Sawa Medical Journal
Reprint from
Vol.2 Issue.1 2026
ISSN : 3007-4738 (online)

Study of Drug Sensitivity and Some Virulence Factors in Epicoccum nigrum and Bipolars sorokiniana Fungi Isolated from Transport Vehicles

Saadiyah Abed Jaddoa, Majid Kadhim Abboud

AL-Qadisiyah University/ College of Education/Department of Biology/Microbiology

Article Information
Received: 27-2-2026
Accepted: 5-3-2026
Published: 1-4-2026

Abstract

A total of 230 samples were collected from various modes of transportation (private and public cars and trains), two isolates of the fungus *Epicoccum nigrum* and two isolates of the fungus *Bipolaris sorokiniana* were obtained. These isolates were identified based on the morphological characteristics of the fungal culture as well as microscopic characteristics, and their diagnosis was confirmed molecularly based on the 18SrRNA gene using PCR technology. Some virulence factors (protease enzyme, lipase enzyme, and biofilm) were investigated in two isolates of the fungus *Epicoccum nigrum* and two isolates of the fungus *Bipolaris sorokiniana*, The results of the phenotypic detection of the protease enzyme showed that all isolates gave a positive result, i.e., they showed their ability to secrete this enzyme. The gene responsible for the secretion of this enzyme was detected molecularly, and the phenotypic detection of lipase secretion was positive in all isolates studied. that all these isolates contained the lipase secretion gene through molecular detection. The results were negative in the morphological detection of biofilm formation in all isolates. I conducted a sensitivity test on the *Epicoccum nigrum* and *Bipolaris sorokiniana* fungal isolates, both morphologically and molecularly, for a number of antifungal agents, namely fluconazole, nystatin, and amphotericin-B. None of the *Bipolaris sorokiniana* isolates showed resistance to any of these antifungals, while one of the *Epicoccum nigrum* isolates showed resistance to the antifungals Nystatin and Amphotericin-B, and the second isolate of this fungus showed resistance to Fluconazole. The results of molecular detection of the fluconazole resistance gene showed that one of my *Epicoccum nigrum* isolates contained this gene, while molecular detection confirmed that neither of my *Bipolaris sorokiniana* isolates contained the gene under investigation.

Keyworrds: Fungi **;** *Epicoccum nigrum* **;** *Bipolaris sorokiniana***;**Virulence factor

Introduction

Fungi are microscopic organisms that are either saprophytic or parasitic, obtaining nutrients from dead organic matter or from the cells of their host organisms (Kohler et al.,2006). Road transport is the most common mode of transport in the world, with cars being the primary means of transportation. More than 70% of trips around the world are made by private cars or taxis, and thousands of people spend long hours every day in various types of road transport vehicles, whether buses or private cars (Wu *et al*.,2016). Vehicles are an important microenvironment for environmental exposure, as many individuals spend a significant portion of their day and week traveling in them. In addition, some individuals, such as drivers of taxis, public buses, or trucks, spend much more time inside these vehicles (Lee and Jo,2005). Fungal structures or their byproducts are pollutants in car air, and these structures are transmitted through the air stream that passengers breathe, causing them illness (Al-Bader et al.,2021).

The causes of diseases, including fungi, depend on various factors, some of which are related to the host (its immune status) and the pathogen itself, which possesses virulence factors such as adhesion, biofilm formation, production of pigments and low molecular weight non-protein metabolites, and secretion of degradative enzymes such as proteases, lipases, phospholipases, and other enzymes that enable it to obtain nutrients (Tomee and Kauffman,2000). Virulence factors enable the pathogen to grow at high temperatures and facilitate its adhesion to and penetration of the host, spreading within the body and enabling it to resist innate immunity through phagocytosis. They also enable it to bypass adaptive immune defenses and necrotic factors, in addition to surviving metabolic processes represented by digestion, enabling these pathogenic microbes to remain alive while interacting with the host. Virulence factors are produced from genetic factors (proteins) or through complex biosynthetic pathways such as polysaccharides and lipids (Khan et al.,2010).

