Journal of Applied and Natural Science
16(2), 722 - 729 (2024)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

Molecular Identification of *Aspergillus* spp. isolated from different vegetables in markets of Dohuk city/Iraq

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Article Info
https://doi.org/10.31018/jans.v16i2.5449
Received: February 06, 2024
Revised: May 09, 2024
Accepted: May 15, 2024

How to Cite
Al-Ameri, H. A. (2024). Molecular Identification of *Aspergillus* spp. isolated from different vegetables in markets of Dohuk city/Iraq. *Journal of Applied and Natural Science*, 16(2), 722 - 729. https://doi.org/10.31018/jans.v16i2.5449

Abstract
Vegetables are important preventive foods and are useful for maintaining health and preventing diseases and some fungi, such as *Penicillium* spp., *Aspergillus* spp. and *Fusarium* spp. grown on vegetables and contaminated it. Therefore, the aim of the study was to isolation and identification of fungi contaminating different types of vegetables infected with rot collected from local markets of Duhok city/Iraq, and Molecular identification of *Aspergillus nidulans* using Polymerase Chain Reactions (PCR), by Design of the primer, Extraction of DNA, Nucleotide sequencing of amplified pieces using DNA sequencing which was done at Psomagene sequencing company (Maryland/ USA) and The phylogenetic tree was constructed by bootstrap (100X) analysis using the MEGA-11 software. Results referred to all vegetable samples under study contaminated with different genera of fungi, green bell pepper contaminated with *Rhizopus stolonifer* in (43.2%), *Aspergillus nidulans* was isolated from red bell pepper in (19.2%), while it was the most frequent isolates from eggplant, pumpkin, cucumber and cauliflower in percentage of 43.3%, 46.8%, 31.5% and 58.9% respectively. According to morphological and microscopic identification *A. nidulans* was chosen for molecular study because of its high frequency in most vegetable samples. Molecular identification of *A. nidulans* using 18 S rRNA, a pair of specialized primers (ITS1) and (ITS2) was adopted. In electrophoresis on agarose gel at a concentration of 2%, the results showed a package size of 600 bp. belonging to *Aspergillus nidulans,* and identical results were obtained for the isolate with the standard isolates installed in the Basic Local Alignment Search Tool (BLAST) program database. The evolutionary relationship was found between the local isolate in this study and the one registered in NCBI under the name *Aspergillus nidulans* HAA78. The isolate *Aspergillus nidulans* HAA78 was 100% identical to the isolate *Aspergillus nidulans* strain ATCC (10074). It was concluded that all vegetables isolated from local markets of Duhok City,Iraq were contaminated with many genera of fungi. The molecular identification of the main and most common fungi collected from these vegetables was *Aspergillus nidulans.* The study would help understand the important role of molecular identification in addition to Manual methods in identifying fungi.

Keywords: *Aspergillus nidulans,* Vegetables, *Vigna sinensis*, 18S rRNA, HAA78

INTRODUCTION

Vegetables are important preventive foods and are very useful for maintaining health and preventing diseases. It provides a variety of pharmacological and therapeutic substances in addition to significant nutritional components that are necessary for the normal function of the body; minerals and vitamins content in high percentage differs with the differentiation of its types: leaves, stems, roots, seeds and fruits, possibly edible of them and every group makes a unique contribution to the diet (Jinajali, 2017; Cecilia *et al*., 2017).

Many people are realizing how important vegetables are to food security and nutrition, and vegetable consumption is linked to a reduced risk of many diseases (Salvin, 2013). Annual reports in Basra city/Iraq markets showed that 20% of vegetables produced are spoiled, especially during the post-harvest stages. This has been associated with spoilage fungi that can be toxic or pathogenic. The toxin-producing fungi such as *Aspergillus flavus*, *Aspergillus niger* , *Penicillium* spp. and *Alternaria alternaria* have been identified and isolated from spoiled vegetables (Ali *et al*., 2023). One of the limiting factors affecting the economic value of fruits and vegetables is the relatively short shelf life resulting from attack by pathogens (Mula Abed and Al-Ameri, 2022; Nji *et al*., 2023).

The consumption of vegetable products has increased by more than 30% globally in the previous few decades (Odelade and Oladeji, 2020). Especially fresh, as the

percentage of consumption of fresh vegetables and fruits reached 32.6% and 25.8%, respectively, and greatly outpaced the rise in manufactured products (Srilakshmi and Venkata Reddy, 2022).Consumption of raw vegetables without any exposure to processes that can be relied upon to eliminate contamination with pathogens. Although it can lower fungal levels, washing vegetables and fruits with water chlorinated does not guarantee the removal of pathogens (Odelade and Oladeji, 2020; Gera *et al*., 2023).

Food is contaminated with different microorganisms (viruses, parasites and bacteria). Consequently, there are numerous varieties of food-borne infections. According to estimates of microbial deterioration, 20% of vegetables in the local government area of Kankara, Katsina state, grown for human consumption are lost. The yeasts, mold and bacteria are the first causative agents of microbial spoilage (Yaradua *et al*., 2018). Microorganisms that cause spoiling can enter a crop on the seed itself, during the crop's growth in the field, during harvest and handling post-harvest or during distribution and storage (Sani *et al*., 2018).

