

# Morphometric Analysis of Populations of *Fusarium oxysporum* f.sp. *vasinfectum* (Atk.) Snyder & Hansen, the Causal Agent of Cotton Fusarium Wilt in Côte d'Ivoire

Abenan N'Guessan Marie-France Kouamé<sup>1,2</sup>, Nogbou Ferdinand Amangoua<sup>2</sup>, Blé Gbacla Flora Dominique Yao<sup>1\*</sup>, Diane Estelle Gnapi<sup>2</sup>, Malanno Kouakou<sup>2</sup>, Louise Akanvou<sup>2</sup>, Esoi N'Guessan<sup>2</sup>, Marc Giband<sup>3</sup>, N'Goran Mathurin Koffi<sup>1</sup>, Kouabenan Abo<sup>4</sup>

<sup>1</sup>Research Unit in Genetics and Molecular Epidemiology (URGEM), Jean Lorougnon Guede University, Daloa, Côte d'Ivoire

<sup>2</sup>National Center for Agronomic Research (CNRA), Bouake, Côte d'Ivoire

<sup>3</sup>International Cooperation Center for Agronomic Research for Development (CIRAD), UMR AGAP Institute, Montpellier, France

<sup>4</sup>Plant Biology and Health Research Unit, Joint Research and Innovation Unit in Agronomic Sciences and Processing Technologies (UMRI-SAPT), Félix Houphouët-Boigny National Polytechnic Institute (INP-HB), Yamoussoukro, Côte d'Ivoire  
Email: \*gbaclaflora@gmail.com

**How to cite this paper:** Kouamé, A.N.M.-F., Amangoua, N.F., Yao, B.G.F.D., Gnapi, D.E., Kouakou, M., Akanvou, L., N'Guessan, E., Giband, M., Koffi, N.M. and Abo, K. (2026) Morphometric Analysis of Populations of *Fusarium oxysporum* f.sp. *vasinfectum* (Atk.) Snyder & Hansen, the Causal Agent of Cotton Fusarium Wilt in Côte d'Ivoire. *Agricultural Sciences*, 17, 30-45.

<https://doi.org/10.4236/as.2026.171003>

**Received:** December 7, 2025

**Accepted:** January 20, 2026

**Published:** January 23, 2026

Copyright © 2026 by author(s) and Scientific Research Publishing Inc.

This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).

<http://creativecommons.org/licenses/by/4.0/>



Open Access

## Abstract

Cotton plays a crucial role in the socio-economic development of communities within its production regions. However, it is susceptible to numerous pathogens that cause significant yield losses. Among these, Fusarium vascular wilt of cotton, caused by *Fusarium oxysporum* f.sp. *vasinfectum* Snyder & Hansen (Fov) stands out as one of the most destructive and threatening diseases in Côte d'Ivoire. Extensive research efforts have been devoted to Fov to identify effective control strategies against this fungus. This study focuses on a morphometric analysis of Fov populations collected throughout the Ivorian cotton basin to characterize their diversity. On Potato dextrose agar (PDA) medium, the isolates exhibited three distinct mycelial appearances: 1) Cottony and dense; 2) Cottony and short; 3) Fine and short. Also, the five colony colorations were observed: 1) Purple; 2) Lavender; 3) Salmon; 4) Pink; 5) White. Furthermore, the composition of the culture medium was found to significantly influence both mycelial growth and sporulation of the isolates. The results indicate that the pathogen demonstrated the ability to grow and sporulate on both complex and synthetic media, but to varying extents. PDA medium proved more conducive to sporulation, whereas CDA medium favored mycelial growth. The

results of this study reveal significant phenotypic variability in mycelial appearance and colony coloration among Fov isolates. They also suggest that the nutritional requirements for mycelial growth and sporulation, two critical aspects of fungal development, differ substantially.

### Keywords

Cotton, *Fusarium oxysporum* f.sp. *vasinfectum*, Sporulation, Morphometric Analysis, Mycelial Growth

---

## 1. Introduction

Cotton is a perennial shrub, yet it is cultivated as an annual crop throughout sub-humid and semi-arid areas [1]. It plays an indispensable role in the agricultural economy and the socio-economic development of populations within production areas. In Côte d'Ivoire, cotton cultivation extends over more than 444,870 ha and involves over 132,419 smallholder farmers [2]. Cotton represents one of the main sources of State revenue, with an estimated production of 539,623 tons during the 2022 campaign [2]. Moreover, it contributes to improving the livelihoods of rural populations, structuring the cooperative movement and fostering industrial development [3] [4]. Indeed, the cotton sector accounts for approximately 1.7% of the country's gross domestic product [5]. However, cotton cultivation faces a significant threat from Fusarium wilt caused by the soil-borne fungus *Fusarium oxysporum* f.sp. *vasinfectum*. A pronounced incidence of Fusarium wilt has been documented in the southern part of the Ivorian cotton basin, alongside its progressive spread to regions previously unaffected, notably the localities of Diawala and Ouangolodougou in the SECO zone, situated in the northern sector of the country's cotton basin [6]. These pathogens inflict severe damage, both ecologically [7] and economically, given the critical role of cotton exports in generating substantial foreign exchange earnings for the Ivorian economy [8]. Control strategies against vascular diseases caused by soil-borne pathogens remain very limited or almost non-existent [9]. Regarding Fusarium wilt management, various strategies have been deployed, including chemical control, which currently predominate. However, this method is increasingly criticized due to its associated toxicity, environmental pollution, adverse health effects and even biodiversity loss [10]. The development of cultivars resistant to Fov appears as the most appropriate and sustainable control strategy. Effective management of cotton Fusarium wilt requires the breeding of resistant varieties. To this end, it is imperative to elucidate the diversity of the pathogen populations present in cotton-growing areas in order to assess the stability of fundamental fungal traits across numerous isolates and to evaluate isolate variability on different culture media. This study thus seeks (i) to evaluate the cultural characteristics of *Fusarium oxysporum* f.sp. *vasinfectum* population and (ii) to investigate the effect of culture media on mycelial growth

and sporulation of isolates collected from the Ivorian cotton.

