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# Phylogeny of *Aspergillus clavatus* isolated from primary schools of Iraq and detection of its ability for production of Patulin

**Maysoon M. AL-Jobouri**
Muthanna Education Directorate, Iraq. E-mail: myswnmjyd766@gmail.com

**Ihsan Ali Alzamily**
*Corresponding author. Department of Applied Biotechnology, College of Biotechnology, Al-Qasim Green University. Babylon 51013, Iraq. E-mail: ihsan@biotech.uoqasim.edu.iq

**Majid Kadhim Al-Shibly**
Department of biology, College of Education, Al-Qadisiyah University, Iraq. E-mail: majid.shibly@qu.edu.iq

## *Abstract*

**Objective**

Schools are among of the most essential environments of children. Children spend an extensive period of time at school. However, indoor air pollution in schools might be dangerous. Fungi's specific health impacts in restricted areas can lead to acute health problems, particularly among school students. The current study carried out in the primary schools of Samawa city that included the selection of several schools randomly for isolation of fungi from the floors, air, door handles and seats in a direct isolation manner then diagnosed by traditional methods in addition to PCR technique. The current study aimed to record *Aspergillus clavatus* as an etiological agent in schools of Iraq and investigating existence of *PatA* gene which is responsible of production of Patulin toxin.

**Materials and Methods**

The samples collection included floors, handles, seats and air. They were collected in a direct isolation method by cotton swabs. Using Bio-Rad Laboratories' Prep-a-Gene system, low-melting-point agarose was purified to get the amplification products needed for sequencing. The dideoxy technique was used to DNA sequencing.

**Results**

The isolated fungi belonging to five genera, Aspergillus, Penicillium, Alternaria, Rhizopus and Cladosporium with the predominance of the genus Aspergillus where it represents 20 isolates and

five species, *Aspergillus niger*, *A. flavus*, *A. candidus*, *A. terreus* and *A. clavatus*. The fungus Penicillum came second represented by seven isolates, *Penicillium chrysogenum*, *Penicillium digitatum* and *P. candidus*. The genus Alternaria represented by four isolates, *A. alternata* and *A.oryzae*. Then the genus Cladosporium with *C. cladosporioides* and *C. herparum* and finally *Rhizopus stolonifer*. Molecular identification was done for the suspected isolate and confirmed that is belonging to *A. clavatus* and the phylogenetic tree was drawn to support the findings. Also, the study included the determination of *PatA* gene that is responsible for producing Patulin in this species.

**Conclusion**

Phylogenetic tree analysis showed a clear convergence between the sequences of fungus under study and other species registered on the NCBI gene bank.

**Key words**: *Aspergillus clavatus*, PatA, Patulin, phylogenetic tree, primary school

**Paper Type:** Research Paper.

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## Introduction

Schools are among the most crucial environments for children (Daisey et al., 2003). Children spend the majority of their time at school (Mendell et al., 2005). However, indoor air pollution in schools can have negative health consequences (Fouladi Fard et al., 2018). According to Hu et al. (2019), monitoring of indoor air quality helps of raise public awareness of indoor air quality and its interaction with students at home. Because of their peculiar characteristics in closed areas, fungi have the potential to create acute health concerns, particularly in schoolchildren. Furthermore, whether fungus or particles, they are identified as two significant causes of upper respiratory infections in children (Liu et al., 2014). Furthermore, exposure to fungi-containing particles has been associated to an increase in allergy reactions (Moon et al., 2014). Similarly,

research has revealed a link between asthma and fungus and in schools (Cai et al., 2011). Over the last decade, clinicians and the general public have become more aware of the critical role that mold sensitivity plays in the development of allergic diseases such as hypersensitivity pneumonitis, allergic rhinitis, allergic fungal sinusitis, bronchopulmonary mycoses, and allergic asthma (Dziadzio and Bush, 2001). Fungal vast distribution and ubiquity account for their presence throughout the biosphere. Air plays a vital role in their dissemination. Because fungal spores are so small, anemochory is assumed to play the most important function in their transmission. Fungal spores can spread with even the tiniest air movement and, due to their small size, can remain in the air we breathe for an extended length of time. These features of fungal spores give rise to their unique dominance over other airborne bio-components, forming a particular bio-colloid with gas serving as a dispersing phase. These features of fungal spores give rise to their unique dominance over other airborne bio-components, forming a particular bio-colloid with gas serving as a dispersing phase. Meteorological factors such as temperature, humidity, air shifts, and atmospheric pressure can also affect the duration of fungal spore retention in the atmosphere. The smaller the spores, the slower they are falling and the greater their tendency to be drifted with Brownian motions, and thus to diffuse in different directions (Pepper & Gerba, 2015; Grinn-gofro, 2011). *Aspergillus clavatus* grows quickly, producing a velvety, rather dense felt that is seen to be bluish-grey green in color. When the conidial heads first emerge, they are big and clavate, and they soon separate into prominent, compact diverging columns (Waksman et al., 1943). It is a saprophyte fungus isolated from soil, grains, air and door handles, characterized by a thin, transparent, divided and branched mycelium, but what distinguishes it is the conidian head, which is characterized by being an elongated club clavate and arise on it phialide, which in turn gives chains of blackboards with the color of bluish green and this color is due to the pigments produced by the fungus specifically in the wall of conidia (Hajji et al., 2007). Animal feces and soil are popular places to find *Aspergillus clavatus*. It causes hypersensitivity pneumonitis, commonly referred to as malt-worker's lung, and is allergic, also it can secret mycotoxins as Patulin (Lopez-Diaz and Flannigan,1997a). *Aspergillus clavatus*, which is linked to a particular neurotoxicosis in ruminants, produce patulin, a cyclic γ-lactone. Patulin has been demonstrated to be poisonous to mammals, plants, bacteria, protozoa, fungi, viruses, in addition to its neurotoxic effects (Bérdy, 2005; Fouillaud & Dufossé, 2022). Moreover, biochemical tests are incapable of distinguishing different types of microorganisms, such as bacteria, viruses, and parasites (Ahsani et al. 2010; Mohammadabadi et al. 2004; Khabiri et al., 2025). Genomic techniques such as PCR and sequencing are the most modern practical technology in diagnosing infectious diseases and compared with classical techniques, it has been shown to be more rapid, with results obtained in a few hours, and also more reliable

