Iraqi Journal of Market Research and Consumer Protection
(2025) 17(3): 198-208.
DOI:
Talib & Abdalshaheed

المجلة العراقية لبحوث السوق
وحماية المستهلك

DETECTION OF *ASPERGILLUS FUMIGATUS* BY POLYMERASE CHAIN REACTION

***Noor Their Talib*[1], *Dalia Abdalkareem Abdalshaheed*[2]**

1Lecturer Department of Microbiology, College of Veterinary Medicine, University of Baghdad, talibnoorthier@gmail.com;

2Professor PhD. Department of Microbiology, College of Veterinary Medicine, University of Baghdad, Correspondence, dalia@covm.uobaghdad.edu.iq

Received 11/ 2/ 2024, Accepted 5/ 8/ 2024, Published 31/ 12/ 2025

ABSTRACT

This research was aimed to isolate and diagnose *Aspergillus species* from this - infected animals collected. From several Baghdad areas, Fifty samples were taken from animals with respiratory illnesses. Molds were isolated and identified using conventional methods (fungal culture on Czapek-Dox agar incubated at 37°C for 3–7 days, then macroscopic, microscopic examination of the colony with lactophenol cotton blue stain) and molecular methods (PCR for identification). Partial ribosomal DNA (rDNA) from different *Aspergillus strains* was aligned to find the optimum primers for gene and species identification. A PCR approach for identifying *Aspergillus fumigatus*-related species was developed utilizing specific primers. To examine the specificities and sensitivities of such primers, 14 putative *Aspergillus fumigatus* isolates were PCR-tested. Only 3 were verified as having Glip P toxin. The PCR technique was sensitive and detected *A. fumigatus* isolates and closely related species. Our findings show that these primers can identify *A. fumigatus*. In a phylogenetic tree study, we compared our ITS sequence (Iraq), OR578448.1 to global sequences. Our sequence was more similar to Russia and Brazil, OR578448.1 and OR727320.1, but more different from Portugal, KF367498.1 and Pakistan, MT316338.1. Detecting the gliP gene in A. fumigatus was especially fascinating because of its medicinal involvement in gliotoxin production. PCR detects *Aspergillus fumigatus* in aspergillosis quickly, precisely, and sensitively.

Keywords: Small ribosomal gene (ITS), *Aspergillus fumigatus*, PCR Technique, Sequencing, Phylogenetic tree.

تشخيص فطر الرشاشيات الدخناء بواسطة تقنية PCR

***نور ثاير طالب*[1] , *داليا عبد الكريم عبد الشهيد*[2]**

[1]المدرس، فرع الاحياء المجهرية، كلية الطب البيطري،جامعة بغداد، بغداد، العراق، talibnoorthier@gmail.com
[2]الاستاذ الدكتور، فرع الاحياء المجهرية، كلية الطب البيطري، جامعة بغداد، بغداد، العراق، dalia@covm.uobaghdad.edu.iq

الخلاصة

هدف هذا البحث الى عزل وتشخيص الفطر التنفسي الرشاشيات الدخناء من الابقار المصابة بأعراض تنفسية و فحص امكانية انتاج الكليوتوكسين من هذا الفطر من خلال تحديد الجين المسؤول عن انتاج السم, حيث تم اخذ خمسون عينة من عدة مناطق في بغداد. وتم العزل والتشخيص باستخدام الطرق التقليدية بزراعة العزلات على الاوساط الزرعية المخصصة لزرع الفطريات وحضنها بدرجة 37 درجة مئوية لمدة 3-7 أيام وكذلك استخدام الطرق الجزيئية لتشخيص الفطر، بعدها تم اجراء الفحص العياني والمجهري للمستعمرة باستخدام صبغة اللاكتوفينول الزرقاء حيث تم عزل (30 عزلة موجبة), كما وتم استخدام الطرق الجزيئية لتحديد البرايمر المناسب لتشخيص الجين المسؤول عن انتاج السم. حيث فحصت 14 عزلة لفطر الرشاشيات الدخناء وكانت 3 عزلات فقط تحتوي على الجين (glip gene) المسؤول عن انتاج الكليوتوكسين بالفحص الجزيئي وكذلك تم تثبيت الشجرة الوراثية لفطر الرشاشيات الدخناء بالعراق ومقارنتها مع الدول حيث وجد ان العزلات الاكثر تشابه كانت في روسيا والبرازيل واكثر الاختلاف كانت في البرتغال وباكستان. ومن خلال هذه

الدراسة وجد ان استخدام الفحص الجزيئي تعتبر طريقة سريعة ودقيقة لتشخيص فطر الرشاشيات الدخناء و تحديد الجين المسؤول عن انتاج سم الكليوتوكسين.

