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Research Article
Isolation and Molecular Identification of *Fusarium* Fungi from Some Egyptian Grains

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Abstract
Background and Objective: *Fusarium* sp. are considered one of the most important fungal genera; responsible for a broad range of plant diseases. The occurrence of *Fusarium* sp. in grains represents a problem in many countries around the world. Therefore, the aim of this work was to isolate and identify *Fusarium* sp. in several grains obtained from different Egyptian governorates by using the molecular technique. **Materials and Methods:** One hundred and fifty samples, 30 of each grain (wheat, white corn, yellow corn, feed corn, barley and rice) were obtained from different local markets from the following governorates; Cairo, Alexandria, Giza, Qena and Ghrbiya. **Results:** Data showed that all the grains were infested to various degrees with storage fungi. *Fusarium* sp., as well as several fungal species were isolated from different grains. *Fusarium* species were identified morphologically and then molecularly using polymerase chain reaction. The results revealed that the first strain exhibited a high level of 18S rRNA similarity (99%) with *Fusarium verticillioides* isolate (GenBank accession No. KJ207389.1), whereas, the second fungal strain of the sequenced 18S rRNA gene was identified as a close relative (99%) to *Fusarium* sp. (GenBank accession No. KJ190248.1). **Conclusion:** The partial or total sequencing of the 18S ribosomal DNA (rRNA) gene showed a fast technique for fungal classification.

Key words: Isolation, identification, fungal classification, feed corns, grains, PCR, *Fusarium* fungi

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The growth of toxigenic fungi can adversely affect grain quality and produce mycotoxins, which should be monitored and controlled during grain storage[1]. *Fusarium* is considered one of the most important fungal genera, where it includes many species which are plant pathogens responsible for a broad range of plant diseases[2]. Other species of *Fusarium* were distinguished as contaminants of human foods and animal feeds[3]. Accordingly, different *Fusarium* sp. are considered the most harmful fungi worldwide[4].

The most common *Fusarium* mycotoxins are deoxynivalenol (DON), 3-acetyl deoxynivalenol (3-ADON), 15-acetyl deoxynivalenol (15-ADON), nivalenol (NIV) and fusarenon X (Fus-X); T-2 toxin, HT-2 toxin, neosolaniol (NEO) and diacetoxyscirpenol (DAS); zearalenone (ZEN), fumonisin B_1 (FB_1), fumonisin B_2 (FB_2) and fusaric acid[4,5]. The widespread presence of fungi and mycotoxins in pre-harvest infected plants or in stored grains are of great concern for human and animal health[6].

Numerous investigations have been carried out on cereal grains contamination all over the world[7]. Previously in Egypt, Abd Alla[8] isolated 79 *Fusarium* strains belonging to 9 different species from Egyptian cereals. In Ecuador, Pacin *et al.*[9] isolated fungi associated with food and feed commodities. The most prevalent fungi on pelleted feed were *Fusarium graminearum*. In northern Croatia, Cvetnić *et al.*[10] isolated *Fusarium* species from non-harvested maize left in the field overwinter in 1999 and 2003. *Fusarium verticillioides* was the dominant species found in 12.5% (1999) and 35.7% (2003) of maize samples, respectively.

Arino *et al.*[11] studied the natural occurrence of *Fusarium* sp. in organic maize grown in Spain. Sixty samples of corn from both conventional and organic farms were tested for internal fungal contamination. Molds were identified to genus and those belonging to the genus *Fusarium* were identified to species. Four members of the *Fusarium graminearum* species complex were isolated from 150 samples of freshly harvested wheat grains collected in three regions of Brazil[12].

Recently, Jedidi *et al.*[13] identified fungal genera based on morphological features. *Fusarium* species were identified by species-specific PCR assays complemented with DNA sequencing. The most frequent fungi isolated from wheat were *Fusarium* sp., especially in freshly harvested samples.

The development of fungal-specific primers for amplification of the internal transcribed spacer (ITS) region of rRNA genes introduces the possibility of molecular characterization and identification of different fungi[14] and has been recommended as the universal fungal barcode sequence[15]. Other researchers indicated that ITS region is the most frequently sequenced genetic marker of fungi and it is routinely used to address research questions relating to the identification of strains[16]. Recently, Mahmoud and Shehata[17] isolated and identified different fungal isolates using the molecular level by ITS-rDNA regions amplification.

