

# Study of Cultural and Morphometric Characters of *Fusarium xylarioides* Strains Isolated from Coffee Trees Infected with Coffee Wilt Disease Collected from Eastern, North Kivu and Equateur Provinces, Democratic Republic of Congo

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## Authors' contributions

This work was carried out in collaboration between all authors. Authors PTD and AKM designed the study and wrote the protocol. Author CM conducted the lab experiments and wrote the first draft of the manuscript in collaboration with author ANN. Author MMM completed the data analysis, the literature review and wrote the final manuscript. All authors read and approved the final manuscript.

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## ABSTRACT

**Aim:** To determine cultural and morphometric characters of different strains of *Fusarium xylarioides* isolated from coffee trees collected from Eastern, North Kivu and Equateur provinces of Democratic Republic of Congo (DRC).

**Study Design:** The study was performed using a completely randomized design with three replications.

**Place and Duration of the Study:** The study was conducted in the laboratory Unit of Phytopathology, Faculty of Agronomy, University of Kinshasa, between January and February 2005.

**Methodology:** Seventeen *F. xylarioides* strains collected from the eastern, North Kivu and Equateur province were isolated from coffee trees infected with CWD. They were grown in Synthetic Nutrient Agar (SNA) and Potato Glucose Agar (PGA) culture medium. For each strain, characteristics based on radial growth, mycelial pigmentation and contour of disc, size and morphology of conidia, and density of sporulation were studied.

**Results:** The results obtained showed that different strains of *F. xylarioides* are characterized with rapid rate of growth (3.76 – 4.14 mm/day), slow rate of growth (3.36 – 3.74 mm/day) and very slow rate of growth (2.78 – 2.98 mm/day). In general, 70.5% of strains had cream pigmentation, 23.5% had purple and 6% had cream to purple pigmentation. Seventy-six percent of strains presented a slightly sinuous mycelial disc, while 24% had sinuous disc. Different strains produced 84.9% of sickle conidia, while 15% of conidia were curved; and 67.7% of microconidia had allantoid form, while 32.1% had a reniform shape. Microscopic observations revealed that macroconidia size varied from 7.1 – 13.31 x 1.7 – 2.45 µm, and microconidia varied from 3.55 – 6.15 x 1.65 – 2.42 µm. All macroconidia presented a single partition, while microconidia were devoid of any partition. The strains studied produced an average 200 – 800 conidia/ml.

**Conclusion:** The results of the present study revealed that *F. xylarioides* presents a plasticity for different characters studied.

*Keywords: Coffee wilt disease; Fusarium xylarioides; cultural and morphometric characters; Democratic Republic of Congo.*

## 1. INTRODUCTION

In Democratic Republic of Congo (DRC), coffee cultivation constitutes an important economic activity and represents a vital interest for smallholders. Overall, the operating system is shared between farmers' plantations and large agribusiness farms, the first occupying about 86% of total coffee acreage [1]. *Coffea canephora* Pierre and *C. arabica* (L.) are the most cultivated coffee species. The *robusta* variety of the first species represents 87.5% of the cultivated area against 12.4% for arabica [1]. The cultivation of *robusta* occupies the regions of lower altitudes while Arabica is grown in high altitude areas of eastern DRC. Another variety of *C. canephora*, the Kouillou is grown locally in the Mayombe forest region in the province of Kongo Central (formerly Bas-Congo) [2].

Currently in the DRC coffee plantations located in Eastern, Equateur and North Kivu provinces are seriously affected by Coffee Wilt Disease (CWD), a vascular system degenerative disease. It is caused by a fungus named *Fusarium xylarioides* Steyaert (teleomorph: *Gibberella xylarioides* Heim and Saccas). In DRC, CWD was reported for the first time in 1948 [3], and it became a serious problem in coffee production in several countries, including Ivory Coast, Central African Republic and DRC [4]. Its impact has been mitigated in the establishment of efficient

breeding programs. The disease reappeared in the DRC at 1986 in the triangle, Isiro – Wamba – Mungbere, in Eastern Province. It has evolved into areas with very diversified ecological conditions, first to the province of North Kivu, and to that of Equateur [5].