There is no doubt that microorganisms have led to an increase in the number of documented outbreaks (Mailafia *et al*., 2017). When raw vegetables are treated with disinfectants or cleaned, pathogenic microorganisms can be eliminated, lowering the disease risk (Gerke *et al*., 2022; Ibrahim *et al*., 2022). However, the hydrophobic cutin, variable abrasions and surface morphology in the skin of vegetables and fruits limit the effectiveness of the treatment (Jinajali, 2017).

Fungi can produce enzymes inside and outside cells, depending on other organisms' carbon sources. Molecules such as monosaccharides or disaccharides, amino acids and fatty acids can break down larger complex compounds such as cellulose, amylase, pectinase and protease, and fungi secrete extracellular enzymes. Fungal extracellular enzyme production facilitates degradation (Vinayachandran *et al*., 2020).

Some previous studies have focused on many fungi, such as Penicillium spp., Aspergillus spp., and *Fusarium* spp. grown on vegetables (Nji *et al*., 2023), Especially *A. flavus*, *A. niger, A. parasiticus, P. digitatum.* and *P. citrinum* (Srilakshmi and Venkata reddy, 2022), *Syncephalastrum racemosum*, *Rhizopus stolonifer* and *Mucor* spp. (Vinayachandran *et al*., 2022).

The study aimed to isolate and identify fungi contaminating different types of vegetables infected with rot collected from local markets of Duhok City, Iraq, and molecular identification of *A. nidulans* using Polymerase Chain Reactions (PCR).

MATERIALS AND METHODS

Sample collection

Vegetables samples such as Green bell pepper (*Capsicum annuum*), Red bell pepper (*Capsicum annuum*), Beans (*Phaseolus vulgaris*), Cowpea (*Vigna sinensis*), Tomato (*Solanum lycopersicum*), Eggplant (*Solanum melongena*), Cucumber (*Cucumis sativus*), Pumpkin (*Cucurbita moschata*), Zucchini (*Cucurbita pepo*) and Cauliflower (*Brassica oleracea* var. *botrytis*) infected with rot were collected from local markets of Duhok city/Iraq. They were transported to the Biology Department/College of Science/Mosul University laboratory in separate sterile polyethylene bags for fungal isolation.

Isolation and identification

Sterilized the surface of vegetables by exposing them to sodium hypochlorite for 3 minutes with a concentration of 1% and wished it with sterile distilled water three times. Small pieces of sample tissues (3 cm-5cm) were placed on Potato Dextrose Agar (PDA) media containing streptomycin in 0.005 mg/ml and incubated at 25± 2^{o} C for 7 days, were by sub-cultured transplanting to a new set using PDA to purification of fungal isolates . The primary identification of colonies that appeared was made using morphological and microscopical features using fungal identification keys (Nelson *et al*., 1983; Domsch *et al.*, 1993; Pitt and Hooking, 2009). To save fungal samples, they were cultured on the slant.

Determination of percentage frequency of occurrence

The frequency of occurrences of different types of isolated fungal contaminants associated with vegetable spoilage was determined using the formula (Al-Ameri, 2022).

$$\% \text{ Frequency} = \frac{\text{The number of fungus colonies in the samples}}{\text{Total number of colonies}} \quad \text{Eq. 1}$$

Molecular identification of *Aspergillus* spp. using Polymerase Chain Reactions (PCR)

Design of the primer

ITS1 (5’ TCCGTAGGTGAACCTGCGG 3’) and ITS4 (5’ TCCTCCGCTTATTGATATGC 3’) were used to amplify the internal transcribed spacers in *Aspergillus* spp. PCR was conducted in a 20 µL volume reaction using GoTaq G2 Green Master Mix (2X) supplied by Promega (USA). The master mix was used in 1X concentration and the primers were used in 1µM. The final volume was completed to 20 µL using nuclease-free water.

The conditions used were: 1 cycle at 95°C for 5 min, followed by 35 cycles with a denaturation step at 95°C for 30 s, an annealing step at 55°C for 1 min, and an extension step at 72°C for 1 min, followed by 1 cycle at 72°C for 6 mins. PCR products were run on agarose gel 2% containing the Redsafe DNA stain (Intron, China). The size of the amplicon was determined by com-

paring the band's location with the bands of the 100bp DNA ladder (Transgene, Taiwan).

The primer was obtained from Macrogene Company (Korea), which specialized in primer synthesising and sequencing.

Extraction of DNA

DNA was extracted using the Geneaid™ DNA Isolation Kit (Yeast) according to the steps recommended by the company. DNA concentration was measured using a nanodrop device at the College of Science/ University of Mosul.

Nucleotide sequencing of amplified pieces using DNA sequencing

Sanger sequencing was done at Psomagene sequencing company (Maryland/ USA).

Phylogenetic tree

The Nucleotide Basic Local Alignment Search Tool (BLASTn) program (Altschul *et al*., 1990) was used to search for homology to the input sequence against entire sequences available on the sequence National Centre for Biotechnology Information (NCBI) GenBank database (https://www.ncbi.nlm.nih.gov/genbank/). The phylogenetic tree was constructed by bootstrap (100X) analysis using the MEGA-11 software (Tamura *et al*., 2021).

Ethical approval

The local Ethical Committee at the University of Mosul/ College of Science approved the project (Approval No. 450 dated 19 September 2023).

