Molecular identification and control of some pathogenic*Fusarium* species isolated from maize in Egypt

Amal S. Hathout¹, Nevien A. Abo-Sereih², Bassem A. Sabry¹, Ahmed F. Sahab³, Soher E. Aly¹*¹

¹Food Toxicology and Contaminants Department, ²Microbial Genetics Department, ³Plant Pathology Department, National Research Centre, Dokki 12622, Cairo, Egypt

**Abstract:** *Fusarium* species are among the most common fungal pathogens on maize causing decrease of the yield and accumulation of one or more of *Fusarium* toxins. The current morphology based taxonomical system for *Fusarium* is inadequate, and detection and identification procedures are both time consuming and error-prone. Thus the aim of this study was to isolate and identify pathogenic *Fusarium* species as well as studying the use of some plant extracts to control the *Fusarium* species. The molecular characterization was performed with partial 18S rRNA sequencing using primer (5’-AGAGTTTGATCCTGGCTCAG) targeted to internal transcribed spacer (ITS) region of rDNA complex. Sequence analysis identified the isolates as *Fusarium solani* and *Fusarium oxysporum* and the species identification based on sequence data correlated well with the morphotaxonomic classification. The growth of *F. oxysporum* and *F. solani* species were completely inhibited using 100 ppm of rocket and star anise extracts. The highly active extract of rocket and star anise was followed by fennel and rosemary in descending order. *Fusarium oxysporum* was more sensitive for the plant extracts used. The MIC and IC50 of plant extracts were also determined. In conclusion, the sequence variation within the ITS region allowed reliable and faster discrimination of the isolates at both the genus and species level. On the other hand, the plant extracts used in this study showed high antifungal activity against the *Fusarium* species.

**Key words:** *Fusarium* species, maize, plant extract, antifungal activity, 18S rDNA.

**Introduction**

Maize (*Zea mays* L.) is a very versatile grain that serves as starting raw material for food and feed products. Its prone to pre- and pro-harvest fungal infection and is often unavoidable and is considered a worldwide problem. The susceptibility of maize grains to various fungi has been very well documented, whereas these fungi belong to the genera *Fusarium*, *Aspergillus* and *Penicillium*. The genus *Fusarium* comprises a diverse array of fungi which are distributed world-wide as important plant pathogens, as well as opportunistic colonizers of plant and agricultural commodities or as saprophytes on debris and cellulosic plant material¹. It has a wide range of host plants, such as tomatoes, potatoes, legumes, clove and grasses such as wheat, barley, oats, maize and sugarcane². In the last ten years, the increased level of colonization and infection by *Fusarium*, particularly of ripening ears of cereals, has attracted much attention: firstly, because of the significant effects on yield and the quality of harvested grains, and secondly because of the ability of *Fusarium* species to produce a wide range of mycotoxins which can enter the human and animal food chains⁴, ⁵, ⁶.

*Fusarium solani* are host-specific pathogens of a number of agriculturally important plants, including pea, cucurbits, and sweet potato⁷. Moreover, they are increasingly associated with opportunistic infections of humans and other animals, causing systemic infections with a high mortality rate⁸, as well as localized infections in the skin and other body parts⁹. It is also associated with serious invasive mycoses in immune compromised and immune suppressed patients¹⁰.*Fusarium oxysporum* is a soil-borne facultative parasite that
causes disease in more than 100 plant species, including important agricultural crops\textsuperscript{11}. The fungus is a morpho-species that is divided into specialized groups according to the hosts they attack, and subdivided into races according to the susceptibility of specific host cultivars\textsuperscript{12}.

The routine analysis of maize and other cereals for toxigenic \textit{Fusarium} species is hindered by difficulties associated with standard methods for isolating and identifying \textit{Fusarium} species\textsuperscript{13}. Traditionally \textit{Fusarium} species have been differentiated by morphological characteristics such as presence or absence of micro-conidia, shape and size of macro-conidia, colony morphology, growth rates and pigmentation. Morphological identification of fungi is the first and the most difficult step in the identification process and is time consuming and requires considerable expertise and skill\textsuperscript{13}. This is especially true for \textit{Fusarium} species which have been differentiated by morphological characteristics, although differentiation of closely related cultures requires extensive molecular techniques\textsuperscript{14}. Molecular techniques represent an important genetic resource for biotechnology study. Several recent studies have shown that genetic methods can be successfully used in the studies of pathogenic fungi. DNA-based techniques, particularly PCR can detect minute quantities of a pathogen\textsuperscript{15, 16}. These methods have provided new opportunities to study and understand, for example, the biology of plant pathogenic fungi, pathogen population structure and dynamics, host-pathogen interactions, etc.\textsuperscript{17, 18, 19}. Thus, today \textit{Fusarium} species are usually identified by combining the morphological, biological, and molecular data.