## 2. Material and Methods

### 2.1. Sampling Symptomatic Plants

Surveys of cotton fields were conducted throughout the cotton-growing region of Côte d'Ivoire to collect diseased cotton plants. Plant fragments (roots and stems) were collected from specimens showing browning of the stem vessels, a hallmark symptom of cotton tracheomycosis. Sampling was performed under strict aseptic conditions, ensuring no direct contact between different samples. Each sample was placed in a large polyethylene bag, clearly labeled with pertinent information including sampling location (date, geographical coordinates and cotton variety) and subsequently stored in a cold until further analysis.

### 2.2. Fungal Isolation and Culture Purification

Symptomatic plant samples were aseptically sectioned into small stem fragments measuring approximately 0.5 to 1 cm in length. These fragments were immersed for 3 minutes in a 15% diluted sodium hypochlorite solution at 8°C, followed by three successive rinses in sterile distilled water. Subsequently, the fragments were placed on Potato Dextrose Agar (PDA) medium. The PDA medium consisted of 20 g bacteriological agar, 20 g glucose and 20 g potato flakes dissolved in 1000 ml distilled water, sterilized by autoclaving at 121°C for 30 minutes. Petri dishes of 90 mm diameter were incubated in the dark at 25°C for 48 hours prior to subculturing. After 3 to 4 successive subcultures on PDA medium, pure fungal cultures were obtained and stored at 25°C in the dark within an incubator.

### 2.3. Identification of *Fusarium oxysporum*

Identification of phytopathogenic strains was primarily conducted through the examination of their macroscopic and microscopic characteristics [11]. The principal diagnostic features employed included:

- Cultural characteristics (color, mycelia appearance, etc.)
- Hyphal types (septate)
- Presence of macroconidia, abundant microconidia in the false head
- Presence of terminal or intercalary chlamydospores
- Phialide characteristics (monophialides)
- Phialide size (short)

Microscopic observations and descriptions of the colonies were performed on isolates cultured on PDA medium for 7 days at 25°C.

Descriptions were based on observation using a light microscope equipped with an integrated camera. For this purpose, a fragment of the fungal colony was aseptically transferred using a sterile platinum loop onto a microscope slide, stained with methyl blue and covered with a coverslip. Observations were carried out under the light microscope at  $G \times 40$  magnifications [12]. Cultural features such as colony appearance and pigmentation were recorded.

## 2.4. Single-Spore Isolation

For the collection of fungal isolates, it is essential to obtain single-spore cultures [13] [14]. The serial dilution technique was used for this purpose. Five ml of sterile distilled water were added to the surface of each culture maintained on PDA medium in 5 cm diameter dishes. The surface of the saturated cultures was gently scraped using the tip of a pipette. The resulting suspension containing conidia and mycelium fragments was filtered through sterilized muslin cloth. The filtrate was collected in tubes, and a series of successive tenfold dilutions was performed up to  $10^{-6}$ . From each dilution, 100  $\mu$ l of homogenized suspension was inoculated onto malt medium (composition) in 9 cm diameter Petri dishes (13 ml of medium per dish). After incubation for one to two days at 25°C in the dark, unicellular conidia that were sufficiently isolated and had formed germ tubes, were identified under a magnifying glass and subcultured at a rate of one colony per dish, onto malt medium supplemented with acid and an antibiotic (250 mg/l citric acid, 50 mg/l chlorine tetracycline, 100 mg/l streptomycin). Three successive subcultures on this medium ensure the bacterial purity of the monospore culture while fungal purity was guaranteed by the monospore subculturing itself. Ninety-one (91) isolates were thus purified and monosporic.

## 2.5. Evaluation of the Growth Rate of Fov Isolates

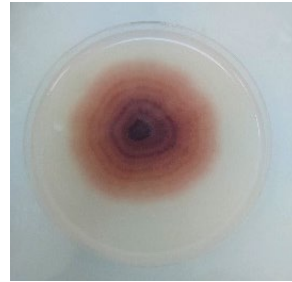
Due to the variability in the characteristics of *Fusarium oxysporum* isolates according to their geographic and plant origin [15], it was considered pertinent to characterize a subset of this isolate collection. For this purpose, 24 isolates from different geographic regions were selected based on morphological characteristics (morphotypes) revealed on culture media for further analysis. These isolates were chosen to be representative of all the morphotypes observed in the collection of 91 isolates. The growth rate of the selected isolates was evaluated on three different culture media over a period of 10 days. The media used were as follows:

- PDA (Potato Dextrose Agar) medium, consisting of 20 g of potato flakes, 20 g of glucose, 20 g of bacteriological agar, and 1 liter of distilled water. Before distributing the medium into 90 mm diameter Petri dishes under a fume hood, 0.25 g of citric acid was added to prevent bacterial growth.
- Czapeck-Dox Agar (CDA) medium, consisting of 3 g of NaNO<sub>3</sub>, 1 g of KH<sub>2</sub>PO<sub>4</sub>, 0.5 g of KCl, 0.5 g of MgSO<sub>4</sub> 7H<sub>2</sub>O, 0.01 g of FeSO<sub>4</sub> 7H<sub>2</sub>O, 30 g of sucrose, 20 g of bacteriological agar, and 1 liter of distilled water.
- Malt medium, the composition of which is 20 g of malt extract, 20 g of bacteriological agar and 1 liter of distilled water.

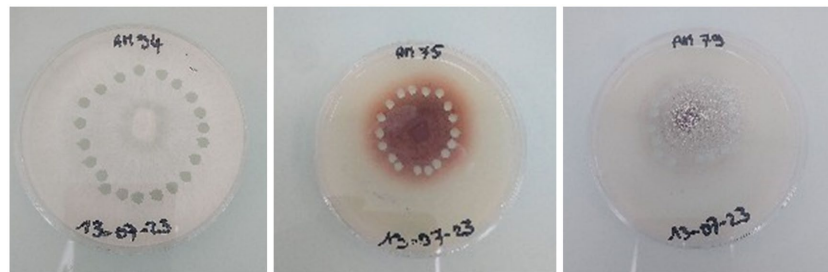
All isolates used for the growth rate evaluation were of the same age and were cultured on the same date. To standardize this, a fragment of purified mycelial colony was aseptically transferred using a sterile loop onto PDA medium in Petri dishes. The plates were incubated at 28°C in the dark for 10 days (**Figure 1**).