Therefore, the aim of this work was to isolate and identify *Fusarium* sp. in several grains obtained from different Egyptian governorates by using the molecular technique.

MATERIALS AND METHODS

Chemicals and reagents: All chemicals were of analytical grade and were directly used without further purification (Merck, Kenilworth, NJ07033, USA).

Sample collection: One hundred and fifty sample of grains (wheat, white corn, feed corn, yellow corn, barley and rice) were obtained from different local markets from the following governorates; Alexandria, Cairo, Giza, Ghrbiya and Qena. The samples were packed in polyethylene bags and stored at 4°C until analysis.

Isolation of fungi: The grains were surface sterilized by dipping in 1% aqueous sodium hypochlorite solution for 1 min, followed by three successive rinses in sterile distilled water. The grains were blotted dry in between sterile Whatman No. 1 filter papers and plated on Potato Dextrose Agar (PDA, Difico Laboratories, USA) at the rate of 10 grains per plate and incubated at a temperature of 25±2°C for 5 days[18].

Morphological identification of *Fusarium* fungi: The isolated fungi were identified according to colony morphology and microscopic examination[19-22]. Fungal colonies were transferred on to PDA slants for species identification and were identified in the Plant Pathology Department, National Research Centre.

Molecular identification of *Fusarium* fungi

Extraction of DNA: Fungal mycelium was produced in 20 mL of Potato Dextrose Broth (PDB, Difco Laboratories, USA). Mycelium was harvested by filtration through mesh sieves (40 µm), washed with sterile water and deposited on to Whatman filter paper to remove excess water. Mycelium was ground to a fine powder in liquid nitrogen using a mortar and DNA was extracted by the method of Abd-Elsalam *et al.*[23].

ITS-PCR conditions: The PCR amplifications were carried out in a total volume of 25 µL, containing 20 ng genomic DNA, 1X PCR buffer (20 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$, 0.1% Triton X-100), 0.2 mM of each of dNTPs,

0.2 unit of *Taq* DNA polymerase (Roche Holding AG, Basel, Switzerland) and 10 pmol of ITS1/ITS4. The sequences of the ITS1 and ITS4 primers were 5'-TCCGTAGGTGAACCTGCGG-3' and 5-TCCTCCGCTTATTGATATGC-3 respectively[24]. The PCR amplification was carried out according to the following temperature profile: An initial step of 2 min at 94°C, 40 cycles of 60 sec at 94°C, 90 sec at 52°C and 2 min at 72°C and a final step of 7 min at 72°C.

Gel electrophoresis: Electrophoresis of PCR-amplified products was performed in 1.5% agarose gels[25] (low melting) for 1.5 h at 7.0 V cm^{-2}. The PCR products were stained with 0.5 g mL^{-1} of ethidium bromide and visualized with 305 nm ultraviolet light.

PCR product purification: Amplified DNA was purified using a specific purification kit (AccuPrep PCR DNA Purification Kit, K-3034-1, Bioneer Corporation, South Korea). First, 48 mL of absolute ethanol was added to wash buffer (Washing Buffer). Five volumes of buffer PB (PCR binding Buffer) were added to one volume of PCR product (45 mL PCR product was removed and the volume was brought to 225 mL) and mixed thoroughly. Then a column of Binding was inserted into a 2 mL tube of Eppendorf and sample was poured into the column. The sample was centrifuged with the rate of 13.000 rpm at room temperature. Excess solution was discarded and 500 mL washing buffer was added to the column and was centrifuged for a minute speed at 13.000 rpm at room temperature. Then the excess solution was discarded and the second column was inserted in a 2 mL tube of Eppendorf and 500 mL washing buffer was added and were centrifuged for a minute at 13,000 rpm speed at room temperature. The binding column was put within a 1.5 mL tube of Eppendorf and a 30 µL samples were inserted in TE buffer or sterile distilled water two times a minute in the middle column of binding placed on a metal rack were to remain constant. Binding column and tube was centrifuged for a minute at a speed of 13.000 rpm. Then the column was removed and the DNA purified product was collected in tubes at -20°C freezer was stored for subsequent studies.

