Journal of Plant Protection Research ISSN 1427-4345

ORIGINAL ARTICLE

New fungi causing postharvest spoilage of cucumber fruits and their molecular characterization in Egypt

El Sayed Hussein Ziedan[1]*, Abd El-Nasser Abd El-Hafez Khattab[2], Ahmed Farahat Sahab[1]

[1] Plant Pathology Department, National Research Centre, Cairo, Egypt

[2] Genetics and Cytology Department, National Research Centre, Cairo, Egypt

Vol. 58, No. 4: 362–371, 2018

DOI: 10.24425/jppr.2018.124650

Received: November 15, 2017
Accepted: November 26, 2018

*Corresponding address:
ziedanehe@yahoo.com

Abstract

This work was carried out during two successive seasons (2016 and 2017) on cucumber fruits from a plastic greenhouse and from open field cultivation in El Gharbeia and El Giza Governorates, Egypt. Isolation trials from spoilage fruit samples of plastic greenhouse cultivation recorded high frequency of *Alternaria tenusinium*, *Fusarium* spp. and *Pleospora alli*. The most common fungi of rotten cucumber fruits from an open field were *Galactomyces* spp. and *Fusarium* spp. Pathogenicity tests proved that, *Fusarium solani* from El-Gharbeia followed by *A. tenusinium* from El-Giza were the most frequent isolates responsible for rot of cucumber fruits from plastic greenhouse cultivation. Moreover, the most frequent isolates causing postharvest disease of cucumber fruits of the open field were *Galactomyces candidium* from El-Giza followed by *Geotrichum* sp. and *F. fujikuroi* from El-Gharbeia Governorates, respectively. This is the first report of several fungi causing postharvest fruit rot disease of cucumber i.e., *G. candidium, Geotrichum* sp., *A. tenusinium*, *P. alli* and *Fusarium* spp. (*F. fujikuroi*, *F. verticiolides, F. solani, F. geraminearium* and *Fusarium incarnatum*). Fungal isolates were identified according to cultural, morphological and molecular characterization based on sequencing of internal transcribed spacer1 (ITS1). All the ITS nucleotide sequences of fungi were applied and conserved in GenBank.

Keywords: cucumber, fruit rot, fungi, internal transcribed spacer (ITS), molecular characterization

Introduction

Cucumber (*Cucumis sativus* L.) is one of the most important economic vegetable crops all over the world. It is cultivated in open fields and protected houses in Egypt for both local consumption and export. The occurrence of fungal spoilage of fruits is recognized as a potential health hazard to man due to their production of mycotoxins (Effiuvwevwere 2000). Cucumber plants are subject to attack by several fungal diseases that affect the yield quantity and quality including *Alternaria tenuis, A. alternata, Botrytis cinerea, Choanephora cucurbitarum, Didymella bryoniae, Fusarium oxysporum, Geotrichum candidum, Penicillium oxalicum, Phytophthora capsici, Rhizopus nigricans* and *Sphaerotheca fuliginea* (Blancard *et al*. 2005; Farrag *et al.* 2007; Sani *et al*. 2015; Ziedan and Saad 2016). Fruit rot pre- and postharvest caused by *B. cinerea* (An and Ma 2005–2006; Soliman *et al*. 2015), *Geotrichum candidum* was reported as a fruit rot causal pathogen on carrot, cucumber, tomato and pumpkin in South Korea (Kim *et al*. 2011), *Monilinia* spp. on peach, pear and apple fruits (Di Francesco *et al*. 2015) and *Galactomyces reessii* on tomato fruits (Suwannarach *et al.* 2016). Recently, *G. candidum* was reported on peach fruit (Alam *et al*. 2017). Molecular biology has offered a number of insights into the detection and enumeration of fungal pathogens and information on identifying unknown species from their DNA sequences. A rapid assay and accurate identification of fungal pathogens

can be important for initiating treatment in the earliest stages of infection and for guiding antifungal therapy (Khot *et al.* 2009). The interest in ribosomal genes for species identification comes from the concerted fashion in which they evolve, showing a low intraspecific polymorphism and a high interspecific variability (Li 1997). Previous results have demonstrated that the internal transcribed spacer (ITS) of the complex regions (non-coding and variable) and the 5.8S rDNA gene (coding and conserved) are useful in measuring close fungus phylogenetic relationships, since they exhibit far greater interspecific differences than the 18S and 26S rDNA genes (Kurtzman 1992; Cai *et al.* 1996; James *et al.* 1996). The ITS has been used in numerous systematic studies at genus and species levels of a wide array of plant taxa (Sang *et al.* 1995; Alice and Campbell 1999). ITS-1 and ITS-2 are two internal spacers which are located between genes encoding the 18, 5.8 and 28S nuclear ribosomal RNA (nrRNA) subunits. In addition, the 5.8S nrRNA are referred to as nrDNA ITS region (Baldwin 1992). This investigation was aimed at surveying the incidence of postharvest diseases on cucumber fruits and identification of fungal isolates by molecular methods based on sequencing of ITS1 and 5.8S rDNA regions.