After 10 days of incubation, mycelial discs were excised from the periphery of the culture (**Figure 2**) using a 4 mm diameter punch and aseptically transferred

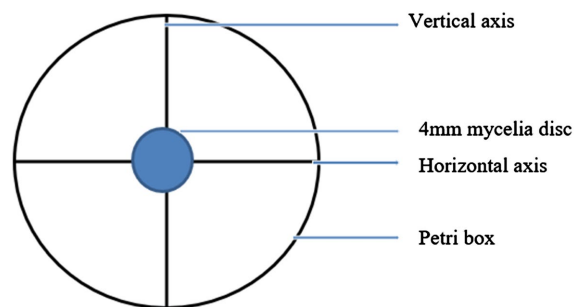
to the center of sterile Petri dishes containing the different solid culture media (PDA, CDA, MALT). Seven replicates were performed for each culture medium and for each isolate [16]. The diameter of the fungal colonies was measured daily along two perpendicular axes (**Figure 3**).



**Figure 1.** 10-day-old Fov isolate.



**Figure 2.** Cultures showing discs of mycelia taken.



**Figure 3.** Measurement of the diameter of a mycelia colony of Fov isolates.

Radial growth was calculated according to the formula of Sofi and collaborators [17].

$$Cr(mm) = (Cn - Cn_{-1})$$

**Cr:** Radial growth

**Cn:** Diameter observed on a given day (mm)

**Cn<sub>-1</sub>:** Colony diameter on day n – 1 (mm)

## 2.6. Determination of Spore Concentration of Isolates

The spore concentration of *Fusarium oxysporum* f.sp. *vasinfectum* isolates was de-

terminated from 10-day-old cultures. Five ml of distilled water were added to each Petri dish, and the surface was gently scraped using a Pasteur pipette. A drop of the resulting suspension was then placed on a blade of Malassez. Spore counts for each culture medium were repeated three times by enumerating spores along the diagonals. The spore concentration of the isolates was calculated using the formula:

$$Q = 2n \times 10^6 \text{ spores/ml}$$

$n$  is the average number of spores counted in the smallest square of the blade.

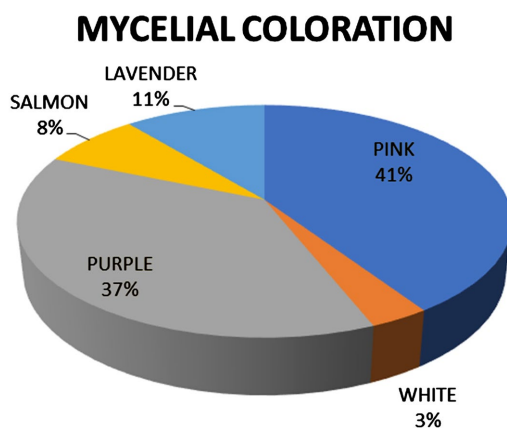
## 2.7. Statistical Analysis

All data were recorded in Excel 2007 which was used to generate graphs and pie charts. The raw data collected during this study were analyzed using STATISTICA 7.1 software. A one-way analysis of variance (ANOVA) was performed for the parameters. When a significant difference was detected among means ( $P < 0.05$ ), pairwise comparisons were performed using Fisher's LSD tests at the 5% significance level.

## 3. Results

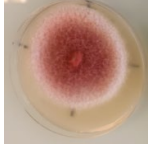
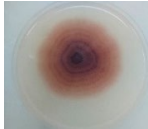
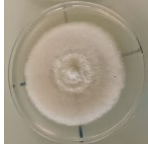


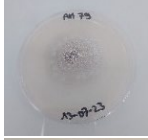
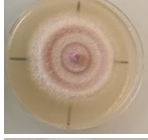
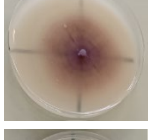
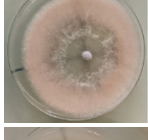

### 3.1. Cultural Characteristics

For the cultural characterization of Fov isolates, the following criteria were considered: mycelia appearance and colony color on PDA culture medium. After isolation, isolates were subcultured on PDA medium, after the fourth subculture showed variability in mycelial appearance and colony pigmentation. **Figure 4** shows the percentage of different mycelial colorations observed among the isolates post-isolation. Of all strains 37 isolates (41%) displayed a pink coloration, 34 isolates (37%) exhibited a purple coloration, 3 isolates were white, and 7 isolates showed a salmon coloration and 10 isolates presented lavender mycelial pigmentation (**Figure 4**). The analysis of the pivot table encompassing mycelium appearance parameters and colonies coloration allows the classification of the isolates into homogeneous groups. Based on the different characteristics assessed, ten (10) groups of isolates were identified (**Table 1**).



**Figure 4.** Percentage of different mycelia colorations of fungal isolates.

**Table 1.** Composition of the different groups and their characteristics.

	Characteristics	Number of isolates	List of isolates	Figures
<b>Group 1</b>	Pink colonies; Cottony dense mycelium	21	AM 10, AM 12, AM 16, AM 17, AM 18, AM 23, AM 25, AM 26, AM 27, AM 30, AM 49, AM 55, AM 56, AM 65, AM 67, AM 80, AM 83, AM 84, AM 115, AM 42, AM 45	
<b>Group 2</b>	Pink colonies; Short cottony mycelium	16	AM 15, AM 19, AM 48, AM 52, AM 53, AM 57, AM 73, AM 72, AM 75, AM 76, AM 78, AM 79, AM 82, AM 105, AM 47, AM 122.	
<b>Group 3</b>	White colonies, Cottony dense mycelium	3	AM 97, AM 98, AM 94.	
<b>Group 4</b>	Purple colonies, Cottony dense mycelium	23	AM 1, AM 3, AM 4, AM 13, AM 21, AM 29, AM 50, AM 51, AM 58, AM 62, AM 66, AM 71, AM 74, AM 91, AM 93, AM 95, AM 96, AM 100, AM 101, AM 44, AM 121, AM 123, AM 124.	
<b>Group 5</b>	Salmon colonies, Fin and short mycelium	1	AM 9	
<b>Group 6</b>	Lavender colonies, Short cottony mycelium	5	AM 2, AM 33, AM 116, AM 117, AM 118	
<b>Group 7</b>	Lavender colonies; Cottony dense mycelium	5	AM 20, AM 22, AM 24, AM 59, AM 120	
<b>Group 8</b>	Purple colonies, Short cottony mycelium	11	AM 1.1, AM 11, AM 14, AM 54, AM 60, AM 69, AM 70, AM 90, AM 41, AM 46, AM 119	
<b>Group 9</b>	Salmon colonies, Cottony dense mycelium	3	AM 81, AM111, AM 112.	
<b>Group 10</b>	Salmon colonies, Short cottony mycelium	3	AM 5, AM 28, AM 61	