CWD is manifested by various symptoms. Studies conducted by Frassel [4], Delassus [6], Heim and Saccas [7] and Buddie et al. [8] showed that the CWD resulting in partial or complete yellowing of foliage, then unilateral or general browning of leaves of young shoots, and their leaves tense up, curl up, wither, turn brown and fall. Gradually withering extends to all parts of the shrub that dies. In DRC, epidemiological surveys and samples have led to the isolation of many strains of the pathogen in all areas where the disease occurs, and a collection of strains of the pathogen was constituted. Keeping in mind the interaction that may exist between environment, host plant and pathogen, the three main factors of the triangle of disease, this study was oriented towards the knowledge of the pathogen population. It is in this context that the present study is aimed to determine cultural and morphometric characters of different strains of *F. xylarioides* isolated from coffee trees collected from Eastern, North Kivu and Equateur provinces, the 3 main coffee-producing regions of DRC.

## 2. MATERIALS AND METHODS

### 2.1 *Fusarium xylarioides* Strains Studied

In the present study, 17 strains of *F. xylarioides* were used (Table 1). They were isolated from stems of coffee trees infected with CWD collected during epidemiological surveys made in 2003 in Eastern, Equateur and North Kivu provinces of DRC. Different strains of *F. xylarioides* were stored as parent strains in test tubes on the medium Synthetic Nutrient Agar (SNA) under paraffin. These strains were first taken to their revival. This consisted of transplanting streaked on medium Synthetic Nutrient Agar (SNA) in Petri dishes with a view to obtain strains which were subcultured on SNA culture medium whose chemical composition described by Tuite [9] was  $\text{KH}_2\text{PO}_4$ : 1 g;  $\text{KNO}_3$ : 1 g;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ : 0.5 g;  $\text{KCl}$ : 0.5 g; Glucose: 0.2 g; Sucrose: 0.2 g; Agar Merck®: 20 g; and  $\text{H}_2\text{O}$ : 1000 ml.

### 2.2 Preparation of Culture Medium Used

According to Tshilenge-Djim et al. [10], the culture medium containing potato is favorable to the expression of macroscopic characters of *Fusarium* spp. In this study, the composition of the culture medium used was inspired by Tuite [9] with slight modifications, where dextrose was replaced by glucose to obtain the Potato Glucose Agar (PGA) culture medium. The final composition of the culture medium was: dehydrated potato powder: 20 g; glucose: 20 g; agar: 20 g; and distilled water: 1000 ml. The pH measured before sterilization was equal to 5. The culture medium used was prepared using the following procedure: potato was peeled, washed with tap water, cut into slices and rinsed with distilled water. The slices were dried in an oven at 65°C until constant weight, and then they were crushed to mill brand "Thomas Scientific (USA)" and "Grinder (Fritsch, Germany)". The culture medium was then autoclaved at 120°C for 20 minutes. After sterilization, culture medium was distributed, under laminar flow hood near a Bunsen burner flame, in Petri dishes measuring 9 cm in diameter. After cooling, Petri dishes were returned to prevent condensation on the lids [10,11].

### 2.3 Macroscopic Characterization

For macroscopic characterization, subcultures were obtained from transplanting a pellet of 5 mm of diameter cut with a cork borer outskirts mycelium of the cultures of stock-girls previously