Epicoccum nigrum fungus may cause allergic reactions and respiratory problems after inhaling its spores. Laboratory studies have shown that the glycoprotein found in its fungal filaments and spores can promote inflammation. Due to the prevalence of this fungus in indoor and outdoor air in urban communities, it is considered one of the most important fungi associated with respiratory allergies. Research has shown that 5-7% of the global population is allergic to species of the genus Epicoccum and that up to 36% of allergy sufferers respond to skin tests using specific fungal extracts (D Halewyn and Chevalier,2019). *Bipolaris sorokiniana* is a plant pathogen with high morphological, physiological, and genetic variability, making it difficult to control (Poloni et al.,2009). There is a correlation between the virulence of this fungus and the morphological appearance of its colonies; isolates that formed black fungal hyphae were found to be the most virulent (Pandey et al.,2008). The fungus *Bipolaris sorokiniana* causes root rot, leaf spot, seedling blight, head blight, and black point in wheat and barley crops. It is the cause of the most dangerous leaf diseases affecting both crops in warm regions, causing significant losses. High temperatures and high relative humidity contribute to the spread of the disease (Kumar et al.,2002). Infected seedlings show dark brown necrotic lesions on the roots, crowns, and lower leaf sheaths. Leaf infection develops as oval to rectangular spots ranging from light to dark brown, causing infected plants to dry out without producing any seeds (Mehta,1998).

Materials and methods

2.1. Isolation and diagnosis of *Epicoccum nigrum* and *Bipolaris sorokiniana*

After collecting the samples, they were cultured on SDA and PDA basic culture media. Isolates belonging to the fungi *Epicoccum nigrum* and *Bipolaris sorokiniana* were identified based on the morphological characteristics of the fungal colony, represented by its color on the upper and lower surfaces and its growth pattern, in addition to its microscopic characteristics, after made microscopic slide by taking a sample from the colony and staining them with methylene blue. Microscopic characteristics, especially those of conidia, are among the most important distinguishing features, based on references(Samson,2002; st-Germain and Summerbell,2011).

2.2.Phenotypic detection of virulence factors

For the purpose of phenotypic detection of virulence factors, the following agricultural media were used:

2.2.1.Skimmed-milk agar media

Prepared according to the reference Bilinsk (1987) Dissolve 5 g of skimmed milk powder in 100 ml of distilled water in a flask and sterilize in a water bath. Dissolve 10 g of agar in 400 ml of distilled water in another flask and sterilize in an autoclave at 151 °C and 15 psi for 15 minutes, then cool to 45°C. Mix well and add 250 mg/L of the antibiotic chloramphenicol to the mixture. Then pour the medium into Petri dishes and leave to solidify until ready for use. This medium was used to detect the ability of fungal isolates to secrete protease.

2.2.2Tween 80 agar medium

was prepared according to the reference Sierra (1957) by dissolving 10 g of peptone, 5 g of sodium chloride, and 0. 1 g of calcium dichloride dihydrate and 15 g of agar in one liter of distilled water in a glass flask, then add 5 ml of Tween 80. After shaking the flask slightly, place it in an autoclave for sterilization at a temperature of 121°C and under a pressure of 15 pounds for 15 minutes. After the sterilization time is over, cool to 45°C, then add 250 mg/liter of the antibiotic chloramphenicol to the mixture. Then pour the medium into Petri dishes and leave to solidify so that it is ready for use. This medium was used to detect the presence of the enzyme lipase in fungal isolates.

2.2.3.Congo red agar medium

Prepare the medium according to the method described by the reference Freeman et al (1989) by dissolving 28 g of nutrient agar powder and 50 g of glucose in 800 ml of distilled water in a glass beaker and dissolving 0. 8 g of Congo red dye powder in 200 ml of distilled water in another glass beaker and sterilized in an autoclave at a temperature of 121 °C and under a pressure of 15 pounds for 15 minutes. After sterilization, the medium was cooled to a temperature of 45 °C, then the dye solution was added and mixed well. The medium was then poured into Petri dishes and left to solidify in preparation for use in detecting the ability of the fungal isolates under study to form a biofilm.

2.3.Antifungal Resistance Test:

The antifungal sensitivity of the fungal isolates under study was tested on Mueller-Hinton agar medium using antifungal discs (Fluconazole, Nystatin, and Amphotericin-B) as described in the reference Casals (1979).

2.4.A phylogenetic tree analysis for some isolates

The PCR products of the genes 18S rRNA for one isolate from *Epicoccum nigrum* and one isolate from *Bipolaris sorokiniana* were sent to Macrogen company in South Korea, and a phylogenetic trees for these isolates were designed.