### 3.2. Effect of Culture Media on Radial Growth of Fov Isolates

To characterize Fov isolates, their mycelial growth on various culture media (PDA, MALT, and CZAPECK) and spore concentrations were evaluated using 24 representative isolates from the collection (**Table 2**).

**Table 2.** List of representative isolates from the collection.

Order Number	Isolates	Place of origin	Mycelia appearance	Colony coloring
1	AM 61	Madoukaha (M)	Cottony and short	Salmon
2	AM 20	Kounahiri	Cottony and dense	Lavender
3	AM 2	Nakaha (bouaflé)	Cottony and short	Lavender
4	AM 55	Tchatchatcha	Cottony and dense	Pink
5	AM 3	Ténéféro	Cottony and dense	Purple
6	AM 48	Gona	Cottony and short	Pink
7	AM 75	Pinguékaha	Cottony and short	Pink
8	AM 12	Tomono	Cottony and dense	Pink
9	AM 105	Sinématialy	Cottony and short	Pink
10	AM 94	Gnonkaha (napié)	Cottony and dense	White
11	AM 97	Napié	Cottony and dense	White
12	AM 16	Tiéma	Cottony and dense	Pink
13	AM 9	Soudougouba	Fin and short	Salmon
14	AM 116	Niakara	Fin and short	Lavender
15	AM 98	Napié	Cottony and dense	White
16	AM 33	Nianon (boundialy)	Cottony and short	Lavender
17	AM 74	Tiépadougou	Cottony and dense	Purple
18	AM 47	Sarhala	Cottony and dense e	Pink
19	AM 90	Ouangolo	Cottony and short	Purple
20	AM 79	Marabadjassa	Cottony and short	Pink
21	AM 72	Gbeko	Cottony and short	Pink
22	AM 81	Kongassou	Cottony and dense	Salmon
23	AM 96	Napié	Cottony and dense	Purple
24	AM 28	Tiéningboué	Cottony and short	Salmon

The mean radial growth rates of Fov isolates were significantly greater on CDA and Malt medium compared to PDA medium (**Table 3**).

At day 8, the growth rates of Fov isolates on the three culture media ranged from  $33.91 \pm 3.35$  to  $86.00 \pm 0.00$  mm (**Table 3**). Specifically, on PDA medium, mycelial expansion varied between  $37.75 \pm 3.76$  to  $86.00 \pm 0.00$  mm; on MALT, it ranged from  $35.66 \pm 3.14$  to  $86.00 \pm 0.00$  mm. The growth rates of isolates on CDA medium ranged from  $33.91 \pm 3.35$  to  $86.00 \pm 0.00$  mm. Isolates AM 94 and AM

98 exhibited the highest mycelial growth across PDA, MALT, and CDA media. Statistical analysis revealed a highly significant difference between isolates ( $P < 0.0001$ ). The maximum average growths are respectively  $64.39 \pm 14.58$  mm on CDA, followed by  $57.88 \pm 14.04$  mm on malt and  $56.11 \pm 11.90$  mm on PDA medium. Statistical analysis revealed that, after 8 days of incubation, a highly significant difference ( $P < 0.0001$ ) was observed among the different media. The radial growth of the fungus was found to vary significantly depending on the type of medium.

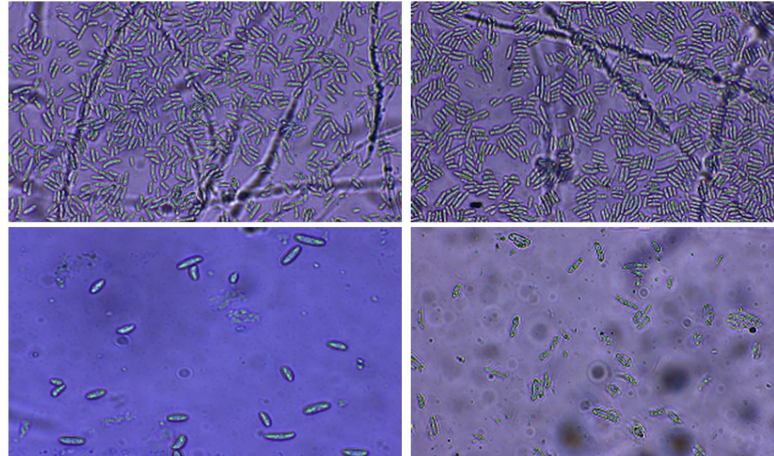
**Table 3.** Radial growth of Fov isolates on different culture media.