The presence of toxigenic fungi in grains present a potential hazard to human and animal health. Currently, there is a strong debate about the safety aspects of chemical preservatives, and consumers tend to be suspicious of chemical additives and thus demand for more natural and more acceptable preservatives to been intensified\textsuperscript{20}. Natural metabolites do not have any indiscriminate hazardous effects like synthetic fungicides. They are normally found in leaves and stem and concentrate in regions such as leaves, bark or fruit\textsuperscript{21}. Bhardwaj\textsuperscript{22} tested the aqueous extracts of twenty plants for their antifungal activity against \textit{F. solani}, whereas Bajpai and Kang\textsuperscript{23} studied the antifungal activity of \textit{Magnolia liliflora} against several pathogenic fungi including \textit{F. solani}.

Rocket (\textit{Eruca sativa} L.) have gained greater importance as a salad vegetable and spice, especially among Middle Eastern populations and Europeans, and possess diversified medicinal and therapeutic properties including inhibition of tumorigenesis, anti-ulcer, and hepatoprotective activities\textsuperscript{24}. Fennel (\textit{Foeniculum vulgare} L.) a plant belonging to the family Apiaceae, has a long history of herbal uses. Traditionally, fennel seeds are used as anti-inflammatory, analgesic, carminative, diuretic and antispasmodic agents. Recently there has been considerable interest in the antioxidant potential and antimicrobial activities of fennel seed extracts\textsuperscript{25}. It is also used as a constituent of cosmetic and pharmaceutical products\textsuperscript{26}. Rosemary (\textit{Rosmarinus officinalis} L.) is a common household plant grown in many parts of the world. It is used for flavouring food, as well as in cosmetics and in folk medicine. Extract of rosemary relaxes smooth muscles of trachea and intestine, and has choleric, hepatoprotective and anti-tumorogenic activity. The most important constituents of rosemary are caffeic acid and its derivatives such as rosmarinic acid\textsuperscript{27}. Star anise (\textit{Illicium verum}) is an aromatic evergreen tree bearing purple-red flowers, growing almost exclusively in southern China and Vietnam. Its fruit is an important traditional Chinese medicine as well as a commonly used spice that closely resembles anise in flavour, obtained from the star-shaped pericarp\textsuperscript{28}.

In the present investigation morphological and molecular techniques were used to identify some \textit{Fusarium} species isolated from pre-harvest maize, as well as evaluating the antifungal activity of some plant extracts to control the of the identified strains.

Materials and Methods

Isolation of \textit{Fusarium} species

Fifteen samples of pre-harvest maize (each of three maize ears) were collected from Faculty of Agriculture, Cairo University. Each maize sample was individually surface sterilized in 1% sodium hypochloride solution for 1 min, and then rinsed three times in sterile distilled water. Surface sterilization was necessary to curtail the development of only potential contaminants which would affect the recovery of molds. On Potato Dextrose Agar (PDA) (Difco, Detroit, MI)\textsuperscript{10} grains were plated and incubated at 25±2°C for 7 days. After incubation, the cultures were transferred onto PDA for species identification.
Morphological identification

The fungal species were identified according to the morphology using the descriptions of Nelson et al.\textsuperscript{2}, Thom and Raper\textsuperscript{29}, Gilman\textsuperscript{30}, and Barnett and Hunter\textsuperscript{31}. The isolates were grown on PDA at 25±2°C for 7 days in order to describe colony morphology and pigmentation as secondary criteria for identification. The isolates were morphologically identified as \textit{F. solani} and \textit{F. oxysporum} and were confirmed by polymerase chain reaction (PCR).