RESULTS AND DISCUSSION

Isolation of fungi

Table 1 shows 5 species of fungi isolated from green bell pepper: *R. stolonifer*, *Mucus* spp. *, A. parasiticus, P. citrinum* and *P. digitatum* with percentage frequency 43.2%, 15.6%, 12.7%, 10% and 18.5%, respectively. *A. nidulans* was isolated from red bell pepper in 19.2%, while it was the most frequent isolate from eggplant,

Table 1. Fungi isolated from different vegetables and their percentage of frequency

Vegetables samples	Fungal isolates	% Frequency
Green bell pepper (*Capsicum annuum*)	*Rhizopus stolonifer*	43.2
	Mucus spp.	15.6
	Aspergillus parasiticus	12.7
	Penicillium citrinum	10.0
	Penicillium digitatum	18.5
Red bell pepper (*Capsicum annuum*)	*Rhizopus stolonifer*	58.1
	Aspergillus parasiticus	10.2
	Aspergillus niger	12.5
	Aspergillus nidulans	19.2
Beans (*Phaseolus vulgaris*)	Curvularia spp.	22.2
	Alternaria alternata	30.4
	Aspergillus dimatatus	14.9
	Aspergillus nidulans	32.5
Cowpea (*Vigna sinensis*)	*Rhizopus stolonifer*	22.6
	Aspergillus parasiticus	39.9
	Geotrichum candidum	37.5
Tomato (*Solanum lycopersicum*)	*Geotrichum candidum*	67.4
	Aspergillus parasiticus	24.7
	Rhizopus stolonifer	7.9
Eggplant (*Solanum melongena*)	Helminthosporium spp.	25.1
	Alternaria alternata	28.9
	Stemphylium spp.	2.7
	Aspergillus nidulans	43.3
Cucumber (*Cucumis sativus*)	*Rhizopus stolonifer*	22.1
	Aspergillus parasiticus	29.9
	Aspergillus nidulans	31.5
	Aspergillus flavus	11.5
	Aspergillus fumigatus	5.0
Pumpkin (*Cucurbita moschata*)	*Penicillium digitatum*	41.2
	Aspergillus nidulans	46.8
	Aspergillus fumigatus	12.0
Zucchini (*Cucurbita pepo*)	*Aspergillus flavus*	44.3
	Aspergillus parasiticus	29.9
	Alternaria alternata	25.8
Cauliflower (Brassica oleracea var. botrytis)	Mucus spp.	3.5
	Penicillium digitatum	11.7
	Aspergillus parasiticus	25.9
	Aspergillus nidulans	58.9

pumpkin, cucumber and cauliflower with 43.3%, 46.8%, 31.5% and 58.9%, respectively, but in tomato, the most frequent fungi isolated were *Geotrichum candidum* in 67.3% .Five fungi isolated from cucumber, the highest frequented was *A. nidulans* at 31.5%, the lowest frequented isolate was *A. fumigatus* 5%, while *A. flavus* was the most frequent isolate from zucchini, with 44.3%. *Alternaria alternata* was isolated from beans and eggplant only in percentages 32.4% and 28.9%, respectively. *Aspergillus nidulans* was chosen for molecular study because of its high frequency in most vegetable samples.

Molecular identification of *Aspergillus nidulans* using 18 S rRNA

A. nidulans was identified using PCR technology. The ITS gene was detected and the ability of the primers to bind to specific sites in the fungus DNA was tested. A pair of specialized primers, Forward (ITS1) and Reverse (ITS2), were adopted, and the amplification products were used. The electrophoresis results on agarose gel at a concentration of 2% showed a package with a size 600 bp. belonging to *A. nidulans* (Fig. 1). This indicated that the designed primers bind to the target gene present within the genetic sequence of the fungus mentioned in the methodology.

Analysis of the nucleotide base sequence of chromosomal DNA

The results of the polymerase chain reaction were sent to (Psomagene sequencing company, USA), and it was shown that the isolate belongs to the genus *Aspergillus*, specifically *A. nidulans*. The sequences of the isolate's nitrogenous bases were compared with the isolates registered in NCBI. The isolate was found to be 100% genetically identical to the isolates registered internationally (Fig. 2), and identical results were obtained for the isolate with the standard isolates installed in Basic Local Alignment Search Tool (BLAST) program database. Sample H1 was 100% identical to *Aspergillus nidulans* strain AS27.

Aspergillus nidulans isolate was registered in the Gene Bank and given a special serial number, shown in Fig.3.

As a result of the nitrogen base sequence analysis sent to the NCBI, the study registered it as a new strain using the code HAA78 (Fig. 4).

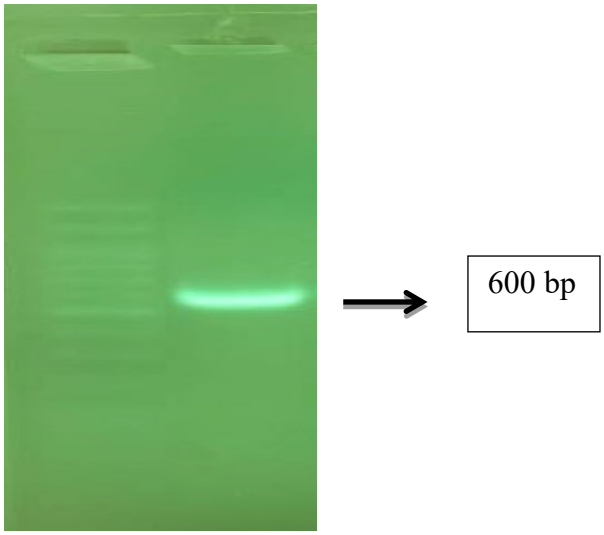

Fig. 1. *Showing separate PCR amplification products from Aspergillus spp. isolates using 2% agarose gel electrophoresis*