Results

3.1. Morphologic and molecular identification

After isolation, only two isolates were obtained, belonging to the fungus *Epicoccum nigrum*. Colonies of *Epicoccum nigrum* appeared white or yellowish-brown on the culture medium, then became dark, with irregular edges. The upper surface was dense and orange, and the dorsal surface was reddish-orange or yellowish on top and brown underneath. Microscopic characteristics showed short conidiophores and dark, spherical conidia with multiple septa (Figure 1), which is completely consistent with what was reported by Li et al. (2022). Two isolates of the fungus *Bipolaris sorokiniana* were obtained. Colonies of *Bipolaris sorokiniana* appeared on PDA medium, white to black with white edges and an irregular white-gray border with a black base. They were fast-growing with a prominent woolly texture and showed mixed black and white fungal threads and hyphae. The conidia are simple, single or clustered, contiguous or erect, brown in color, and conidia from their apex are olive-brown, ovoid with pointed ends, with slightly curved septa, numbering 4-9 septa, with smooth and thick walls at the septa. (Figure 2) This is consistent with the description by Navathe et al. (2020).

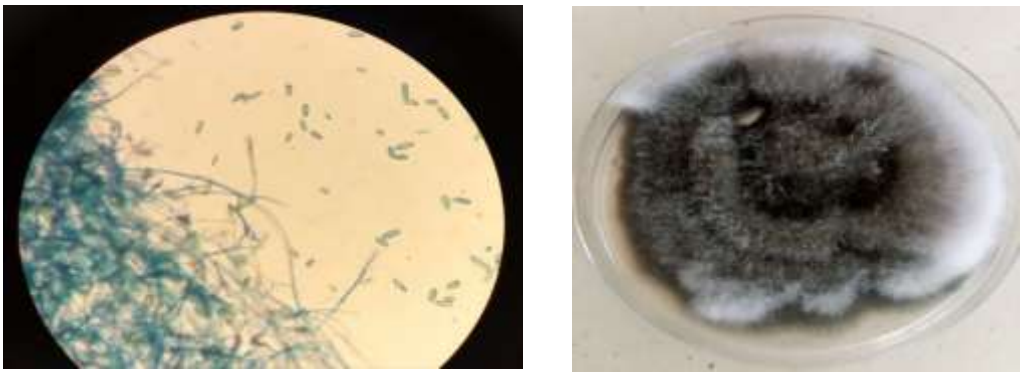

Figure (1) *Epicoccum nigrum*

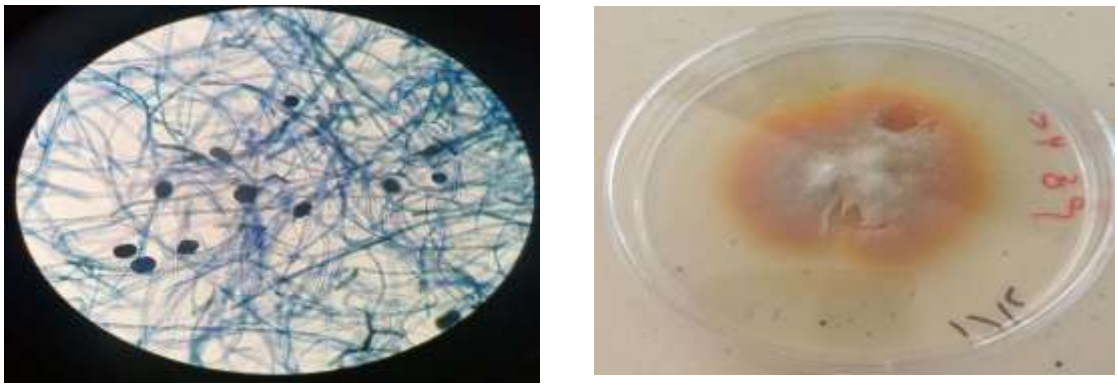

Figure (2) *Bipolaris sorokiniana*

3.2. The genetic diagnosis of isolates based on the gene 18S rRNA

After electrophoresis, the results of 18**S** rRNA gene detection showed that, this gene is present in both *E.nigrum* isolates and *Bipolaris sorokiniana*, with 550 base pairs (bp) size, figure (3),(4)

Figure (3): electrophoresis results of 18S rRNA.of *E.nigrum* using 1.5% concentration of agarose, and 70 volt for an hour a half, and the size standard of the DNA band (550bp).

Figure (4) electrophoresis results of 18S rRNA gene polymerization of *Bipolaris sorokiniana* fungus on 1.5% agarose gel at 70 volts for an hour and a half, and the size standard of the DNA band (550bp).

