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Identification and Phylogenetic Analysis of Nine Plant Pathogenic Fungi by Application of ITS marker

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Fungi are a large and diverse group of organisms which possess great importance in agriculture and natural plant communities. Phytopathogenic fungi cause large number of plant diseases. Fungi are able to infect any tissue at any stage of plant growth and storage conditions. The main objective of this study was to investigate the taxonomic identities and phylogenetic relationships of devastated pathogenic fungi isolated from some infected crops, vegetables and fruits collected from Saudi Arabia, using molecular approach. Ten fungal isolates were selected for molecular phylogenetic analysis by PCR amplification of the ITS region using universal primers (ITS1 and ITS4). The amplicons were sequenced and aligned with ex-type strain sequences using BLAST and FASTA search tools. Sequence data revealed that we have nine fungal isolates. Phylogenetic analyses of the nine fungal isolates revealed that fungi were closely related to nine species: three *Fusarium* spp., *Rhizoctonia solani,* three *Aspergillus* spp. and two *Penicillium* spp. The species *P. chrysogenum*, *F. solani*, *R. solani*, *A. fumigatus* and *A. oryzae* exhibited 97, 96, 95, 95 and 88% similarities. These five species are suggested to be new type strains with potential novelty. Meanwhile, the overlap of species in phylogenetic trees for each one of the four genera suggested a common ancestor of each genus. Conclusively, analysis of ITS region is rapid and reliable technique for fungal taxonomy, but it is recommended to be assured by additional confirmatory tests to prove species novelty.

Keywords: DNA sequencing; Fungal Taxonomy; ITS locus; Fungal phylogeny.

INTRODUCTION

Fungi are considered the main disease-causing organisms of plants. Some fungi cause many changes during the host development even after harvest. Many fungal diseases of vegetables and fruits cause quality-related issues; such as organoleptic characteristics, dietary esteem, and short shelf life (Agrios, 2004). Up to 30% of stored grains is lost due to the microbial bio-deterioration phenomenon (Raja et al., 2017). Fungi, especially *Aspergillus*, *Fusarium* and *Penicillium*, destroy about 120 grains and food stuffs during storage, making them unfit for human use by reducing their nutritional value and/ or by producing mycotoxins (Ajayi-Oyetundea and Bradley, 2018; Geiser, 2009). More than 300 fungal metabolites were notified to be toxins of animals and human beings, as well. In addition, more than 25% of the world cereals were reported to be sullied with mycotoxins (Ali et al., 2016). Many harmful effects of fungal metabolites and mycotoxin have been demonstrated including genetic, nephrotic and hepatic toxicities, carcinogenicity, teratogenicity, immune suppression and reproductive disorders (Ali et al., 2016).

Precise recognition and early diagnosis of plant pathogens is one of the foremost critical approaches for controlling plant infections to design curative and/ or

preventive programs. Early diagnosis of infections in seeds, mature plants and propagative plant materials are very crucial to maintain a strategic distance from further dissemination of new pathogens (Goud et al., 2003). To make accurate and timely decision of disease control, we are in bad need to fast, sensitive and economically effective tools of diagnosis. Traditionally, morphological identification of plant infections was the most predominant methodology. Such techniques are frequently laborious, time-consuming, and entail broad awareness of classical taxonomy. Troubles in the *in vitro* cultivation of some species, and failure to precisely quantify the pathogen are additional limitations (Goud et al., 2003). On the other hand, advantages of genetic and molecular methods have been previously reported (minute quantity of a pathogen is required for detection and/ or identification) in the studies of pathogenic fungi (Edgar, 2018). These methods have provided new opportunities to investigate and understand many research subjects. For example, study of phylogenetic analysis, fungal biology, structure and dynamics of pathogen population and host-pathogen interactions have been achieved by optimizing techniques (Robbertse et al., 2017; Mayayo et al., 1999). Because there are many molecular markers, selecting the proper marker gene is the cornerstone of successful studies. Sequencing of internal transcribed spacers (ITS) and ribosomal DNA (rDNA) has been effectively applied to fungal taxonomic studies (Edgar, 2018; Badotti et al., 2017; Raja et al., 2017; Robbertse et al., 2017; Higgins et al., 2007; Crozier et al., 2006; Duong et al., 2006; Promputtha et al., 2005).

The main objectives of the current study were to investigate the biosignature of pathogenic fungi associated with many crop, vegetable and fruit diseases in Saudi markets and cultivations, using ITS gene as molecular marker.

MATERIALS AND METHODS

Sample collection and preparation of inocula

Based on the apparent symptoms, the infected plant parts were collected from the following plants: banana fruits, potato tubers and leaves, tomato fruits and leaves, corn silage and seeds, peanuts, wheat brans, orange fruits, and apple fruits. The pre and post-harvest infected plant materials were collected from Aljouf and Jazan regions, Saudi Arabia, between January and August, 2017. Table (1) provides detailed information about the fungal isolate, source plant, the infected plant part, date of collection and source of collection.