obtained on SNA. Each pellet was then placed in the center of the Petri dish containing culture medium previously prepared with potato. The Petri dishes were returned, labeled and tightly closed with parafilm, and then incubated for 12 days at laboratory temperature ( $\pm 27^\circ\text{C}$ ). The observations were recorded daily in daylight, and were focused on radial growth, contour of disc and pigmentation of mycelium. The diameter of the mycelial disc was measured every day until the 12<sup>th</sup> day corresponding to the total occupation of the Petri dish. This parameter was used to calculate the daily rate expressed in  $\text{mm}\cdot\text{day}^{-1}$ . Taking mycelial growth is facilitated by tracing two perpendicular lines on the reverse side of the Petri dishes. Using a transparent ruler (mm), we made two steps in diameter, in the horizontal and vertical direction before calculating the average value. The study was conducted in a Completely Randomized Design (RCD) with three replications. Data recorded were submitted to analysis of variance using R software (2.12.0 version). The comparison of means was done by Least Significant Difference test at 5% of probability level. After total occupation of the Petri dish, the contour of mycelial disc was described according to the diagram presented by Ainsworth [12]. The color of aerial mycelium was described on the total occupation of the Petri dish using the color scale described by Nelson et al. [13].

### 2.4 Microscopic Characterization

The study related to microscopic characters was conducted from subcultures incubated at laboratory temperature ( $\pm 27^\circ\text{C}$ ) for 7 days on culture medium SNA described by Tuite [9]. From girls-strains obtained after revitalization of parent strains, production of subcultures was completed by transplanting streaked with a platinum loop on culture medium SNA. Morphometric characters of conidia were observed on microscopic sections collected from subcultures where, a preliminary observation in microscope revealed a high density of conidia. The sections were mounted in a drop of lactophenol blue and then observed under a microscope (Olympus BX 40) equipped with a micrometer scale and the 40x objective. Data were recorded on a sample of 30 conidia, and were focused on the length and width of macroconidia and microconidia. The length and width of a conidium were measured using an ocular micrometer. Number of partitions of macroconidia, and shape of microconidia and their frequency were also described. The length

**Table 1. List of different strains of *Fusarium xylarioides* studied**

Strain	Province	Locality	Date of harvest by <sup>1</sup>	Date of isolation by <sup>2</sup>	Identified by <sup>3</sup>
B10101(2)J	North Kivu	Beni-Mutwanga	02/12/02 K.T. D.	02/01/03 T.	T.
MUCL 14186	Eastern	Yangambi	1960 Meyer	Meyer	Meyer
MUCL35223	Eastern	Isiro	Nov. 1992 P.	1992 Decock	Decock
SR01B/10a	Eastern	Yangambi (L147)	15/12/02 K.T. D.	21/05/03 L.	L.
SR12B/01a	Eastern	Isiro	17/12/02 K.T.D.	21/05/03 L.	L.
SR22/01a	North Kivu	Butembo	12/9/02 NV	17/12/02 L.	L.
Bunduki	Equateur	Near Eastern	09/09/2004 K.D.	20/11/2004 T.	T.
Isangi	Equateur	Lisala	17/09/2004 K.D.	20/11/2004 T.	T.
Lisanza	Equateur	Loeka	15/09/2004 K.D.	20/11/2004 T.	T.
Mangbakapale	Equateur	Not determined	15/09/2004 K.D.	20/11/2004 T.	T.
Mindembo-17	Equateur	Lisala	17/09/2004 K.D.	22/11/2004 T.	T.
Mongene	Equateur	Itimbiri	10/10/2004 K.D.	22/11/2004 T.	T.
Notre-Dame	Equateur	Loeka	15/09/2004 K.D.	22/11/2004 T.	T.
Vil-basalaka	Equateur	Itimbiri	11/09/2004 K.D.	22/11/2004 T.	T.
Yangoyi	Equateur	Itimbiri	11/09/2004 K.D.	20/11/2004 T.	T.
Yeboka	Equateur	Itimbiri	09/09/2004 K.D.	20/11/2004 T.	T.
Zobolia	Equateur	Loeka	15/09/2004 K.D.	20/11/2004 T.	T.

<sup>1, 2, 3</sup> K = Kalonji; T = Tshilenge-Djim; D = Dibue; P = Pochet; NV = Ndungo Vigheri; L = Lepoint

consisted of the straight distance between the two ends of the conidium. The width of a conidium was measured at its widest section. The number of partitions of conidia was counted by determining the limits between different cells thereof. Characters studies were described according to the morphological description proposed by Ainsworth [12].

