ISSN NO. 2320-5407

Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/21971
DOI URL: http://dx.doi.org/10.21474/IJAR01/21971

RESEARCH ARTICLE

MORPHOLOGICAL CHARACTERIZATION OF ALTERNARIA BRASSICICOLA STRAINS COMBINED WITH CABBAGE CULTIVATION IN COTE D'IVOIRE

N'guessan Wilfried Yao, Assiri Elloh Patrice Kouame, Marie Noel Yeyeh Toualy, Ollo Dominique Kambou, and Hortense Atta Diallo

1. Universite Nangui Abrogoua, Natural Science Department, Laboratory of Plant Protection, G4 Street, Abobo-Adjame, 02 Bp 801 Abidjan 02,Abidjan, Cote D'ivoire.

Manuscript Info

Manuscript History
Received: 13 August 2025
Final Accepted: 15 September 2025
Published: October 2025

Key words:-
cabbage, pathogen, Alternaria brassicicola, morphological characteristics

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 yiel d and market value. It is caused by fungi of the Alternariagenus, particu larly A. Brassicicola,which has several morphologies. This study theref ore aims at investigating the morphology of five A. Brassicicolastrains 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. Brassicicolastrains in Côte d'Ivoire.

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 (Sugieret 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

Corresponding Author:-Nguessan Wilfried Yao
Address:-Universite NanguiAbrogoua, Natural Science Department, Laboratory Of Plant Protection, G4 Street, Abobo-Adjamé, 02 Bp 801 Abidjan 02,Abidjan, Cote Divoire.

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 brassicaeand 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 (Blagiojevićet 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 brassicicolafungal colonies. This study was conducted in order to study the morphological diversity of previously isolated A. Brassicicolastrains.

Material and Methods:-

Culture of fungal strains on PDA medium:-

The different A. Brassicicolastrains (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 straincolonydiameters:-

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:

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

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 µm mesh sieve and then with filter paper. Next, 100 µl 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 [0.01×10^{-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 brassicicolastrains:-
The study conducted on the five Alternaria brassicicolastrains isolated from cabbage leaves revealed macroscopic and microscopic characteristics typical of the Alternaria genus, but with some differences between isolates.
Macroscopic characteristicsThe 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:-
The strains showed cylindrical to elongated ellipsoidal conidia, with a tapered end that was often slightly narrowed. The conidia were brown or olive-brown in the center, contrasting with lighter, even hyaline ends. Multicellular and septate conidia, with 1 to 5 transverse septa, sometimes accompanied by one or two longitudinal septa, were observed (Figure 1). They developed on simple, erect, brownish conidiophores, often arranged in short or isolated chains.