الكلمات المفتاحية: الرايبوسومات صغيرة الجين , فطر الرشاشيات الدخناء,تفاعل البلمرة المتسلسل,التسلسل الجيني ,الشجرة الوراثية.

INTRODUCTION

Fungi play a very significant role in our life (**Rukaibaa *et al.*, 2020**). *Aspergillus Fumigatus* It is a prevalent fungus with a worldwide spreading, which can be identified in air and soil samples, and even on the international place Station **(Kyung & Janyce, 2013; Adriana *et al.*, 2019).** This prevalence is because it is greatly adaptative; able to colonise a wide diversity of environments because of its metabolic diversification, broad stress, and thermal condonation; and has the capability to spread its conidia simply **(Kyung & Janyce, 2013; Caroline *et al.*, 2017; Fadwa & Geoffrey, 2014; Nansalmaa *et al.*, 2014).** Also, the hypha of *Aspergillus fumigatus* have the ability to avoid the innate immune protection by cilia and the mucosal covering of the respiratory tract. For this reason, the hypha first start to enter the outer blood vessels, movable through the endothelial cells in the inner portion of the blood vessels throughout growth of hyphae. However, signs and symptoms involve asthma, pneumonia, sinusitis, or quickly progressing systemic **disease (Khayria, 2021)**. It was also find that, *Aspergillus flavus* was the second usual etiological factor of invasive aspergillosis (IA) *following Aspergillus fumigatus* **(Shivaprakash *et al.*, 2019)**. Pulmonary disease that was resulted by *Aspergillus, A. mainly fumigatus,* exhibit with a spectrum of clinical syndromes inside the lung**. (Taqwa & Zainab, 2020).** Symptoms of aspergillosis are described by respiratory problems, Skin diseases, **(Taqwa *et al,* 2022).** Put simply, any harmful fungus metabolite is a mycotoxin. As a consequence of their metabolism, fungi create a wide range of secondary metabolites; those that have the potential to harm other species are referred to as mycotoxins **(Mohmad & Zainab, 2017).** Numerous toxins, including gliotoxin, that are generated by *A. fumigatus* hyphae have been investigated as possible virulence factors. With a molecular weight of 326.4 g/mol, the gliotoxin C13H14N2O4S2 has been the subject of much research. It is thought to have a role in virulence because it suppresses the immune system by inhibiting phagocytosis and inducing death in macrophages. Recently, pathogenic *Aspergillus spp*. One of the diagnostic methods for *A.fumigatus* is Polymerase Chain Reaction (PCR), which can amplify some specific fragment of DNA into millionsofcopies**(Nawras *et al,* 2015).** have been detected and identified using PCR-based techniques. In addition to the 28S rRNA region (D1–D2), the internal transcript spacer (ITS1–ITS4) is a particular area found in rDNA that is frequently utilized to distinguish *Aspergillus* spcies **(Muna & Halima, 2017).** The target DNA in the majority of published data was either 18S rDNA or 28S rDNA; ITS sections were seldom employed. But because these sequences are conserved, the resolving power (specificity) is poor, making it possible to identify *Aspergillus species* only at the genus level. An further, labor-intensive, and time-consuming step is needed for further separation **(Kappe *et al.*, 1996)** accepted variations in the ITS region's length for the purpose of identifying therapeutically significant fungus, according to **(Turenne *et al.*, 1999).** Even though sequencing is very precise, its application in clinical laboratories is limited by its high cost and manpower requirements**(**The ITS region is sufficiently diverse, according to data released thus far, to distinguish between distinct species **.**As a result, it appears that the ITS region is a great tool for classifying fungal species**(Melchers *et al.*, 1994)** .The chosen primers were given careful consideration in the current investigation since they impact the PCR's specificity and

sensitivity. by employing certain primers designed to target the ITS region. If the fast method is applied for amplification, this is a simple, economical test with good sensitivity that may be completed in 4-5 hours **(Einsele *et al*., 1997; Fletcher *et al*., 1998).** The current study demonstrate virulence factors of *Aspergillus species* in samples by detecting Gliotoxin, which is the source of Glip P toxin, as well as study the fungus in most molecular types compared with other global types.