Materials and Methods

Disease survey

A survey of postharvest fruit rot diseases of cucumber in El-Giza and El-Gharbeia governorates, Egypt was performed during the winters of 2016 and 2017. Disease incidence and their severity were determined using the following formula:

$$\text{Disease incidence} = \frac{\text{No. of infected plants}}{\text{Total no. of plants assessed}} \times 100\ [\%].$$

Disease severity was assessed as a percentage of rotten tissue of cucumber fruits using a linear scale from 0 to 4 according to Cohen *et al.* (1991) as follows:

0 = healthy fruits;
1 = 1–25% soft rot of fruit;
2 = 6–50% soft rot of fruit;
3 = 51–75% soft rot of fruit;
4 = 76–100% soft rot of fruit.

Isolation of fungi associated with postharvest fruit rot

Samples of rotten fruit tissue were washed thoroughly with tap water, then cut into small pieces and rinsed with 5% chlorox (sodium hypochlorite) for 3 min. They were then cultured on potato dextrose agar (PDA) medium. Five pieces of rotten tissue were placed in individual Petri dishes. Plates were incubated at 25 ± 2°C for 5 days and subsequently colonies were counted. Frequency occurrence of isolated fungi was recorded using the following formula:

$$\text{Fungal frequency} = \frac{\text{No. of fungal genera of each location}}{\text{Total fungal colonies of each location}} \times 100\ [\%].$$

Fungal identification

Different fungal colonies were isolated and purified using single spore and hyphal tip methods and identified according to their cultural and morphological characteristics (Booth 1971; Ellis 1971; Nelson *et al.* 1983; Barnett and Hunter 1998).

Pathogenicity test

The pathogenicity of the isolated fungi was tested by artificially infecting sterilized cucumber fruits from an open field (cv. Beta alfa) and a greenhouse (cv. Golden) by sodium hypochlorite (1%) for 1 min then dried under sterilized conditions. Ten fruits were sprayed with spore suspensions 1×10^4 spore · ml^{-1} of each fungal isolate tested. Cucumber fruits were incubated at 25 ± 2°C for 20 days. Percentage of diseased fruits and disease severity were determined 20 days after infestation by each fungal isolate as previously mentioned.

Molecular identification

DNA extraction

Genomic DNA was extracted from pure cultures of fungal strains isolated from rotten cucumber fruit samples from open field and plastic greenhouse cultivation grown on PDA using i-genomic BYF DNA extraction Mini Kit (iNtRON Biotechnology Inc., South Korea) following the manufacturer´s instructions (Sambrook *et al.* 1989).

PCR partial amplification and sequencing of 18S r DNA

Identification of the fungal isolates was based on molecular genetic analysis using the initials ITS. Partial sequences of the isolates18S rDNA were obtained using a strategy based on Boekhout *et al.* (1994). A divergent domain of the gene was amplified using three different primers:

– the first primer (ITS1) sequence: 5'-TCCGTAGG TGAACCTGCGG-3';

- the second primer (ITS2) sequence: 5'-GCT GCGTTCTTCATCGATGC-3';
- the third primer (ITS4) sequence: 5'-TCCTC CGCTTATTGATATGC-3'.

All primers were supplied by Operon Technologies Company, Netherlands. To each polymerase chain reaction (PCR) bead, 12 ng of the used primer and 40 ng of the purified DNA sample were added. The total volume of the amplification reaction was completed to 25 µl using sterile distilled water. The amplification protocol was carried out as follows: denaturation at 95°C for 5 min (each of the 35 cycles consisted of the following segments: denaturation at 95°C for 1 min; primer annealing at 55°C for 2 min and incubation at 72°C for 2 min for DNA polymerization). Finally, the PCR was kept at 4°C till analysis. The amplified DNA products were electrophorated on 1.0% agarose gel and 1X TBE (Tris-borate-EDTA) buffer at a constant 100 V for about 2 h. The different band sizes were determined against 100 bp ladder (Vivantis # NL 1407-Malaysia) and the separated bands were stained with 0.5 $\mu g \cdot ml^{-1}$ ethidium bromide and photographed using the Gel Documentation System with UV Transeliminator.