(Mohammadabadi et al. 2011; Khabiri et al. 2023; Mohammadabadi et al., 2025). Genomic techniques allow a faster identification directly from clinical samples (Shahdadnejad et al. 2016; Mohammadabadi et al. 2024). The current study aimed to record *Aspergillus clavatus* for the first time in Iraq and investigating the presence of the *PatA* gene which is responsible for the production of Patulin toxin.

## Materials and methods

**Samples collection and cultivation:** The study carried out in the laboratory of Biology dep.at science college of Muthana university in the period between March 2023 to July 2023. Samples were collected from a number of primary schools in the city of Samawa, where the collection included floors, handles, seats and air. They were collected in a direct isolation method by cotton swabs. As for the air, the fungi were isolated from it by preparing Petri dishes containing the medium Sabouraud dextrose Agar, they were opened in the air of rooms and halls and left for 3 minutes, after which the dishes were closed and sent to the laboratory for incubation at a temperature of 28 ° C for a week, after which the growth were observed and the numbers and nature of the growth were recorded (Alshibly et al., 2019; Ejdys et al., 2013).

**Fungi identification:** Traditional methods: including macroscopic characters as color,texture and speed of growth of the colony and microscopic examination to note mycelium, spores and conidiophore. These features were compared with standard criteria in taxonomic keys and atlases (Harris, 2001; Sciortino, 2017; Watanabe, 2010).

**DNA extraction:** DNA was extracted by following Bioneer kit's instructions. Subsequently, partially combine with ethidium bromide dye on the 1.5% agarose gel prepared in 100 mL TBE buffer solution at a concentration of (1X) mL provided by Bio Basic in order to confirm its purity.
**PCR product:** A (50 µL) reaction mixture was used for the PCR, which included 0.5 p.g of DNA from each Aspergillus isolate , 10 mM Tris-HCl (pH 9.0 at 25°C), 10 mM KCl, 1.4 mM MgCl2, 0.1% Triton X-100, 50 pmol of each of the two primers,0.2 mM (each) deoxynucleoside triphosphates (dNTPs), 0.2 U of SuperTaq DNA polymerase (AccuPower® PCR PerMix kit (Bioneers) that have been denatured at 94°C for five minutes. Subsequently, amplification was carried out in 30 cycles, which involved denaturing for 1 minute at 94°C, primer annealing for 1 minute at 42°C, and permitting elongation for 3 minutes at 72°C. A product of amplification that produced about 1,800 bp when it was successful. Every product was subjected to 1.5% agarose gel electrophoresis for analysis.

**DNA sequencing:** Using Bio-Rad Laboratories' Prep-a-Gene system, low-melting-point agarose was purified to get the amplification products needed for sequencing. The dideoxy

technique was used to sequence DNA. The process of annealing a primer to the template involved heat denaturation of the double-stranded PCR products while a primer is present, followed by immediate freezing in an ethanol bath cooled by carbon dioxide. Sequencing primers were derived from multiple conserved areas of 18S RNA. A VAX computer performing a sequence analysis program developed by the University of Wisconsin's Genetics Computer Group aligned the resulting sequences to those of organisms previously described. specific sequences of Aspergillus were then selected and their specificity tested (Sanger et al., 1977).

**Investigation of patulin production:** Concerning fungal toxins, especially patulin toxin, was investigated by using the primer for this test, as shown in the Table 1 from the same company (White and Barnes, 2008). Electrophoresis of the product of polymerization reactions: mixing 10 µL of gene amplification products with 3 µL of loading buffer on the surface of a clean glass plate. The mixture was put into a designated hole in the agarose gel at a concentration of 1.5%, where the samples were electrocuted with a voltage of 80 volts and 100 mA for a period of 55 Compared to the standard (100-2000 bp) DNA Ladder, the gel sheet was lifted from the device and exposed to ultraviolet light with a wavelength of (320) nm by a UV box, and the gel image was taken using a camera (Diba et al., 2014).