Apparently infected samples were cut with a sterilized knife from the advancing edges of the lesion. 85% ethanol was applied for 2 minutes to disinfect the cut portion of the lesion, then the cut portions were rinsed in three different changes of distilled water. Each cut part was homogenized in a sterilized test tube using a sterile glass rod.

Propagation of the fungal isolates

Freshly prepared Potato Dextrose Agar (PDA) media, containing 30 mg/l Chloramphenicol (to prevent the growth of bacteria), were inoculated with 0.1 ml aliquots of homogenate sample and incubated at 28 ˚C in dark.

Morphological separation of the fungal isolates

After 5 to 7 days, the fungal isolates were morphologically identified using lactophenol cotton blue stain method described by Fawole and Oso (1995). Features such as colony morphology and hyphal characteristics and asexual reproductive structure were observed and recorded. Isolates were transferred to PDA medium and maintained at 28 ˚C. After seven days, growth of fungal colonies on PDA was counted and recorded in a cfu/ml (colony forming unit per milliliter). Ten isolates were selected for molecular identification and species confirmation according to the infected part of the plant (Table 1).

Molecular identification and phylogeny

DNA extraction

Thirty sterilized conical flasks (250 ml) containing PD broth were seeded with the ten selected fungal isolates (three repeats for each isolate) and incubated without shaking at 28 ± 2 ˚C. After 5 to 7 days, approximately 100 mg of mycelial biomass were harvested (Lu et al., 2012). Genomic DNA of each isolate was extracted using Biospin Fungus Genomic DNA Extraction Kit (Bioer Technology Co., Ltd., Hangzhou, P. R. China), following the manufacturer's protocol. Purified DNAs were transferred into new tubes and stored at -20 ˚C until processing. Concentration of DNA was determined at OD_{260} and purity of DNA was assessed by measuring OD_{260}/OD_{280} and OD_{260}/OD_{230} ratios (White et al., 1990).

Table 1. A key-table showing the main information of the ten isolates of pathogenic fungi.

Isolate No.	Isolate genus	Source plant	Infected plant part	Date of collection	Location	Supplier
1	*Fusarium*	Banana	Leaves	Aug, 2017	Jazan	Private Farm
2	*Fusarium*	Potato	Tubers	Jan, 2017	Aljouf	A farmer AlSalam Co.
3	*Fusarium*	Tomato	Fruits	Feb, 2017	Aljouf	A farmer AlSalam Co.
4	*Fusarium*	Tomato	Leaves	Feb, 2017	Aljouf	A farmer Aljouf Co.
5	*Rhizoctonia*	Potato	Leaves	Jan, 2017	Aljouf	A farmer Aljouf Co.
6	*Aspergillus*	Corn	Silage	April, 2017	Aljouf	A farmer Aljouf Co.
7	*Aspergillus*	Peanut	Seeds	Aug, 2017	Aljouf	Market Shelf
8	*Aspergillus*	Wheat	Bran	April, 2017	Aljouf	A farmer Aljouf Co.
9	*Penicillium*	Orange	Fruit	June, 2017	Aljouf	Market Shelf
10	*Penicillium*	Apple	Fruit	July, 2017	Aljouf	Market Shelf

Table 2. Sequences of the primers used in amplification of ITS rDNA region of the selected 9 fungal isolates.

Primer name	Sequence 5'→3'	Expected product in bp	Reference
ITS1 (Forward)	TCTGTAGGTGAACCTGCGG	600	Shahid et al. (2013)
ITS4 (Reverse)	TCCTCCGCTTATTGATATGC		

Oligonucleotides (primers*)*

The ITS region of the rDNA repeat from the 3′ and 5' ends of the 28S gene was amplified using the two primers, ITS-1 and ITS-4 which were synthesized by MWG-Biotech, Germany on the basis of conserved regions of the eukaryotic rRNA gene (Aihua et al., 2013). Details of the primers are clarified in Table (2).