3.3.Sensitivity of fungal isolates to certain antifungal

The results shown in (Table 1) indicate that both isolates of *Bipolaris sorokiniana* were sensitive to all antifungal used, while one isolate of *E. nigrum* showed resistance to fluconazole. Molecular detection of the fluconazole protein gene -1, the results showed that it possessed this gene, as shown in Figure (5). The second isolate of this fungus showed resistance to the antifungal Nystatin and Amphotericin-B and was sensitive to Fluconazole, as it did not possess the Fluconazole resistance gene, as determined by molecular detection, Figure (6).

Table (1) Sensitivity of fungal isolates to certain antifungal

Inhibition zone	Antifungal	Number of isolate	Fungi
23	Amphotericin-B	1	E.nigrum
0	Fluconazol		
17	Nystatin		
0	Amphotericin-B	2	E.nigrum
13	Fluconazol		
0	Nystatin		
24	Amphotericin-B	1	Bipolaris sorokiniana
9	Fluconazol		

15	Nystatin		
21	Amphotericin-B	2	Bipolaris sorokiniana
12	Fluconazol		
9	Nystatin		

Figure (5) Amplification results of Fluconazole protein-1 for *E. nigrum* isolates, migrated on 1.5% agarose gel at 70 V for 1.5 hours, and DNA band size (400 bp).

Figure (6) Amplification results of Fluconazole protein-1 for *Bipolaris sorokiniana* isolates, migrated on 1.5% acarose gel at 70 V for 1.5 hours, and DNA band size (400 bp).

3.4.Virulence Factors:

Table (2) Virulence Factors of isolates under study

Biofilm formation	Precipitation zone of Lipase	Clear zone of Protease	Number of isolate	Fungi
-	7	13	1	*E.nigrum*
-	5.2	8	2	*E.nigrum*
-	8	10	1	*Bipolaris sorokiniana*
-	6.2	4	2	*Bipolaris sorokiniana*

- Negative result

The results of the morphological investigation showed that all isolates are capable of producing the protease enzyme, as a clear zone formed around the fungal colonies, Figure (7). This is due to the fact that they possess the gene for secreting this enzyme, Figures (8) and (9).

Figure (7) Positive test for protease enzyme

Figure (8) shows the amplification results of the protease gene for *E. nigrum* isolates, migrated on 1.5% agarose gel at 70 V for 1.5 hours, and the size of the DNA band (402 bp).

Figure (9) Amplification results for the protease gene of *Bipolaris sorokiniana* isolates, migrated on 1.5% agarose gel at 70 V for 1.5 hours, and the size of the DNA fragment (402 bp).

The results of the morphological detection showed that all isolates are capable of producing lipase enzyme, as a precipitation zone formed around the fungal colonies, Figure (10). This is due to the fact that they possess the gene that secretes this enzyme, Figures (11) and (12).

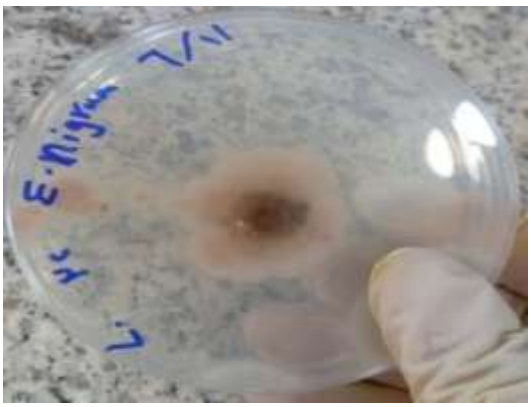

Figure (10) Positive test of lipase enzyme

Figure (11) Amplification results for Lipase genes from *E. nigrum* isolates, migrated on 1.5% agarose gel at 70 V for 1.5 hours, and DNA band size (763 bp).

Figure (12) Amplification results for Lipase genes from *Bipolaris sorokiniana* fungal isolates, migrated on 1.5% agarose gel at 70 V and 80 mA for 1.5 hours, and the size of the DNA band (763 bp) .

The results of the morphological detection showed that none of the isolates tested positive for the morphological detection of biofilm formation, as the color and texture of the medium did not change, as shown in Figure (13).

Figure (13)
The negative result of biofilm formation

Discussion

4.1.Identification

The identity of the fungi examined in the current study was confirmed by molecular identification, Molecular diagnosis based on DNA sequences of specific molecular regions is an effective and rapid method for species identification (Tekpinar and Kalmer ,2019).

