# Morphological Characterization of Alternaria Brassicicola Strains Combined with Cabbage Cultivation in Côte d\'Ivoire

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# Morphological Characterization of *Alternaria Brassicicola* Strains Combined with Cabbage Cultivation in Côte d'Ivoire

.


## Abstract

Cabbage (*Brassica oleraceae* L.) is a leaf vegetable of global importance due to its high nutritional and economic value. However, its cultivation is threatened by Alternaria leaf spot, a disease that impacts cabbage yield and market value. It is caused by fungi of the *Alternaria*genus, particularly *A. brassicicola*, which has several morphologies. This study therefore aims at investigating the morphology of five *A. brassicicola*strains isolated from cabbage in Côte d'Ivoire. To this end, these previously isolated strains were cultured on PDA culture medium, and their cultural and microscopic characteristics were determined. Thus, the diameter of the fungal colonies was measured at 24-hour intervals over 12 days so as to determine their growth rate. Microscopic observation also revealed specific characteristics related to conidia production. Macroscopic observations revealed colonies that were greenish-brown in the center and light brown at the edges, with a dense, carpet-like appearance. Mycelial growth rate varied significantly from one strain to another, with mean diameters ranging from 7.01 to 8.50 cm after 12 days of incubation. Strain A5 showed the fastest growth. In terms of sporulation, strain A5 also showed the highest spore production, followed by strain A4. These results provide an essential basis for understanding the epidemiological behavior of these different *A. brassicicola*strains in Côte d'Ivoire.



**Keywords**: cabbage, pathogen, *Alternaria brassicicola*, morphological characteristics


## Introduction

Cabbage (*Brassica oleracea* L.) is a vegetable species native to the Mediterranean region, now cultivated in many regions worldwide. It occupies an important place in the human diet due to its high content of vitamins (A, C, K), antioxidants, and dietary fiber (Wang *et al*., 2022). This gives it both recognized nutritional and therapeutic properties. Cabbage is also an essential part of the human diet due to its affordability and availability in local markets (Sugier*et al*., 2023).

In Côte d'Ivoire, cabbage is one of the main vegetable crops consumed locally. Although official sector statistics remain incomplete, overall estimates indicate a yield of more than 740 000 tons of fresh vegetables, including cabbage, in 2023 (RVO, 2023). Cabbage contributes to

rural and urban household incomediversification in Côte d'Ivoire through short marketing channels in urban areas such as Abidjan, Yamoussoukro, and Bouaké. It thus contributes to national food security, poverty reduction, and the economic resilience of smallholder farmers (Kouakou, 2024).

Despite its economic importance and numerous virtues, cabbage cultivation is subject to severe pest pressure, particularly from fungi (Meena *et al*., 2017).

Among these diseases, Alternaria leaf spot, mainly caused by *Alternaria brassicae*and *Alternaria brassicicola*, is one of the most feared. These necrotrophic fungi cause circular leaf lesions, which lead to defoliation, reduced photosynthesis, and ultimately significant yield losses (Blagiojević*et al*., 2020). Moreover, these pathogens also affect crop quality, reducing cabbagemarket value and limiting the yield of high-quality products (Goyal *et al*., 2020). *Alternaria* sp. spores are preserved in plant debris, seeds, and soil. They are spread by rain splashes or air currents, thus promoting rapid reinfection of cultivated plots. Studies have shown that climatic conditions characterized by high relative humidity (>90%) and moderate temperatures (20–25°C) are conducive to Alternaria leaf spot epidemiology (Kumar *et al*., 2019).

In Côte d'Ivoire, leaf symptoms typical of Alternaria leaf spot have been observed on cabbage plants (*Brassica oleracea* L.). Isolates from infected tissue made it possible to get several *Alternaria brassicicola*fungal colonies. This study was conducted in order to study the morphological diversity of previously isolated *A. brassicicola*strains.