**Table 1. Characterization of used primers to identify *A. clavatus* and PatA**

| Name of primers | | Primer sequence ('3→5') | Reference |
|---|---|---|---|
| ITS | F | CAGAGCCGGAAAGTTGGTCA | Diba et al., 2014 |
| ITS-4 | R | CCTACAAGAGCGGGTGACAA | |
| PatA | F | ATGGAAGTGGTATCGTGCTG | |
| | R | TTACGGACCCACGAAGGTTA | |

## Results

The isolated fungi distributed to five genera Aspergillus, Penicillium, Alternaria, Cladosporium and Rhizopus, the genus Aspergillus has the predominance for other genera as it represents 20 isolates and five species which are *Aspergillus niger*, *A. flavus*, *A. candidus*, *A. terreus* and *A. clavatus* by 8, 6, 3, 2 and 1 isolates respectively. The fungus Penicillum came second represented by seven isolates, four of which were *Penicillium chrysogenum*, two *Penicillium digitatum* and one *P candidus*. The genus Alternaria represented by four isolates three of them were *A. alternata* and one was *A. oryzae*. Cladosporium with two isolates one belongs to C.*herparum* and the other was *C. cladosporioides* and finally *Rhizopus stolonifer* with one isolate (Table 2). The fungus has a consistent color that tends toward green or olive, becoming grayish with age. Under a microscope, it is characterized by club-shaped, elongated sacs and thin, spindle-shaped filaments (Figure 1).

**DNA sequencing:** The sequential sequencing technique was used to isolate the diagnosed after the completion of the PCR test, the test result was sent to the company Macrogen in the southern Korea to conduct the sequence of nitrogen bases using the AB DNA sequencing device, and then the results were compared with fungal sequences registered on the NCBI gene bank using the MEGA10 program (Figure 3).

**Table 2. Characterization of isolated fungi from primary school**

| Fungi | No. of isolates | Percentage (%) | Species |
|---|---|---|---|
| Aspergillus | 20 | 66.7 | *A. niger* (8), *A. flavus* (6), *A. candidus* (3), *A. terreus* (2), *A. clavatus* (1) |
| Penicillium | 7 | 23.3 | *P. chrysogenum* (4), *P. digitatum* (2), *P. candidus* (1) |
| Alternaria | 4 | 13.3 | *A. alternata* (3), *A. oryzae* (1) |
| Cladosporium | 2 | 6.7 | *C. cladosporioides* (1), *C. herparum* (1) |
| Rhizopus | 1 | 3.3 | *R. stolonifer* |

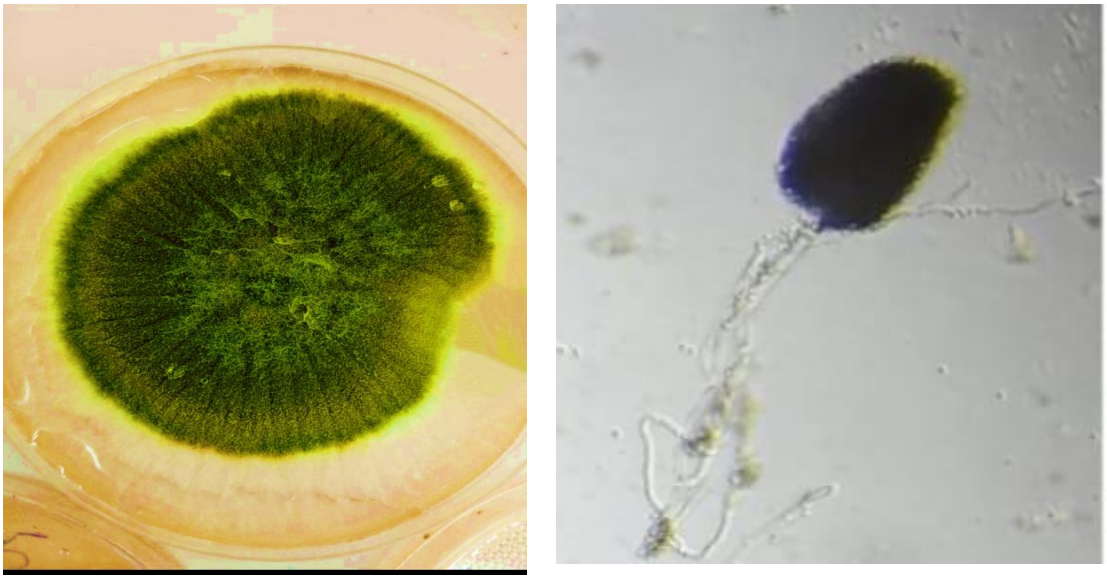

**Figure 1. Morphology of *A. clavatus* (colony and conidial head)**

**Molecular identification of *A. clavatus*:** The PCR test was conducted for the purpose of diagnosing *A. clavatus* .The results of the study showed that among the suspected isolates there is one isolation belonging to this species, where it appeared with a base pair of 210 has differed from the rest of the isolates phenotypically and genetically and we looked at local sources and found that this fungus was isolated for the first time at Iraq (Figure 2). Genetic tree analysis results showed a clear convergence between sequences of fungus under study and other species registered on the NCBI GenBank and the phenomenon in the genetic tree analysis, and the percentage of

correspondence for the sequence of nitrogen bases for the isolates recorded globally (OK090868.1) was 99% as in (Figure 4).