Amplification of DNA using ITS primers

Primers were designed to amplify 600 bp fragment of ITS gene. PCR master mix (2.5 µl PCR buffer, 1.5 mM $MgCl_2$, 200 µM dNTPs, 1 U *Taq* DNA polymerase (AmpliTaq, Perkin-Elmer), 8.5 µl distilled water/ reaction) for all reactions was prepared in biosafety cabinet. PCR tube contains 7.5 µl master mix, 4 µl of DNA (40 ng), 2.5 µl of 10 pmol forward primer, and 2.5 µl of 10 pmol reverse primer, to reach a 25 µl final volume. A no-template PCR tube was used as negative control. PCR amplification was carried out in a thermocycler ABI GeneAmp 9700 (Applied Biosystems, USA), which was programmed for one cycle at 94 ˚C for 5 min (initial denaturation), followed by 40 cycles of denaturation for 1 min at 94 ˚C, annealing for 1 min at 55 ˚C, extension for 1 min at 72 ˚C, and a final extension for 10 min at 72 ˚C. PCR products were displayed on 1.5% agarose gel and photographed utilizing gel documentation system. Molecular size of PCR products was determined in relation to 1 Kb ladder (MBI, Fermentas, USA) as a marker. On a UV-transilluminator, the specified bands were cut from the gel with least amount of gel fraction. DNAs were eluted and purified using QIAGEN gel extraction kit (QIAGEN, GmbH, Germany) as described in the manufacturer's manual.

DNA sequencing of fungal ITS rDNA gene

For each fungal isolate, two positive PCR products were sent for sequencing (to exclude PCR errors certainly) using the specific ITS-1 (forward) and ITS-4 (reverse) primers (Table 2). Sequencing was accomplished using ABI PRISMTM 3100 DNA sequencer (Applied Biosystems) and Big Dye terminator sequencing kit (Version 3.1, Applied Biosystems, USA).

Sequence analysis and phylogeny

The consensus sequences of 10 fungal isolates obtained from both ITS1 and ITS4 primers were first edited and subjected to BLAST search to assign putative identity with similar sequences using database of NCBI http://blast.ncbi.nlm.nih.gov/Blast.cgi. Each fungal isolate was then designed to its operational taxonomic unit (OTU) based on measures of sequence similarities, and inferences of phylogenetic trees. Sequences were then aligned with other similar sequences downloaded from GenBank using ClustalX (Thompson et al., 2002), BioEdit (Hall, 1999) and Molecular Evolutionary Genetics Analysis (MEGA) software ver. 6.0 (Tamura et al., 2011). Alignments were manually edited where necessary. All nucleotide sequences of the pathogenic fungi have been submitted to GenBank for accession numbers.

Sequences obtained were split into different datasets in order to assess phylogenetic relationships at the familial and species level. Phylogenetic trees were constructed using neighbor Joining (NJ) method and molecular evolutionary analyses were conducted using MEGA software, ver. 6.0 (Tamura et al., 2011).

RESULTS

Preliminary morphological investigation of the different tissues of infected plant materials (banana, orange, apple, wheat, corn, tomato, potato and peanuts) has led to isolation of ten isolates of pathogenic fungi. Molecular analyses were carried out using ITS-rDNA sequences to confirm the identification of nine different fungal species from their hosts (isolates number 3 and 4 were identified as the same species).

PCR identification

In the present study, the ITS region of ribosomal genes was used to synthesize specific primer set; ITS1 and ITS4. They were successfully employed in PCR to produce positive amplicons with approximate molecular sizes of 600 bp (Fig. 1).

Sequence alignment

Firstly, sequences of two positive PCR products of each isolate were aligned to ensure the correctness of the sequence. The intraspecific identity of the ten sequences was examined to ensure the number of different sequences. Sequence analyses of the ITS region of the ten isolates revealed that nine different pathogenic fungi were identified (3 *Fusarium* spp., 3 *Aspergillus* spp., 2 *Penicillium* spp. and one *Rhizoctonia* sp.). Based on sequence similarity of ≥98 %, the isolates number 3 and 4 were identified as the same fungal species (*Fusarium brachygibbosum*). Although the ITS1 and ITS4 primers amplified bands of approximately 600 bp in relation to marker size, the obtained readable sequences were between 323 and 541 bp in size (Table 3). The nine fungal species were separately subjected to BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi?) and FASTA (https://www.genome.jp/tools/fasta/) searches for comparisons with potentially related sequences. The nine sequences have displayed significant alignments (88–99%) with other related sequences in the NCBI GenBank database. Satisfactory percentage of similarity with the ex-type strains were observed, too. The results of sequence alignment analyses are summarized in Table (3). Briefly, analyses of the nine different ITS sequences of fungal isolates revealed that studied isolates belong to three fungal families (Nectriaceae, Ceratobasidiaceae and Trichocomaceae) and four genera (*Fusarium, Rhizoctonia, Aspergillus* and *Penicillium*). Out of the nine fungal species, five species created less than 98% similarity with their corresponding species. 97, 96, 95, 95 and 88% similarities were exhibited by *Penicillium chrysogenum*, *Fusarium solani*, *Rhizoctonia solani*, *Aspergillus fumigatus* and *Aspergillus oryzae*, respectively (Table 3).

Molecular phylogeny

Due to the huge number of sequences, phylogenetic trees were generated using MEGA 6.0 software (Figs. 2-5). A separate phylogenetic tree was constructed for each genus. The most similar sequences were utilized to build a phylogenetic tree of the specified genus.