For studying the density of conidia produced by each strain of *Fusarium xylarioides*, suspensions of conidia were first prepared from subcultures incubated during 7 days. Three milliliters of Tween 20<sup>®</sup> collected using a sterile pipette were placed in each Petri dish containing the culture of each strain in order to facilitate the recovery of conidia. The culture medium was the superficially scraped by smooth movements with a sterile Pasteur pipette for snatch conidia of mycelial mass. This operation was conducted near the Bunsen burner flame under a laminar flow hood. After 15 minutes or rest, the scraping movement was then repeated. Currently, polysaccharides surrounding conidia have time to swell in the suspension and conidia easily detached from the mycelium. The suspension of conidia was then collected using a sterile pipette and filtered through a sterile cloth whose meshes measured 75 µm in diameter. The filtrate was collected in the Erlenmeyer flask. The amount of the filtrate obtained was determined in an Eppendorf tube of

2 ml of capacity. The sporulation of each strain of *F. xylarioides* was measured using the direct method of counting with the hemocytometer or Thoma cell as described by Tshilenge et al. [11].

### 3. RESULTS

Results related to the mycelial growth of *F. xylarioides* strains studied are illustrated in Fig. 1. Results based on the contour of mycelial disc and the mycelial pigmentation of *F. xylarioides* strains, after 12 days of growth in PGA medium are presented in Table 2. Data related to morphology of macro- and micro- conidia of different strains of *F. xylarioides* studied are reported in Table 3. Results related to dimension of macro- and microconidia of different strains of *F. xylarioides* studied are reported in Table 4. The density of sporulation was calculated by the abundance of micro- and macroconidia produced in SNA medium. Results related to this parameter after observations on the Thoma cell are illustrated by Fig. 2.

#### 3.1 Macroscopic Characters

##### 3.1.1 Radial growth of mycelium

In general, examination of Fig. 1 revealed that the radial growth varies among strains used. From 24h of incubation, it emerged three groups of *F. xylarioides* strains which differed in their

growth. Analysis of variance revealed significant differences ( $P = .05$ ) among strains used. The first group is characterized by rapid growth, and includes strains Bunduki, Isangi, Mangbakapale, Mindembo-17, MUCL14186 and Zobolia. The second group, characterized by slower growth includes strains B10101(2)J, Lisanza, Mongene, MUCL35223, Notre-Dame, SR01B/10a, SR12B/01a, Vil-Basalaka and Yangoyi. The third group is characterized by very slow growth and includes strains Yeboka and SR22/01a.

**Table 2. Mycelial pigmentation and contour of mycelial disc of different strains of *F. xylarioides* studied**

Strains	Mycelial pigmentation	Contour of mycelial disc
B10101(2)J	Cream	Slightly sinuous
Bunduki	Cream	Slightly sinuous
Isangi	Cream	Slightly sinuous
Lisanza	Cream to purple	Slightly sinuous
Mangbakapale	Cream	Slightly sinuous
Mindembo-17	Cream	Slightly sinuous
Mongene	Purple	Sinuous
MUCL14186	Cream	Slightly sinuous
MUCL35223	Cream	Sinuous
Notre-Dame	Cream	Slightly sinuous
SR01B/10a	Cream	Slightly sinuous
SR12B/01a	Cream	Sinuous
SR22/01a	Cream	Slightly sinuous
Vil-Basalaka	Purple	Slightly sinuous
Yangoyi	Purple	Slightly sinuous
Yeboka	Purple	Slightly sinuous
Zobolia	Cream	Sinuous

### 3.1.2 Contour of disc and pigmentation of mycelium

Results reported in Table 2 show three different color: cream, cream to purple, and purple. Strains B10101(2)J, Bunduki, Isangi, Mangbakapale, Mindembo-17, MUCL14186,

MUCL35223, Notre-Dame, SR01B/10a, SR12B/01a, SR22/01a and Zobolia presented the color cream in their mycelial pigmentation, while the strain Lisanza presented the color cream to purple, and Mongene, Vil-Basalaka, Yangoyi and Yeboka strains were characterized by the color purple. In addition, it appeared that Mongene, MUCL35223, SR12B/01a and Zobolia strains presented a sinuous contour of mycelial disc, while for other strains, it was slightly sinuous.