MATERIAL AND METHODS

Collection of samples

Fifty samples were collected from cattle respiratory infections cases in various locations within Baghdad city. The samples were immediately culture on Cazpek Dextrose Agar and peti dish were incubated for 3-7 days at 37°C, appearance the developed of fungal growth. Fungal isolates were purified by parts that move from the edges of the colonies using a clean needle to Petri dishes containing Cazp Dextrose Agar **(Roqia & Neran, 2022; Ali & Neran, 2023) .**The separation and identification of *Aspergillus fumigatus* Depend upon morphological features according to **(Samanta, 2015; Saraa & Dalia, 2021).**Cultural and morphological characteristics of Fungi isolates were carried out on PDA using lactophenol blue dye (according to the taxonomic keys) which were described **(Mohamed & Elham, 2022)** Microscopic examination was carried out to determine the type of the associated fungi, and then the common genera was diagnosed under the small power of the compound microscope based on the spores and the sexual and asexual structures that the fungus formed**. (Dareen *et al*., 2023)**

DNA preparation

A primer unique to *A. fumigatus* genes was employed in PCR method to identify the gene responsible for pathogenic *A. fumigatus*. To validate the pathogenicity of *A. fumigatus* isolated from a cow's respiratory illness, the Afumi gene was exposed to testing. Fungal DNA isolation procedures were employed as stated by **(Heng *et al*., 1993)**. In summary, "500 microliters of extraction DNA buffer (100 mM Tris-HCl (pH 9.0), 40 mM EDTA), 60 milliliters of 20% sodium dodecyl sulfate, and 300 milliliters of benzyl chloride were added to each sample after mycelia and spores were carefully scraped from cultures". To ensure that the two phases have combined, each reaction mixture was vortexed, incubated for 40 minutes in a water bath at 50°C, and then shaken for 10 minutes. After adding 60 ml of 3M sodium acetate (pH 5.0), the tube was placed on ice for a duration of 20 minutes. DNA was precipitated using "isopropanol (1:1) after the supernatant was collected following a 15-minute centrifugation at 3,500 g at 4°C. A 300 ml of TE buffer (10 mM Tris-HCl (pH 7.4)–1 mM EDTA) were used to re suspend the DNA pellet, and 1.5 ml of RNase (10 mg/ml) were added". The samples were extracted using "phenol-chloroform (1:1 (vol/vol)) after 5 minutes, and isopropanol was used to precipitate the chloroform extract". After suspending the DNA pellet in 200 milliliters of TE buffer, 3 to 5 microliters were electrophoresed.

PCR amplification

All PCR were the same for each template and set of primers used. Each of the reaction mixtures contained "2.5 ml of 103 PCR buffer (100 mM Tris-HCl [pH 9.0] at 25°C, 15 mM

MgCl2, 500 mM KCl, 1.0% TritonX-100), 0.5 U of *Taq* DNA polymerase (Promega), 1 ml of deoxynucleoside triphosphates (dATP, dCTP, dGTP, and dTTP [10 mM each]; GmbH, Mannheim, Germany), 20 pmol of each primer, and 1 ml of sample DNA. Ultrapure sterile water was added to a final volume of 25 ml. Primers ITS1, and GlipP were used for amplifications. The DNA samples used for the PCR are indicated in Table (1). When doing the PCR, the specific primers used were designed as mentioned in Table (1).PCR was performed in a GeneAmp PCR system 9600 instrument (Perkin-Elmer Applied Biosystems, Foster City, Calif.), for ITS1 amplification, and ,GlipP at "95°C for 5 min for denaturation, 95°C for 30 s for denaturation, 59°C for 30 s for annealing, and 72°C for 30 s for primer extension for 30 cycles, with 5 min of extension at 72°C used for the final cycle.

Agarose gel electrophoresis

The PCR results were separated by electrophoresis on 2% (wt/vol) agarose gels stained with 0.5 mg of ethidium bromide per milliliter in order to examine the DNA fragments. In each lane, volumes containing 10 ml of the PCR product and 2 ml of the Blue/Orange 63 loading dye were added. Electrophoretic conditions were 100 V for 45 min.,TAE buffer (Tris-acetate, EDTA electrophoresis buffer concentrated stock solution which comprises, per liter, 242 g of Tris base, 57.1 ml of glacial acetic acid, and 100 ml of 0.5 M EDTA; pH 8.0). In order to approximate the sizes of the PCR products, markers are also run in parallel.

Multiple sequence alignments and primer design

The Pileup and Pretty programs from the Multiple Sequence Analysis program group (included in Web ANGIS, the fourth iteration of ANGIS (Australian National Genomic Information Service) were used to make multiple sequence alignments. Using reference data and sequence alignment, specific primers were created.

Specificities of the primers

The species listed in Table 1 were used to evaluate the primers' specificities. The DNA sample was amplified using the suitable primers to guarantee that no PCR inhibitors were present.With the right primers, a single PCR may amp up a segment of about 308 bp from the ITS gene, and a PCR with the inner specific primers can amp up a fragment of 178 bp. As previously mentioned, the GlipP amplicon from
Aspergillus fumigatus was extracted, purified, and sequenced to ensure that it had the predicted size from a gel.

