Journal homepage
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Anbar Journal of Agricultural Sciences
(University of Anbar – Agriculture College)

STUDY OF MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF SOME TYPES OF FUNGUS ASPERGILLUS SPP.

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Article info

Received: 2023-09-08
Accepted: 2023-10-31
Published: 2023-12-31

DOI-Crossref:
10.32649/ajas.2024.143268.1083

Cite as:
Abdulmageed, L. H. (2023). Study of morphological and physiological characteristics of some types of fungus aspergillus spp. Anbar Journal of Agricultural Sciences, 21(2): 386-395.

Abstract

Aspergillus is considered the most genera of fungi that pollutes vegetables and is of great importance as it is used in the food and pharmaceutical industries, on the other hand, some of them are harmful to human health. In this study, the morphological and physiological characteristics of *A. niger*, *A. flavus*, *A. fumigatus*, *A. tubingensis*, and *Aspergillus spp.* that were isolated from onions, carrots, peppers, cucumbers, and tomato were studied and grown in potato dextrose agar for a week. After that study the macroscopic characteristics of the colony with the naked eye and microscopic examination. One of the most important results that have been reached is the speed of growth of these species and their adaptation to the surrounding conditions. The similarity between the types of black Aspergillus and the contrast between the other types. In addition, the relationship between the morphological and physiological characteristics of these species is represented by their ability to secrete enzymes, toxins, vital metabolites, and metabolism. Given the importance of the morphological characteristics and their relationship to the physiological characteristics of the species of Aspergillus, we recommend that the studies be expanded to include more types with different environmental conditions and different regions because of the effect that this has on those characteristics and to obtain more accurate and comprehensive information.

Keywords: Aspergillus, Morphological, Physiological, Microscopic Examination.

دراسة الخصائص المورفولوجية والفيسيولوجية لبعض أنواع فطر الاسبيرجيلس

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الخلاصة

يعتبر فطر *Aspergillus* من أكثر أنواع الفطريات التي تلوث الخضروات وله أهمية كبيرة اذ يُستخدم في صناعات الغذاء والصيدلة. ومن ناحية أخرى، بعضها يكون ضارًا لصحة الإنسان. في هذه الدراسة، تم دراسة الخصائص المورفولوجية والفيسيولوجية لبعض أنواع هذا الفطر مثل *A. niger*، *A. flavus*، *A. fumigatus*، *A. tubingensis*، وأنواع *Aspergillus* أخرى تم عزلها من ثمار الخضراوات (البصل، الجزر، الفلفل، الخيار، والطماطم) وزُرعت في وسط بطاطس الديكستروز لمدة أسبوع. بعد ذلك، تم دراسة الخصائص الماكروسكوبية للمستعمرة بالعين المجردة والفحص المجهري. وقد تم التوصل إلى واحدة من أهم النتائج وهي سرعة نمو هذه الأنواع وتكيُّفها مع الظروف المحيطة. والشبه بين أنواع أسبيرجيلوس السوداء والتباين بين الأنواع الأخرى. بالإضافة إلى ذلك، يتمثل العلاقة بين الخصائص المورفولوجية والفسيولوجية لهذه الأنواع في قدرتها على إفراز الإنزيمات، والسموم، والمركبات الحيوية الأساسية، والأيض. نظرًا لأهمية الخصائص المورفولوجية وعلاقتها بالخصائص الفيسيولوجية لأنواع أسبيرجيلوس، نوصي بتوسيع الدراسات لتشمل المزيد من الأنواع في ظروف بيئية مختلفة ومناطق متعددة بناءً على التأثير الذي يمثله ذلك على هذه الخصائص وللحصول على معلومات دقيقة وشاملة أكثر.

كلمات مفتاحية: أسبيرجيلوس، مورفولوجي، فيسيولوجي، فحص مجهري.

Introduction

Many types of research have emerged concerned with studying and identifying the characteristics of fungi, especially Aspergillus species (5, 15 and 28). Currently, the focus on detecting and identifying fungi is essential, because types of fungi may appear that have physiological properties and have a serious impact on humanity. In addition, some types of *Aspergillus* such as *Aspergillus fumigatus* and *Aspergillus flavus* were detected in the lungs of some people infected with the Coronavirus, which led to the deterioration of their health condition and led to the death of some patients (4 and 24). Some types of Aspergilli are pathogenic and may infect humans or plants. In addition, some of them (*A. flavus, A. parasiticus, and A. niger*) have the ability to produce secondary metabolites known as mycotoxins that lead to damage to agricultural crops such as fruits and vegetables and thus infect humans and animals (15, 19 and 27). Aspergillus is distinguished from other genera of fungi in that it

possesses physiological, biological and morphological characteristics that make it widespread as it is found in different environments such as air, soil, and plants. Due to its ability to adapt and live according to the environment in which it is located and also Aspergillus has the ability to decompose organic matter and considers its substrates which live on (2).