4.2.Antifungal resistance

There are several mechanisms behind fungal resistance to antifungal agents, including fluconazole, which modifies ergosterol synthesis, the main target of this agent (Bhattachary et al.,2020) and increased activity of efflux pumps, which is associated with antifungal resistance, especially azoles. These pumps facilitate the transport of antifungal agents out of the cell, preventing their accumulation inside the cells (Ramage et al.,2002a).

4.3.Virulence factors

Fungi can cause disease and overcome host immunity because they possess many genes that encode a number of proteins associated with their ability to cause disease, which are referred to as virulence factors. These factors protect fungi in unfavorable conditions and promote the spread of fungal infection in tissues (Mezher and Bandar,2015). *E. nigrum* isolates possessed the ability to secrete protease, which is consistent with Lumi Abe et al (2015), where all isolates of E. nigrum (three isolates) showed the ability to secrete protease.

The two isolates of *Bipolars sorokiniana* fungus also had the ability to secrete protease enzymes as a result of possessing the protease secretion gene. Our findings were similar to those of Poloni et al. (2009), as 74% of their total isolates of *Bipolars sorokiniana* fungus had this ability.

Calcium ions were added to the lipase detection medium, where an increase in the activity of this enzyme was observed in their presence (Katiyar and Ali,2013). The surfactant Tween 80 enhances lipase activity when incorporated into the growth medium, and its enhancing effects vary according to fungal strains (Geoffry and Achar,2018). The results showed that the two isolates of *E. nigrum* possessed the ability to secrete lipase, which was consistent with Saleem (2008) and Xu et al. (2022). The results showed that the two isolates of the fungus *B. sorokiniana* possessed the lipase enzyme secretion gene, and our results were highly similar to those obtained by Poloni et al. (2009), as 34 out of 35 isolates of the fungus *Bipolaris sorokiniana* produced the lipase enzyme.

Protease is one of the virulence factors in fungi, enabling them to break down the host's cell membrane proteins, which facilitates adhesion to host cells and then invasion, destroying cells and immune system molecules to avoid host resistance (Naglik et al.,2003). As with other digestive enzymes, including lipase, it is essential for providing soluble substances that can be absorbed as food (Ogorek et al.,2016).

Conclusion

Fungi are living organisms that possess a number of virulence factors that enable them to invade living cells and obtain food, causing disease in living organisms, including humans and plants. The excessive use of antifungal agents has produced fungal strains that possess genes resistant to these agents.

References

Al-Bader, S. M., Ismael, L. Q., Ali, H. S., Al-Tamimi, A. S., & Saleh, A. A. (2021). Antifungal activity of essential oils vapors against fungi isolated from car air-conditioner filters. Medico-Legal Update, 21(4), 301-306.

Bhattacharya, S., Sae-Tia, S., & Fries, B. C. (2020). Candidiasis and mechanisms of antifungal resistance. Antibiotics, 9(6), 312.

Bilinsk, E. A. (1987). Proteinases and beer production. Journal of Applied and Environmental Microbiology.53:495-499.

Casals, J. B. (1979). Tablet sensitivity testing on pathogenic fungi. Journal of clinical pathology, 32(7), 719-722.

D'Halewyn, M., & Chevalier, P. (2019). Epicoccum purpurascens.

Freeman, D. J., Falkiner, F. R., & Keane, C. T. (1989). New method for detecting slime production by coagulase negative staphylococci. Journal of clinical pathology, 42(8), 872–874.

Geoffry, K., & Achur, R. N. (2018). Screening and production of lipase from fungal organisms. Biocatalysis and agricultural biotechnology, 14, 241-253.

Katiyar, M., & Ali, A. (2013). Effect of metal ions on the hydrolytic and transesterification activities of Candida rugosa lipase. Journal of Oleo Science, 62(11), 919-924.

Khan, M.S.A., Ahmad, I., Aqil, F., Owais, M., Shahid, M., Musarrat, J. (2010). Virulence and Pathogenicity of Fungal Pathogens with Special Reference to Candida albicans . In: Ahmad, I., Owais, M., Shahid, M., Aqil, F. (eds) Combating Fungal Infections. Springer, Berlin, Heidelberg.

Köhler, G. A., Brenot, A., Haas-Stapleton, E., Agabian, N., Deva, R., & Nigam, S. (2006). Phospholipase A2 and phospholipase B activities in fungi. Biochimica et biophysica acta, 1761(11), 1391–1399.