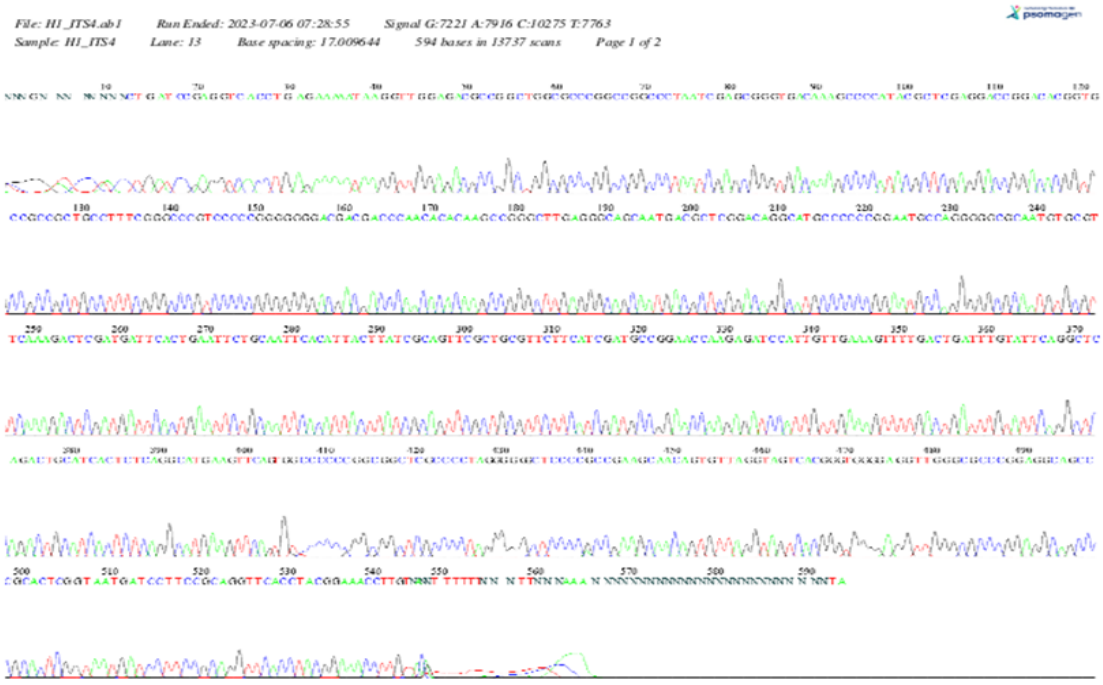

Fig. 2. *Sequence analysis of isolate HAA78 based on the 18S rRNA gene*

Aspergillus nidulans isolate AS27 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

Sequence ID: MT328535.1 Length: 523 Number of Matches: 1

Range 1: 7 to 493 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
900 bits(487)	0.0	487/487(100%)	0/487(0%)	Plus/Plus

```
Query  1    GTGCGGGCTGCCTCCGGGCGCCCAACCTCCCACCCGTGACTACCTAACACTGTTGCTTCG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  7    GTGCGGGCTGCCTCCGGGCGCCCAACCTCCCACCCGTGACTACCTAACACTGTTGCTTCG  66

Query  61   GCGGGGAGCCCCCTAGGGGCGAGCCGCCGGGGACCACTGAACTTCATGCCTGAGAGTGAT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  67   GCGGGGAGCCCCCTAGGGGCGAGCCGCCGGGGACCACTGAACTTCATGCCTGAGAGTGAT  126

Query  121  GCAGTCTGAGCCTGAATACAAATCAGTCAAAACTTTCAACAATGGATCTCTTGGTTCCGG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  127  GCAGTCTGAGCCTGAATACAAATCAGTCAAAACTTTCAACAATGGATCTCTTGGTTCCGG  186

Query  181  CATCGATGAAGAACGCAGCGAACTGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  187  CATCGATGAAGAACGCAGCGAACTGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATC  246

Query  241  ATCGAGTCTTTGAACGCACATTGCGCCCCCTGGCATTCCGGGGGGGCATGCCTGTCCGAGC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  247  ATCGAGTCTTTGAACGCACATTGCGCCCCCTGGCATTCCGGGGGGGCATGCCTGTCCGAGC  306

Query  301  GTCATTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGTCGTCGTccccccGGGGGACGGG  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  307  GTCATTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGTCGTCGTCCCCCCCGGGGGACGGG  366

Query  361  CCCGAAAGGCAGCGGCGGCACCGTGTCCGGTCCTCGAGCGTATGGGGCTTTGTCACCCGC  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  367  CCCGAAAGGCAGCGGCGGCACCGTGTCCGGTCCTCGAGCGTATGGGGCTTTGTCACCCGC  426

Query  421  TCGATTAGGGCCGGCCGGGCGCCAGCCGGCGTCTCCAACCTTATTTTTCTCAGGTTGACC  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  427  TCGATTAGGGCCGGCCGGGCGCCAGCCGGCGTCTCCAACCTTATTTTTCTCAGGTTGACC  486

Query  481  TCGGATC  487
            |||||||
Sbjct  487  TCGGATC  493
```

Fig. 3. *Ratio of nitrogenous base sequence similarity between Aspergillus nidulans and the standard strain previously recorded at NCBI*