The *Fusarium* tree was constructed by our three species and other 6 *Fusarium* species (Fig. 2). Tree's topology displayed that the nine sequences are divided into two major lineages. Lineage I includes two divisions. Division I subdivides into two subdivisions. Subdivision I comprises two phylogenetic sister clades and Subdivision II comprises monophyletic sister clade. Meanwhile, division II subdivides into two subdivisions. Each of which contains one species in a separate sister clade. However lineage II divides into two divisions. Each division subdivides into two subdivisions. Each subdivision comprises monophyletic sister clade (Fig. 2).

Table 3. A summary showing the size of readable sequences, GenBank accession numbers, the highest percentage identity, and summarized information about the identical species published in GenBank.

Identified as	Product size in bp	GenBank Acc#	Highest % Identity	Acc# of the identical species
Fusarium oxysporum* (1)	477	KJ831189	99	LT746252, 463/468, coverage 97%, 3 gaps, 2 subs
***Fusarium solani* (2)**	389	KJ831188	96	KM229706, 379/393, coverage 100%, 4 gaps, 10 subs
***Fusarium brachygibbosum* (3 & 4)**	376	KJ831190	99	MH517365, 331/336, coverage 88, 3 gaps, 2 subs
***Rhizoctonia solani* (5)**	541	KJ831186	95	KU360151, 519/549, coverage 96, 29 gaps
***Aspergillus fumigatus* (6)**	459	KJ831194	95	KU882055, 427/448, coverage 95, 10 gaps
***Aspergillus flavus* (7)**	530	KJ831193	99	KC907367, 526/528, coverage 99, 1 gap
***Aspergillus oryzae* (8)**	491	KJ831192	88	MH191254, 426/486, coverage 95, 16 gaps
***Penicillium chrysogenum* (9)**	455	KJ831195	97	MH517445, 385/398, coverage 86, 5 gaps
***Penicillium commune* (10)**	323	KJ831196	99	KM063203, 315/319, coverage 98, 2 gaps

*: Number of the isolate based on Table (1).

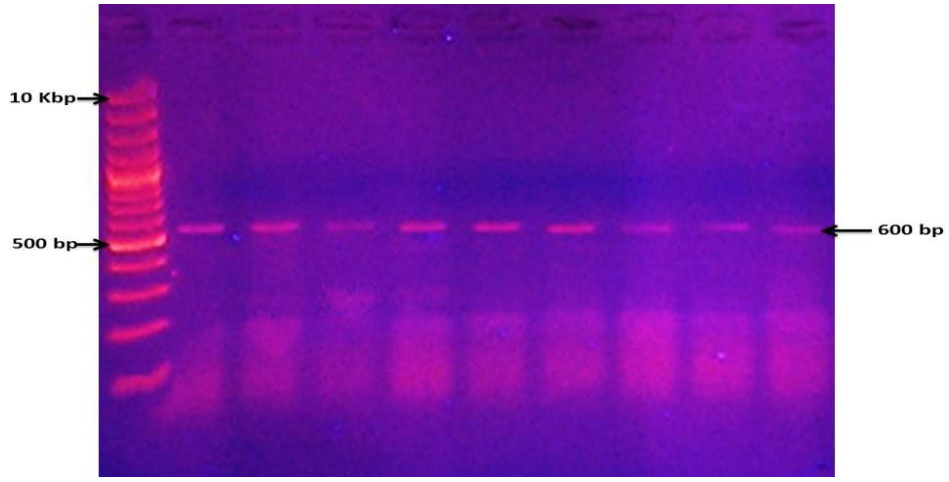

Figure 1. PCR amplification of ITS region of nine fungal isolates: Lane M, 10 kb DNA marker & lanes 1-9 represent the nine fungal isolates.