Isolates	MIDDLE PDA	MIDDLE MALT	MIDDLE CDA
AM 9	$42.41 \pm 7.17^l$	$35.66 \pm 3.14^l$	$46.33 \pm 4.67^{ij}$
AM 61	$56.00 \pm 5.47^{efg}$	$55.91 \pm 2.15^{ghi}$	$61.83 \pm 4.15^{fg}$
AM 72	$46.08 \pm 2.15^{kl}$	$52.83 \pm 2.48^{hij}$	$71.83 \pm 8.47^{cd}$
AM 20	$55.16 \pm 2.04^{efgh}$	$51.00 \pm 3.16^{ijk}$	$58.91 \pm 6.65^{gh}$
AM 81	$54.16 \pm 2.63^{fghi}$	$53.50 \pm 2.73^{hij}$	$64.08 \pm 8.89^{efg}$
AM 12	$56.83 \pm 5.84^{def}$	$60.91 \pm 15.93^{efg}$	$74.75 \pm 9.96^{bc}$
AM 90	$37.75 \pm 3.76^m$	$38.58 \pm 2.97^l$	$33.91 \pm 3.35^k$
AM 3	$57.41 \pm 5.88^{def}$	$70.41 \pm 4.56^{bc}$	$72.50 \pm 5.00^{cd}$
AM 55	$54.08 \pm 6.39^{fghi}$	$58.41 \pm 3.92^{fgh}$	$67.83 \pm 8.01^{cdef}$
AM 33	$57.00 \pm 1.26^{def}$	$72.5 \pm 4.54^b$	$70.33 \pm 6.71^{cde}$
AM 48	$49.16 \pm 2.13^{jk}$	$46.33 \pm 9.28^k$	$49.25 \pm 8.62^i$
AM 16	$50.33 \pm 3.82^{hijk}$	$47.33 \pm 3.61^{jk}$	$52.16 \pm 7.30^{hi}$
AM 79	$51.00 \pm 6.25^{hij}$	$56.58 \pm 7.90^{ghi}$	$72.66 \pm 5.16^{cd}$
AM 75	$49.66 \pm 4.96^{ijk}$	$45.66 \pm 4.96^k$	$41.08 \pm 2.53^{jk}$
AM 74	$46.00 \pm 0.00^{kl}$	$37.66 \pm 2.58^l$	$47.66 \pm 4.08^{ij}$
AM 105	$51.58 \pm 3.55^{ghij}$	$54.66 \pm 4.16^{hi}$	$70.66 \pm 9.00^{cde}$
AM 28	$57.33 \pm 3.55^{def}$	$65.00 \pm 1.26^{cde}$	$65.5 \pm 11.89^{defg}$
AM 47	$52.66 \pm 6.83^{fghij}$	$51.08 \pm 4.86^{ijk}$	$61.75 \pm 4.77^{fg}$
AM 96	$59.33 \pm 4.08^{cde}$	$63.58 \pm 4.22^{def}$	$71.00 \pm 5.54^{cde}$
AM 97	$67.66 \pm 5.16^b$	$66.33 \pm 7.11^{bcde}$	$81.00 \pm 0.00^{ab}$
AM 2	$61.50 \pm 0.83^{cd}$	$69.33 \pm 5.78^{bcd}$	$72.66 \pm 9.83^{cd}$
AM 94	$86.00 \pm 0.00^a$	$86.00 \pm 0.00^a$	$86.00 \pm 0.00^a$
AM 98	$86.00 \pm 0.00^a$	$86.00 \pm 0.00^a$	$86.00 \pm 0.00^a$
AM 116	$63.66 \pm 3.38^{bc}$	$63.91 \pm 4.73^{def}$	$65.66 \pm 2.58^{defg}$
Average	<b><math>56.11 \pm 11.90</math></b>	<b><math>57.88 \pm 14.04</math></b>	<b><math>64.39 \pm 14.58</math></b>
Probability	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>
CV%	<b>21.20</b>	<b>24.25</b>	<b>22.64</b>

*For each average, the values bearing the same letters (a, b, c, and d) in the same column are statistically identical to the 5% threshold.*

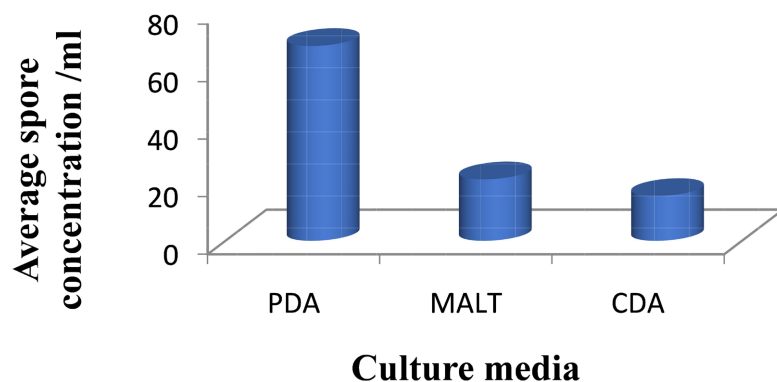
### 3.3. Spore Concentration of Fov Isolates

Fov isolates cultured on PDA, MALT, and CDA media showed different sporulation depending on the culture medium. **Figure 5** shows some microscopic views of the spores of Fov



**Figure 5.** Microscopic views of spores of some Fov isolates.

The spore concentration of Fov isolates varied across all culture media from 0 spores/ml to  $2.69 \times 10^8$  spores/ml (**Figure 6**).



**Figure 6.** Effect of different culture media on spore concentration of Fov isolates.

On PDA and MALT media, concentrations were 0 spores/ml to  $2.69 \times 10^8$  spores/ml and 0 spores/ml to  $1.18 \times 10^8$  spores/ml, respectively. On CDA medium, it was 0 to  $2.05 \times 10^7$  spores/ml. Isolates AM 98, AM 90, AM 9, AM 94, and AM 61 produced fewer spores across all three-culture media (**Table 4**). The highest spore counts came from isolates AM 16 on PDA medium, AM 12 on MALT medium, and AM 105 on CDA medium.

Statistical analysis showed a highly significant difference between isolates ( $P < 0.001$ ) and between culture media ( $P < 0.001$ ).

PDA medium allowed the isolates to produce a large number of spores. This medium revealed greater variability in spore concentrations among Fov isolates,

with up to fifteen (15) homogeneous groups. MALT and CDA media only presented eight (8) and seven (7), respectively (**Table 4**).