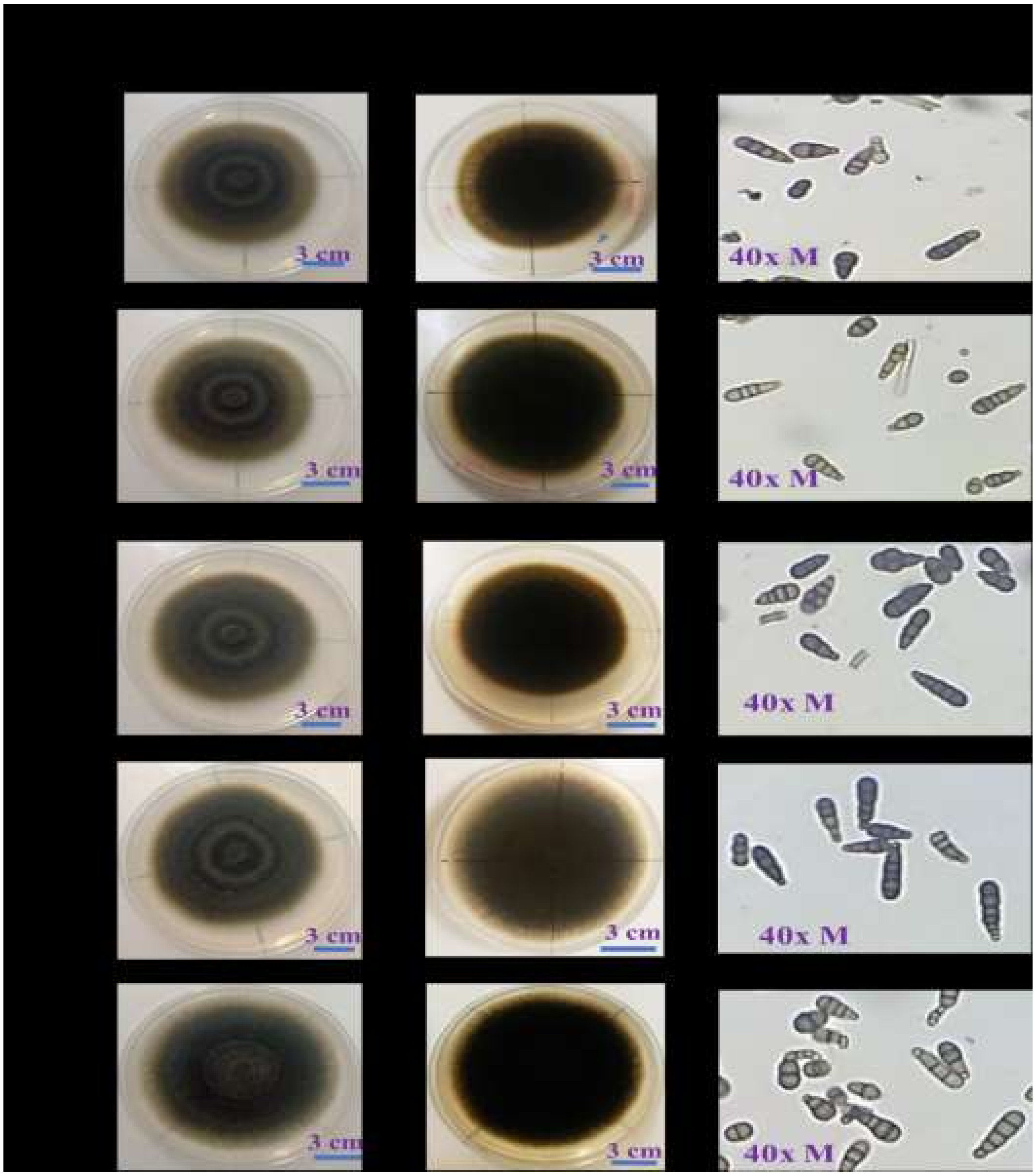

Figure 1: mycelial colonies and spores of the five Alternaria brassicicolastrains of after 12 days of culture on PDA medium.

Diameter of strain mycelial colonies:-

Assessment of the mycelial growth of the five Alternaria brassicicolastrains 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 1.26×10^6 to 2.64×10^6 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 2.64×10^6 conidia/ml. Strain A4 also showed significant conidial production, estimated at approximately 2.0×10^6 conidia/ml, ranking second behind strain A5. However, strains A1, A2, and A3 showed significantly lower levels of sporulation, with concentrations ranging from 1.26×10^6 to 1.40×10^6 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

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

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

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 brassicicolastrains showed morphological diversity at both macroscopic and microscopic levels. This diversity reflects the intraspecific variability frequently observed in phytopathogenic fungi (Blacuttet 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 1.26×10^6 to 2.64×10^6 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 Attrassiet al. (2005), whose work has shown that conidia and mycelia develop under different conditions (Attrassiet 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.