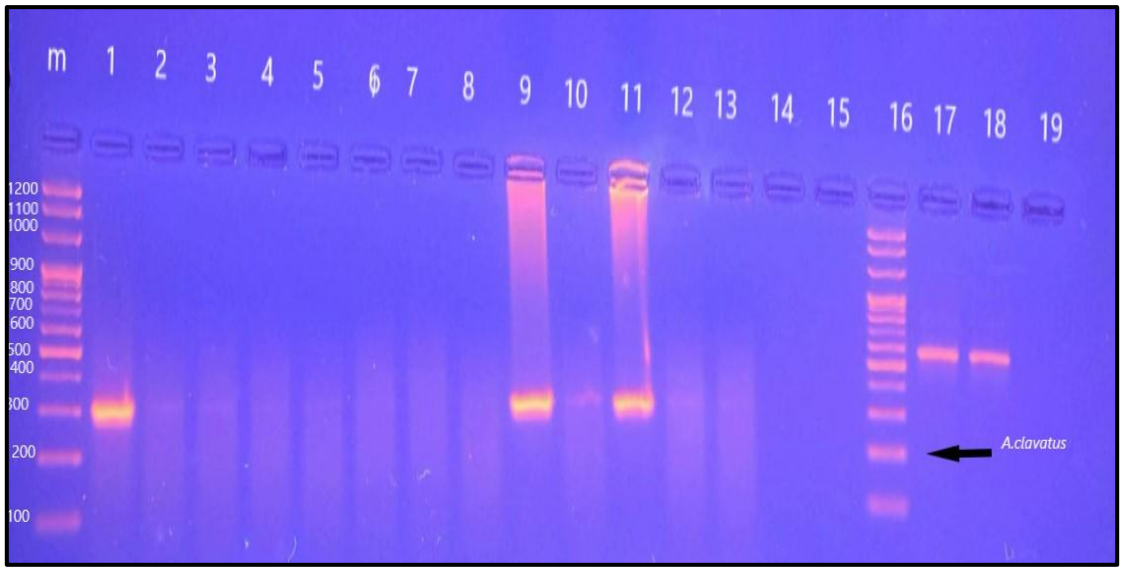


**Figure 2. Electrophoresis results for the polymerization of the 18S rRNA region of *Aspergillus* sp, 1x TBE buffer, 1.5% agarose gel concentration for an hour and a half, size scale of the DNA bundle (100bp-1200bp)**


**Figure 3. Sequences of the nitrogen bases of a gene 18SrRNA of *A. clavatus* compared with fungi recorded in NCBI**

**Investigating *PatA* gene responsible for producing Patulin in *A. clavatus*:** The current study included the investigation of the gene responsible for the production of the Patulin as one of the important toxins. The results of electrophoresis show the presence of this gene in the isolate of this species, which indicates the seriousness of this isolate, especially since schools frequented

by young students who are ignorant of dealing with dangers, especially the threat of fungal contaminants (Figure 5).

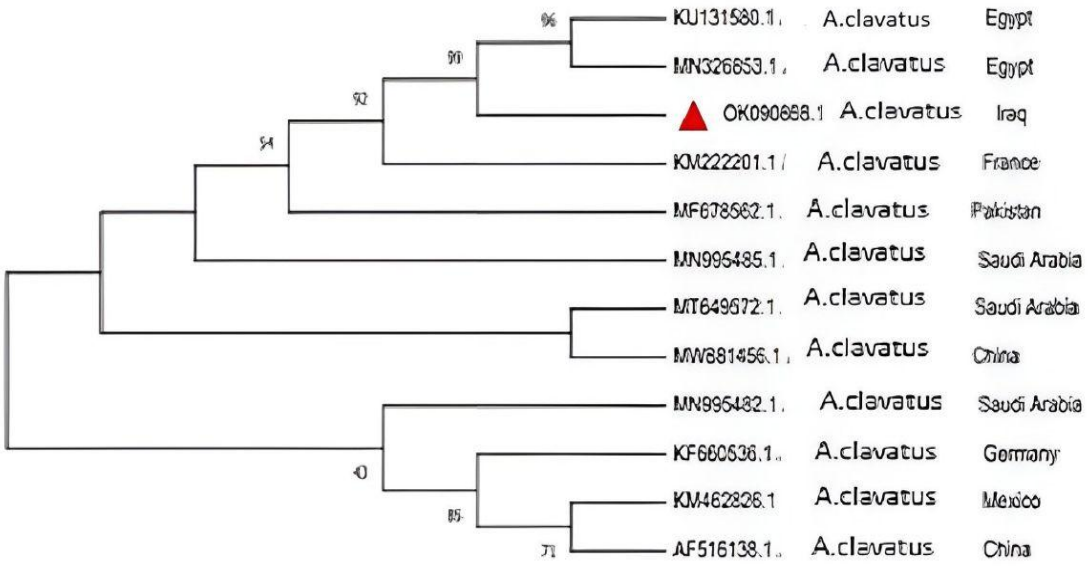


**Figure.4 shows the phylogenetic position of *Aspergillus clavatus* OK090888.1. DNA sequences for A. clavatus OK090888.1 Aspergillus section Clavati strains was utilized for comparison to strain type. MEGA 6 software was used to generate a phylogenetic tree using the greatest likelihood technique. The bootstrap analysis was carried out with 1,000 replications. Bar represents 0.05 substitutions for each nucleotide position. Just values more than 70% are indicated.**

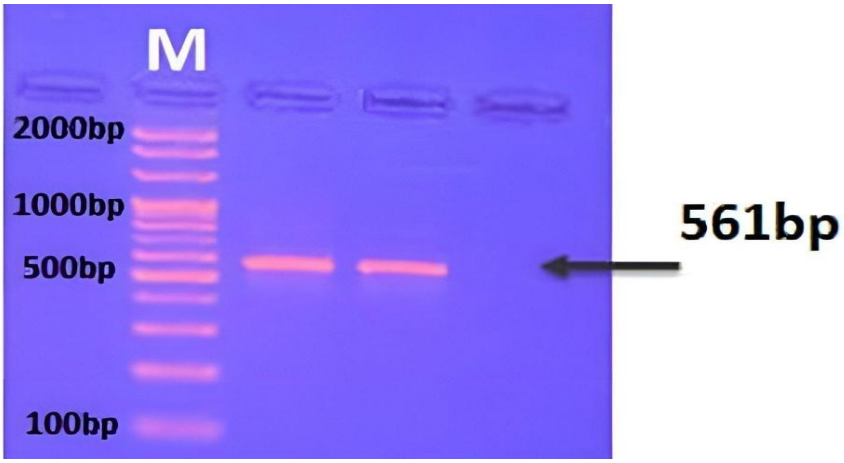


**Figure 5. Electrophoresis results for the polymerization of the gene of *PalA gene* belong to *A. clavatus* agarose gel concentration 1.5% for an hour and a half, using 1x TBE buffer and the size scale of the DNA bundle (100bp-2000bp)**