Molecular identification

\textbf{DNA extraction}

Genomic DNA was extracted from pure mycelial cultures of the \textit{Fusarium} isolates; grown on PDA using Extract-N-Amp Plant PCR Kit (Sigma-Aldrich Co., USA) following the manufacturer’s instructions. The crude lysate (freshly prepared) was subjected to 18SrDNA PCR partial amplification by use protocol of Gene Jet genomic DNA purification kit\textsuperscript{32}.

\textbf{PCR partial amplification and sequencing of 18S rDNA}

Identification of the fungal isolates was performed based on molecular genetic analysis using the internal transcribed spacer region (ITS). Partial sequences of the isolates 18S rDNA were obtained using a strategy based on Boekhout et al.\textsuperscript{33}. A divergent 5’ domain of the gene was amplified using primers forward (5’- AGAGTTTGATCCTGGCTCAG) and reverse (5’-GGTTACCTTGTTACGACTT). DNA amplification involved the following 25 cycles: initial 1 min denaturation at 94°C, 2 min annealing at 50°C, 1.5 min extension at 74°C and a final 5 min extension at 74°C. PCR amplification was performed using a PTC-100 thermal cycler (MJResearch Inc., Watertown, MA, USA). Amplified products were isolated with a silica matrix (Geneclean II Kit; Bio 101).

\textbf{Identification of isolates}

Sequencing results were individually inputted online into the nucleotide BLAST program (BLASTN 2.2.29) through the NCBI database (http://blast.ncbi.nlm.nih.gov/) to identify the isolate\textsuperscript{34, 35}.

\textbf{Phylogenetic analysis}

Sequencing results of the isolates were also inputted into a sequence alignment program called ClustalW to determine the phylogenetic relatedness of the different species. They were aligned using the UPGMA algorithm, which considers the rate of evolution to be constant between species, to develop a phylogenetic tree based on sequence homology. The resulting alignment was opened into a program called Tree View which allowed the phylogenetic tree to be viewed\textsuperscript{36}.

\textbf{Plant materials}

Four plants viz., rocket (seeds), fennel (fruits), rosemary (leaves), and star anise (fruits) were used in this study, and they were purchased from local markets in Egypt. The plants were identified by the Department of Botany, Faculty of Science, Cairo University.

\textbf{Preparation of plant extracts}

All plant samples (seeds, fruits, and leaves) were thoroughly washed, air dried under forced circulation of heated air at 40°C and ground to powder. The plant materials were entailing extracted for 8 h with ethanol using a Soxhlet apparatus. Then the ethanol was evaporated using Rotary evaporator under vacuum at 30°C. The plant extracts were kept in a freezer at -20°C until use.

\textbf{Antifungal activity}

The inhibitory effect of the plant extracts was calculated against the linear growth of \textit{F. solani} and \textit{F. oxysporum}. For each extract, seven concentrations 0, 50, 100, 200, 300, 400 and 500 ppm were prepared into sterilized molten and cooled PDA medium. Later 15mL of the molten medium was poured into sterilized Petri plates and then inoculated with a mycelium disc of 5 mm size and placed at the centre. Three replications were used for each concentration. The plates were incubated at 25±2°C and the radial growth was measured when fungus attained maximum growth in control plates.
Statistical analysis

Statistical analysis was performed using SPSS statistical program for windows (Version 16) (SPSS Inc., Chicago, IL, USA). All data were statistically analysed using analysis of variance.

Results

Fungal isolates

In this study, a total of 50 fungal isolates (data not shown) were obtained from the pre-harvest maize samples. Among the *Fusarium* isolates two isolates were selected to identify their morphological and molecular characteristics.

Morphological characterization

The first isolate grown on PDA produced aerial mycelium (dense and floccose), white colony appearance, greenish or brownish shades on reverse, and fast growing (2.5 to 5.0 cm after 4 days). The isolate also had longer macroconidia (3-4 septates), moderately curved 27x5.0 µm. Morphological observation revealed the taxonomic identity of the fungus, and the above characteristics designated the fungus to be *F. solani*. The second *Fusarium* isolate grown on PDA produced aerial mycelium, blue or purple colony colour, dark blue or dark purple shades on reverse, and fast growing. The isolate also had abundant macrocondia, only slightly sickle shaped, with foot shaped basal cell (3-5 septates). Microconidia were abundant, generally single celled, oval, and produced only in false heads. Morphological observation revealed the taxonomic identity of the fungus, and the above characteristics designated the fungus to be *F. oxysporum*.