Fungal DNA purification

The PCR product was cleaned up using GeneJET™ PCR Purification Kit (Thermo K0701).

Identification of isolates

The DNA sequencing of the purified PCR products was done with ABI 3730xl DNA sequencer (GATC Company, Germany) by using forward primer.

Phylogenetic analysis

The DNA sequences of the fungal isolates were compared with the sequences available by the Basic Local Alignment Search Tool (BLAST) in the NCBI, GenBank database (http://www.ncbi.nlm.nih.gov). The sequences were aligned together with those of reference taxa retrieved from public databases. The evolutionary distances were generated based on parameter model (Jukes and Cantor 1969) and phylogenetic trees were constructed by using the neighbor-joining method (Saitou and Nei 1987).

Data analysis

The ITS nucleotide sequences for each isolate were then compared to those in the public domain databases NCBI (National Center for Biotechnology Information; www.ncbi.nih.gov) using the Basic Local Alignment Search Tool for Nucleotide Sequences (BLASTN). Alignment of ITS DNA sequences was done using Clustal_W program [30]. A phylogenetic tree was created using CLC Sequence Viewer Version 6.3 based on UPGMA (unweighted pair-group method for arithmetic analysis). The confidence of the branching was estimated by bootstrap analysis.

Statistical analysis

The obtained data were statistically analyzed according to Snedecor and Cochran (1980). Means were compared by using the LSD test at 0.05 level.

Results and Discussion

Incidence and severity of cucumber fruit rot

Different colonies were observed at the end of the procedure necessary for the isolation and identification of fungi associated with cucumber fruit rot. The fungal colonies spoiled the cucumber fruits, causing their deterioration. Mixed colonies were obtained when the fungi were first isolated on PDA medium. Pure cultures of the spoilage fungi were observed afterwards when each colony of the fungi was subcultured on freshly prepared medium. Data in Table 1 and Figure 1 indicated that postharvest fruit rot of cucumber was observed in open field and greenhouse cultivations during storage. The percentage of fruit rot of open field cultivation was less than fruits from greenhouse cultivation. Severity of fruit rot, represented as the percent of symptomatic cucumber fruits of the total fruit number, varied from 1.0 to 3.6%. Data also showed that a high percentage of cucumber fruits was recorded in El-Dokki followed by El-Gharbeia. Also, syndromes on cucumber fruits from an open field differed from greenhouse cultivation (Fig. 1).

Table 1. Mean fruit rot incidence and disease severity of cucumber from open field and greenhouse cultivation

Cultivation	Location	Fruit rot incidence of cucumber	
		%	disease severity
Open field	El Gharbeia	5.0 c	1.0 d
	El-Dokki	7.0 c	1.5 c
Greenhouse	El Gharbeia	60.0 b	2.8 b
	El-Dokki	100.0 a	3.6 a

Values followed by the same letter are not significantly different at $p \leq 0.05$ according to Duncan's multiple range

Fig. 1. Cucumber fruits from open field cultivation. Healthy (A) and rotten fruit (B) as well as rotten cucumber fruits from plastic greenhouse cultivation (C)

Pathogens associated with cucumber fruit rot

The frequency of occurrence of fungal isolates associated with the spoilage of cucumber fruits is shown in Table 2. A total of six fungal genera were obtained from spoiled cucumber fruits. The fungal genera were identified as *Fusarium, Galactomyces, Mucor, Aspergillus, Alternaria, Pleospora,* of which *Fusarium* spp. and *Galactomyces* spp. were the most common fungi associated with cucumber fruits from El-Gharbeia open field cultivation, 50 and 25%, respectively. This was followed by *Mucor* spp. and *Aspergillus niger* as saprophytic fungi. *Galactomyces* spp. (40%) and *Mucor* spp. (30%) were recorded in El-Giza Governorates. Data in Table 2 also showed that *Alternaria* had the highest frequency occurrence (65.0%) on cucumber fruits of greenhouse cultivation in El-Gharbeia Governorate, followed by *Pleospora allii* (20.0%), then *Fusarium* spp. (14.0%). *Alternaria* and *Fusarium* spp. (90.0 and 10.0%), respectively, were detected in El-Giza Governorate. These results are in agreement with Kim *et al.* (2011), Di Francesco *et al.* (2015), Sani *et al.* (2015), Ziedan and Saad (2016), Suwannarach *et al.* (2016), and Alam *et al.* (2017). It is worth mentioning that the two fungal isolates of *Galactomyces* spp. and *Pleospora allii* were isolated and recorded for the first time from spoiled cucumbers in Egypt.