**Discussion**

The study of Ejdys et al. (2013) demonstrated that the most commonly isolated fungi were Aspergillus (36 species), including *Aspergillus clavatus* (12 isolates), Penicillium (26 species) and Candida (14 species) were isolated from schools (Ejdys et al., 2013). According to study of Fouladi-Fard et al. (2023) Aspergillus was shown to be the primary cause of fungal growth in school buildings and *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus candidus* being the most common species. In a study that was carried out in a few Egyptian governorates revealed that the most common fungal species of found in samples was Aspergillus spp., which was followed by Penicillium spp. and Alternaria spp. (Abdel-Nasser et al., 2022). Aspergillus species accounted for the largest percentage of occurrences (23%), followed by Cladosporium species (21%), *Fusarium oxysporum* (19%), and Pencillium species (17%). Conversely, *Alternaria alternaria* recorded 11% and Trichophyton spp., 9% (Jasim et al., 2021). According to Charles et al. (2009), Aspergillus spp. are the most prevalent fungi, followed by Cladosporium spp. Cladosporium is a common source of allergies and can be found in both indoor and outdoor air. A reason for the prevalence of Aspergillus spp. due to its capacity to thrive in diverse environments. The fungus can produce a huge number of asexual reproductive units, while certain varieties have sclerotia bodies that are resistant to harsh climatic conditions and a sexual phase (Odebode et al., 2020). Penicillium spp. is a fungus that thrives in both indoor and outdoor environments, allowing it to adapt and live despite food shortage (Park et al., 2013). Alternaria spp. is a fungus that has been linked to human health issues (Davis, 2001). Fusarium spp. and other thrush fungi disseminate their spores in the air. All fungal strains may produce germs and are present in the air we breathe, whether it's outside or inside. The current study showed that the first record of the fungus *A. clavatus*, which was isolated from the air of one of the schools under study, a saprophytic fungus that decomposes organic matter and is known to sometimes cause respiratory diseases for people with weak immunity as well as famous for the production of toxins, especially patulin toxin, which causes digestive and neurological problems and causes kidney and liver failure, cancers and fetal malformations (Lopez-Diaz and Flannigan, 1997b; Tomee and Kauffman, 2000). The *A. clavatus* genome has recently been found to have a 15 genes cluster of utilized in production of patulin. A 40 kb region contains all of the genes. Genes encode not just the particular regulatory factor and transporters but also the enzymes required for the toxin's production. Three transporter genes are found in this cluster: an acetate transporter, an (Adenosine triphosphate ATP) binding cassette transporter, and an MFS (Major Facilitator Superfamily) transporter (Puel et al., 2010). Although data on genotoxicity was inconsistent, most assays with mammalian cells were positive, while those with bacteria were mostly negative. Patulin has been shown in several experiments to inhibit DNA synthesis. Its genotoxic effects may be linked to its

capacity to react with groups of sulfhydryl resulting in oxidative damage (Liu et al., 2007). However, based on existing data, the WHO concluded that patulin is genotoxic. Several in vitro investigations have shown that patulin suppresses various macrophage activities. Sorenson et al. [89] found that in vitro exposure of alveolar rat macrophages to patulin reduced production of protein and changed functions of membrane. Patulin inhibited O2- generation, microbiological activity in mice macrophages phagosome-lysosome fusion, lysosomal enzyme, and phagocytosis (Puel et al.,2010).

**Conclusions:** Microfungi isolated from school rooms can persist on building materials and in the bioaerosol of rooms, despite the fact that their primary source of building contamination is the external environment. Because of their high environmental flexibility and possible harmful qualities, thermophilic isolates should receive particular attention when it comes to prevalence and species abundance of fungi in schools, especially some species producing toxins such as *A. flavus* and *A. clavatus* and the ability of these fungi of patulin production has been proven. Gene cluster function of patulin is still unknown, despite the fact that the majority of the patulin intermediates have been structurally described. As has already been proven for the regulation of aflatoxin biosynthesis, numerous environmental factors have been shown to affect patulin biosynthesis. As a result, multiple layers of regulation are engaged in patulin biosynthesis. Future research on long-term monitoring, effects of various ventilation systems and remedies including air purification and quality management programs in schools is advised.

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**Author contributions**

The first author wrote the abstract and introduction, the second author write the materials and method section and results and discussion section, the third author write the conclusion and arrangement of references with translation.

**Data availability statement**

The data supporting this study's findings are not publicly available, but are available from the corresponding author upon reasonable request, subject to approval by the relevant institutional review board.

**Ethical considerations**

The study was carried out with integrity, with no fabrication, falsification, plagiarism, or scientific misconduct.

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**Conflicts of interest**

The author has declared that no conflicts of interest.