## Material andmethods

### Culture of fungal strains on PDA medium

The different *A. brassicicola*strains (A1, A2, A3, A4, and A5) that were isolated were transferred to PDA (Potato Dextrose Agar) culture medium for the purpose of observing their mycelial growth. Before inoculation, two perpendicular lines were drawn on the back of each 90-mm diameter Petri dish so as to mark the axes for measuring the radial development of the colonies. A volume of 10 ml of PDA medium was poured into each dish. For each strain, a fungal inoculum was taken using a sterile agar punch in the form of a 7-mm diameter mycelial disc. The inoculum was cut from the edge of 7-day-old colonies previously cultured on PDA. The inoculum disc was then placed in the center of the solidified PDA medium, at the intersection of the two perpendicular diameters, with the mycelial side in contact with the medium. The Petri dishes were then hermetically sealed with paraffin film.

The cultures were incubated at laboratory room temperature (25 ± 2 °C). Regular monitoring of fungal growth was carried out for fourteen (14) days, at 24-hour intervals, in order to observe the evolution of the colonies in terms of growth rate, texture, and mycelium coloration. These observations made it possible to characterize the different morphological aspects of each strain. Six Petri dishes (n = 6) were used for each fungal strain. The mycelial growth measurements were then used to determine the mean radial growth rate of the colonies on the PDA medium.

**Measurement of fungal straincolony diameters**

Fungal colony diameters were measured daily using a graduated ruler along the two straight lines (Figure 6). Measurements were stopped when a strain had completely colonized the culture medium contained in a Petri dish. The mean colony diameter was calculated for each strain using the following formula:

$$\mathrm{Md} = \frac{1}{n}\sum\left(\frac{d1+d2}{2}\right) (1)$$

Md: Mean diameter (cm), d1: diameter along axis 1, d2: diameter along axis 2, n: number of dishes per strain.

**Description of the cultural and microscopic characteristics of fungal strains**

The macroscopic characteristics were described on 14-day-old strains cultured on PDA medium. These macroscopic characteristics were the size, color, appearance, and texture of the mycelial colonies. The strains were described using the identification keys of Bany and Barnett (1972) and Botton *et al*. (1990).

Next, a fungal inoculum was taken from each strain and placed in a drop of distilled water on a slide and covered with a coverslip. The design was mounted on an optical microscope for observation of the fungal structures. The microscopic description took into account the shape of the conidia, the presence or absence of septa, color, branching, and the presence or absence of mycelium septa. This identification was made using the identification keys of Bany and Barnett (1972) and Botton *et al*. (1990). Microscopic observations were made at 400× magnification (40x M).

**Assessment of fungal strain sporulation**

In order to assess strain sporulation, three 7-mm diameter inocula were taken from each 12-day-old fungal colony on PDA medium. One inoculum was taken from the center of the dish. The second was taken from the periphery of the dish and the third from halfway between the first two inocula. These inocula were placed in 10 ml of distilled water in a sterile tube. The

tube was vortexed for 30 seconds so as to detach the conidia. The suspension was filtered using a 2 $\mu$m mesh sieve and then with filter paper. Next, 100 $\mu$l of the spore suspension was taken and placed on a Malassez slide, which was then mounted on an optical microscope. The spore concentration (number of conidia/ml) was estimated using the formula below:

$$C = \frac{N}{n \times v} \times Df (2)$$

C: Spore concentration per unit volume, N: Number of conidia per unit volume, Df: Dilution factor, n: Number of counting units counted, v: Volume of a counting unit [$0.01 \times 10^{-3}$ ml].

**Statistical analyses**

R software version 4.3.0 was used to analyze the collected data. Before data analysis, a test of homogeneity of variances was performed.

One-way analysis of variance (ANOVA 1) was used to compare spore concentration, mean diameter, and mean lesion severity depending on strains.

In cases of significant differences at 5% threshold, Fischer's LSD test was performed to determine the different homogeneous groups. For the non-parametric test, the Kruskal-Wallis test was used to compare the mean colony diameter of the strains. In cases of significant differences, the Mann-Whitney U test was performed to determine the homogeneous groups.

## Results and discussion

### Morphological characteristics of the different *Alternaria brassicicola*strains

The study conducted on the five *Alternaria brassicicola*strains isolated from cabbage leaves revealed macroscopic and microscopic characteristics typical of the *Alternaria* genus, but with some differences between isolates.