References:-

1. Blacutt AA, Gold SE, Voss KA, Gao M, Glenn AE. (2018).Fusarium verticillioides: Advancements in Understanding the Toxicity, Virulence, and Niche Adaptations of a Model Mycotoxigenic Pathogen of Maize. Phytopathology, 108, 312–326.
2. Pryorbm, Michailides TJ. (2002). Morphological, pathogènic and molecular caractérization of Alternariaisolats associated with Mildiou of pistachio. Phytopathology, 92 : 406-41.
3. Attrassik,Selmaoui k, ouazzanita, Badoc A, Douira A. (2005).Biologie et physiologie des principaux agents fongiques de la pourriture des pommes en conservation et luttechimique par l'azoxystrobine. Bull. Soc. Pharm. Bordeaux, 144 : 47-62.
4. Kushwaha U, Arsia SK. (2024). Cultural and morphological variability of Alternaria brassicicola causes leaf spot of cauliflower at East Nimar Khandwa. International Journal of Advanced Biochemistry Research, 8(10): 1387-1392.
5. Wang J, Liu Z, Dou J, Lv J, Jin N, Jin L, Li Z, Zhang B, Tang Z, Yu JA.(2022).Comparative Study on the Nutrients, Mineral Elements, and Antioxidant Compounds in Different Typesof Cruciferous Vegetables. Agronomy, 12 :31-21.
6. Sugier D, Sugier P, Jakubowicz-Gil J,Gawlik-Dziki U, Zaja CA, Król B, Chmiel S, Konczak M, pim, Paduch R.(2023).Nitrogen Fertilization and Solvents as Factors Modifying the Antioxidant and Anticancer Potential of Arnica montana L. Flower Head extracts. Plants, 12, 1-17. Https://doi.org/10.3390/plants12010142.
7. Uuh-Narváez O, Umaña DE et Moreno JP. (2021).Cabbage (Brassica oleracea var. Capitata): A food with functional properties aimed at type 2 diabetes prevention and management. Journal of Food Science, 86(7), 3100–3113. Https://doi.org/10.1111/1750-3841.15939.
8. FAOSTAT (2023). Https://www.fao.org/faostat/en/#data/QCL, consulté le 19/10/2025.
9. Tabima JF, Rodríguez DJ,López-Bautista JM.(2024). The current status, challenges, and future perspectives for managing diseases of brassicas. Frontiers in Plant Science, 15, 10392840. Https://doi.org/10.3389/fpls.2024.10392840.
10. Atlasbig. (2023). Https://www.atlasbig.com/fr-fr/pays-par-production-de-chou
11. RVO (2023). Https://www.rvo.nl/files/file/2023-03/Scoping-Study-Horticulture-Sector Northern-Cote-d%27Ivoire.pdf.
12. Kouakou P A K.(2024). Analyse du potentielproductif des productrices de chou à Korhogo. Revue Marocaine des Sciences Agronomiques et Vétérinaires, 12(2) : 132–139.
13. Blagiojević JD, Vukojević JB,Ivanović ZS. (2020).Occurrence and characterization of Alternariaspecies associated with leaf spot disease in rapeseed in Serbia. Plant Pathology, 69 : 883–900.
14. Goyal P, Shanmugam V, Sinha AK, Nandi D, Kaur B, Bansal KC, Sharma TR. (2020). Complexity of Brassica oleracea–Alternaria brassicicola susceptible interaction: deciphering the role of pathogen secretome. BMC Genomics, 21, 45. Https://doi.org/10.1186/s12864-019-6414-6.
15. Kumar P, Rajarammohan S, Pental D, Kaur J.(2019). Comparative genomics of Alternaria species provides insights into the pathogenic lifestyle of Alternaria brassicae a pathogen of the Brassicaceae family. BMC Genomics, 20 : 10-36.
16. Bany BB, Barnett HL. (1972). Illustrated genera of imperfecti fungi. Third edition. Burgess Publishing Company. Minapolis, USA. 241 p.
17. Botton B, Breton A, Gauthier S, Guy PH, Larpent JP, Remond P,sanglierjj.(1990).Moisissuresutiles et nuisibles, importance industrielle. Collection biotechnologique, 2[è]édition, Masson, Paris, 498 p.