DNA sequencing: The DNA sequencing was carried out (Macrogen Incorporation, Seoul, South Korea). All inter transcribed spacer sequencing work was carried out on both strands of the submitted DNA fragments. The sequences were assembled, edited and aligned by using the DNA STAR SeqMan (DNA STAR Incorporation, Wisconsin, USA) and the CLC sequence viewer.

Identification of isolates using BLAST: Forward and reverse DNA strand sequence was aligned using BLAST (bl2seq) program available at NCBI. The finalized sequence of amplified 18S rDNA fragment from each isolate was blasted against the collection of non-redundant nucleotide sequence database of NCBI. The isolates were identified based on hits analysis from mega blast (highly similar sequences) output. The hits of 18S rDNA sequences were used in phylogenetic analysis of 18S rDNA sequences of isolates to determine 18S rDNA sequence-based evolutionary relationship among the isolates and hit. The 18S rRNA gene fragments sequencing and identification of isolates were carried out using routinely used techniques[26].

RESULTS AND DISCUSSION

Isolation and frequency distribution of fungi in collected grains from different governorates: The study showed that all the grains (wheat, white corn, feed corn, yellow corn, barley and rice) obtained from five governorates (Alexandria, Cairo, Giza, Gharbia and Qenna) were infested to various degrees with fungi (Table 1). The results indicated the isolation and identification of fungal strains that belonged to five genera of *Aspergillus*, *Alternaria*, *Fusarium*, *Penicillium* and *Rhizopus*. These results are considered similar to those reported by Aly *et al.*[27], who isolated five fungal genera from peanut samples. Similar observations were reported by Mohammed *et al.*[28]. The most common genera isolated were

Table 1: Frequency distribution of total fungal count in different grains collected from all Egyptian governorates

	Wheat		White corn		Feed corn		Yellow corn		Barley		Rice	
Fungi	TFC	Percentage	TFC	Percentage	TFC	Percentage	TFC	Percentage	TFC	Percentage	TFC	Percentage
A. flavus	18	16.33	33	30.00	61	48.41	40	42.00	14	20.89	14	21.53
A. niger	26	23.63	17	15.45	9	7.14	14	14.73	19	28.35	15	23.07
A. ochraceus	5	4.500	5	4.54	6	4.76	1	1.05	1	1.49	6	9.23
A. parasiticus	11	10.00	16	14.54	14	11.11	22	23.15	12	17.91	6	9.23
Alternaria	14	12.72	6	5.45	1	0.79	1	1.05	2	2.98	2	3.07
Fusarium	13	11.81	13	11.81	13	10.31	3	3.15	12	17.91	13	20.00
Penicillium	22	20.00	15	13.63	21	16.66	12	12.63	5	7.46	8	12.31
Rhizopus	1	0.90	5	4.54	1	0.79	2	2.10	2	2.98	1	1.53
TFC	110	100.00	110	100.00	126	100.00	95	100.00	67	100.00	65	100.00