**References**

Abdel-Nasser, A., Fathy, H. M., Badr, A., Hathout, A., & Barakat, O. S. (2022). Prevalence of aflatoxigenic fungi in cereal grains and their related chemical metabolites. *Egyptian Journal of Chemistry, 65*(10), 455-470. https://doi.org/10.21608/ejchem.2022.122494.5487

Ahsani, M. R., Mohammadabadi, M. R., & Shamsaddini, M. B. (2010). Clostridium perfringens isolate typing by multiplex PCR. *Journal of Venomous Animals and Toxins Including Tropical Diseases, 16*(4), 573-578. https://doi.org/10.1590/S1678-91992010000400006

Alshibly, M. K., Al Zamily, I. A., & Fazil, N. (2019). Survey of fungi found in books on the shelves of the libraries of the University of Qadisiyah—Iraq. *IOP Conference Series: Materials Science and Engineering, 571*(1), Article 012042. https://doi.org/10.1088/1757-899X/571/1/012042

Bérdy, J. (2005). Bioactive microbial metabolites. *The Journal of Antibiotics, 58*(1), 1-26. https://doi.org/10.1038/ja.2005.1

Cai, G. H., Hashim, J. H., Hashim, Z., Ali, F., Bloom, E., Larsson, L., ... Norbäck, D. (2011). Fungal DNA, allergens, mycotoxins and associations with asthmatic symptoms among pupils in schools from Johor Bahru, Malaysia. *Pediatric Allergy and Immunology, 22*(3), 290-297. https://doi.org/10.1111/j.1399-3038.2010.01127.x

Charles, B., Freddy, P., Minati, D., & Jay, P. (2009). Alternaria and Cladosporium fungal allergen epitopes are denatured by sodium hypochlorite. *World Allergy Organization Journal, 2*(12), 296-302. https://doi.org/10.1097/WOX.0b013e3181c4c98f

Daisey, J. M., Angell, W. J., & Apte, M. G. (2003). Indoor air quality, ventilation and health symptoms in schools: An analysis of existing information. *Indoor Air, 13*(1), 53-64. https://doi.org/10.1034/j.1600-0668.2003.00153.x

Davis, P. J. (2001). *Molds, toxic molds and indoor air quality*. California Research Bureau, California State Library.

Diba, K., Mirhendi, H., Kordbacheh, P., & Rezaie, S. (2014). Development of RFLP-PCR method for the identification of medically important Aspergillus species using single restriction enzyme MwoI. *Brazilian Journal of Microbiology, 45*(2), 503-507. https://doi.org/10.1590/S1517-83822014000200018

Dziadzio, L., & Bush, R. K. (2001). Assessment and control of fungal allergens. *Current Allergy and Asthma Reports, 1*(5), 455-460. https://doi.org/10.1007/s11882-001-0033-3

Ejdys, E., Dynowska, M., Biedunkiewicz, A., & Sucharzewska, E. (2013). An overview of the species of fungi occurring in school rooms—A four-year study. *Polish Journal of Environmental Studies, 22*(6), 1665-1672.

Fouillaud, M., & Dufossé, L. (2022). Microbial secondary metabolism and biotechnology. *Microorganisms, 10*(1), Article 123. https://doi.org/10.3390/microorganisms10010123

Fouladi Fard, R., Hosseini, M. R., Faraji, M., & Omidi Oskouei, A. (2018). Building characteristics and sick building syndrome among primary school students. *Sri Lanka Journal of Child Health, 47*(4), 332-337. https://doi.org/10.4038/sljch.v47i4.8595

Fouladi-Fard, R., Vaezi, N., Mahvi, A. H., & Beigi, M. Z. (2023). Airborne culturable fungi in primary schools: Building characteristics and environmental factors in Qom, Iran. *Journal of School Administration Research and Development, 8*(2), 97-108. https://doi.org/10.32674/jsard.v8i2.4319

Grinn-Gofroń, A. (2011). Airborne Aspergillus and Penicillium in the atmosphere of Szczecin, (Poland) (2004-2009). *Aerobiologia, 27*(1), 67-76. https://doi.org/10.1007/s10453-010-9177-8

Hajji, M., Kanoun, S., Nasri, M., & Gharsallah, N. (2007). Purification and characterization of an alkaline serine-protease produced by a new isolated Aspergillus clavatus ES1. *Process Biochemistry, 42*(5), 791-797. https://doi.org/10.1016/j.procbio.2007.01.011

Harris, J. L. (2001). [Review of the book *Atlas of clinical fungi* (2nd ed.), by G. S. de Hoog, H. Gene, & M. J. Figueras. Centraalbureau voor Schimmelcultures]. *Mycopathologia, 152*(2), 159-160. https://doi.org/10.1023/A:1013183715057

Hu, J., Li, N., Zou, S., Yoshino, H., Yanagi, U., Yu, C. W., & Qu, H. (2019). Indoor environmental conditions in schoolchildren's homes in central-south China. *Indoor and Built Environment, 29*(7), 956-971. https://doi.org/10.1177/1420326X19875185

Jasim, A. A., Abaed, S. A., & Hassoni, A. A. (2021). Isolation and identification of the air fungi present inside the schools' buildings in Babylon province. *Biochemical & Cellular Archives, 21*(2), 4201-4206. https://connectjournals.com/03896.2021.21.4201