Molecular characteristics

PCR amplification of ITS region produced about 800 bp of DNA fragments. The fragments were then sequenced to determine the species of fungus based on the similarity with other references of identified species. The sequences of the isolates studied were compared with those of NCBI databases using BLAST network. The partial sequences of 18S rRNA obtained from the first *Fusarium* isolates was aligned with the available 18S rRNA sequences in GenBank database, and the fungus was found to have 99% similarity with *Fusarium solani* strain (Accession Number Gen Bank: KF572456.1) and *Fusarium solani* CEF-325 (Accession Number Gen Bank: KF999012.1) (Table 1). The second *Fusarium* isolate was found to have 99% similarity with *Fusarium oxysporum* isolate (Accession Number Gen Bank: AY928414.1) (Table 2).

<table>
<thead>
<tr>
<th>Accession</th>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query coverage</th>
<th>E value</th>
<th>Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>KF572456.1</td>
<td><em>Fusarium solani</em> strain</td>
<td>953</td>
<td>953</td>
<td>97%</td>
<td>0.0</td>
<td>99%</td>
</tr>
<tr>
<td>KF999012.1</td>
<td><em>Fusarium solani</em> strain CEF-325</td>
<td>952</td>
<td>952</td>
<td>96%</td>
<td>0.0</td>
<td>99%</td>
</tr>
<tr>
<td>HG798753.1</td>
<td><em>Fusarium solani</em> strain TUFs8</td>
<td>948</td>
<td>948</td>
<td>96%</td>
<td>0.0</td>
<td>98%</td>
</tr>
<tr>
<td>KC808261.1</td>
<td><em>Fusarium keratoplasticum</em> f101</td>
<td>946</td>
<td>946</td>
<td>96%</td>
<td>0.0</td>
<td>95%</td>
</tr>
<tr>
<td>JF740923.1</td>
<td><em>Fusarium lacertarum</em> isolate 091029</td>
<td>946</td>
<td>946</td>
<td>96%</td>
<td>0.0</td>
<td>94%</td>
</tr>
<tr>
<td>GQ505464.1</td>
<td><em>Fusarium</em> NRRL45994 isolate FUS</td>
<td>946</td>
<td>946</td>
<td>96%</td>
<td>0.0</td>
<td>94%</td>
</tr>
<tr>
<td>KC808252.1</td>
<td><em>Fusarium petroliphilum</em> isolate Fs2</td>
<td>944</td>
<td>944</td>
<td>96%</td>
<td>0.0</td>
<td>94%</td>
</tr>
</tbody>
</table>
Table 2: Sequence producing significant alignments for the second Fusarium isolate

<table>
<thead>
<tr>
<th>Accession</th>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query coverage</th>
<th>E value</th>
<th>Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>AY928414.1</td>
<td>Fusarium oxysporum isolate</td>
<td>959</td>
<td>959</td>
<td>97%</td>
<td>0.0</td>
<td>99%</td>
</tr>
<tr>
<td>JN400705.1</td>
<td>Fusarium oxysporum f101</td>
<td>959</td>
<td>959</td>
<td>96%</td>
<td>0.0</td>
<td>98%</td>
</tr>
<tr>
<td>KC808261.1</td>
<td>Fusarium keratoplasticum f101</td>
<td>959</td>
<td>959</td>
<td>96%</td>
<td>0.0</td>
<td>97%</td>
</tr>
<tr>
<td>JF322999.1</td>
<td>Fusarium solani isolate Fs1</td>
<td>957</td>
<td>957</td>
<td>96%</td>
<td>0.0</td>
<td>95%</td>
</tr>
<tr>
<td>GQ505464.1</td>
<td>Fusarium NRRL45994 isolate FUS</td>
<td>957</td>
<td>957</td>
<td>96%</td>
<td>0.0</td>
<td>95%</td>
</tr>
<tr>
<td>JF740923.1</td>
<td>Fusarium lacertarum isolate 091029</td>
<td>957</td>
<td>957</td>
<td>95%</td>
<td>0.0</td>
<td>94%</td>
</tr>
<tr>
<td>KC808252.1</td>
<td>Fusarium petrophilum isolate Fs2</td>
<td>957</td>
<td>957</td>
<td>95%</td>
<td>0.0</td>
<td>94%</td>
</tr>
</tbody>
</table>

The 18S rRNA partial sequence of the F. solani is presented in Fig (1), whereas the 18S rDNA partial sequence of the F. oxysporum is presented in Fig (2). As a result, phylogenetic trees were mapped using the neighbour joining method, and are shown in Fig (3 and 4). From data in Table (1) and Fig (3), it can be noticed that the first Fusarium isolates was closely related to the Fusarium solani strain, while data in Table (2) and Fig (4), showed that the second Fusarium isolate is closely related to the Fusarium oxysporum. Data in Table (3) showed a comparison between the two identified isolates.