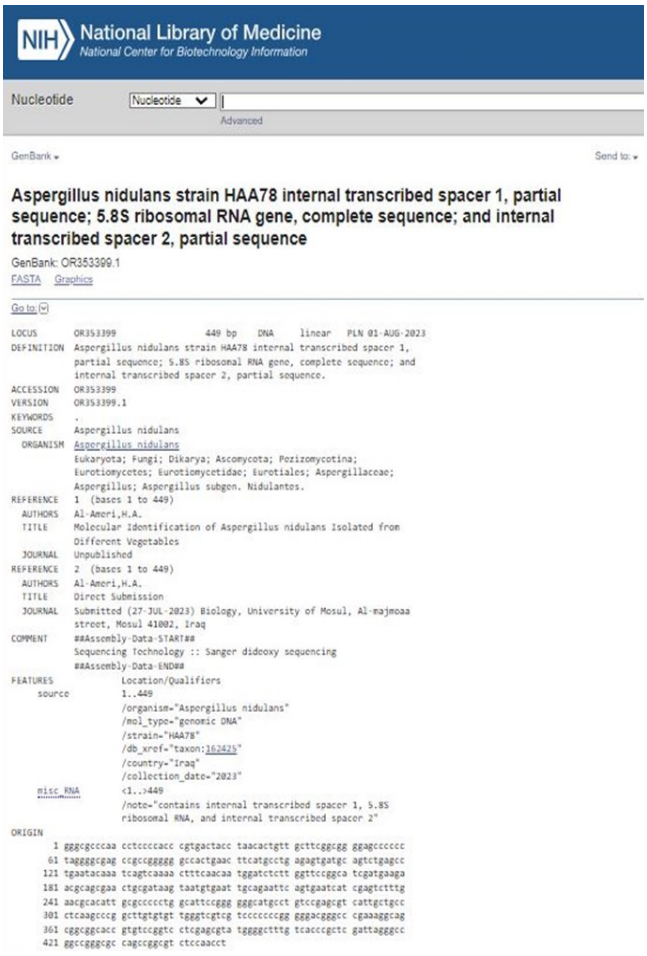

Fig. 4. *Genebank of the National Center for Biotechnology Information isolate code Aspergillus nidulans, National Ceter for Biotechnology Information*

Phylogenetic tree

BLASTn program was used to obtain the tree diagram. The sequence of the nitrogenous bases obtained using the 18S rRNA gene was searched for homology to confirm the diagnosis of the *A. nidulans* isolate with its corresponding strains registered in the NCBI Gene Bank database. The phylogenetic tree of *A. nidulans* was constructed using bootstrap analysis (100X) using MEGA software.

It was observed through the evolutionary tree diagram shown in Table 2 and Fig. 5 that there is an evolutionary relationship between the local isolate in this study and registered in NCBI under the name *Aspergillus nidulans* HAA78. The isolate *Aspergillus nidulans* HAA78 was 100% identical to the isolate *Aspergillus nidulans* strain ATCC 10074 (NG 064803.1).

There are several connections between microorganisms and the food eaten, which may affect our food's quantity, quality, and availability. Naturally occurring foods, like fruits and vegetables, typically include some microorganisms and can pick up more during handling (Al-Ameri, 2023). It is also estimated that about 20% of all vegetables and fruits are lost yearly due to contamination that causes an annual loss of 20% or so of all fruits and vegetables (Chaudhary, 2019). It is known that microorganisms, especially fungi, destroy vegetables, reducing the amount allocated for consumption and the profits obtained from vegetable sales. Therefore, It is necessary to identify these microorganisms, especially human disease causative agents, to reduce the risks of infection and contamination arising from