TFC: Total fungal count

Table 2: Nucleotide sequence of 18S rRNA gene of *Fusarium* species

Fusarium name	Sequence
Fusarium verticillioides	CGACGTGACCGCCAATCAATTTGGGGAACGCGATTTGACTCGCGAGTCCCAACACCAAGC CGACGTGACCGCCAATCAATTTGGGGAACGCGATTTGACTCGCGAGTCCCAACACCAAGC TGGGCTTGAGGGTTGAAATGACGCTCGAACAGGCATGCCCGCCAGAATACTGGCGGGCGC CGACGTGACCGCCAATCAATTTGGGGAACGCGATTTGACTCGCGAGTCCCAACACCAAGC CGACGTGACCGCCAATCAATTTGGGGAACGCGATTTGACTCGCGAGTCCCAACACCAAGC CGACGTGACCGCCAATCAATTTGGGGAACGCGATTTGACTCGCGAGTCCCAACACCAAGC TGGGCTTGAGGGTTGAAATGACGCTCGAACAGGCATGCCCGCCAGAATACTGGCGGGCGC TGGGCTTGAGGGTTGAAATGACGCTCGAACAGGCATGCCCGCCAGAATACTGGCGGGCGC GTCCCGTTTTACCGGGAGCGGGCTGATCCGCCGAGGCAACAATTGGTATGTTCACAGGGG GTCCCGTTTTACCGGGAGCGGGCTGATCCGCCGAGGCAACAATTGGTATGTTCACAGGGG AATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCAT AATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCAT TTTGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTGATTTA TTTGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTGATTTA TTTATGGTTTTACTCAGAAGTTACATATAGAAACAGAGTTTAGGGGTCCTCTGGCGGGCC TTTATGGTTTTACTCAGAAGTTACATATAGAAACAGAGTTTAGGGGTCCTCTGGCGGGCC TTTGGGAGTTATTAACCCGGTTAATGATCCCACCTTTGGGAGTTGTAAACTCGGTAAATGATCCCTCC
Fusarium sp.	GCCCGCCAGAGGACCCCTAAACTCTGTTTCTATATGTAACTTCTGAGTAAAACCATAAAT GCCCGCCAGAGGACCCCTAAACTCTGTTTCTATATGTAACTTCTGAGTAAAACCATAAAT AAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAA AAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAA TGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTG TGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTG CGCCCGCCAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCCCAG CGCCCGCCAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCCCAG CTTGGTGTTGGGACTCGCGAGTCAAATCGCGTTCCCCAAATTGATTGGCGGTCACGTCGA CTTGGTGTTGGGACTCGCGAGTCAAATCGCGTTCCCCAAATTGATTGGCGGTCACGTCGA GCTTCCATAGCGTAGTAGTAAAACCCTCGTTACTGGTAATCGTCGCGGCCACGCCGTTAA GCTTCCATAGCGTAGTAGTAAAACCCTCGTTACTGGTAATCGTCGCGGCCACGCCGTTAA ACCCCAACTTCTGAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATA ACCCCAACTTCTGAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATA

Aspergillus, *Penicillium* and *Fusarium*. Among the *Aspergillus* sp., *A. flavus*, *A. parasiticus*, *A. niger* and *A. ochraceus* were identified. The presence of *Aspergillus* sp. that include *A. flavus* and *A. parasiticus*, which are able to produce aflatoxins and *A. ochraceus* and *A. niger* strains known to produce ochratoxin A could pose a risk to consumer health[29].

The results of the present study showed the percentage of *Fusarium* sp. isolated from wheat (11.81%), white corn (11.81%), feed corn (10.31%), yellow corn (3.15%), barley (17.91%) and rice (20.00%) on PDA medium using the grain-plate method (Table 1). It was noticed that the frequency of occurrence of *Fusarium* sp. depended on the type of grains[30].

Data in Table 1 also showed the frequency occurrence of fungi in different grains, whereas high percentage of *A. niger* (23.63%) were isolated from wheat grains, whereas in white corn high percentage of *A. flavus* (30.00%) was detected. On the other hand, in both feed corn and yellow corn, high percentage of *A. flavus* (48.41 and 42.00%) was detected respectively. *Aspergillus niger* (28.35 and 23.07%) was detected in both barley and rice grains, respectively. These results are similar to those reported by Jedidi *et al.*[13], who found that *A. flavus* was the most fungal species isolated in corn. The occurrence of *A. flavus* is considered vital because they are known to produce aflatoxins which are considered the most potent carcinogenic to human and animals[31].

Results in Fig. 1(a-f) showed the total fungal count in different governorates for wheat, white corn, feed corn, yellow corn, barley and rice. It was noticed that wheat samples obtained from Giza governorate were highly contaminated, followed by Cairo governorate. Data also showed that white corn obtained from Alexandria governorate were highly contaminated followed by Giza governorate. Feed corn, yellow corn and rice obtained from Giza governorate were highly contaminated, whereas, barley obtained from Alexandria governorate was highly contaminated. It could be noticed that many of the cereal grains obtained from Giza governorate were highly contaminated by fungi. These results could be due to climate condition which is one of the most important factors that have a great effect on fungal growth as Giza governorate was considered one of the highest governorates in temperature averages[32].