## 3.2 Microscopic Characters

### 3.2.1 Morphology of conidia

In general, it appeared two forms of macroconidia: curved and sickle, and two other forms of microconidia: allantoid and reniform. In general, analysis of the Table 3 revealed that sickle and allantoid shapes presented highest values than curved and reniform shapes, for macro- and microconidia respectively. Observations made on macroconidia indicated that the highest frequency of curved and sickle shape (27.7%) and (99.0%), and the lowest frequency (72.2%) and (1.0%) were respectively recorded on MUCL14186 and Zobolia. For microconidia, the highest frequency of allantoid and reniform shape (86.6%) and (50.0%), and the lowest frequency (50.0%) and (13.3%) were respectively recorded SR01B/10a and Notre-Dame.

### 3.2.2 Dimension of conidia

According to biometric traits reported in Table 4, it appears that different strains of *F. xylarioides* presented macroconidia measuring 7.1 – 13.31  $\mu\text{m}$  in length and 1.6 – 2.45  $\mu\text{m}$  in width, and microconidia measuring 3.55 – 6.15  $\mu\text{m}$  in length and 1.65 – 2.42  $\mu\text{m}$  in width. All macroconidia showed typically a single partition for all strains, while in microconidia, partition and the widest section were absent. Analysis of variance showed significant differences among strains for macro- ( $P = .05$ ) and micro- conidia size.

### 3.2.3 Density of sporulation

The analysis of Fig. 2 shows that different strains of *Fusarium* studied produced a variable number of conidia which can be grouped in 3 categories. The first, characterized by low production of conidia (200 – 380 conidia/ml) comprises the strains B10101(2)J, Mongene, MUCL35223, SR01B/10a, SR12B/01a, SR22/01a and Yeboka.

**Table 3. Frequency (%) of different forms of macro- and micro- conidia of different strains of *F. xylarioides* studied**

Strains	Macroconidia		Microconidia	
	Curved	Sickle	Allantoid	Reniform
B10101(2)J	26.9	73.1	66.4	33.7
Bunduki	12.2	87.8	56.7	43.7
Isangi	17.6	82.4	76.4	23.6
Lisanza	13.6	86.4	56.5	43.5
Mangbapakale	12.5	87.5	70.0	30.0
Mindembo-17	10.6	89.4	70.0	30.0
Mongene	19.6	80.4	52.3	47.7
MUCL14186	27.7	72.2	79.2	21.8
MUCL35223	26.7	73.3	80.0	20.0
Notre-Dame	3.7	96.3	50.0	50.0
SR01B/10a	13.3	86.7	86.6	13.3
SR12B/01a	18.6	81.4	56.6	43.3
SR22/01a	12.1	87.9	67.0	33.0
Vil-Basalaka	16.6	83.3	63.3	36.7
Yangoyi	9.8	90.2	73.3	26.7
Yeboka	12.9	87.1	63.6	33.4
Zobolia	1.0	99.0	83.3	16.7

**Table 4. Averages length and width ( $\mu\text{m}$ ) of macro- and micro- conidia of different strains of *F. xylarioides* studied, produced on SNA culture medium**