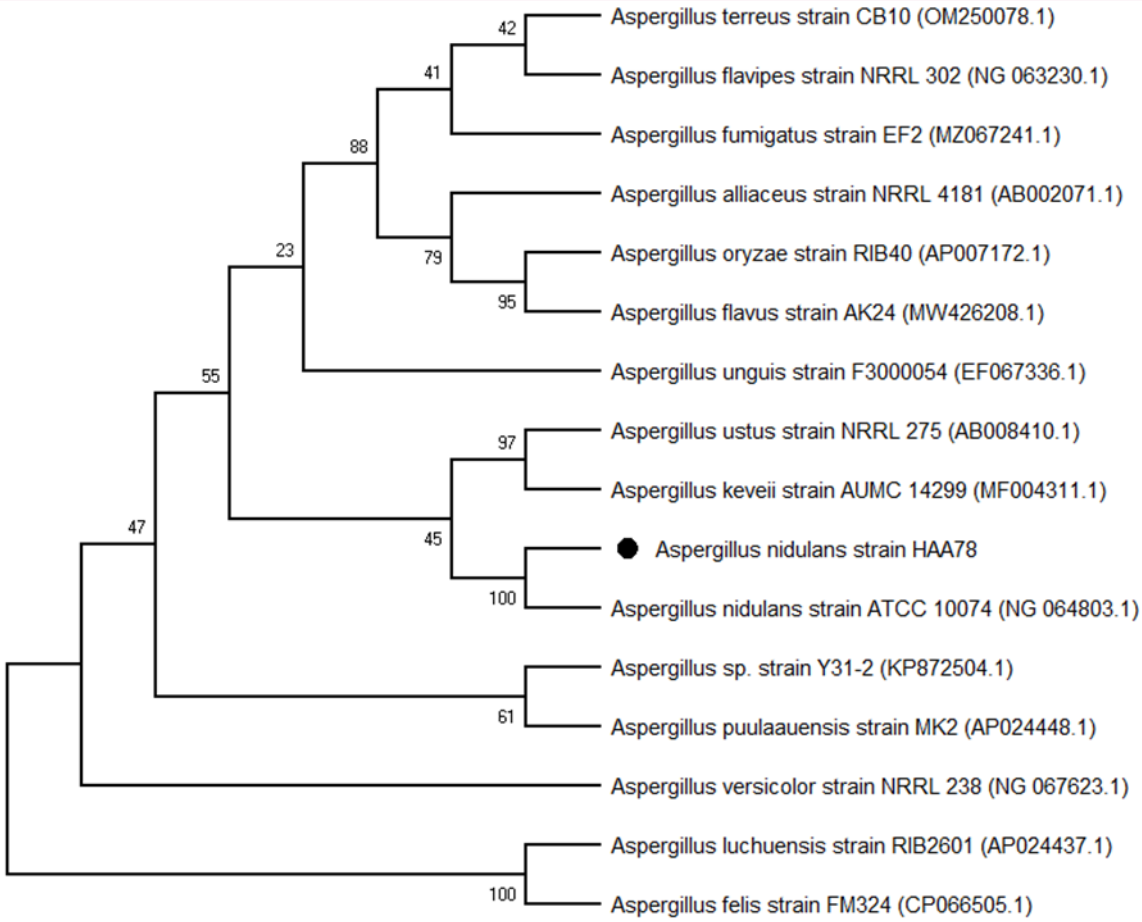

Fig. 5. *Neighbor-Joining phylogenetic trees showing the relationship between the strain Aspergillus nidulans HAA78 (indicated in black circle) and the related species of Aspergillus based on ITS sequences using MEGA-11 software; Percentage of replicate trees in which the associated strains clustered together in the bootstrap test (100 replicates) shown next to the branches*

Table 2. Most related *Aspergillus* species with their accession numbers showing homology with *Aspergillus nidulans* HAA78 strain retrieved from the NCBI database.

	Species name	Query Coverage%	E-value	Identity %	Accession No.
1	Aspergillus nidulans strain ATCC 10074	100	0.0	100	NG_064803.1
2	Aspergillus keveii strain AUMC 14299	100	0.0	99.48	MF004311.1
3	Aspergillus ustus strain NRRL 275	100	0.0	99.48	AB008410.1
4	Aspergillus puulaauensis strain MK2	100	0.0	99.37	AP024448.1
5	Aspergillus sp. strain Y31-2	100	0.0	99.31	KP872504.1
6	Aspergillus versicolor strain NRRL 238	100	0.0	99.08	NG_067623.1
7	Aspergillus unguis strain F3000054	99	0.0	99,36	EF067336.1
8	Aspergillus flavipes strain NRRL 302	100	0.0	98.96	NG_063230.1
9	Aspergillus oryzae strain RIB40	100	0.0	98.85	AP007172.1
10	Aspergillus alliaceus strain NRRL 4181	100	0.0	98.85	AB002071.1
11	Aspergillus flavus strain AK24	99	0.0	98.84	MW426208.1
12	Aspergillus terreus strain CB10	100	0.0	98.73	OM250078.1
13	Aspergillus felis strain FM324	100	0.0	98.67	CP066505.1
14	Aspergillus fumigatus strain EF2	100	0.0	98.62	MZ067241.1
15	Aspergillus luchuensis strain RIB2601	100	0.0	98.56	AP024437.1

dealing with fruits and their consumption (Yaradua *et al*., 2018).

The results in Table 1 include different vegetables (Green bell pepper, Red bell pepper, Beans, Cowpea, Tomato, Eggplant, Cucumber and Pumpkin). It was found that different fungi contaminated vegetables in different proportions as well, and these results are similar to Srilakshmi and Venkata Reddy (2022) refer to when examining samples of vegetables (infected and healthy) to isolate and identify the developing species of fungi present. The vegetables are tomatoes, pepper, cucumber and onion collected from local farms around Guntur market area. All samples showed contamination with fungi, spatially *Rhizopus stolonifer, Aspergillus flavus, A. parasiticus, A. niger, Penicillium citrinum*, *P. digitatum*, *Mucor* spp., highest occurrence rate was *A. parasiticus* then *P. digitatum* and *A. flavus* while *Rhizopus stolonifer*, *Mucor* spp. was the lowest and also similar with the study of Sani *et al*.(2018) which was conducted to the isolation of fungal contamination, especially cabbage, tomatoes, onions spoilage sold at the Dutse-Ultra Modern Market in Dutse Metropolis, Jigawa State, Nigeria. Thirty fungal isolates were obtained from tomato, onion and cabbage samples *R. stolonifer* (27.27%), *A. niger* (36.36%), *A. flavus* (18.18%), *Penicillium* spp. and (9.10%) *Mucor* spp. (9.10%). In tomato contamination, it has been noted that fungi can cause perishable foods like onions, cabbage and tomatoes to deteriorate, perhaps because these organisms' spores are easily spread through the air, which could cause these vegetables' spoilage.

Vinayachandran *et al*. (2022), when investigating fungi associated with some vegetables in Mattancherry Market, India, indicated the presence of *Aspergillus flavus, Aspergillus niger*, *Curvularia, Fusarium, Mucor, Mucor mucedo*, *Pencillum, Rhizopus, Rhizomucor, Syncephalastrum acemosum* was found.