Kumar, J., Schäfer, P., Hückelhoven, R., Langen, G., Baltruschat, H., Stein, E., … & Kogel, K. H. (2002). Bipolaris sorokiniana, a cereal pathogen of global concern: cytological and molecular approaches towards better control. Molecular plant pathology, 3(4), 185-195.

Lee, J. H., & Jo, W. K. (2005). Exposure to airborne fungi and bacteria while commuting in passenger cars and public buses. Atmospheric environment, 39(38), 7342-7350.

Li, T., Im, J., & Lee, J. (2022). Genetic Diversity of Epicoccum nigrum and its Effects on Fusarium graminearum. Mycobiology, 50(6), 457-466.

Lumi Abe, C. A., Bertechini Faria, C., Fernandes de Castro, F., De Souza, S. R., dos Santos, F. C., Novais da Silva, C., … & Barbosa-Tessmann, I. P. (2015). Fungi isolated from maize (Zea mays L.) grains and production of associated enzyme activities. International journal of molecular sciences, 16(7), 15328-15346.

Mehta, Y. R. (1998). Helminthosporium blights of wheat: spot blotch and tan spot. Constraints on the integrated management of spot blotch of wheat. Mexico, DF, Mexico: CIMMYT, 18-27.

Mezher, M. A., & Bandar, K. I. (2015). Identification study some virulence factors of invasive mold infections isolated from patients undergoing chemotherapy in Tikrit teaching Hospital. Egyptian Academic Journal of Biological Sciences, G. Microbiology, 7(1), 1-11.

Naglik, J. R., Challacombe, S. J., & Hube, B. (2003). Candida albicans secreted aspartyl proteinases in virulence and pathogenesis. Microbiology and molecular biology reviews, 67(3), 400-428.

Navathe, S., Yadav, P. S., Chand, R., Mishra, V. K., Vasistha, N. K., Meher, P. K., … & Gupta, P. K. (2020). ToxA–Tsn1 interaction for spot blotch susceptibility In Indian wheat: An example of inverse gene-for-gene relationship. Plant disease, 104(1), 71-81.

Ogórek, R., Dyląg, M., & Kozak, B. (2016). Dark stains on rock surfaces in Driny Cave (Little Carpathian Mountains, Slovakia). Extremophiles : life under extreme conditions, 20(5), 641–652.

Pandey, S. P., Sharma, S., Chand, R., Shahi, P., & Joshi, A. K. (2008). Clonal variability and its relevance in generation of new pathotypes in the spot blotch pathogen, Bipolaris sorokiniana. Current microbiology, 56(1), 33-41.

Poloni, A., Pessi, I. S., Frazzon, A. P. G., & Van Der Sand, S. T. (2009). Morphology, physiology, and virulence of Bipolaris sorokiniana isolates. Current microbiology, 59(3), 267-273.

Ramage, G., Bachmann, S., Patterson, T. F., Wickes, B. L., & López-Ribot, J. L. (2002). Investigation of multidrug efflux pumps in relation to fluconazole resistance in Candida albicans biofilms. Journal of Antimicrobial Chemotherapy, 49(6), 973-980.

Saleem, A. R. (2008). Effect of some food preservatives on the lipolytic activity of beef luncheon fungi. Mycobiology, 36(3), 167-172.

Samson, R. A. (2002). Introduction to food and airborne fungi. American Society Microbiology.

Sierra, G. (1957). A simple method for the detection of lipolytic activity of micro-organisms and some observations on the influence of the contact between cells and fatty substrates. Antonie van Leeuwenhoek, 23, 15-22.

St-Germain, G., & Summerbell, R. C. (2011). Identifying fungi: a clinical laboratory handbook. (No Title).

Tekpinar, A. D., & Kalmer, A. (2019). Utility of various molecular markers in fungal identification and phylogeny. Nova Hedwigia, 109(1-2), 187-224.

Tomee, & Kauffman. (2000). Putative virulence factors of Aspergillus fumigatus. Clinical & Experimental Allergy, 30(4), 476-484.

Wu, Y., Chen, A., Luhung, I., Gall, E. T., Cao, Q., Chang, V. W. C., & Nazaroff, W. W. (2016). Bioaerosol deposition on an air-conditioning cooling coil. Atmospheric Environment, 144, 257-265.

Xu, X., Li, J., Yang, X., Zhang, L., Wang, S., Shen, G., … & Xiang, W. (2022). Epicoccum spp. Causing maize leaf spot in Heilongjiang province, China. Plant Disease, 106(12), 3050-3060.