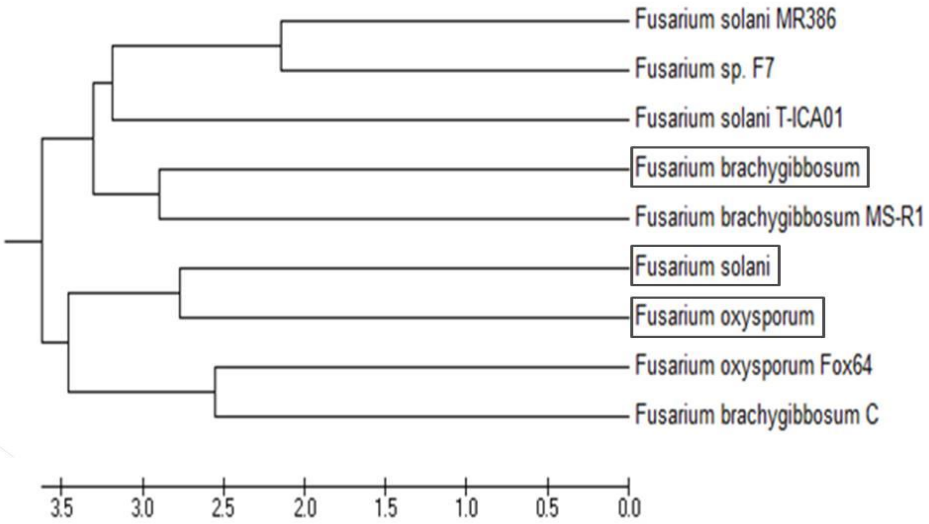

Figure 2. Phylogram based on the ITS- rDNA region of three *Fusarium* isolates and their phylogenetic analysis conducted using the UPGMA method with other ex-type isolates of *Fusarium* spp. by MEGA 6.0 software.

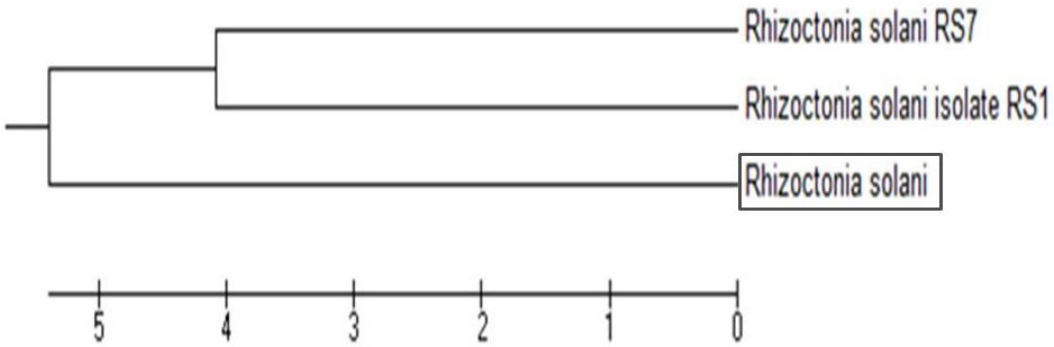

Figure 3. Phylogram based on the ITS- rDNA region of one *Rhizoctonia* isolate and their phylogenetic analysis conducted using the UPGMA method with other ex-type isolates of *R. solani* by MEGA 6.0 software.

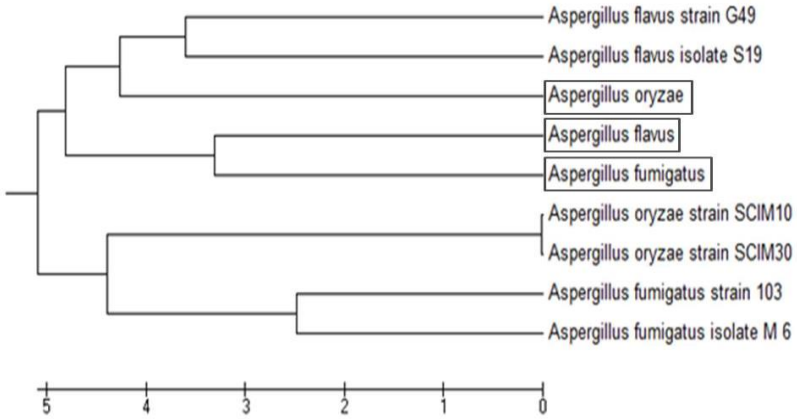

Figure 4. Phylogram based on the ITS- rDNA region of three *Aspergillus* isolates and their phylogenetic analysis conducted using the UPGMA method with other ex-type isolates of *Aspergillus* spp. by MEGA 6.0 software