Table (1): The designed primers for ITS AND GlipP gene that used in this study.

Gene Name	Forward primer (5-3)	Reverse primer (5-3)	Length of gene
small subunit ribosomal gene	ACTACCGATTGAATGGCTCG	CATACTTTCAGAACAGCGTTCA	308bp
Glip P	AAACCCCTGTGAATGCAGAC	CCCCTTGAGATGAAAGGTGA	178bp

RESULTS AND DISCUSSION

Isolation and Identification of fungi

Infectious fungi that were isolated from cows' nasal samples that had upper respiratory tract infections are the subject of the current investigation. The outcome of the morphological identification is dependent upon the kind of mycelium development, unique microscopic traits, and traditional methodology. The fungi that were growing were refined and classified down to the genus level using the calcification key that was provided by **(Wasan &Hurria, 2020).** shown that the fungus was the cause of the disease, as seen in figure (1).

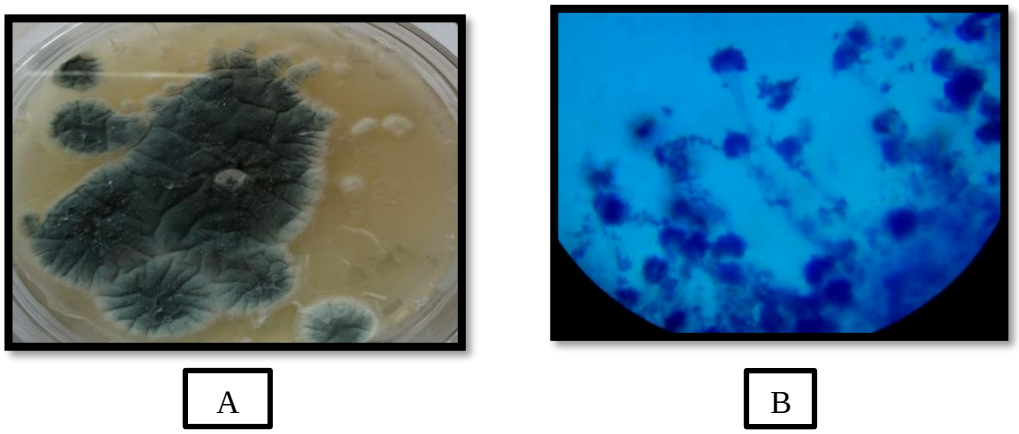

Figure (1): (A) Colony appearance grow on agar media at 37c for 3-7 days (B) The shape of *Aspergillus Fumigatus* and structural branches with magnifying 40X.

MOLECULAR IDENTIFICATION

By using a PCR procedure, the morphological identity of Aspergillus fumigatus was verified. The ITS amplification PCR products were analyzed using the ITS AND GlipP primers, as indicated in table 1.

Specificity of the PCR with the designed primers

Fifty samples were utilized in order to assess the specificity of the PCR to detect A. fumigatus and Glip P toxin, with the particular primers used in the current investigation. The 18 S rRNA gene was searched for sequences that contain both highly variable and highly conserved areas that have been utilized to produce markers specific to species and genus, respectively, in order to develop specific PCR primers **(Bridge &Arora, 1998; Edel, 1998).** According to PCR Figure 3, amplicons of around 308 bp that were seen on agarose gels when exposed to UV light were deemed positive. (Figure, 5), provides a summary of the findings. The findings show that three isolates of A. fumigatus were amplified by the particular primers intended for *Aspergillus fumigatus* identification. Therefore, all *Apergillus fumigatus* isolates and isolates that were closely related were effectively amplified using the primers specifically designed for *A. fumigatus* that were created for this investigation .

These outcomes, ascertained using the Basic Blast software and the particular primers, are consistent with the sequence data in GenBank. After sequencing, the amplicon from A. fumigatus was compared to sequences of the same species that were stored in GenBank. The outcomes demonstrate that the length and position of the amplification are accurate. We

discovered that our sequence matched with the sequences of *A. fumigatus* isolates that were deposited in GenBank by using the sequenced amplicon. Simultaneously, there were no false-positive results reported with other fungal species.

Sensitivity of the PCR

Each PCR result was placed onto agarose gels in an amount of 10 microliters for electrophoresis. The PCR produced discernible bands at DNA dilutions as high as 1:13 to 1:14. This is equivalent to between 10 and 100 ng of DNA sample. As a result, the PCR is more sensitive than other techniques like Southern blotting or Elisa's antigen detection.