Figure (1): Partial sequence of 18S ribosomal RNA gene of the first Fusarium isolate

Figure (2): Partial sequence of 18S ribosomal RNA gene of the second Fusarium isolate
Table 3: Identification percentage between fungal isolates and related species

<table>
<thead>
<tr>
<th>Source of isolate</th>
<th>Fungal isolates</th>
<th>Identified isolates</th>
<th>Identity percentage (%)</th>
<th>Query coverage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-harvest maize</td>
<td>1st isolate</td>
<td>Fusarium solani KF572456.1</td>
<td>99</td>
<td>97</td>
</tr>
<tr>
<td>Pre-harvest maize</td>
<td>2nd isolate</td>
<td>Fusarium oxysporum AY928414.1</td>
<td>99</td>
<td>97</td>
</tr>
</tbody>
</table>

Antifungal activity

Data in Fig (5 and 6) revealed that rocket and star anise extracts showed high antifungal activity and completely inhibited *F. solani* at a concentration of 100 ppm, whereas *F. oxysporum* was completely inhibited at a lower concentration (50 ppm). Results also revealed that the fennel and rosemary extracts completely inhibited the growth of the two isolates under study at 400 and 500 ppm respectively. Data in Table (4) showed the minimum inhibitory concentration (MIC) and inhibitory concentration at 50% (IC50) of the plant extracts. Both rocket and star anise extracts were highly effective against *F. solani* and MIC was recorded as low as 88.00 ppm, while MIC of fennel and rosemary extracts was recorded at high concentrations, 341.76 and 442.61 ppm respectively.
Figure (6): Antifungal activity of some plant extract against *F. oxysporum*

Table 4: Inhibitory concentration at 50% and minimum inhibitory concentration of some plant extracts against *F. solani* and *F. oxysporum* growth

<table>
<thead>
<tr>
<th>Plant extract</th>
<th><em>Fusarium solani</em></th>
<th><em>Fusarium oxysporum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC50*</td>
<td>MIC*</td>
</tr>
<tr>
<td>Fennel</td>
<td>38.79</td>
<td>341.76</td>
</tr>
<tr>
<td>Rocket</td>
<td>29.73</td>
<td>88.00</td>
</tr>
<tr>
<td>Rosemary</td>
<td>221.40</td>
<td>442.61</td>
</tr>
<tr>
<td>Star anise</td>
<td>30.13</td>
<td>88.35</td>
</tr>
</tbody>
</table>

IC50: Inhibitory concentration at 50%
MIC: Minimum inhibitory concentration
*: ppm

Discussion

The importance of this investigation is to shed light on morphological and molecular techniques used to identify some *Fusarium* species isolated from pre-harvest maize, as well as evaluating the antifungal activity of some plant extracts to control the growth of the identified strains. In a recent study, Najim showed that *F. solani* produced deoxynivalenol, fumonisins, zearalenone, and T2 toxin. Moreover, Manisha reported that strains of *F. oxysporum* were able to produce several toxins including fusaric acid. *Fusarium solani*, as defined based on morphology, is actually a diverse complex of over 45 phylogenetic and/or biological species. These morphologically similar species are generally identified broadly under the name *F. solani*. The morphological concept of *F. solani* proposed by Nelson et al. and Snyder and Hansen is characterized by the production of slightly curved, usually three septate macroconidia with a blunt apical cell and foot-shaped basal cell, from usually cream-colored but sometimes green, blue or red sporodochia, abundant resistant chlamydospores, and production of one- or two-celled aerial conidia that vary in shape, from long monophialides that often have a distinct collarette. Concerning *F. oxysporum*, our results revealed that the above-mentioned morphological characters were considered as secondary criteria for the identification of *F. oxysporum*. Meanwhile, Booth considered the conidiogenous cell bearing microconidia as a primary taxonomic criterion and growth rate as a secondary criterion for the identification of *F. oxysporum*. The shape of macroconidia produced in sporodochia is one of the primary defining characteristics of *Fusarium* species and are preferred in identification purpose. The molecular characterization can also be a useful tool to phylogenetically relate the fungi on the basis of their characteristic morphological features. Our results indicated that molecular identification of the organisms exhibited high specificity and sensitivity and can be used for classifying microorganisms at taxonomical level.