### Macroscopic characteristics

The mycelial colonies of the different strains were greenish-brown in the center, turning light brown toward the periphery. Overall, the strains showed dense, slightly velvety, carpet-like colonies. The edges of the colonies had a regular, well-defined outline, characteristic of *A. brassicicola* isolates cultured on PDA. However, a significant difference was observed between strains in terms of growth structures. Isolates A1, A2, A3, and A4 showed concentric ring growth. In contrast, strain A5 showed uniform radial growth without apparent ring formation (Figure 1).

### Microscopic characteristics

126 The strains showed cylindrical to elongated ellipsoidal conidia, with a tapered end that was
127 often slightly narrowed. The conidia were brown or olive-brown in the center, contrasting
128 with lighter, even hyaline ends. Multicellular and septate conidia, with 1 to 5 transverse septa,
129 sometimes accompanied by one or two longitudinal septa, were observed (Figure 1). They
130 developed on simple, erect, brownish conidiophores, often arranged in short or isolated
131 chains.

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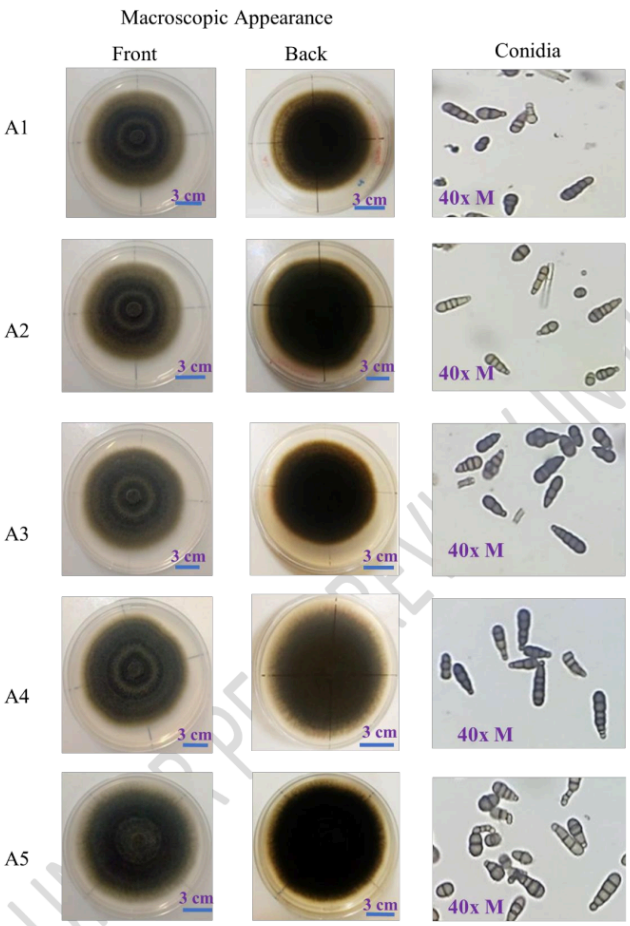


Figure 1: mycelial colonies and spores of the five *Alternaria brassicicola*strains of after 12 days of culture on PDA medium.

**Diameter of strain mycelial colonies**

Assessment of the mycelial growth of the five *Alternaria brassicicola*strains revealed a significant difference after 12 days of incubation at 25 ± 2 °C. The average colony diameters ranged from 7.01 to 8.50 cm.

Statistical analyses showed a significant difference between the mean colony diameters (p = 0.025), making it possible todistinguishthree homogeneous groups.

Strain A5 showed the fastest mycelial growth, with a mean diameter of 8.50 cm. Strains A2, A3, and A4 showed intermediate diameters, ranging from 7.08 to 7.91. In contrast, strain A1 showed the slowest growth, with a mean diameter of 7.01 cm after 12 days of incubation (Table I).

**Quantity of conidia produced per strain**

A significant difference was observed in the sporulation of the five strains. Spore concentrations ranged from $1.26\times10^6$ to $2.64\times10^6$ conidia/mL. Statistical analyses showed a highly significant difference (p = 0.004) between the spore concentrations of the strains with three homogeneous groups. Strain A5 stood out for its maximum spore production, reaching an average concentration of $2.64\times10^6$ conidia/mL. Strain A4 also showed significant conidial production, estimated at approximately $2.0\times10^6$ conidia/mL, ranking second behind strain A5. However, strains A1, A2, and A3 showed significantly lower levels of sporulation, with concentrations ranging from $1.26\times10^6$ to $1.40\times10^6$ conidia/ml (Table II).