Multiple sequence alignments and Phylogenetic tree

Multiple sequence alignments were first developed from the sequence data and information from GenBank, our registration accession number OR727320.1 (Iraq), was aligned with global ITS gene sequences to find out the similarities and differences from the multiple sequence alignment results, we noticed that (i) the Iraqi small-subunit ITS (Iraq), OR578448.1 showed similarity much more with (Russia) OR578448.1 and less similarity to the following OR053856.1 (Pakistan), KT159727.1 (Slovakia), KT159726.1 (Slovakia), KT159725.1 (Slovakia),KR023997.1 (Brazil), ON955910.1 (Czech), (Brazil), OR727320.1 , as well as MF379664.1 (Brazil), Mk267099.1.1 (India), (Nigeria), MK680251.1 , (Pakistan), MN545449, (Pakistan), MT297629.1(Pakistan), MT297633.1.respectively but was more different to (Portugal), KF367498.1 and (Pakistan) MT316338.1. This elucidate the variety among Iraqi *A. fumigatus* isolates and the others countries.

Despite the identification of *A. fumigatus*, several questions remain unanswered. *A. fumigatus* and N. fischeri, a similar telemorph species of *A. fumigatus* section, may be distinguished from one another in certain studies. According to taxonomy, they belong to *A. fumigatus* section fumigati.Additional findings provide credence to the theory that they most likely descended from an *A. fumigatus* common ancestor. Additionally, they highlight issues with the conventional morphology-based naming system and raise questions about whether the target region selected for identification was the best one **(Kawamura *et al*., 1999; Moore *et al*., 2000).**

```
F_primer_                       ACTACCGATTGAATGGCTCG----------------------------------------
small_subunit_ribosomal_gene    actaccgattgaatggctcggtgaggccttcggactggcccagggaggtcggcaacgacc
R_primer_                       ----------------------------------------------------------


F_primer_                       ----------------------------------------------------------
small_subunit_ribosomal_gene    acccagggccggaaagttggtcaaacccggtcatttagaggaagtaaaagtcgtaacaag
R_primer_                       ----------------------------------------------------------


F_primer_                       ----------------------------------------------------------
small_subunit_ribosomal_gene    gtttccgtaggtgaacctgcggaaggatcattaccgagtgagggccctctgggtccaacc
R_primer_                       ----------------------------------------------------------


F_primer_                       ----------------------------------------------------------
small_subunit_ribosomal_gene    tcccacccgtgtctatcgtaccttgttgcttcggcgggcccgccgtttcgacggccgccg
R_primer_                       ----------------------------------------------------------


F_primer_                       ----------------------------------------------------------
small_subunit_ribosomal_gene    gggaggccttgcgccccgggcccgcgcccgccgaagaccccaacatgaacgctgttctg
R_primer_                       ------------------------------------------------TGAACGCTGTTCTG


F_primer_                       --------
small_subunit_ribosomal_gene    aaagtatg
R_primer_                       AAAGTATG
```

Figure (2): Alignment map of our designed primers to the ITS gene (308bp) in *Aspergillus fumigatus.*

```
F_primer_       ----------------------------------------AAACCCCTGTGAATGCAGAC--
Glip_P_gene     gatgggtctgttcctcgatcgcctgcctcttcgcttcaaaaccccctgtgaatgcagactg
R_primer_       ------------------------------------------------------------


F_primer_       ------------------------------------------------------------
Glip_P_gene     cgccaccatcctgcagtctacgcgtgcagcgagccaggcagccgtctgcaattccattcc
R_primer_       ------------------------------------------------------------


F_primer_       ------------------------------------------------------------
Glip_P_gene     attcgagcaggtcctgaacctcctccacctgccgcggaccatccggcaacacccgctgtt
R_primer_       ------------------------------------------------------------


F_primer_       ------------------------------------------------------------
Glip_P_gene     cgaagccatggtcacctttcatctcaaggggcagtggaagattgtctcgccatcgaggg
R_primer_       ------------TCACCTTTCATCTCAAGGGG----------------------------


F_primer_       ------------------------------------------------------------
Glip_P_gene     gctggaggtgaaacgcgagatgtgctttgcgtccggggccaagttcctgctcatgttcga
R_primer_       ------------------------------------------------------------
```

Figure (3): Alignment map of our designed primers to the GlipP gene (178bp) in *Aspergillus fumigatu*s.