Aspergillus is a diverse group of fungi, approximately 260-830 species have been identified and these species are classified into ten teleomorph genera (13). The identification and characterization of Aspergillus types depend mainly on morphological characteristics (colony (shape, color, texture, diameter), color reverse side, color of mycelia) and microscopic features (hyphae shape, conidiophores, conidial heads, vesicles, conidia, spore) (2 and 12). Aspergillus is characterized by being similar in their morphological characteristics, especially black Aspergillus. Thus, to identify these species, we support their morphological characteristics by studying their physiological characteristics. The physiological properties of fungi relate to their ability to secrete mycotoxins, use 2-deoxy D-glucose as a carbon source, produce proteins and polysaccharide hydrolases, secrete hydrolytic enzymes, as well as their ability to perform elastase activities (2, 17, 20, 25 and 26). It is through these physiological characteristics that we can identify the types of Aspergillus more accurately. Moreover, by the physiological characteristics of fungi, we may be able to identify pathogenic and non-pathogenic species. Despite the technological development in the tools and techniques that are used to identify fungi, these techniques cannot give full results without relying on culture, morphological (macroscopic and microscopic features), and physiological characteristics. In view of the importance of these characteristics, the aim of this research paper is to study the morphological and physiological characteristics of some types of fungi that were obtained and isolated from some types of rotting vegetables found in the market in Anbar, Iraq.

Materials and Methods

Sample Collection: Five samples of vegetable fruits (cucumber, onion, tomato, pepper, and carrot) were collected from the market in Anbar, Iraq, and placed in sterile polythene bags. And then made slides of fungal parts, which were found on infected parts of vegetables.

Fungal isolates and morphological characterization: Fungal isolates were cultured on media Potato Dextrose Agar (PDA) (6). Fungal isolates were inoculated in petri-dish plates of PDA media at 27 ± 3 °C for 7 days in a dark place. After that, we observed macroscopic features (colony color and diameter, and exudates) with the naked eye. The morphological examination of pure colonies is carried out by harvesting the spores using sterile needles and then placing them on glass slides. The spore was stained on glass slides with Lacto phenol (cotton blue) and was covered by a slipcover and placed under the microscope lens. Thus, the microscopic characteristics are observed. Digital photography for fungi species colonies was performed using a Sony Cyber-Shot W810 digital camera. Finally, Identification books and classification keys were used to diagnose the fungi isolated microscopically based on

their appearance and the characteristics of their growth in the medium (6, 17, 20, 25 and 26).

physiological characterization: Czapek–Dox Agar medium was modified by the addition of glucose, cellulose as carbon sources, and pectin to identify the ability of these fungi to produce α-galactosidase, cellulase and pectinase (3).

Results and Discussion

The study of morphological characteristics and identification of Aspergillus species: The morphological characteristics of five species out of twenty isolates from five samples of vegetable fruits were studied (cucumber, onion, tomato, pepper, and carrot). The types of fungi that included the genus Aspergillus were identified after they were grown on potato dextrose agar (PDA) media for a whole week and monitored at a temperature ranging from 27 ± 3 °C shown in Figures 1, 2, 3, 4 and 5 in this study. The Aspergillus species identified in this study were moderate to fast-growing on the PDA and the colonies were black, brown, green, and olive, while the color on the back of the plate was colorless to yellow or slightly yellowish. The results of each type of Aspergillus are discussed and explained below.

Macroscopic and microscopic characteristics of *Aspergillus niger*: The *A. niger* was characterized by macroscopic characteristics are rapid growth on the PDA media, the color of the colony is black with white mycelia, in addition to the that the shape of the colony was thin. The colony texture was distinguished by having distinct and clear growth areas, and it was formed as powdery as a result of heavy sporulation. Colony diameters were measured after one week of incubation at 28°C, ranging from 50 to 70 mm. The edges of the colony were surrounded by white mycelia, while the reverse side was colorless to pale yellow and surrounded by mycelia in the form of a white circular halo. All of these macroscopic features are illustrated in Figure 1 (A, B). In addition, this type of Aspergillus has pigments and soluble secretions (2, 12, 18, 23 and 28).