**Table 4.** Spore concentration of Fov isolates on different culture media.

Isolates	MIDDLE PDA	MIDDLE MALT	MIDDLE CDA
AM 9	32.00 ± 0.00 <sup>ghi</sup>	1.33 ± 0.66 <sup>f</sup>	0.00 ± 0.00 <sup>f</sup>
AM 61	5.33 ± 0.66 <sup>ik</sup>	1.33 ± 0.66 <sup>f</sup>	1.33 ± 0.66 <sup>f</sup>
AM 72	172.66 ± 7.42 <sup>b</sup>	66.66 ± 6.76 <sup>b</sup>	2.00 ± 0.00 <sup>f</sup>
AM 20	16.66 ± 4.05 <sup>ijk</sup>	5.33 ± 1.33 <sup>f</sup>	2.66 ± 1.33 <sup>f</sup>
AM 81	166.00 ± 18.33 <sup>b</sup>	16.00 ± 1.15 <sup>de</sup>	1.33 ± 1.33 <sup>f</sup>
AM 12	58.66 ± 2.90 <sup>def</sup>	118.66 ± 1.76 <sup>a</sup>	1.33 ± 0.66 <sup>f</sup>
AM 90	0.00 ± 0.00 <sup>k</sup>	0.00 ± 0.00 <sup>f</sup>	0.00 ± 0.00 <sup>f</sup>
AM 3	43.33 ± 3.71 <sup>fgh</sup>	4.66 ± 0.66 <sup>f</sup>	0.00 ± 0.00 <sup>f</sup>
AM 55	40.66 ± 1.76 <sup>fghi</sup>	20.66 ± 1.76 <sup>d</sup>	64.00 ± 2.30 <sup>c</sup>
AM 33	52.00 ± 4.00 <sup>defg</sup>	6.66 ± 2.40 <sup>ef</sup>	2.00 ± 1.15 <sup>f</sup>
AM 48	16.66 ± 1.76 <sup>ijk</sup>	63.33 ± 3.71 <sup>b</sup>	2.66 ± 0.66 <sup>i</sup>
AM 16	269.33 ± 15.37 <sup>a</sup>	40.00 ± 7.57 <sup>c</sup>	45.33 ± 6.35 <sup>d</sup>
AM 79	20.00 ± 5.03 <sup>hijk</sup>	2.66 ± 6.11 <sup>f</sup>	4.66 ± 0.66 <sup>f</sup>
AM 75	151.33 ± 20.82 <sup>b</sup>	2.66 ± 0.66 <sup>f</sup>	4.66 ± 2.66 <sup>f</sup>
AM 74	74.66 ± 3.71 <sup>cd</sup>	1.33 ± 0.66 <sup>f</sup>	0.00 ± 0.00 <sup>f</sup>
AM 105	174.00 ± 15.62 <sup>b</sup>	44.00 ± 6.92 <sup>c</sup>	104.66 ± 11.85 <sup>a</sup>
AM 28	48.00 ± 0.00 <sup>efg</sup>	20.00 ± 4.61 <sup>d</sup>	6.66 ± 1.76 <sup>f</sup>
AM 47	92.66 ± 9.33 <sup>c</sup>	37.30 ± 2.66 <sup>c</sup>	8.66 ± 2.66 <sup>ef</sup>
AM 96	32.00 ± 13.11 <sup>ghi</sup>	6.00 ± 4.00 <sup>f</sup>	92.00 ± 12.70 <sup>b</sup>
AM 97	29.33 ± 4.66 <sup>ghij</sup>	6.00 ± 1.15 <sup>f</sup>	2.00 ± 0.00 <sup>f</sup>
AM 2	69.33 ± 2.40 <sup>cde</sup>	4.66 ± 1.76 <sup>f</sup>	20.00 ± 8.08 <sup>e</sup>
AM 94	0.00 ± 0.00 <sup>k</sup>	00.00 ± 0.00 <sup>f</sup>	0.00 ± 0.00 <sup>f</sup>
AM 98	2.00 ± 0.00 <sup>k</sup>	0.00 ± 0.00 <sup>f</sup>	0.00 ± 0.00 <sup>f</sup>
AM 116	75.33 ± 8.66 <sup>cd</sup>	8.66 ± 3.71 <sup>ef</sup>	11.33 ± 5.33 <sup>ef</sup>
Average	<b>67.25 ± 8.30</b>	<b>21.30 ± 3.40</b>	<b>15.58 ± 3.54</b>
Probability	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>
CV%	<b>12.34</b>	<b>15.96</b>	<b>22.72</b>

For each average, the values bearing the same letters (a, b, c, and d) in the same column are statistically identical to the 5% threshold.

#### 4. Discussion

Following isolation, variability in cultural traits was observed upon subculturing. This variability indicates the presence of significant heterogeneity within the collected strains, which would be attributed to genetic factor (heritable across gener-

ations) and environment influences such as rainfall, temperature and relative humidity. Tigist and collaborators [18] also identified genotype and the environment components as the main sources of variability in genotypes. Furthermore, this variability may be explained by combinations of evolutionary mechanisms (mutation, natural selection), environmental constraints and biotic interactions.

In addition, variability in cultural traits has been reported by several authors [19] [20] who noted that the transplanted isolates exhibited certain variability in cultural characteristics. This underlines the importance of integrated management approaches to limit the emergence of adaptive pathogenic strains. Furthermore, Sedra and Djerbi [21] attributed this variability in cultural traits to mutations. As a predominantly clonal fungus, *Fusarium oxysporum* relies on mutation and other mechanisms like parasexuality to generate the significant genetic diversity observed in its populations. Indeed, mutations promote the acquisition of considerable genetic variability in these fungi, thereby facilitating the generation of novel genomic when a mutation occurs in a coding region resulting in an amino acid substitution; it can be deleterious to the host plants [22].

In other words, such mutations are advantageous for the fungus, enabling better adaptation to environmental changes, including variations in the host and surroundings. Consequently, the mutant strain gradually out competes the original fungus, involving into a new strain that is generally more aggressive than its predecessor. The high variability of Fov amplifies the risk of overcoming monogenic resistance. This resistance therefore becomes short-lived and threatens the sustainability of cotton varieties, making the development of resistant and durable cultivars necessary. This high variability of Fov strains observed in this study should alert stakeholders to implement stringent control measures against *Fusarium* wilt of cotton, including research into and deployment of cultivars with enhanced resistance to this disease.

Mycelia growth of Fov isolates on three culture media at day 8 ranged from  $33.91 \pm 3.35$  to  $86.00 \pm 0.00$  mm. On PDA medium, mycelial growth ranged from  $37.75 \pm 3.76$  to  $86.00 \pm 0.00$  mm, on MALT, it was from  $35.66 \pm 3.14$  to  $86.00 \pm 0.00$  mm. As for the growth of isolates on CDA medium, it ranged from  $33.91 \pm 3.35$  to  $86.00 \pm 0.00$  mm.