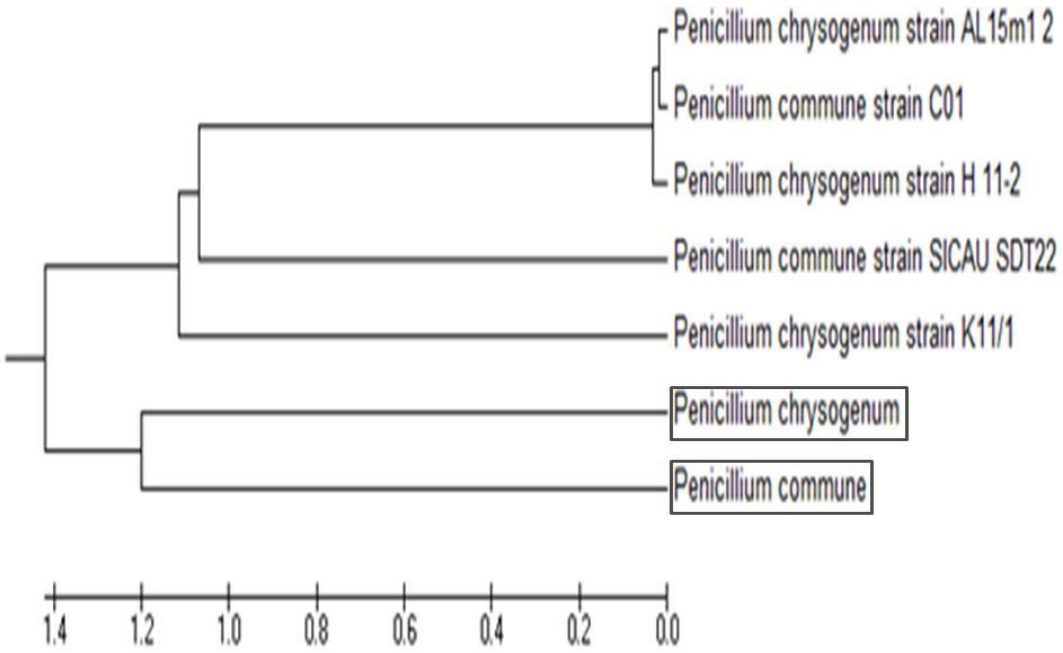

Figure 5. Phylogram based on the ITS- rDNA region of two *Penicillium* isolates and their phylogenetic analysis conducted using the UPGMA method with other ex-type isolates of *Penicillium* spp. by MEGA 6.0 software.

The overlap of species in *Fusarium* evolutionary tree is an evidence of the decline of these species from one common ancestor.

The *Rhizoctonia* tree was constructed by our species and other two *Rhizoctonia* species (Fig. 3). The topology of the tree separated our *Rhizoctonia solani* in a monophyletic sister clade and grouped the other two isolates in the same division (Fig. 3).

The *Aspergillus* tree was constructed by our three species and other 6 *Aspergillus* species (Fig. 4). The tree's topology showed that the nine sequences are divided into two lineages. Lineage I displays maximum divergence, dividing into two divisions, four subdivisions and ending by five sister clades. Meanwhile, lineage II divides into two divisions, four subdivisions and ends by four sister clades (Fig. 4). The overlap of species in *Aspergillus* phylogenetic tree is a clue on descending of these species from a common ancestor.

The *Penicillium* tree was constructed by our two species and other 5 *Penicillium* species (Fig. 5). Topology of the tree indicated that the seven sequences divide into 2 major lineages. Lineage I divides into two divisions. Division I subdivides into two subdivisions, subdivision I divides further into two groups and group I ends into two sister clades. All other divisions and subdivisions of the tree end directly into monophyletic sister clades (Fig. 5). The overlap of species was observed in *Penicillium* phylogenetic tree. This overlap of *Penicillium* species in phylogenetic tree is a sign on descending of these species from a common ancestor (Fig. 5).

DISCUSSION

Classification of fungi is crucial for further physiological, biochemical and economical studies. Therefore, the main objective of the present study was to classify fungi isolated from cultivated and shelf-preserved plant materials using molecular technologies.

Traditional, microscopic and cultural techniques are often available for detecting and enumerating fungal spores. Endophytic fungi are assessed by rolling the surface-sterilized plant tissue samples, and subsequently isolation of fungal endophytes which grow in samples place onto culture media (Devarajan et al., 2006; Bills et al., 1996). However, the methods are time-consuming, laborious and require skilled and highly specialized expertise. Furthermore, microscopy is often fallible for identification of

non-descript spores and cultural techniques are inappropriate for detection of slow growing or non-culturable fungi *in vitro* (Lacap et al., 2003).