Strains	Macroconidia			Microconidia	
	Length	Width	Number of partition	Length	Width
B10101(2)J	11.5 $\pm$ 5.12	2.22 $\pm$ 0.69	1	4.23 $\pm$ 1.33	2 $\pm$ 0.53
Bunduki	9.95 $\pm$ 3.68	2.3 $\pm$ 0.39	1	4.25 $\pm$ 1.48	2 $\pm$ 0.56
Isangi	8.85 $\pm$ 3.03	2.23 $\pm$ 0.53	1	4.8 $\pm$ 1.33	1.95 $\pm$ 0.64
Lisanza	7.8 $\pm$ 3.70	1.7 $\pm$ 0.33	1	4.55 $\pm$ 1.33	2.4 $\pm$ 0.60
Mangbapakale	9.1 $\pm$ 2.55	1.92 $\pm$ 0.50	1	4.6 $\pm$ 1.20	2.12 $\pm$ 0.62
Mindembo-17	9.25 $\pm$ 4.17	2.45 $\pm$ 0.47	1	4.75 $\pm$ 1.31	2.1 $\pm$ 0.63
Mongene	9.1 $\pm$ 2.58	1.85 $\pm$ 0.38	1	4.55 $\pm$ 1.19	2 $\pm$ 0.35
MUCL14186	8.65 $\pm$ 2.31	1.72 $\pm$ 0.34	1	4.15 $\pm$ 1.28	1.85 $\pm$ 0.51
MUCL35223	7.5 $\pm$ 2.12	1.72 $\pm$ 0.34	1	4.45 $\pm$ 1.39	1.7 $\pm$ 0.33
Notre-Dame	11.45 $\pm$ 4.06	2.1 $\pm$ 0.36	1	4.5 $\pm$ 1.06	1.9 $\pm$ 0.42
SR01B/10a	7.88 $\pm$ 3.04	1.6 $\pm$ 0.25	1	3.9 $\pm$ 1.01	1.65 $\pm$ 0.36
SR12B/01a	8.95 $\pm$ 3.30	1.87 $\pm$ 0.42	1	5.7 $\pm$ 1.64	2.42 $\pm$ 0.46
SR22/01a	13.31 $\pm$ 5.88	1.9 $\pm$ 0.46	1	4.85 $\pm$ 1.74	2.4 $\pm$ 0.91
Vil-Basalaka	9.65 $\pm$ 2.18	2.02 $\pm$ 0.40	1	6.15 $\pm$ 1.98	2.4 $\pm$ 0.53
Yangoyi	7.85 $\pm$ 2.93	2.05 $\pm$ 0.39	1	5.3 $\pm$ 1.74	2.15 $\pm$ 0.61
Yeboka	9.65 $\pm$ 2.18	2.3 $\pm$ 0.39	1	4.5 $\pm$ 1.39	1.67 $\pm$ 0.32
Zobolia	7.1 $\pm$ 2.21	1.7 $\pm$ 0.33	1	3.55 $\pm$ 0.96	1.72 $\pm$ 0.34

The second category is characterized by medium production of conidia (400 – 600 conidia/ml), and comprises the strains Isangi, Lisanza, Mindembo-17, Notre-Dame and Vil-Basalaka. The third category is characterized by high production of conidia (605 – 800 conidia/ml), and comprises the strains Bunduki, Mangbapakale, MUCL14186, Yangoyi and Zobolia. Analysis of variance revealed significant differences ( $P = .05$ ) among strains used.

#### 4. DISCUSSION

The study related to cultural and morphometric characters of *Fusarium xylarioides* strains, isolated from coffee trees harvested in 3 main coffee-producing provinces of DRC revealed varied characters. According to macroscopic characters (radial growth, mycelial pigmentation and contour of mycelial disc) studied using PGA culture medium, our results revealed differences