The identification of *Aspergillus Fumigatus* was confirmed by PCR protocol, using ITS gene of DNA. The analysis of PCR products of ITS amplification using the Primers for ITS AND Glip P genes. after electrophoresis using gel containing 1% agarose in 0.5 TAE buffer adjusted time for 40 minutes under 70 volt ,as showed in Figure (5):

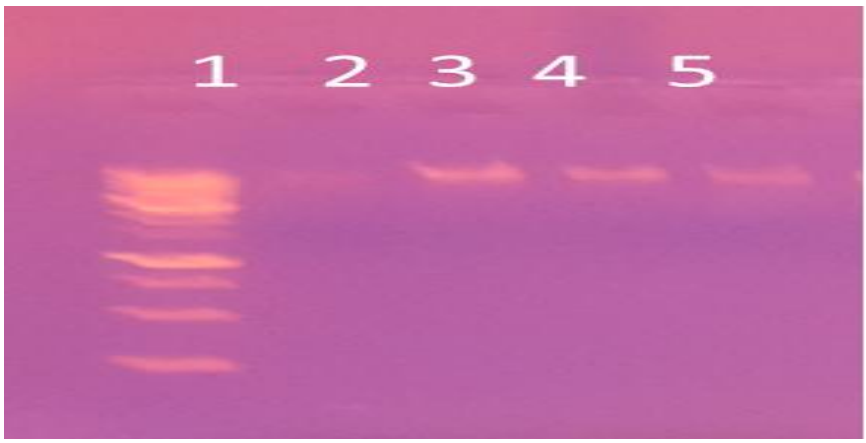

Figure (4): DNA isolation picture after electrophoresis using gel containing 1% agarose in 0.5 TAE buffer adjusted time for 40 minutes under 70 volt. and 3microliter Ethidium.

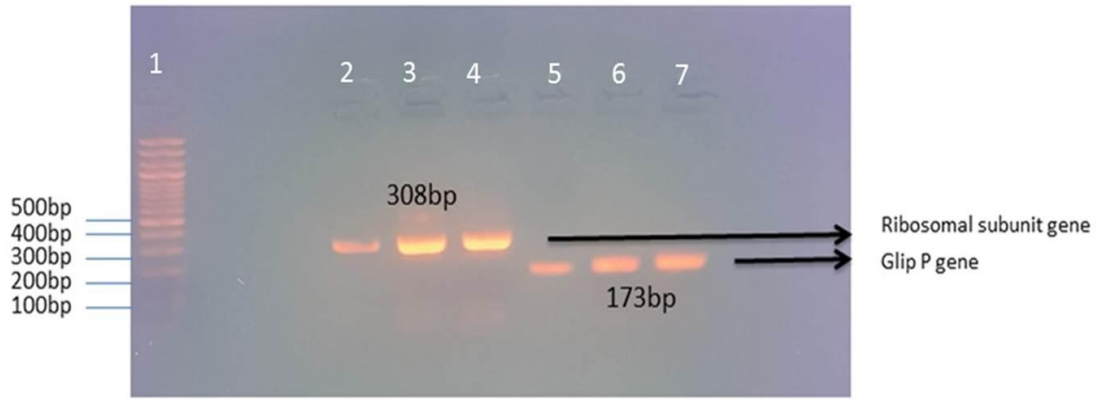

Figure (5): PCR amplification picture for ITS AND Glip P genes. after electrophoresis using gel containing 1% agarose in 0.5 TAE buffer adjusted time for 40 minutes under 70 volt. and 3microliter Ethidium .

Based on DNA sequences between Accession numbers are as follows: MT316338.1 (Pakistan), MT297633.1 (Pakistan), MT297629.1 (Pakistan), MN545449.1 (Nigeria), MK680251.1 (Egypt), Mk267099.1.1 (India), MF379664.1 (Brazil), OR727320.1 (Iraq), OR578448.1 (Russia), OR053856.1 (Pakistan), KT159727.1 (Slovakia), KT159726.1 (Slovakia), KT159725.1 (Slovakia),KR023997.1 (Brazil), ON955910.1 (Czech), KF367498.1

(Portugal). Using MEGA v.5.05, the Maximum Likelihood technique of phylogenetic tree construction was used to create the phylogenetic tree as showed in Figure(6):