Molecular identification of the isolated fungi: Two fungal isolates were identified on the basis of their molecular characteristics. The amplification of 18S rRNA with ITS1 and ITS4 primers has been successfully performed and 18S rRNA gene was chosen as a target for PCR amplification because the sequence data is widely used in the molecular analysis to reconstruct the evolutionary history of organisms. The partial sequences of 18S rRNA and aligned with the available 18S rRNA sequences (Table 2). The phylogenetic tree was

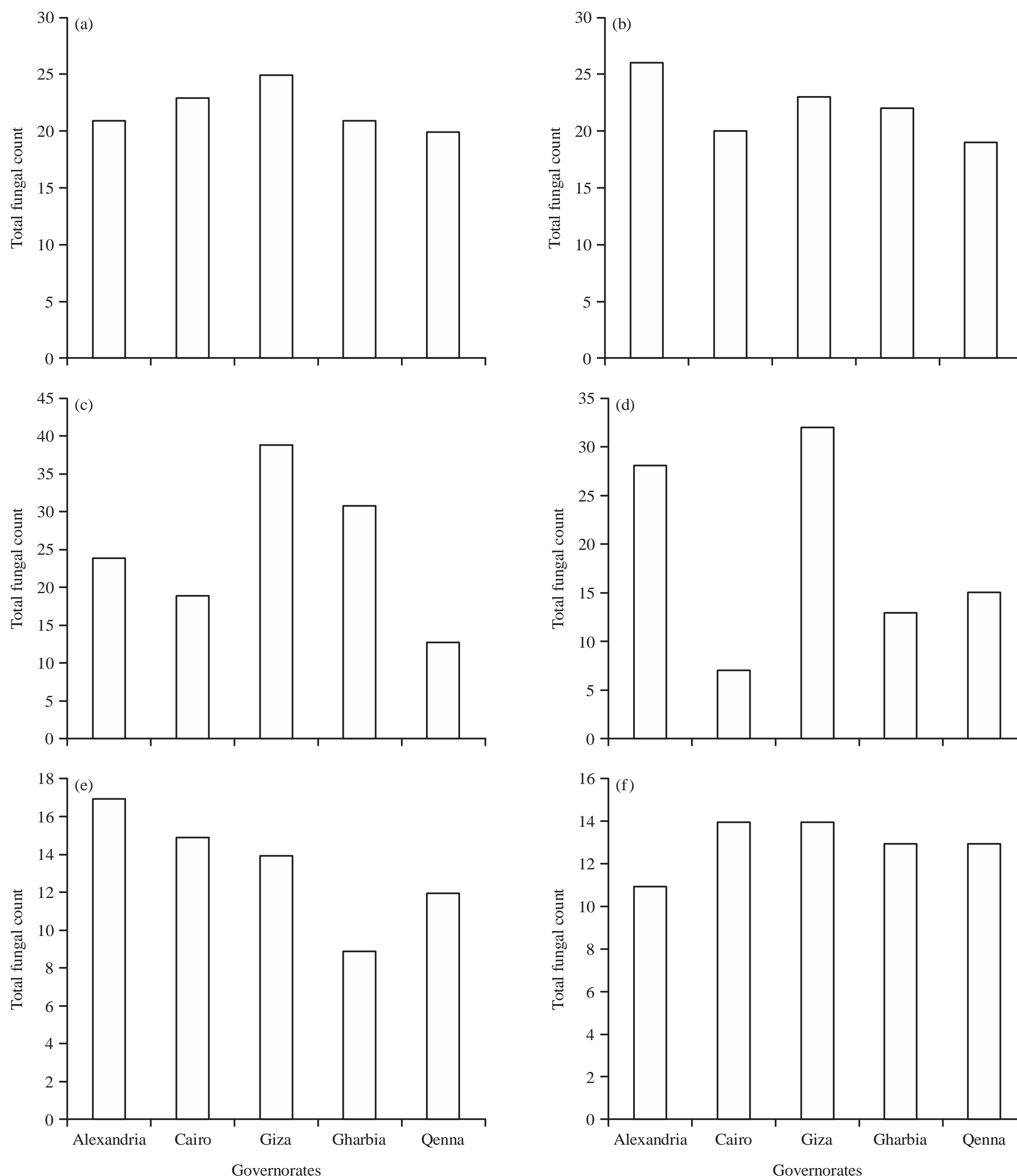

Fig. 1(a-f): Total fungal count in different governorates for, (a) Wheat, (b) White corn, (c) Feed corn, (d) Yellow corn, (e) Barley and (f) Rice