between strains on the radial growth and mycelial pigmentation, while the contour of mycelial disc has not been different for all strains. The difference observed on the radial growth permitted to categorize different strains of *Fusarium* into three groups. The first group is characterized by the rapid rate of growth ranging from 3.76 – 4.14 mm/day; the second is characterized by slow rate of growth ranging from 3.36 – 3.74 mm/day, and the third group consisted to strains with very slow rate of growth ranging from 2.78 – 2.98 mm/day (Fig. 1). The present study corroborates observations made by various authors such as Saccas [14], Booth [15], Girma and Mengistu [16] and Tshilenge-Djim et al. [10] who have revealed various variabilities in macroscopic characteristics of *Fusarium* species. Similarly to this study, trials conducted by Girma [17] and Getachew et al. [18] revealed varying radial growth rate among *F. xylarioides* populations collected from different coffee types and geographical localities in Africa. In addition, a study conducted by Tshilenge et al. [11] on different species of *Fusarium* associated to CWD in DRC revealed that *F. stilboides*, *F. solani*, *F. lateritium* and *F. xylarioides* expressed in general different growth rates depending on the species. Based on the present findings, it appeared that those differences observed are not related to the geographical origin of *F. xylarioides* strains studied.

Microscopic characteristics studied (size, morphology and production of conidia) revealed in general that 70.5% of strains presented a cream pigmentation of mycelium, 23.5% presented a purple pigmentation, and 6% had a cream to purple pigmentation. Analyzing the geographical origin of strains, it appeared that all strains of North Kivu and Eastern provinces presented a cream pigmentation, and purple mycelial pigmentation was only observed on strains derived from Equateur province. The present observations are similar to those reported by Getachew et al. [18] who revealed that, the colony color of *Gibberella xylarioides* varies with origin of the isolates. According to the contour of mycelial disc, it appeared that 76% of strains presented a slightly sinuous disc, while 24% had a sinuous disc (Table 2). The present finding is consistent with that made by Tshilenge et al. [11] who observed that *F. xylarioides* presented a slightly sinuous contour of mycelial disc compared to others species of *Fusarium*. According to the macroconidia form, it appeared

that different strains of *F. xylarioides* studied produced in most sickle conidia with a frequency equal to 84.9%, while 15% of conidia were curved. For microconidia, it noted that 67.7% presented an allantoid form, while 32.1% had a reniform shape (Table 3). The present study corroborates findings reported by Getachew et al. [18] who noted that macroconidia produced by isolates of Ethiopia were cylindrical, slightly curved and curved, and microconidia were allantoid, comma- and U-shape. In addition, Tshilenge et al. [11] observed that *F. xylarioides* produced curved and sickle shape of macroconidia. However, data reported by those authors mentioned high frequency of curved shape produced by macroconidia than sickle form, which is contrary to our results. This can be explained by the diversity of strains from various origins that were used in the present study, while Tshilenge et al. [11] had used only one strain of *F. xylarioides* that has been compared to other species of *Fusarium*.

Observations made on macro- and micro-conidia indicated that size varied respectively from 7.1 – 13.31 x 1.7 – 2.45  $\mu\text{m}$  and from 3.55 – 6.15 x 1.65 – 2.42  $\mu\text{m}$  (Table 4). Macroconidia were characterized by the presence of a single partition, while microconidia were devoid of any partition. Results of the present study corroborate findings reported by Saccas [14], Girma [17], Getachew et al. [18] and Rutherford et al. [19] who founded that macroconidia presented 1 to 3 septate cells. In addition, Booth [15] reported that in their vegetative stage, macroconidia are characterized by the absence or presence of a single partition. Data reported by Roger [20] mentioned that the widest section of the macroconidia presented values range from 5.25 – 10  $\mu\text{m}$ , which are consistent to results reported in Table 4. Based on the conidial morphology, all strains studied showed forms previously described by various authors. In addition, it was demonstrated that macroconidia presented curved, cylindrical, falcate or fusoid shape, while microconidia had cylindrical, curved and allantoid form [7,17,20]. According to the density of sporulation, it appeared in general that different strains of *F. xylarioides* studied produced an average 200 – 800 conidia/ml (Fig. 2). This means that the number of conidia produced by a fungus of the *Fusarium* genus varies among strains or species such as reported in Tshilenge et al. [11].