In contrast, molecular techniques are sensitive and specific for identifying microorganisms. Molecular markers were used for classification and identification of microbial strains (Sette et al., 2006). Among molecular markers, ITS locus is the foremost commonly utilized fungal barcode for species identification. ITS locus has been effectively used to recognize around 70% of fungi (Badotti et al., 2017; Raja et al., 2017; Schoch et al., 2012). In this study, nine fungal species were identified using sequencing and molecular phylogeny of ITS region of the nuclear encoded rDNA. Two tools of multiple alignment search (BLAST and FASTA) were used. The readable sequences showed significant alignments of 88–99% with related sequences in GenBank. Five of our sequences created identities less than 98%. In addition, phylogenetic trees clarified overlap of species and segregation of some species in monophyletic sister clades. The general rule of thumb stated that <97 % sequence identity with closest relative species is an indication of novel species status (Rossello´-Mora and Kampfer, 2004). Based on this rule, four new species were identified in the present study. The closest relatives to the new species are *F. solani*, *R. solani*, *A. fumigatus* and *A. oryzae*. Recently, the identification of fungi by molecular methods was reviewed (Raja et al., 2017). They recommended that, whenever conceivable, both morphology and molecular information be utilized for fungal species identification. An updating of the 97% identity threshold was recently reassessed (Edgar, 2018; 2018a; 2018b). He quantified the relationship between taxonomy and sequence identity. He found that the accuracy of prediction were rapidly reduced to 50% whenever identity became 97%. However, the accuracy becomes twilight at 95% identity (Edgar, 2018). In another study, he clarified that the optimal identity thresholds for full-length ITS sequences were ≈99% for species and ≈90% for genus. For partial sequences of ITS, the optimal identity thresholds are ≈100%. Conclusively, sequencing of two or more hypervariable loci instead of one was recommend (Edgar, 2018a). A 98.65% sequence similarity was suggested as threshold for differentiating two species of bacteria using full-length 16S rRNA gene (Kim et al., 2014). Using the RefSeq ITS database of *Trichoderma* as a case study, Robbertse et al. (2017) recommended the necessity of pairing type material information with up to date taxonomic data for modern fungal taxonomy. In addition, is essential to have more than one reliable marker together with the whole genome analyses to correctly fall a species in its taxonomical unit. Pearson (2013) pointed out that BLAST, FASTA and other tools of similarity search are reliable for inferring homology. Translated DNA and protein sequences were recommended in comparison of sequences, as their better sensitivity than DNA: DNA comparison. Furthermore, E-values and bit scores (bits >50) were preferred than similarity percent for inferring homology (Pearson, 2013).

Fusarium is considered one of the most dangerous fungi as it causes diseases of plant, animal and human worldwide (Abd-Elsalam, 2009). Many *Fusarium* spp. have been isolated in Saudi Arabia: *F. thapsinum* from imported green banana fruit (Abd-Elsalam, 2009), *Fusarium* sp. from roots of *Mentha longifolia* (Ibrahim et al., 2017), many *Fusarium* spp. including *F. oxysporum*, *F. solani* and *F. brachygibbosum* from palm trees (Saleh et al., 2016), *F. oxysporum* and *F. solani* from banana fruits (Alghuthaymi and Bahkali, 2015), *Fusarium* sp. from corn and sorghum (Mahmoud et al., 2013), *F. proliferatum* from roots and leaves of date palm (Abdalla et al., 2000), *F. oxysporum* and *F. solani* from imported wheat, barely, sesame, sorghum, soy and corn (Abd-Elsalam et al., 2016), *F. oxysporum* from cultivated beans (Alwathnani et al., 2012), *F. oxysporum* from tomato, green bean and squash (Nawar, 2015), *F. oxysporum* from polluted soil (Moustafa, 2016), *Fusarium* sp. from blemish of potato tubers (Gashgari and Gherbawy, 2013), *F. oxysporum* from wheat grains (Mahmoud and Shehata, 2017), *F. oxysporum* from shelf-market honey (Nasser, 2004), *F. oxysporum* and *F. solani* from barley, wheat, sorghum and maize (Abdel-Hafez, 1984), *F. oxysporum* from apricot (Khallaf et al., 2017), *F. oxysporum* and *F. solani* from wheat grains (Al-Wadai et al., 2013), *F. oxysporum* from soil (Saadabi, 2006), *F. oxysporum* and *F. solani* from desert soil (Abdel-Hafez, 1982), *Fusarium* sp. from roots of vegetables (Manzelat, 2017), *Fusarium* spp. from air (Vijayakumar et al., 2017), *Fusarium* spp. from food of marine culture (Salama, 2007), *F. solani* from hot desert sands (Murgia et al., 2018), *F. oxysporum* from sands of west coastal region (Moussa et al., 2017), *F. oxysporum* from common spices (Hashem and Alamri, 2010), *F. oxysporum* and *F. solani* from 12 varieties of date palm (Al Hazzani et al., 2014), *F. oxysporum* and

F. solani from squash (Nawar, 2007), *F. oxysporum* from grains (Hamed et al., 2016), *F. oxysporum* from grape and banana (Al-Hindi et al., 2011), *F. oxysporum* from cultivated maize (Dawood and Elshamry, 2015), *F. solani* from watermelon (Mokbel and Alharbi, 2017) and *F. oxysporum* from stored nuts (Nawar, 2008).

Rhizoctonia solani is the foremost critical species within the genus *Rhizoctonia.* It is soil-borne fungus causing serious diseases to many economic plants worldwide (Ajayi-Oyetundea and Bradley, 2018). Isolation of *Rhizoctonia* spp. in Saudi Arabia was previously documented: *R. solani* from green beans (Al-Hazmi and Al-Nadary, 2015), *R. solani* from potato tubers (Alghuthaymi, 2018; Rabbani et al., 2013; Abd-Elsalam et al., 2009), *R. solani* from watermelon (Mokbel and Alharbi, 2017), *R. solani* from tomato, green bean and squash (Nawar, 2015; 2007) and *Rhizoctonia* spp. from blemish of potato tubers (Gashgari and Gherbawy, 2013).