Khabiri, A., Toroghi, R., Mohammadabadi, M., & Tabatabaeizadeh, S. E. (2023). Introduction of a Newcastle disease virus challenge strain (sub-genotype VII.1.1) isolated in Iran. *Veterinary Research Forum, 14*(4), 221-227. https://doi.org/10.30466/vrf.2022.548152.3373

Khabiri, A., Toroghi, R., Mohammadabadi, M., & Tabatabaeizadeh, S. E. (2025). Whole genome sequencing and phylogenetic relative of a pure virulent Newcastle disease virus isolated from an outbreak in northeast Iran. *Letters in Applied Microbiology, 78*(4), Article ovaf049. https://doi.org/10.1093/lambio/ovaf049

Liu, B. H., Wu, T. S., Yu, F. Y., & Su, C. C. (2007). Induction of oxidative stress response by the mycotoxin patulin in mammalian cells. *Toxicological Sciences, 95*(2), 340-347. https://doi.org/10.1093/toxsci/kfl156

Liu, Z., Li, A., Hu, Z., & Sun, H. (2014). Study on the potential relationships between indoor culturable fungi, particle load and children respiratory health in Xi'an, China. *Building and Environment, 80*, 105-114. https://doi.org/10.1016/j.buildenv.2014.05.029

Lopez-Diaz, T. M., & Flannigan, B. (1997a). Production of patulin and cytochalasin E by Aspergillus clavatus during malting of barley and wheat. *International Journal of Food Microbiology, 35*(2), 129-136. https://doi.org/10.1016/S0168-1605(96)01211-1

Lopez-Diaz, T. M., & Flannigan, B. (1997b). Mycotoxins of Aspergillus clavatus: Toxicity of cytochalasin E, patulin, and extracts of contaminated barley malt. *Journal of Food Protection, 60*(11), 1381-1385. https://doi.org/10.4315/0362-028X-60.11.1381

Mendell, M. J., & Heath, G. A. (2005). Do indoor pollutants and thermal conditions in schools influence student performance? A critical review of the literature. *Indoor Air, 15*(1), 27-52. https://doi.org/10.1111/j.1600-0668.2004.00320.x

Mohammadabadi, M. R., Shaikhaev, G. O., Sulimova, G. E., & Rahman, O. (2004). Detection of bovine leukemia virus proviral DNA in Yaroslavl, Mongolian, and Black Pied cattle by PCR. *Cellular and Molecular Biology Letters, 9*(4A), 766-768.

Mohammadabadi, M. R., Soflaei, M., Mostafavi, H., & Honarmand, M. (2011). Using PCR for early diagnosis of bovine leukemia virus infection in some native cattle. *Genetics and Molecular Research, 10*(4), 2658-2663. https://doi.org/10.4238/2011.October.27.2

Mohammadabadi, M., Afsharmanesh, M., Khezri, A., Kheyrodin, H., Babenko, O. I., Borshch, O., Kalashnyk, O., Nechyporenko, O., Afanasenko, V., Slynko, V., & Usenko, S. (2025). Effect of mealworm on GBP4L gene expression in the spleen tissue of Ross broiler chickens. *Agricultural Biotechnology Journal, 17*(2), 343-360. https://doi.org/10.22103/jab.2025.25277.1714

Mohammadabadi, M., Babenko, O. I., Borshch, O., Kalashnyk, O., Ievstafiieva, Y., & Buchkovska, V. (2024). Measuring the relative expression pattern of the UCP2 gene in different tissues of the Raini Cashmere goat. *Agricultural Biotechnology Journal, 16*(3), 317-332. https://doi.org/10.22103/jab.2024.24337.1627

Moon, K. W., Huh, E. H., & Jeong, H. C. (2014). Seasonal evaluation of bioaerosols from indoor air of residential apartments within the metropolitan area in South Korea. *Environmental Monitoring and Assessment, 186*(4), 2111-2120. https://doi.org/10.1007/s10661-013-3521-8

Odebode, A., Adekunle, A., Stajich, J., & Adeonipekun, P. (2020). Airborne fungi spores' distribution in various locations in Lagos, Nigeria. *Environmental Monitoring and Assessment, 192*(2), Article 87. https://doi.org/10.1007/s10661-019-8038-3

Park, M., Do, E., & Jung, W. H. (2013). Lipolytic enzymes involved in the virulence of human pathogenic fungi. *Mycobiology, 41*(2), 67-72. https://doi.org/10.5941/MYCO.2013.41.2.67

Pepper, I. L., & Gerba, C. P. (2015). Aeromicrobiology. In *Environmental microbiology* (pp. 89-110). Academic Press. https://doi.org/10.1016/B978-0-12-394626-3.00005-3

Puel, O., Galtier, P., & Oswald, I. P. (2010). Biosynthesis and toxicological effects of patulin. *Toxins, 2*(4), 613-631. https://doi.org/10.3390/toxins2040613

Sanger, F., Nicklen, S., & Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences, 74*(12), 5463-5467. https://doi.org/10.1073/pnas.74.12.5463

Sciortino, C. V., Jr. (2017). *Atlas of clinically important fungi*. John Wiley & Sons. https://doi.org/10.1002/9781119069720

Shahdadnejad, N., Mohammadabadi, M. R., & Shamsadini, M. (2016). Typing of Clostridium perfringens isolated from broiler chickens using multiplex PCR. *Genetics in the Third Millennium, 14*(4), 4368-4374.