constructed by the neighbor-joining (N-J) method based on the 18S rRNA sequences. The 18S rRNA gene sequence analyses showed that strains were most closely affiliated with members of the genus *Fusarium*.

In the phylogenetic tree, the first strain exhibited a high level of 18S rRNA similarity (99%) with *Fusarium verticillioides* isolate (GenBank accession No. KJ207389.1) (Table 3, Fig. 2). On the other hand, the second fungal strain of the sequenced 18S rRNA gene was identified as the 18S rRNA sequence analysis revealed that the isolate is a close relative (99%) of *Fusarium* sp. (GenBank accession No. KJ190248.1) (Table 4, Fig. 3).

It is well-known that molecular classification is a fast procedure which requires minimal management of pathogens and also helps in distinguishing morphologically, similar fungal species[33]. Similar applications of PCR technology were used for the identification and detection of fungi, by using an internal transcribed spacer (ITS)[34-37]. The genomic DNA

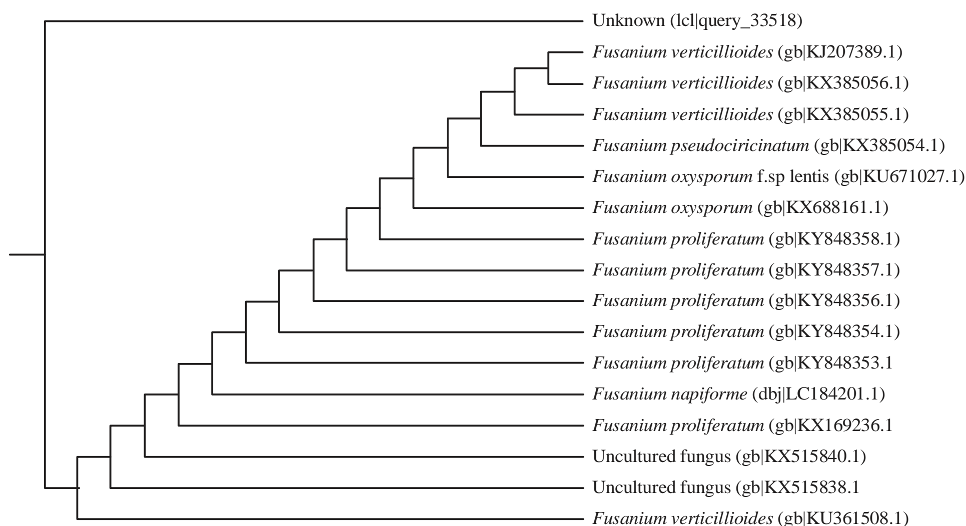

Fig. 2: Phylogenetic tree showing the relationship of closely related species constructed using the neighbor-joining method and based on 18S rRNA gene sequences. Isolate is closely related to *Fusarium verticillioides*

Table 3: Sequence producing significant alignments for the *Fusarium verticillioides*