The genus *Aspergillus* consists of hundreds of species. Members of this genus are able to grow in various environments worldwide (Geiser, 2009). Many serious diseases are caused by *Aspergillus* species (*e.g. A. fumigatus* and *A. flavus* produce aflatoxins). Meanwhile, other species (*A. niger* and *A. oryzae*) play important roles in environment, biotechnology and industry (Geiser, 2009). Many members of the genus *Aspergillus* have been isolated in Saudi Arabia: *A. fumigatus* and *A. flavus* from shelf-market honey (Nasser, 2004), from green coffee beans (Bokhari, 2007), from air and human sputum (Alqurashi, 2009), from soil (Saadabi, 2006), from desert soil (Abdel-Hafez, 1982), from 12 varieties of date palm (Al Hazzani et al., 2014), from wheat (Al-Hazmi, 2011), from hairs of sheep (Nasser and Abdel-Sater, 1997), from coastal region of red sea (Alwakeel, 2017), from food of marine culture (Salama, 2007), from common spices (Hashem and Alamri, 2010), from air (Vijayakumar et al., 2017), *Aspergillus* spp. from roots of vegetables (Manzelat, 2017), from sand of west coastal region (Moussa et al., 2017), from corn and sorghum (Mahmoud et al., 2013), from tomato, green bean and squash (Nawar, 2015), *A. flavus* from barley, maize, sorghum and wheat (Abdel-Hafez, 1984), from plum, cherry, apricot and peach (Khallaf et al., 2017), from wheat grains (Al-Wadai et al., 2013), from petroleum-polluted soil (Bokhary and Parvez, 1993), from grains (Hamed et al., 2016), from mango (Al-Hindi et al., 2011), from cultivated maize (Dawood and Elshamry, 2015), from stored nuts (Nawar, 2008), from currency papers and cell phones (Alwakeel and Nasser, 2011), *A. oryzae* from market apple samples (Alwakeel, 2013), from orange (Al-Hindi et al., 2011), from air and human sputum (Alqurashi, 2009), *A. niger* from soil (Alwathnani et al., 2012), *A. flavus* and *A. niger* from market wheat grains (Mahmoud and Shehata, 2017) and from polluted soil (Moustafa, 2016).

The genus *Penicillium* contains over 300 species (Kirk et al., 2008). These species are important from the environmental and economical point of view, as well. The importance and utilization of *Penicillium* spp. has been extensively reviewed (Ali et al., 2016). Some species of *Penicillium* have been previously isolated from Saudi Arabia: *Penicillium* spp. from corn and sorghum (Mahmoud et al., 2013), from tomato, green bean and squash (Nawar, 2015), from market wheat grains (Mahmoud and Shehata, 2017), from roots of vegetables (Manzelat, 2017), from air (Vijayakumar et al., 2017), from food of marine culture (Salama, 2007), from currency papers and cell phones (Alwakeel and Nasser, 2011), from blemish of potato tubers (Gashgari and Gherbawy, 2013), *P. chrysogenum* from market green coffee (Bokhari, 2007), from desert soil and from barley, wheat, sorghum and maize (Abdel-Hafez, 1984; 1982), from air and human sputum (Alqurashi, 2009), from soil (Saadabi, 2006), from wheat (Al-Hazmi, 2011), from petroleum-polluted soil (Bokhary and Parvez, 1993), from hairs of sheep (Nasser and Abdel-Sater, 1997), from coastal region of red sea (Alwakeel, 2017), from market apple samples (Alwakeel, 2013), from polluted soil (Moustafa, 2016) and *P. citrinum* from soil (Alwathnani et al., 2012).

CONCLUSION

Conclusively, the present study recoded the isolation and identification of nine fungal species from Saudi Arabia. Eight species were previously isolated in Saudi environment. This study reported *P. commune* as first record on apple fruit collected from a market in Saudi Arabia, Aljouf. Accounting on sequence data of ITS using two reliable identity searching tools (BLAST and FASTA), phylogenetic analyses, the rule of thumb and all recent revisions, four type strains (*F. solani, R. solani, A. fumigatus, P. chrysogenum*) and one new type species (*A. oryzae*) are reported herein and should be re-examined for novelty confirmation. In future studies, it is highly recommended to enhance isolation of new fungal species from Saudi Arabia where large areas, multi-environments and wide variation of climates

exist.

CONFLICT OF INTEREST

The author declares no conflict of interest. All the experiments undertaken in this study comply with the current laws of the country where they were performed.

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AUTHOR CONTRIBUTIONS

SE designed and performed the experiments and also wrote the manuscript. She read and approved the final version of the manuscript.

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