Microscopic characteristics of *A. niger* include: conidia sub-globose to globose in shape, and their sizes range between 4-6 μm. The conidia were brown in color with a smooth to rough surface. In addition, the conidia were small in size. While conidiophores are hyaline, long globose, and brown in color with a smooth surface (2, 12, 18, 23 and 28). The spore was dense with the wall smooth (23). All of these microscopic features are shown in Figure 1 (B, C).

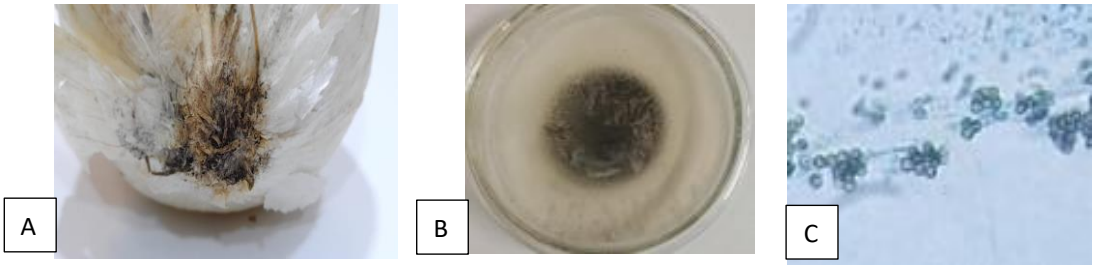

Figure 1 Characteristics of *Aspergillus niger* (A) *A. niger* growth on onion; (B) macroscopic characteristics; (C) microscopic characteristics.

Macroscopic and microscopic characteristics of *Aspergillus flavus*: The *Aspergillus flavus* was characterized by macroscopic features including the colony was rapid growth on the PDA media and diameters 50 mm - 80 mm were measured after 7 days at 28 ± 2°C. The colony has a light green color with mycelia having bright green color and white boundaries. The colony was moderately sporulated and formed in rings. The colony has a powdery texture and the reverse color was colorless to pale yellow as shown in Figure 2 (A, B). *A. flavus* has soluble pigments (10, 18 and 28).

Microscopic characteristics of *A. flavus* are represented by conidia which have a green color with a smooth to the finely rough surface, its shape was sub-globose to globose, with size arranged from 2 - 6µm, and ornamentation almost smooth. The conidia head seriation was uniseriate (Figure 2 (C)). Conidiophores are colorless and hyaline, short globose to subglobose with a rough surface, and their size of 15 - 36 µm (2, 10, 18 and 28). The spore was dense with the wall rough. (2 and 10).

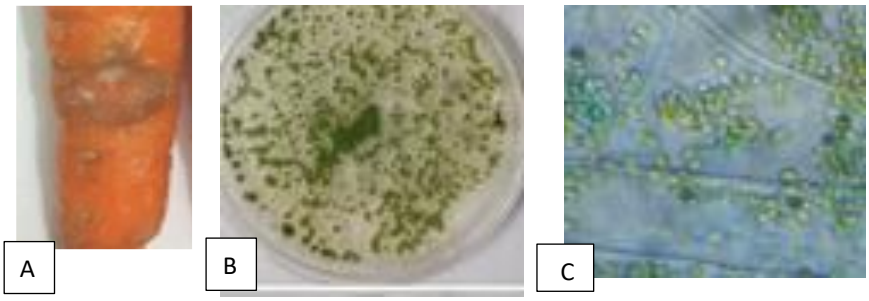

Figure 2 Characteristics of *Aspergillus flavus* (A) *A. flavus* growth on carrots; (B) macroscopic characteristics; (C) microscopic characteristics.

Macroscopic and microscopic characteristics of *Aspergillus fumigatus*: The *Aspergillus fumigatus* has macroscopic characteristics such as rapid growth on the PDA media, the color of the colony is greenish-grey with white regular margins, and colourless mycelia. The colony diameter after incubation at 28˚C for one week was 50 - 80 mm, and the colony texture was powdery because the sporulation was dense. The reverse side was greyish, and there was a presence of soluble pigments and exudates Figure 3 (A, B) (1, 10 and 14). *A. fumigatus* was distinguished from other Aspergillus with clear morphological characteristics and different from other Aspergillus species. In addition to that, it grows in different environments and at different temperatures, which helps in its adaptation to life in any environment and the speed of growth when appropriate conditions are available.