The antifungal activity of the plant extracts under study to control the growth of *F. solani* and *F. oxysporum* was measured as the inhibition %. It was noticed that the extent of inhibition of fungal growth varied
depending on the concentrations of plant extract used. The results also revealed that the plant extracts can be divided into two groups according to their antifungal activity to high antifungal activity (rocket and star anise) and low antifungal activity (fennel and rosemary). The high antifungal activity of rocket extract was confirmed by Rani et al.\textsuperscript{42}. Similar results were reported by Sabry\textsuperscript{43} who found that rocket extract and essential oil had the most powerful effect on dry mycelium weight and/or aflatoxin production by \textit{A. flavus} even at a concentration as low as 50 ppm.

In the present study, the effectiveness of fennelethanolic extract against both \textit{Fusarium} species was recorded. Our results are in agreement with those reported by Thakur et al.\textsuperscript{44} who reported the antifungal activity of the alcoholic and the aqueous extracts of the \textit{F. vulgaris} seeds against several species of fungi. Results are also confirmed by Prabha et al.\textsuperscript{45} who reported the ability of \textit{F. vulgaris} extracts to inhibit the growth of \textit{Fusarium oxysporum}. On the other hand, several authors reported the efficacy of \textit{F. vulgaris} essential oil as antifungal agent\textsuperscript{26,46, 47}. Similar results were reported for star anise extract, which was found to be highly effective and caused 100% reduction of fungal growth. Yazdani et al.\textsuperscript{48} reported that star anise fruits extract inhibited the growth of \textit{A. niger}, one of the most important saprophytic fungi known to be associated with mycotoxin production in agricultural products and foods, at 16 mg/mL. In the same trend, Singh et al.\textsuperscript{49} showed that the star anise essential oil prevented the growth of \textit{Fusarium moniliforme} at 6 µl dose.

The antifungal activity of rosemary against both \textit{Fusarium} species is similar to results reported by Dellavalle et al.\textsuperscript{50} who found that rosemary exhibited antifungal effects against \textit{Alternaria} species even at low concentrations.

The antifungal activities of plant extracts are most likely due to the presence of chemical compounds with antifungal properties. Particularly worth noting is erucin, which accounted for approximately 78.69% of the rocket extract which play an important role as an antifungal agent\textsuperscript{43}. On the other hand, the antifungal activity of fennel and star anise extracts may be due to presence of anethole\textsuperscript{51, 52}. Moreover, De et al.\textsuperscript{53} indicated that anethole was effective against bacteria, yeast as well as fungal strains. In a recent study Aly et al.\textsuperscript{54} identified trans-anethole as the main component of star anise essential oil (83.32%). Concerning rosemary extract, Okamura et al.\textsuperscript{55} reported that the antifungal effects of \textit{Rosmarinus officinalis} oil can be attributed to the Monoterpens combination and in particular \textit{α}-pinene whose antifungal effects of this combination has been proved. Similar results were reported by Moghtader and Afzali\textsuperscript{56} who revealed that the antimicrobial impacts of rosemary essential oil can be related to the high percentage of \textit{α}-pinene, camphor, verbenone and 1, 8-cineole. On the other hand, the difference in the sensitivity of the \textit{Fusarium} isolates to the plant extracts might be due to the genetic differences between these isolates as shown in Figs (1, 2, 3 and 4).

\section*{Conclusion}

Molecular characterization showed significant promise in allowing precise and rapid identification of fungal species. Furthermore, complex studies (microbiological, biochemical and molecular) are essential, when the identification of new fungal isolates is the purpose of the investigation.

\section*{Acknowledgement}

This research was funded by the National Research Centre (NRC), Cairo, Egypt under grant No. S90404. The authors, therefore, would like to thank NRC for their financial support.

\section*{References}


*****