**Tables**

**TableI:**Mean diameters of mycelial colonies of *Alternaria brassicicola* strains on PDA medium after 14 days of growth

| ***Alternariabrassicicola*strains** | **Meandiameters(cm)** |
|---|---|
| A1 | 7.01 ± 1.25 **c** |
| A2 | 7.58 ± 0.38 **ab** |
| A3 | 7.08 ± 1.25 **ab** |
| A4 | 7.91 ± 0.13 **ab** |
| A5 | 8.50 ± 0.00 **a** |
| H | 11.06 |
| P | 0.025 |

Values with the same letters are statistically identical according to the Kruskall-Wallis test at 5% threshold (α=5%); H: Kruskal-Wallis statistics; P: probability value

| ***Alternariabrassicicola*strains** | **Spore concentrations** |
|---|---|

| | (10$^6$conidia/ ml) |
|---|---|
| A1 | 1.26 ± 0.29 **b** |
| A2 | 1.28 ± 0.25 **b** |
| A3 | 1.40 ± 0.17**b** |
| A4 | 2.0 ± 0.15 **ab** |
| A5 | 2.64 ± 0.19 **a** |
| F | 4.46 |
| P | < 0.00449 |

**TableII:**mean spore concentration of *Alternaria brassicicola* 14 days after culture on strains PDA medium

Values assigned the same letter in the same columns are statistically identical according to Fischer's LSD test at 5% threshold; F: Fischer's value; p: probability



**Discussion**

The five *Alternaria brassicicola*strains showed morphological diversity at both macroscopic and microscopic levels. This diversity reflects the intraspecific variability frequently observed in phytopathogenic fungi (Blacutt*et al*., 218). The presence of concentric rings in strains A1, A2, A3, and A4 suggests discontinuous mycelial growth, probably linked to alternating active and resting phases in fungal metabolism, which are often influenced by nutrient availability or microenvironmental conditions. In contrast, strain A5, characterized by uniform radial growth, may have a greater ability to exploit the substrate homogeneously, reflecting a physiological or enzymatic difference.

Microscopic observations also confirm the morphological characteristics typical of the *Alternaria* genus, notably multicellular, septate conidia that are brownish in the center and hyaline at the ends, arranged in short chains or isolated. The number of septa (1 to 5) observed is consistent with the descriptions reported by Pryor and Michailides (2002) for *A. brassicicola*. Such variations in conidia morphology may reflect genetic differences between isolates, but also influences from the culture medium and the age of the colonies.

The results relating to sporulation confirm this variability between strains. Spore concentrations ranged from $1.26\times10^6$ to $2.64\times10^6$ conidia/mL after 14 days of culture, with a highly significant difference ($p = 0.004$). Strain A5, which showed the strongest mycelial growth, also produced the largest quantity of conidia.

Indeed, rapid growth would be accompanied by increased metabolic activity, promoting the formation of reproductive structures such as conidia. This positive correlation between mycelial growth and sporulation is not shared by some authors, such as Attrassi*et al*. (2005), whose work has shown that conidia and mycelia develop under different conditions (Attrassi*et al*., 2005).

The high production of conidia in certain strains could constitute a major adaptive capacity by increasing their ability to spread and, consequently, increasing the infectious power of the fungus.

The morphological variability observed between isolates could be attributed to factors such as geographical origin and intraspecific genetic diversity.

Such intraspecific morphological variation has also been observed in other species of the *Alternaria* genus, as demonstrated by Umashankar and Arsia in 2024.

**Conclusion**

This study made it possible to demonstrate intraspecific diversity within *A. brassicicola* strains based on morphological characteristics. The differences observed, both in terms of mycelial growth and sporulation, highlight significant morphological diversity within the species. Strain A5 was distinguished by rapid growth and a high sporulation capacity, suggesting greater physiological vigor and infectious potential.

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