Tomee, J. F. C. M., & Kauffman, H. F. (2000). Putative virulence factors of Aspergillus fumigatus. *Clinical & Experimental Allergy, 30*(4), 476-484. https://doi.org/10.1046/j.1365-2222.2000.00796.x

Waksman, S. A., Horning, E. S., & Spencer, E. L. (1943). Two antagonistic fungi, Aspergillus fumigatus and Aspergillus clavatus, and their antibiotic substances. *Journal of Bacteriology, 45*(3), 233-248. https://doi.org/10.1128/jb.45.3.233-248.1943

Watanabe, T. (2010). *Pictorial atlas of soil and seed fungi: Morphologies of cultured fungi and key to species* (3rd ed.). CRC Press. https://doi.org/10.1201/EBK1439804193

White, P. L., & Barnes, R. A. (2008). Aspergillus PCR. In *Aspergillus fumigatus and aspergillosis* (pp. 373-388). ASM Press. https://doi.org/10.1128/9781555815523.ch29

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# فیلوژنی قارچ *Aspergillus clavatus* جداشده از مدارس ابتدایی عراق و بررسی توانایی آن در تولید پاتولین

**میسون م. الجبوری**

اداره آموزش استان مثنی، عراق. ایمیل: myswnmjyd766@gmail.com

**احسان علی الزاملی**

*نویسنده مسئول. گروه زیست‌فناوری کاربردی، دانشکده زیست‌فناوری، دانشگاه سبز القاسم، بابل ۵۱۰۱۳، عراق. ایمیل: ihsan@biotech.uoqasim.edu.iq

**ماجد کاظم الشبلی**

گروه زیست‌شناسی، دانشکده علوم تربیتی، دانشگاه القادسیه، عراق. ایمیل: majid.shibly@qu.edu.iq

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## چکیده

**هدف:** مدارس از مهم‌ترین محیط‌های زندگی کودکان به شمار می‌روند و کودکان مدت زمان قابل‌توجهی را در مدرسه سپری می‌کنند. با این حال، آلودگی هوای داخل مدارس می‌تواند خطرناک باشد. اثرات بهداشتی خاص قارچ‌ها در محیط‌های بسته می‌تواند منجر به مشکلات حاد سلامت، به‌ویژه در میان دانش‌آموزان، شود. مطالعه حاضر در مدارس ابتدایی شهر سماوه انجام شد که در آن چندین مدرسه به‌صورت تصادفی انتخاب شدند و جداسازی قارچ‌ها از کف، هوا، دستگیره درها و نیمکت‌ها به روش جداسازی مستقیم انجام گرفت. سپس شناسایی قارچ‌ها با استفاده از روش‌های سنتی و همچنین تکنیک PCR صورت پذیرفت. هدف این مطالعه، ثبت *Aspergillus clavatus* به‌عنوان عامل اتیولوژیک در مدارس عراق و بررسی وجود ژن *PatA* به‌عنوان ژن مسئول تولید سم پاتولین بود.

**مواد و روش‌ها:** نمونه‌برداری از کف، دستگیره‌ها، نیمکت‌ها و هوا انجام شد. نمونه‌ها با استفاده از سوآپ‌های پنبه‌ای و به روش جداسازی مستقیم جمع‌آوری گردیدند. به‌منظور خالص‌سازی محصولات تکثیر مورد نیاز برای تعیین توالی، از سیستم Prep-a-Gene شرکت Bio-Rad Laboratories و آگاروز با نقطه ذوب پایین استفاده شد. تعیین توالی DNA با روش دیدئوکسی انجام گرفت.

**نتایج:** قارچ‌های جداشده به پنج جنس شامل *Aspergillus*، *Penicillium*، *Alternaria*، *Rhizopus* و *Cladosporium* تعلق داشتند که جنس *Aspergillus* غالب بود و شامل ۲۰ ایزوله و پنج گونه *Aspergillus niger*، *A. flavus*، *A. candidus*، *A. terreus* و *A. clavatus* می‌شد. جنس *Penicillium* در رتبه دوم قرار داشت و با هفت ایزوله شامل *Penicillium chrysogenum*، *Penicillium digitatum* و *P. candidus* شناسایی شد. جنس *Alternaria* با چهار ایزوله شامل *A. alternata* و *A. oryzae* مشاهده گردید. سپس جنس *Cladosporium* با گونه‌های *C. cladosporioides* و *C. herbarum* و در نهایت *Rhizopus stolonifer* شناسایی شدند. شناسایی مولکولی برای ایزوله مشکوک انجام شد و تعلق آن به *A. clavatus* تأیید گردید. همچنین درخت فیلوژنتیکی برای پشتیبانی از یافته‌ها ترسیم شد. علاوه بر این، وجود ژن *PatA* که مسئول تولید پاتولین در این گونه است، مورد بررسی قرار گرفت.

**نتیجه‌گیری:** تحلیل درخت ژنتیکی همگرایی آشکاری را بین توالی‌های قارچ مورد مطالعه و سایر گونه‌های ثبت‌شده در پایگاه داده ژن‌بانک NCBI نشان داد.

**کلمات کلیدی:** پاتولین، درخت فیلوژنتیکی، مدرسه ابتدایی، *Aspergillus clavatus*، PatA

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**استناد:** میسون م. الجبوری، احسان علی الزاملی، ماجد کاظم الشبلی (۱۴۰۵) فیلوژنی قارچ Aspergillus clavatus جداشده از مدارس ابتدایی عراق و بررسی توانایی آن در تولید پاتولین. *مجله بیوتکنولوژی کشاورزی*، ۱۸(۲)، ۱۷۳-۱۸۸.

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