A. fumigatus has microscopic characteristics that are represented in hyphae size were 2.5- 8 mm, septate, branching like a tree, and hyaline. Conidia are globose to ellipsoidal in shape, and their sizes range between 2-4 µm. The conidia were pale blue in color with a smooth to finely rough wall. Conidial heads were uniseriate, smooth-walled, and columnar conidial arrangement. While conidiophores are hyaline, erect, long, globose to ellipsoidal, and have a smooth wall (Figure 3 (C)) (1, 10, 14 and 16).

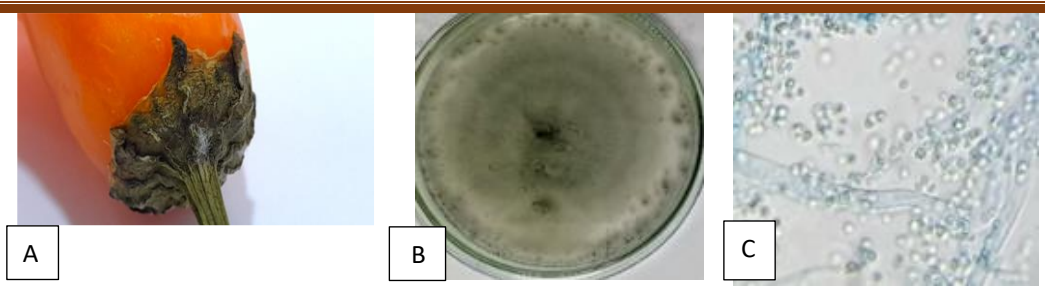

Figure 3 Characteristics of *Aspergillus fumigatus* (A) *A. fumigatus* growth on peppers; (B) macroscopic characteristics; (C) microscopic characteristics.

Macroscopic and microscopic characteristics of *Aspergillus tubingensis*: The *A. tubingensis* was characterized by macroscopic characteristics which are the colony was rapid growth on the PDA media and diameters of the colony were 40 mm - 70 mm were measured after 7 days at 28°C, the color of the colony is greyish black to black with white mycelia, white margins. In addition to that, the shape of the colony has a powdery texture. The reverse side was colorless to pale yellow. *A. tubingensis* has pigments and soluble secretions (14 and 28). All of these macroscopic features are illustrated in Figure 4 (A, B). *A. tubingensis* has nearly similar macroscopic features to *A. niger* with very minor differences that appear in the microscopic features (28).

Microscopic characteristics of *A. tubingensis* include: conidia color was brown, globose to elliptical in shape with a rough-walled, and their sizes range between 4-5 µm. The biseriate conidial heads of the conidia and it has phialides. While conidiophores are hyaline, large, long globose, and brown to black in color with sclerotia production Figure 4 (C) (12, 14 and 28).

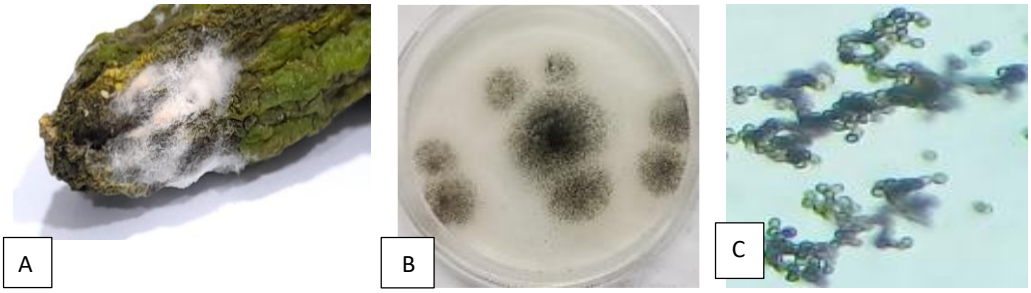

Figure 4 Characteristics of *Aspergillus tubingensis* (A) *A. tubingensis* growth on cucumber; (B) macroscopic characteristics; (C) microscopic characteristics.

Macroscopic and microscopic characteristics of *Aspergillus spp.*: *Aspergillus spp.* was characterized by macroscopic characteristics. where it has rapid growth on the PDA media at 28°C after incubation for one week, the color of the colonies was brown to black and the diameters of the colony were 50 - 80 mm. The colony's reverse side colour was colorless to pale yellow. The colony texture was powdery and abundant sporulation (14). *Aspergillus spp.* has soluble pigments and all of these macroscopic features are shown in Figure 5 (A, B).