Description	Max score	Total score	Query cover (%)	E-value	Ident (%)	Went Accession
Fusarium verticillioides isolate 141WS 18S ribosomal RNA gene. partial sequence; internal transcribed spacer	701	701	99	0.0	99	KJ207389.1
Fusarium owsporum strain H L52 small subunit ribosomal RNA gene, partial sequence: internal transcribed spacer	699	699	99	0.0	99	KX688161.1
Fusarium proliferatum isolate 14F small subunit ribosomal RNA gene, partial sequence: intern al transcribed spacer	699	699	99	0.0	99	KY848358.1
Fusarium proliferatum isolate 12F small subunit ribosomal RNA gene, partial sequence: internal transcribed spacer	699	699	99	0.0	99	KY848357.1
Fusarium proliferatum isolate 11 F small subunit ribosomal RNA gene, partial sequence: internal transcribed spacer	699	699	99	0.0	99	KY848356.1
Fusarium proliferatum isolate 9F small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer	699	699	99	0.0	99	KY848354.1
Fusarium proliferatum isolate 6F small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer	699	699	99	0.0	99	KY848353.1
Fusarium verticillioides isolate DET-51 18S ribosomal RNA clone, partial sequence; internal transcribed spacer	699	699	100	0.0	98	KX848353.1
Fusarium verticillioides isolate DET-3 183 ribosomal RNA gene, partial sequence; internal transcribed spacer	699	699	100	0.0	98	KY848353.1
Fusarium pseudocircinatum isolate DET-46 18S ribosomal RNA gene, partial sequence: internal transcribed spacer	699	699	100	0.0	98	KX385056.1
Fusarium napiforme genes for 18S rRNA, ITS1. 513 rRNA, ITS2., 28S rRNA, partial and complete sequence	699	699	99	0.0	99	KX385055.1
Fusarium proliferatum strain R41 188 ribosomal RNA gene, partial sequence; internal transcribed spacer 1	699	699	99	0.0	99	KX385054.1
Fusarium verticillioides isolate FM& 18S ribosomal RNA gene. partial sequence; intern al transcribed spacer 1	699	699	98	0.0	99	LC184201.1
Fusarium owsporum f. sp_ lentis isolate FLS 5 internal transcribed spacer 1, partial sequence; 5.83 ribosomal	699	699	100	0.0	98	KX169236.1
Uncultured fungus clone ZSYN2131307-25 small subunit ribosomal RNA gene. partial sequence; internal transaction	699	699	99	0.0	99	KU361508.1
Uncultured fungus clone ZSYN201307-24 grog subunit ribosomal RNA gene. partial sequence; internal transaction	699	699	99	0.0	99	KU671027.1

Table 4: Sequence producing significant alignments for the *Fusarium* species

Description	Max score	Total score	Query cover (%)	E-value	Ident (%)	Went Accession
Fungal so. SK11 internal transcribed spacer 1, partial sequence: 5.83 ribosomal RNA gene and internal transaction	808	808	99	0.0	99	KP893212.1
Fusarium sp. CC2 internal transcribed spacer 1, partial sequence; 5.38 ribosomal RNA gene and internal transaction	808	808	99	0.0	99	KJ190248.1
Fungal endophyte sp. P1717C internal transcribed spacer 1, partial sequence: 5.8S ribosomal RNA gene	806	806	98	0.0	100	EU977254.1
Gibberella monilfformis isolate FKCB-009 18S ribosomal RNA gene. partial sequence; internal transcribed pacer	806	806	99	0.0	99	EU314975.1
Fusarium so. Fs219TNW-T internal transcribed spacer 1, partial sequence: 5.83 ribosomal RNA gene and into	804	804	99	0.0	99	KF293355.1
Gibberella moniliforrnis strain P3A82b internal transcribed spacer 1, partial sequence: 5.8S ribosomal RNA gene	804	804	100	0.0	99	JN672602.1
Fusarium sp. Lit-001 18S ribosomal RNA gene, partial sequence: internal transcribed spacer 1, 5.38 ribosomal	804	804	99	0.0	99	HQ025928.1
Gibberella moniliformis strain bxg41213 188 ribosomal RNA gene, partial sequence: internal transcribed spacer	804	804	99	0.0	99	EF556217.1
Fusarium verticillioides isolate 1,1038 Cl 5a internal transcribed spacer 1, partial sequence: 5.38 ribosomal	802	802	99	0.0	99	KX681583.1
Fusarium verticillioides isolate H023 C2 4 internal transcribed spacer 1. partial sequence; 5.38 ribosomal RNA	802	802	99	0.0	99	KX681582.1
Fusarium subfilutinans isolate M038 Cl 1 internal transcribed spacer 1, partial sequence: 5.8S ribosomal RNA	802	802	99	0.0	99	KX681580.1
Fusarium subfilutinans isolate MO28 Al 92 internal transcribed spacer 1, partial sequence: 5.88 ribosomal	802	802	99	0.0	99	KX681579.1
Fungal sp. voucher ARIZ:PS0943 internal transcribed spacer 1, partial sequence; 5.38 ribosomal RNA gene	802	802	99	0.0	99	KU977979.1
Fungal sp. voucher ARIZ:P30863 internal transcribed spacer 1, partial sequence; 5.83 ribosomal RNA gene	802	802	99	0.0	99	KU977939.1
Fungal sp. voucher ARIZ:P30770 internal transcribed spacer 1, partial sequence; 5.83 ribosomal RNA gene	802	802	99	0.0	99	KU977911.1
Fungal sp. voucher ARIZ:PS0660 internal transcribed spacer 1, partial sequence; 5,83 ribosomal RNA gene	802	802	99	0.0	99	KU977871.1
Fungal sp. voucher ARIZ:PS0507 internal transcribed spacer 1, partial sequence; 5.38 ribosomal RNA gene	802	802	99	0.0	99	KU977794.1