Table 2. Frequency of isolation of different fungi associated with postharvest fruit rot of cucumber from different locations

Cultivation	Location	Fungal name	Frequency [%]
Open field	El Gharbeia	*Fusarium* spp.	50.0 c
		Galactomyces spp.	25.0 f
		Aspergillus niger	12.5 h
		Mucor spp.	12.5 h
	El-Giza	*Galactomyces* spp.	40.0 d
		Mucor spp.	30.0 e
		unknown	30.0 e
Greenhouse	El Gharbeia	*Alternaria* spp.	65.0 b
		Pleospora allii	20.0 g
		Fusarium spp.	14.0 h
	El-Giza	*Alternaria* spp.	90.0 a
		Fusarium spp.	10.0 i

Values followed by the same letter are not significantly different at $p \leq 0.05$ according to Duncan's multiple range

Pathological potential of isolated fungi

Differences were observed between the isolated fungi in their aggressiveness on cucumber fruits. Data in Table 3 and Figures 2 and 3 indicated that the common fungi, i.e., *G. candidium* from El-Giza and *G. candidium*, *Geotrichum* sp., *F. fujikuroi* and *F. verticiolides* from El-Gharbeia were highly pathogenic, causing cucumber fruit rot. *Galactomyces candidium* from El-Giza was highly pathogenic, followed by *Geotrichum* sp. and *F. fujikuroi* from El-Gharbeia. *Fusarium verticiolides* from El-Gharbeia Governorate caused the least fruit rot. In addition, fungi isolated from cucumber fruit, cultivated under a protective greenhouse, i.e., *Alternaria tenussium* (No. 2) *F. geraminearum,* and *P. allii*, *Fusarium solani* and *F. incarnatum* from El-Gharbeia were pathogenic to cucumber fruit (Cv. Golden) and *A. tenussium* (No. 1) was non-pathogenic. Symptoms in most of the inoculated fruits were characterized by yellowing of the tissue, followed by browning and fruit rot. The pathogenic fungi which induced symptoms in the inoculated fruits were re-isolated from symptomatic tissue. These results are in agreement with Blancard *et al*. 2005; Kim *et al*. 2011; Di Francesco *et al*. 2015; and Sani *et al*. 2015. Recently, in Pakistan, peach, fruit decay was caused by *Geotrichum candidum* (Alam *et al*. 2017). Also, Al-Sadi *et al.* (2011) reported that *Alternaria alternata, F. equiseti, F. solani, Cladosporium tenuissimum, Corynespora cassiicola, Aspergillus* spp., *Curvularia* sp. and *Bipolaris* sp. were isolated from diseased cucumber fruits.

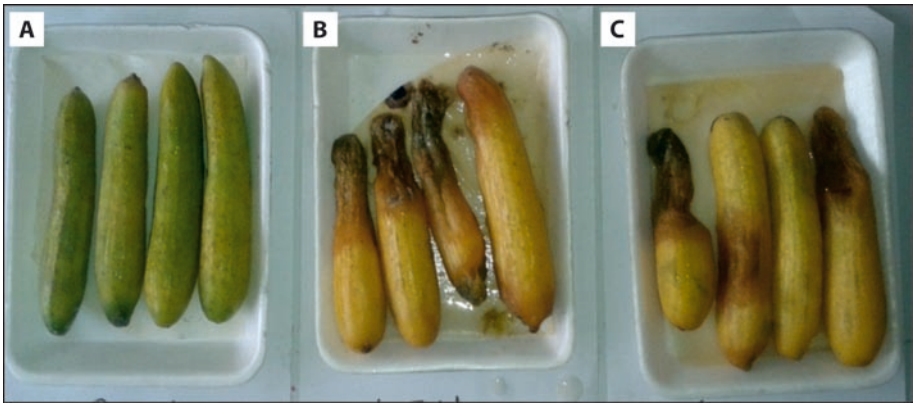

Fig. 2. Fruit rot incidence of cucumbers from open field cultivation 20 days after infestation. Control (A), *Galactomyces candidium* (B) and *Geotrichum* sp. (C)

