genera observed probably originate from the fact that farmers get stock of seeds through markets which are places from where contaminated seeds are dispersed. In the same way, Montes-Hernandez and Eguiarte (2002) showed that sharing seeds among farmers is one of the main causes of the reduction of genetic differentiation in several cropped plant species.

From all the leaves sample collected, 17 fungal genera were identified, the 7 were found in all the localities. Only one genus, *Pestalotiopsis*, was found to be specific to one locality, Divo. The variability in symptom types does not necessarily reflect variability in fungus genera. According to Howell (2003) and Mouria *et al.* (2003), one lesion can be provoqued by one pathogen species that can be thereafter colonized by others. Also, attack by different pathogen agents can result in similar symptom and attack by a specific pathogen agent can result in variable symptoms, owing to the conditions. Thus, one type of symptom is not necessarily specific of one fungus genera.

The statistical analyses performed on the data revealed a significant difference between mean isolation frequencies of the 17 genera encountered on the *L. siceraria* leaves. The frequencies varied between as well as within localities. In addition, the presence of the fungal genera was found to be linked to the climatic factors such as rainfall and temperature as reported by Anahosur (1992). For all of the localities, monthly rainfall amounted to 250 mm and the temperature, to 27°C. Such conditions are favorable to fungus development and dissemination.

The isolation frequency of the genera *Aspergillus*, *Colletotrichum* and *Fusarium* didn't vary among localities; this can be explained by the fact that these are soil-born genera which breed through spore dissemination. Indeed, soil-born fungi disseminate the best through drainage or surface run-off during rain periods (Bieysse *et al.*, 2002; Oostland and Pacico, 2007).

Conclusion

This study allowed the identification of numerous fungus genera that parasite *L. siceraria* leaves on farm, based on their morphological characteristics. The isolation frequencies of the fungal genera varied between as well as within localities. The presence of a fungus genus was found to be greatly influenced by the prevailing climatic conditions.

Fungal genera responsible of devastating plant disease such as *Fusarium* wilt, blight and powdery mildew were isolated. Since the symptoms were not specific to the fungus genera encountered on the *L. siceraria* leaves, the identification may have suffered from some inaccuracy. Thus, further studies including pathogenicity test are needed to make more accurate link between fungus genera and plant disease.

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