containing 18S rRNA was the right candidate for the detection of fungus as it is a multi-copy gene which evolves slowly and is conserved among fungi. The present study proves that the genomic DNA containing 18S rRNA-based PCR is suitable for probing a large range of significant fungi owing to its higher level of analytical sensitivity and specificity[38].

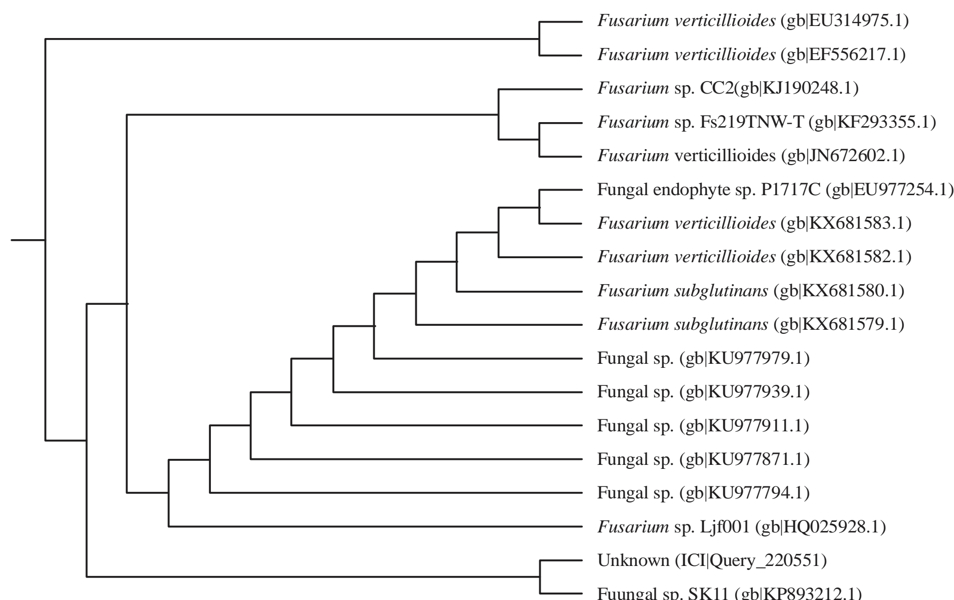

Fig. 3: Phylogenetic tree showing the relationship of closely related species constructed using the neighbor-joining method and based on 18S rRNA gene sequences. Isolate is closely related to *Fusarium* sp.

CONCLUSION

Fusarium and other fungal species were isolated from different grains collected from different governorates in Egypt. The isolated species were first identified morphologically. Then the tested *Fusarium* species were identified genetically by sequencing of 18S rRNA gene using ITS1 and ITS4 primers.

SIGNIFICANCE STATEMENT

This study confirmed the fungal contamination including *Fusarium* sp. of different cereal grains that were obtained from different Egyptian governorates. *Fusarium* sp. were identified using morphological and molecular methods. The study contributes to the effective monitoring of fungal contamination and raising awareness on the hazards of fungi and their mycotoxin on human and animal health.

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