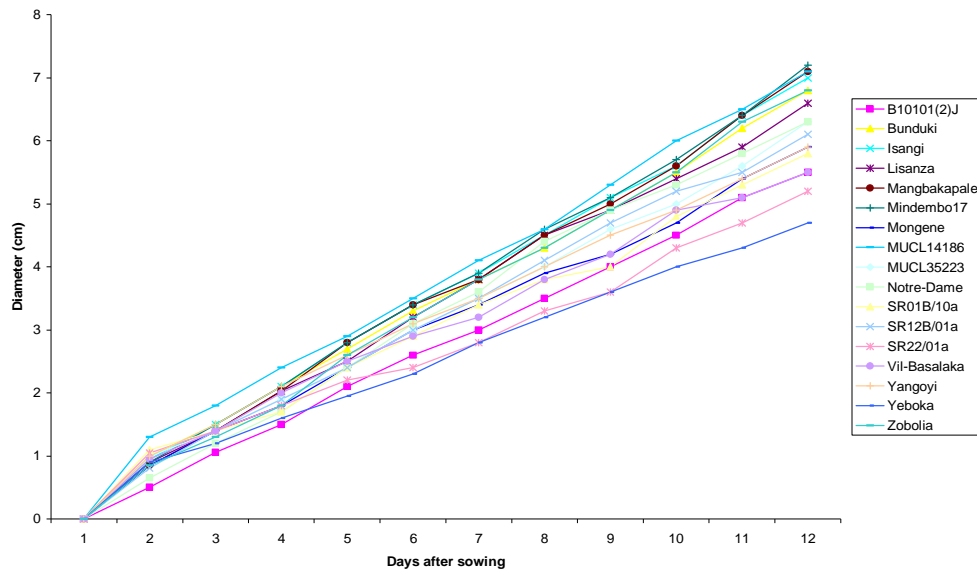


Fig. 1. Mycelial growth of different strains of *F. xylarioides* on PGA

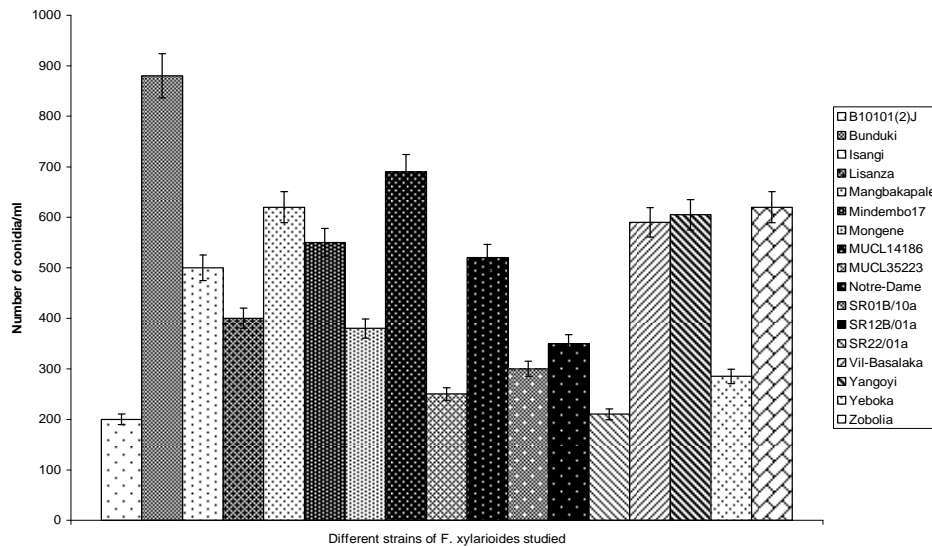


Fig. 2. Number of conidia produced by different strains of *F. xylarioides*

## 5. CONCLUSION

The results of the present study revealed that *F. xylarioides* presents a plasticity for different characters studied. Cultural and morphometric characters observed are similar to those described by various authors cited in the literature. It is imperative to establish a relationship between variability in the observed characters and pathogenicity of different strains to determine the precise role of the pathogen in the resurgence of CWD in DRC.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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