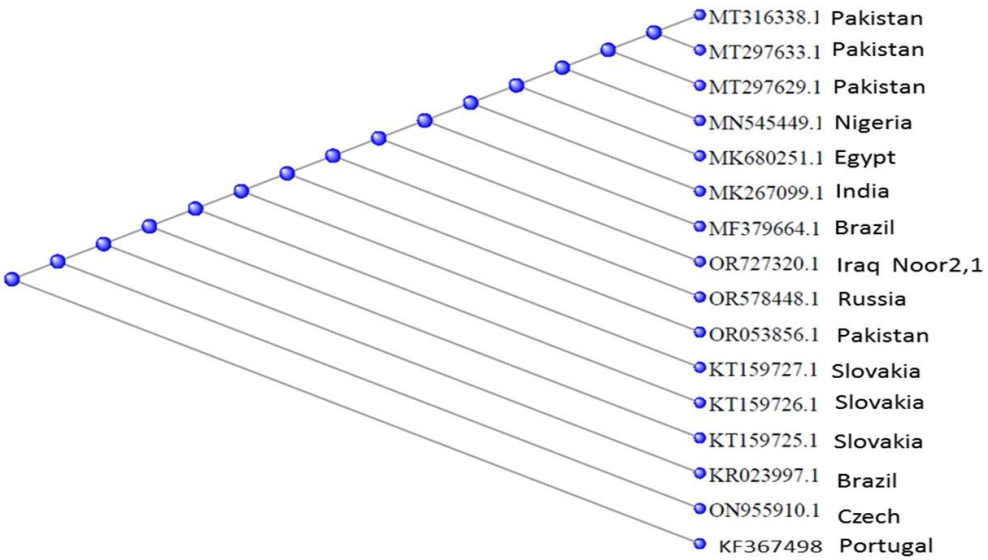

Figure (6): Phylogenetic tree Construction.

CONCLUSION

1-The most important conclusion was that fungi are the cause of respiratory diseases, especially the Aspergillus species.
2-Aspergillus species are ubiquitous fungi, present in many environments, and it is a potentialpathogen of importance in animals (cows) and humans.
3-Mycotoxins cause immunosuppression and cause economic losses from their effects on animals

RECOMMENDATIONS

1- Animals, especially cows, are the primary source of proteins and meat for humans, so it is important to keep the animals healthy and free of diseases.
2- Attention and care to animal housing include of ventilation and humidity to reduce the growth of fungi and production of mycotoxin in the fields.

REFERENCES

1. Abad, M.H. & Al-haddad, Z.A.A. (2017). Isolation of aspergillus flavus from maize and sesame with detection of aflatoxin b1 in rural area around baghdad. *Al-Anbar Journal of Veterinary Sciences*, 10(2), 10-16.
2. Abdel-Gawad K.M. (2021). *Aspergillus fumigatus* and Aspergillosis. *American Journal of Biomedical Science & Research*, 14(6),495-499.
3. Abdul Karim, R.R., Aljarah, N.S. (2022). The inhibitory effect of sodium bicarbonate, phylex and seaweed extract against *Penicillium digitatum* causing greenrot disease in local lemon fruits. *Earth and Environmental Science*, 1225(2023), 1-13.
4. AL-Barazanchi, T.A., & Al-Asady, Z.H.A. (2020). Molecular identification of aspergillus fumigatusby detection *Aspergillus* hemolysin asphsgene. *Plant Archives*, 20(1), 3855-3861.
5. Al-Barazanchi, T.A., Abood, Z.H., & Al-Rawi, R.S. (2022). Molecular investigatio of heat shock protein 70 (hsp70) expression levels in aspergilllosis patients. *Iraqi Journal of Agricultural Sciences*, 53(3), 534-541.
6. Ali, M.H. & Neran, S.A. (2023). Seed treatment of okra with humic acid to control pathogen of damping off fusarium solani. *Bionatura*, 8(1),1-12.
7. Alrawi, M.A. & Hussein, H.Z. (2017). Molecular identification of fungi *Aspergillus* flavus which producing toxin (AFB1) in Iraq. *Pakstan Journal Biotechnol*, 14(4), 673-675.
8. Al-Saffy, S.G.A. & Abdulshaheed, D.A. (2021). Effect of *Solanum aculeastrum* on hematological parameters of Al-bino mice infected with *Aspergillus fumigatus*. *Bionatura*, 13,1-5.
9. Alshareef, F. & Robson, G.D. (2014). Prevalence, persistence, and phenotypic variation of *Aspergillus fumigatus* in the outdoor environment in Manchester, UK, over a 2-year period. *Medical Mycology*, 52(4), 367–375.
10. Amarsaikhan, N., O'Dea, E.M., Tsoggerel, A., Owegi, H., Gillenwater, J., Templeton, S.P. (2014). Isolate-dependent growth, virulence, and cell wall composition in the human pathogen *Aspergillus fumigatus*. *PLoS ONE*, 9(6), 1-14.
11. Blachowicz, A., Chiang, A.J., Romsdahl, J., Kalkum, M., Wang, C.C.C. & Venkateswaran, K. (2019). Proteomic characterization of *Aspergillus fumigatus* isolated from air and surfaces of the International Space Station. *Fungal Genetics and Biology*,124, 39-46.
12. Bridge, P.D. & Arora, D.K. (1998). Interpretation of PCR methods for species definition. *Applications of PCR in Mycology*, 63-84.
13. Chechan, R.A., Farhan, E.M., Muslat, M.M. & Abdul-Qader, Z.M. (2020). Morphological characterization,molecular diagnosis and enzymatic activity of some wild mushroom in baghdad province, iraq. *Plant Archives*. 20(2), 7437-7445.
14. Edel, V. (1998). Polymerase chain reaction in my- fungi by using the ITS2 genetic region and an autocology: an overview. *Journal of Clinical Microbiology*, 37(2), 1846–1851.
15. Einsele, H., Hebart, H., Roller, G., Lo¨ffier, J., Rothenho¨fer, I., Muller, C.A., Bowden, R.A., van Burik, J., Engelhard, D., Kanz, L., & Schumacher, U. (1997). Detection and identification of fungal pathogens in blood by Using molecular probes. *Journal Clin Microbiology*, 35:1353–1360.
16. Fletcher, H.A., Barton, R.C., Verweij, P.E., & Evans, E.G. (1998). Detection of Aspergillus fumigatus PCR products by a microtitre plate-based DNA hybridization assay. *Journal Clin Microbiology*, 51, 617–620.