Culture-based methods, in addition to various molecular techniques, were used to identify *Aspergillus nidulans*. Manual methods are not sufficient for the identification of fungal species. Thus, a molecular technique is necessary to identify fungi accurately (Assis *et al*.,2015; Mustafa and Rostam, 2021). ITS regions are most often used to identify the broadest range of fungi. In fact, the ITS region offers a probability of correct identification in the highest percentage and large number of strains of fungi (6, 7, 8, 9). The regions ITS1 and ITS2 were selected as a universal DNA marker suitable for fungal detection at species level (Stefanie *et al*., 2021).

Isolates in this research showed similarity to *Aspergillus nidulans* present in the GenBank database, with a percentage of 100% (Table 2). At the same time, all isolates revealed similarity (100%) to other species of the same genus, such as *Aspergillus variecolor, A. rugulosus, A. ruglovalvus* and *Emericella dentate*, *Aspergillus nidulans* differentiate strains with the closely related species mentioned mainly rely on microscopic characteristics like smooth-walled ascospores bearing two parallel equatorial crests with a width of 0.5 to 1.0 µm used to distinguish them (Purkan *et al*., 2016; Yunasfi and Budi, 2020; Bastos *et al*, 2020).

Conclusion

All vegetables (Green bell pepper, Red bell pepper, Beans, Cowpea, Tomato, Eggplant, Cucumber, Pumpkin, Zucchini and Cauliflower) samples in the present study were contaminated with different fungi, especially *Aspergillus* spp. with different frequent percentages. Molecular identification of *Aspergillus nidulans* using 18 S rRNA and PCR technology showed a package of 600 bp that was sent to the Psomagene sequencing company. The sequences of the isolate's nitrogenous bases were compared with those registered in NCBI. The isolate was found to be 100% genetically identical to the isolates registered internationally due to the nitrogen base sequence analysis sent to NCBI. The study was able to register it as a new strain using the code HAA78, and the isolate *Aspergillus nidulans* HAA78 was 100% identical to the isolate *Aspergillus nidulans* strain ATCC 10074 (NG 064803.1).

ACKNOWLEDGEMENTS

The author thanks the University of Mosul/College of Science/Biology department.

Conflict of Interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Al-Ameri, H. A. (2022). Estimation of phenyl alanine ammonialyase (PAL) and some phytoalexins in *Vicia faba* plants infected with spot disease caused by *Alternaria alternata*. *Journal of Applied and Natural science*, 15 (4), 1499-1504. https://doi.org/10.31018/jans.v15i4.4811.
2. Al-Ameri, H. A. (2022). Defining Mycotoxins Associated with Wheat Grains in Mosul Silo by ELISA, *Journal of Hunan University (Natural Sciences)*, Vol. 49 (4) , 344-352. https://doi.org/10.55463/issn.1674-2974.49.4.36
3. Ali, B. M. , Hamdi, R. H. (2023). Isolation and molecular identification of fungi contaminating fruits and vegetables in cold storages in Al – Anbar city and studying the effect of aqueous extract and dry powder of Eugenia caryophyllata on it., *Journal of University of Anbar for Pure Science* (JUAPS), 17 (1) : 85– 96, DOI: 10.37652/juaps.2023.178867
4. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of molecular biology*, 215(3), 403-410.