This variation in mycelial growth may be explained by the influence of nutritional factors. Indeed, Ansari and collaborators [23] showed that fungal growth is strongly influenced by factors such as nitrogen source, carbon source, substrate pH and temperature. Majumdar and Mandal [24] further revealed that fungal growth largely depends on the quality of the growth medium which plays a major role in determining the anatomical, morphological and physiological characteristics of fungi. Kumara and Rawal [25] revealed that different types of media affect colony growth, morphology, pigmentation and sporulation.

These results are consistent with the findings of the present study, as the same isolate cultured on three different media exhibited variations in growth and colony morphology.

Fungal growth was influenced by the culture medium. The experimental results revealed that after 8 days of incubation, CDA medium was the most conducive to the mycelial development of Fov. The maximum growth rates recorded were  $64.39 \pm 14.58$  mm for CDA, followed by MALT at  $57.88 \pm 14.04$  mm and PDA at  $56.11 \pm 11.90$  mm.

This indicates that CDA medium, which is richer in glucose promotes superior development of Fov. These results corroborate those of Farooq and collaborators [26], who demonstrated that Czapeck-dox medium induced maximum radial growth of *Fusarium oxysporum* f. sp. *Ciceris*.

Furthermore, glucose was identified as the most effective carbon source for optimal mycelial growth. The results indicate that all tested carbon sources support fungal growth to some extent, as fungi can metabolize some complex carbon compounds by converting them into simpler forms [27] [28].

The composition of the culture medium constitutes an important factor for mycelial growth. In addition to the carbon source, other elements present in the medium, such as nitrogen, phosphorus, sulfur, vitamins, and metal ions, iron and magnesium, are essential for fungal growth and development.

Sporulation of Fov isolates varied both among isolates and across culture media. PDA medium was found to be most favorable for sporulation, whereas MALT and CDA media supported comparatively low sporulation compared to PDA medium. Hanlin [29] cited by Majumdar and Mandal [24] indicated that sporulation is a complex process regulated by both environment and genetic factors, which may differ among isolates. These observations are consistent with those of Khan and collaborators [30] who reported that PDL medium induces maximal spore production by *Fusarium oxysporum* f. sp. *ciceris*. They further found that media containing organic components are most conducive to sporulation. Similarly, Atrasi and collaborators [31] confirmed that PDA medium facilitates robust sporulation of apple pathogenic fungi.

Overall, the results indicate that this pathogen is capable of growth, sporulation and metabolite synthesis, albeit to varying degrees depending on the culture conditions.

## 5. Conclusion

This study aimed to evaluate the morphometric characteristics of *Fusarium oxysporum* f.sp. *vasinfectum* populations in Côte d'Ivoire. The results obtained reveal considerable variability in mycelial morphology and colony pigmentation. Furthermore, these findings indicate that mycelial growth and sporulation two key developmental processes of the fungus, exhibit distinct nutritional requirements. The fungus prefers to grow in carbon-rich environments like the CDA medium and sporulates better in environments with organic elements like the PDA medium. This research may have practical applications, such as developing media for high-density inoculum production for resistance screening or for maximizing mycelial biomass for molecular studies. Morphological characterization of Fov

isolates should be completed by molecular characterization to assess the genetic diversity of Fov populations in Côte d'Ivoire. Concurrently, investigating the host-pathogen relationship would be valuable to compare the genetic structure of the pathogen with that of the host plant. Such studies will contribute to elucidating the dynamics of the host-pathogen interactions and to establishing an effective strategy to manage *Fusarium* wilt of cotton.

### Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

### References

- [1] Hussein, K., Perret, C. and Hitimana, L. (2005) Importance économique et sociale du coton en Afrique de l'Ouest: Rôle du coton dans le développement, le commerce et les moyens d'existence. OCDE SAH/D556. 71p.
- [2] ICAC (2022) World Cotton Outlook Report 2022. *Bulletin of the International Cotton Advisory*.
- [3] Aiwa, A.D. (2015) L'impact de la culture du coton sur le développement socio-économique: Étude de cas de la région de Korhogo, au nord de la Côte d'Ivoire. *Européen Scientifique Revue*, **11**, 1857-1881.
- [4] Rathore, K.S., Campbell, L.M., Sherwood, S. and Nunes, E. (2015) Cotton (*Gossypium hirsutum* L.). *Methods in Molecular Biology*, **1224**, 11-23.
- [5] CCIC (2021) Comité Consultatif International du Coton, Communication de la Côte d'Ivoire à la 79<sup>ème</sup> plénière. <https://sports.abidjan.net/>
- [6] Kouame, A., Amangoua, N., Kouadia, A., Akanvou, L., Gnapi, D., Guessan, E., *et al.* (2024) Distribution of Fusarium Wilt of Cotton in Côte d'Ivoire and Evaluation of the Pathogenicity of Isolates of the Causal Agent *Fusarium oxysporum* F.sp. *Vasinfectum* (Atk.) Snyder and Hansen. *Journal of Research in Agriculture and Food Sciences*, **2**, 31-38. <https://doi.org/10.5455/jrafs.20241010032228>
- [7] Sayegh, M. (2009) La résistance du cotonnier *Gossypium hirsutum* à la bactériose causée par *Xanthomonas campestris* pathovar *malvacearum*. Rôle du gène GhLOX1 dans la réaction hypersensible. Thèse de doctorat, Institut National Polytechnique de Lorraine-Nancy, France. 155p.
- [8] ICAC (2018) Cotton: World Statistics. *Bulletin of the International Cotton Advisory*, 4 p.
- [9] El Hassni, M., El Hadrami, A., Daayf, F., Chérif, M., Barka, E.A. and El Hadrami, I. (2007) Biological Control of Bayoud Disease in Date Palm: Selection of Microorganisms Inhibiting the Causal Agent and Inducing Defense Reactions. *Environmental and Experimental Botany*, **59**, 224-234. <https://doi.org/10.1016/j.envexpbot.2005.12.008>
- [10] Faurie, B., Cluzet, S. and Méryllon, J.M. (2009) Implication of Signaling Pathways Involving Calcium, Phosphorylation and Active Oxygen Species in Methyl Jasmonate-Induced Defense Responses in Grapevine Cell Cultures. *Journal of Plant Physiology*, **166**, 1863-1877. <https://doi.org/10.1016/j.jplph.2009.05.015>
- [11] Campbell, C.K., Johnson, E.M. and Warnock, D.W. (2013). Identification of Pathogenic Fungi. Wiley. <https://doi.org/10.1002/9781118520055>
- [12] Chabasse, D., Bouchara, J.P., De Gentile, L., Brun, S., Cimon, B. and Penn, P (2002)