Microscopic characteristics of *Aspergillus spp.* include: sporangium was spherical, conidia were brown in colour, size 3 -4 µm, and sub-globose to globose in shape. The

conidia have a smooth surface. The conidial heads were biseriate (Figure 5 (C)). While conidiophores are hyaline and spiny (10, 11, 14 and 22).

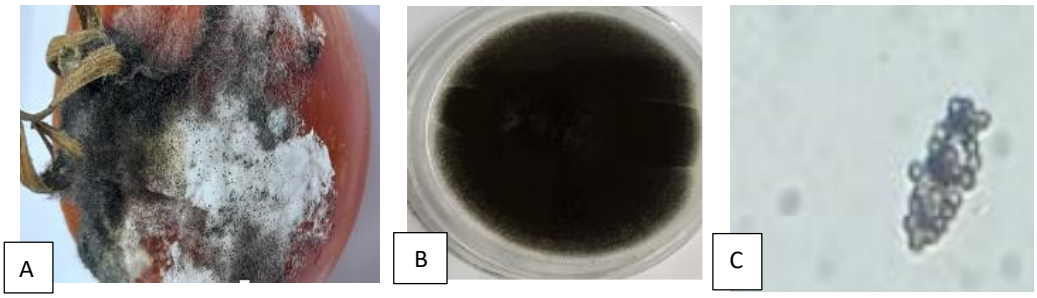

Figure 5 Characteristics of *Aspergillus spp.* (A) *Aspergillus spp.* growth on tomato; (B) macroscopic characteristics; (C) microscopic characteristic.

The study of physiological characteristics of some aspergillus species: The physiological characteristics of fungi are related to their ability to secrete enzymes and metabolites. It plays an important role in the food industry; it was used as a stabilizer and colorant. Moreover, the bioactive metabolites secreted by some types of fungi have been used in the pharmaceutical industry (9). One of the enzymes secreted by *A. niger* is α-galactosidases (9) because *A. niger* has the ability to grow rapidly on vegetables, as we have noticed through its morphological characteristics. We studied the morphological characteristics of the Aspergillus section Nigri to which belongs, *A. niger*, *Aspergillus spp.*, and *A. tubingensis* were studied, and regarding the physiological characteristics of these species, they produce α-galactosidase, cellulase and pectinase. (21 and 22).

In general, the Aspergillus species were distinguished by their various physiological characteristics, as we previously mentioned their ability to secrete toxins, metabolites, and enzymes also has the potential to metabolize and adapt to the surrounding environmental conditions, take advantage of carbon as a source of, and respond to stress (8). These species such as *A. flavus* and *A. fumigatus*. Through our study of the morphological characteristics of five types of Aspergillus, we found that they grow rapidly and with a high density of spores and biomass. This is what made them physiologically characterized by their ability to secrete glycosylation-linked proteases to produce recombinant protein (7).

Many studies are concerned with the physiological characteristics of fungi especially Aspergillus which are present in food such as vegetables and fruits. These studies mentioned the factors that affect these characteristics, the growth of fungi, and their ability to secrete enzymes and toxins, namely pH, temperature, water activity, atmosphere, and preservatives (21).

The study of morphological and physiological features of fungi is very important as it is possible through these features to identify pathogenic and non-pathologic types of fungi. On the other hand, fungi can benefit in many areas after identifying their physiological characteristics. In addition, it is possible to develop anti-fungal therapies for pathogenic fungi such as A. fumigatus and A. flavus.

Conclusion: During our study of the morphological characteristics of five types of Aspergillus, we noticed that the species belonging to Aspergillus nigari (*A. niger, A. tubingensis, Aspergillus spp*.) are very close in macroscopic features, but differ slightly in microscopic features, even if one microscopic feature. While other species such as *A. flavus and A. fumigatus* differ clearly. Among the results that were reached are the speed of growth of these species, the density of spores and biomass, and their adaptation to the surrounding environmental conditions, which helps to spread them more widely and contaminate vegetables with them. Also, there is an important relationship between morphological and physiological characteristics, as the ability of fungi species to adapt and grow rapidly is related to the ability of those species to secrete enzymes, toxins, bio-metabolites, metabolism, and utilize carbon as a source. Based on the results that were reached in this study, we recommend that the studies should be expanded to include a greater number of fungi types and different regions to know the types of fungi present and their impact on human health through their transmission through food.

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