5. Assis, L. J, Ries, L. N. A, Savoldi, M., Reis, T. F., Brown, N. A. and Goldman, G. H. (2015). *Aspergillus nidulans* protein kinase Aplays an important role in cellulase production, de Assis *et al. Biotechnol Biofuels*, 8:213, DOI 10.1186/s13068-015-0401-1 , http://creativecommons.org/licenses/by/4.0/
6. Bastos, R. W., Valero, C. , Silva, L. P., Schoen, T. , Drott , M. , Brauer, V., Silva-Rocha, R. , Lind, A. , Steenwyk , J. L. , Rokas, A. , Rodrigues, F. , Resendiz-Sharpe, A. , Lagrou, K., Marcet-Houben, M., Gabaldón, T., McDonnell, E., Reid, I., Tsang, A., Oakley, B. R., Loures, F. V., Almeida, F., Huttenlocher, A. , Keller, N. P., Ries, L. N. A., Goldman, G. H. (2020). Functional characterization of clinical isolates of the opportunistic fungal pathogen *Aspergillus nidulans*, mSphere 5:e00153-20. https://journals.asm.org/journal/msphere
7. Cecilia, S. C., Carla, L. B., Stella, M. C., Carina, E. M. (2017). Assessment of growth of Aspergillus spp. from agricultural soils in the presence of glyphosate, *Rev Argent Microbiol*.;49(4):384-393. http://creativecommons.org/licenses/by-nc-nd/4.0/
8. Chaudhary, L. (2019). Isolation and identification of fungi from some spoiled vegetables, *Plant Archives*, V. 19 No. 2, pp. 2596-2600,
9. Domsch, K. H.; Gams, W. and Anderson, T. H. (1980). Compendium od soil fungi, Vol. 1 No. 2, Academic press, London.
10. Gera, V. G., Bhavana, G. , Deviprasanna, D., Kowsalyadevi, A., Teja, K. D and Ratna kumar , P.K. (2023). Isolation, identification, and enzyme screening of fungi infested various vegetables in the local markets of Visakhapatnam, Andhra Pradesh , *World Journal of Biology Pharmacy and Health Sciences* , 15(01), 160–174, https://doi.org/10.30574/wjbphs.2023.15.1.0319
11. Gerke, J., Köhler, A. M., Wennrich, J. P., Große, V., Shao, L., Heinrich, A. K., Bode, H. B., Chen, W, Surup, F. and Braus, G. H. (2022). Biosynthesis of Antibacterial Iron-Chelating Tropolones in *Aspergillus nidulans* as Response to Glycopeptide-Producing, Streptomycetes. *Frontiers in Fungal Biology,* V. 2, 777474. www.frontiersin.org . doi: 10.3389/ffunb.2021.777474
12. Ibrahim, A. H., Alatbee, A. H. , Ghanim , A. F. (2022). Isolation and identification of the fungi that cause rotting of fruits and vegetables found in the markets of Basra city , *Eurasian Medical Research Periodical*, V. 13, ISSN 2795- 7624. www.geniusjournals.org
13. Jinajali, N. (2017). In wild vegetables *A .spinosus* contains between 3900-5800mg /100g. *African Journal of Agricultural Research*, 16, 231-254.
14. Mailafia, S., Okoh, G. R., Olabode, H. O. K. and Osanupin, R., (2017). Isolation and identification of fungi associated with spoilt fruits vended in Gwagwalada market, Abuja, Nigeria, *Vet World*. , 10(4): 393–397.
15. Mula Abed, F. N. and Al-Ameri, H. A. (2022). Citrus diseases caused by fungi, *International Journal of Biology Research* , V. 7, Issue 2, 1-6.
16. Mustafa, K., Rostam , S. K. (2021). Molecular Identification of Local Isolates *Aspergillus nidulans* from Erbil Province Using Internal Transcribed Spacers , *International Journal of Applied Biology*, 5(2), , ISSN : 2580-2410 , 24-42.,
17. Nelson, P . E.; Toussoun, T . A . and Marasas, W . F . O. (1983) . *Fusarium* species, an Illustrated manual for identification, *The Pennsylvania state university press*, University park.73:1457-1462 p.
18. Nji, Q.N.; Babalola, O.O.; Mwanza, M. (2023). Soil *Aspergillus* Species, Pathogenicity and Control Perspectives. *J. Fungi*, 9, 766. https://doi.org/10.3390/jof9070766 .
19. Odelade, K. A. , Oladeji , O. L. (2020). Isolation of phytopathogenic fungi associated with the post-harvest deterioration of watermelon fruits , *Scientific African* , 8, e00366, http://creativecommons.org/licenses/by/4.0/.
20. Pitt, J. I. and Hocking, A. D. (2009). Fungi and food spoilage, Academic Press, Sydney, Australia.
21. Purkan, P., Baktir, A., & Sayyidah, A. R. 2016. Produksi Enzim Kitinase Dari Aspergillus Niger Menggunakan Limbah Cangkang Rajungan Sebagai Induser. Jurnal Kimia Riset, 1(1), 34. https://doi.org/10.20473/jkr.v1i1 .2440.
22. Salvin, S. (2013). Dietryfibre as consisting of indigestible carbohydrate and lignin that is intrinsic and intact in plants, *Journal of agricultural science*, 5(4), 1417-1435.
23. Sani, N.M., Salihu, Y., Sai du, A.Y., Yusouf , S., Nuhu, D.Z. & Rabiu, G.A. (2018). Isolation of Fungi associated with Spoilage of Selected Vegetables sold in Dutse-Ultra Modern Market, Jigawa State, Nigeria , *Dutse Journal of Pure and Applied Sciences (DUJOPAS)*, Vol. 4 No. 2, 349, – 356.
24. Srilakshmi, K.. and Venkata reddy, ,K. (2022). Isolation and identification of fungi from some selected vegetables , *The International journal of analytical and experimental modal analysis*, V. XIV, Issue III, ISSN NO: 0886-9367.
25. Stefanie, S., Henriet, V., Verweij, P. E. and Warris, A. (2021) *Aspergillus nidulans* and Chronic Granulomatous Disease: A Unique Host–Pathogen Interaction, Aspergillus nidulans in CGD • *The Journal of Infectious Diseases*, 206:1128–37, https://academic.oup.com/jid/article/206/7/1128/808596
26. Tamura, K., Stecher, G. & Kumar, S. (2021). MEGA11: molecular evolutionary genetics analysis version 11. *Molecular biology and evolution*, 38(7), 3022-3027.
27. Vinayachandran, K. , Arpitha, M. R., Subramanian, M. V., Smitha, N. R., Vineeth Kumar, T. V., Varghese, P. (2020). Isolation And Identification Of Fungi Associated With Local Fruits And Vegetables From Kochi And Mattancherry Market, India, *Bioscience Discovery*, 11(4):239-248. https://biosciencediscovery.com
28. Yaradua, S. S., Kankara1, S. L. & Yusuf, M. (2018). Isolation and identification of fungi from some selected vegetables in Kankara local government area, Katsina state , Ujmr, *Journal of Microbiology Research (UJMR)*, 3 (1),2616 - 0668 . https://www.researchgate.net/publication/340385498
29. Yunasfi, S. S. S., & Budi, U. (2020). Aplikasi Fungi *Aspergillus niger, Aspergillus* sp. 1, *Aspergillus* sp. 2 Untuk *Meningkatkan Pertumbuhan Rhizophora apiculata* Di Kecamatan Pangkalan Susukabupaten Langkat. Talenta Conference Series: *Agricultural and Natural Resources (ANR)*, 3(1). https://doi.org/10.32734/anr.v3i1.841 .