- Les moisissures d'intérêt médical, cahier de formation en biologie médicale No. 25. <https://lesbiologistesmedicaux.fr/>
- [13] Rapilly, F. (1968) Les techniques de mycologie en pathologie végétale. *Annales des epiphyties*, **19**, 102.
- [14] Abo, K., Klein, K.K., Edel-Hermann, V., Gautheron, N., Traore, D. and Steinberg, C. (2005) High Genetic Diversity among Strains of *Fusarium oxysporum* F. Sp. *Vasinfectum* from Cotton in Ivory Coast. *Phytopathology*, **95**, 1391-1396. <https://doi.org/10.1094/phyto-95-1391>
- [15] Djerbi, M. (1990) Méthodes de diagnostic du bayoud. *OEPP/EPPO Bulletin*, **20**, 607-613.
- [16] Monowara, R., Tonu, N.N., Begum, F., Karim, M.M. and Sultana, N. (2017) Morphological and Physiological Variation among Different Isolates of *Alternaria* spp. from Rapeseed-Mustard. *International Journal of Environment, Agriculture and Biotechnology*, **2**, 2433-2442. <https://doi.org/10.22161/ijeab/2.5.21>
- [17] Sofi, T.A., Beig, M.A., Ahmad, M., Hamid, A., Ahangar, F.A., *et al.* (2013) Cultural, Morphological, Pathogenic and Molecular Characterization of *Alternaria* Mali Associated with *Alternaria* Leaf Blotch of Apple. *African Journal of Biotechnology*, **12**, 370-381. <https://doi.org/10.5897/ajb12.2735>
- [18] Tigist, S.G., Sibiya, J., Amelework, A. and Keneni, G. (2023) Agromorphological and Physiological Performance of Ethiopian Common Bean (*Phaseolus vulgaris* L.) Genotypes under Different Agroecological Conditions. *Plants*, **12**, Article 2342. <https://doi.org/10.3390/plants12122342>
- [19] Sedra, M.H. (2003) Le bayoud du palmier dattier en Afrique du nord. Publication du bureau sous régional de la FAQ pour l'Afrique du nord (SNEA) en protection des plantes. 124p.
- [20] Kadri, Y. (2008) Caractérisation de la variabilité biologique et pathologique de *Fusarium oxysporum* f.sp. *albedinis* (Killian et Maire) W.L. Gordon et essai de méthodes de lutte «*in Vitro*». Thèse de Magister en Science Agronomiques, ENSA.
- [21] Sedra, M.H. and Djerbi, M. (1985) Mise au point d'une méthode rapide et précise d'identification *in Vitro* du *Fusarium oxysporum* f.sp. *albedinis*, agent causal du Bayoud. *Annales de l'Institut national de la recherche agronomique de Tunisie*, **2**, 1-12.
- [22] Kostyrka, G. (2018) La place des virus dans le monde vivant. Mémoire de Doctorat en Philosophie. Université Panthéon Sorbonne, 845p.
- [23] Ansari, A., Khanzada, M.A., Rajput, M.A., Maitlo, S., Rajput, A.Q. and Ujjan, A. (2018) Effect of Different Abiotic Factors on the Growth and Sporulation of *Colletotrichum gloeosporioides* Causing Anthracnose of Mango. *Plant Protection*, **2**, 23-30.
- [24] Majumdar, N. and Mandal, N.C. (2018) Effect of Different Modified Growth Media on Postharvest Pathogens. *Research on Crops*, **19**, 520-525.
- [25] Kumara, K.L.W. and Rawal, R.D. (2008) Influence of Carbon, Nitrogen, Temperature and pH on the Growth and Sporulation of Some Indian Isolates of *Colletotrichum gloeosporioides* Causing Anthracnose Disease of Papaya (*Carrica papaya* L). *Tropical Agricultural Research and Extension*, **11**, 7-12. <https://doi.org/10.4038/tare.v11i0.1779>
- [26] Farooq, S., Iqbal, S.H.M. and Abdul Rauf, C.H. (2005) Physiological Studies of *Fusarium oxysporum* f. sp. *Ciceri*. *International Journal of Agriculture and Biology*, **7**, 275-277.

- [27] Bais, B.S., Singh, S.B. and Singh, D.V. (1970) Effect of Different Carbon and Nitrogen Sources on the Growth and Sporulation of *Curvularia pallescens*. *Indian Phytopathology*, **23**, 511-517.
- [28] Mechta, N. and Tassadit, A. (2015) *Fusarium oxysporum* f. sp. Albedinis: Effets du milieu de culture sur la croissance mycélienne, la sporulation et la production de l'acide fusarique. *Algerian Journal of Arid Environment*, **5**, 82-90.
- [29] Ulloa, M. and Hanlin, R.T. (2001) Illustrated Dictionary of Mycology. *Mycological Research*, **105**, 383-384.
- [30] Khan, A.J., Azam, K.M. and Razvi, S.A. (2001) Pesticide Residue Analysis of Date Palm Fruits by Gas Chromatography Mass Spectrophotometry. *2nd International Conference on Date Palms*, Al-Ain, 25-27 March 2001, 211-215.
- [31] Attrassi, K., Selmaoui, K., Ouazzani, T.A., Badoc, A. and Douira, A. (2005) Biologie et physiologie des principaux agents fongiques de la pourriture des pommes en conservation et lutte chimique par l'azoxystrobine. *Bulletin de la Société de Pharmacie de Bordeaux*, **144**, 47-62.