17. Jamel, D.S., Abdul-Razzak, A.K. & Taha, A.S. (2023). Antifungal activity of garlic and neem extracts against cobweb mold dactylium and green rot diseases in agaricus bisporus: antifungal activity of garlic and neem extracts against cobweb mold dactylium and green rot diseases in *Agaricus bisporus*. *Iraqi Journal of Market Research and Consumer Protection*, 15(2), 60-73
18. Kawamura, S., Maesaki, S., Noda, T., Hirakata, Y., Tomono, K., Tashiro, T. & Kohno, S. (1999). Comparison between PCR and detection of antigen in sera for diagnosis of pulmonary aspergillosis. *Journal of Clinical Microbiology*, 37(1), 218-220.
19. Kwok, S., & Higuchi, R. (1989). Avoiding false positives with PCR. Nature *Publishing Group, London*, 339, 237–238.
20. Kwon-Chung, K.J., Sugui, J.A. (2013). *Aspergillus fumigatus*—what makes the species a ubiquitous human fungal pathogen. *PLoS Pathog*, 9(12), 2-15.
21. Melchers, W.J., Verweij, P.E., Van den Hurk, P., Van Belkum, A., De Pauw, B.E., Hoogkamp-Korstanje, J.A. & Meis, J.F. (1994). General primer-mediated PCR for detection of Aspergillus species. *Journal of clinical microbiology*, 32(7), 1710-1717.
22. Mohamed, A.M. & Al–Shamary, E.I. (2022). Isolation and identification of aflatoxin b1 producing fungi from stored wheat in some silos of Baghdad. *Iraqi Journal of Agricultural Sciences*, 53(6), 1427-1436.
23. Moore, C.B., Walls, C.M. & Denning, D.W. (2000). In vitro activity of the new triazole BMS-207147 against *Aspergillus* species in comparison with itraconazole and amphotericin. *Antimicrobial agents and chemotherapy*, 44(2), 441-443.
24. Paulussen, C., Hallsworth, J.E., Álvarez-Pérez, S., Nierman, W.C., Hamill, P.G., Blain, D., Rediers, H. & Lievens, B. (2017). Ecology of aspergillosis: Insights into the pathogenic potency of *Aspergillus fumigatus* and someother Aspergillus species. *Microbiology and Biotechnology*, 10, 296–322.
25. Rassin, N.K., Al-judy, N.J. & Dheeb, B.I. (2015). Molecular identification of *Aspergillus fumigatus* using ISSR and RAPD markers. *Iraqi Journal of Science*, 56(4), 2788-2797.
26. Reponen, T.A.M.M.P., Nevalainen, A., Jantunen, M., Pellikka, M. & Kalliokoski, P. (1992). Normal range criteria for indoor air bacterial and fungal spores in a subarctic climate. *Indoor air*, 2(1), 26-31.
27. Rudramurthy, S.M., Paul, R.A., Chakrabarti, A., Mouton, J.W., & Meis, J.F. (2019). Invasive aspergillosis by *Aspergillus flavus*: epidemiology, diagnosis, antifungal resistance, and management. *Journal of Fungi*, 5(3), 55-63.
28. Turenne, C.Y., Sanche, S.E., Hoban, D.J., Karlowsky, J.A. & Kabani, A.M. (1999). Rapid identification of fungi by using the ITS2 genetic region and an automated fluorescent capillary electrophoresis system. *Journal of clinical microbiology*, 37(6), 1846-1851.
29. Zhu, H., Qu, F. & Zhu, L.H. (1993). Isolation of genomic DNAs from plants, fungi and bacteria using benzyl chloride. *Nucleic Acids Research*, 21(22), 5279.