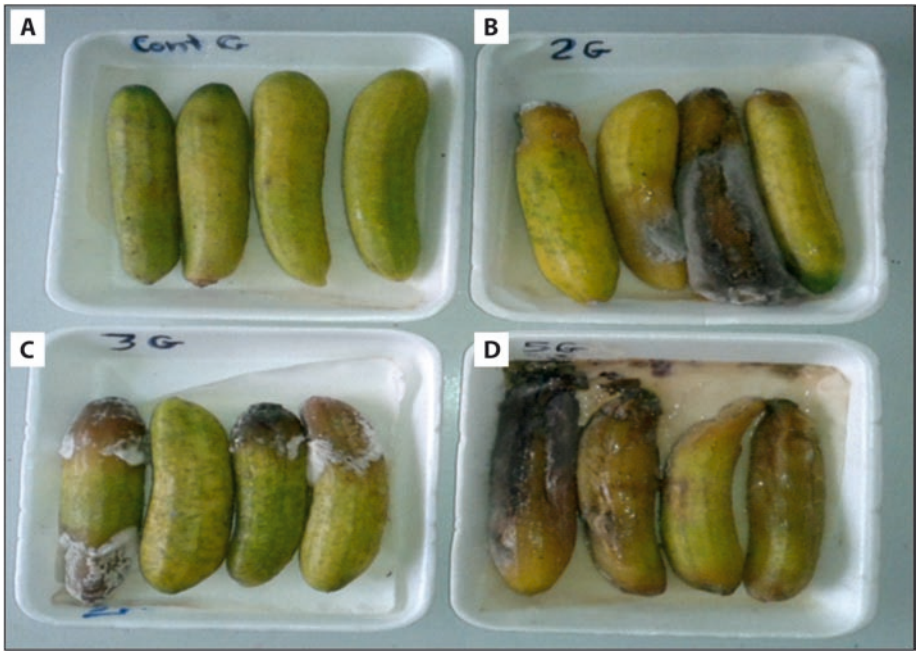

Fig. 3. Fruit rot disease incidence of cucumbers from greenhouse cultivation 20 days after infestation. Control (A), *Alternaria tenussima* (B), *Fusarium geraminearium* (C) and *F. solani* (D)

Table 3. Pathogenicity test of fungal isolates on postharvest disease of cucumber fruits 20 days after infestation

Cultivation	Location	Fungal		Fruit rot incidence	
		no.	name	infection [%]	disease severity
Open field	El-Giza	1	*Galactomyces candidium*	100.0 a	2.0 b
	El-Gharbeia	2	*Fusarium verticiolides*	00.0 d	0.0 g
		3	*F. fujikuroi*	40.0 c	0.1 f
		4	*F. verticiolides*	20.0 d	0.1 f
		5	*G. candidium*	20.0 d	0.8 d
		6	*Geotrichum* sp.	60.0 b	1.2 b
		0	Control	00.0 d	0.0 g
Greenhouse	El-Giza	1	*Alternaria tenuissima*	00.0 d	0.0 g
		2	*A. tenuissima*	100.0 a	1.0 c
		3	*F. geraminearium*	60.0 b	0.8 d
	El-Gharbeia	4	*Pleospora allii*	60.0 b	0.8 d
		5	*F. solani*	100.0 a	2.4 a
		6	*F. incarnatum*	40.0 c	0.4 e
		0	Control	00.0 d	0.0 g

Values followed by the same letter are not significantly different at $p \leq 0.05$ according to Duncan's multiple range

Molecular identification of fungi associated with postharvest diseases

After the DNA isolation from the different fungal pathogenic strains and determination of the concentration by spectrophotometer, the ITS1 and ITS2 primers were used to amplify the region of the rDNA repeat unit that includes the ITS1 from the genomic DNA of the fungal pathogenic strains. After amplification, approximately 150 to 200 bp were obtained as shown in Figure 4. On the other hand, the ITS1 and ITS4 primers were used to amplify the region of the rDNA repeat unit that includes ITS1, 5.8S, ITS2 and 28S from the genomic DNA of the fungal pathogenic strains. After amplification, approximately 450 to 550 bp were obtained as shown in Figures 5 and 6. Only two strains (Z-Kh-F1and Z-Kh-F2) did not produce any products after PCR amplification by ITS1 and ITS4 primers. After the DNA sequencing of the purified PCR products with ABI 3730xl DNA sequencer (GATC Company, Germany) by using forward primer, the 12 obtained DNA sequences (Seq1 to Seq12) with the identified fungal strains were applied and conserved in the GenBank under the following accession numbers:

- Seq1 [organism = *Galactomyces candidum*] Z-Kh-F1, ITS1, partial sequence (GenBank accession number MF373433),
- Seq2 [organism = *Fusarium verticillioides*] Z-Kh-F2, 5.8S ribosomal RNA gene, partial sequence; ITS2, complete sequence; and 28S ribosomal RNA gene, partial sequence (GenBank accession number MF373434),

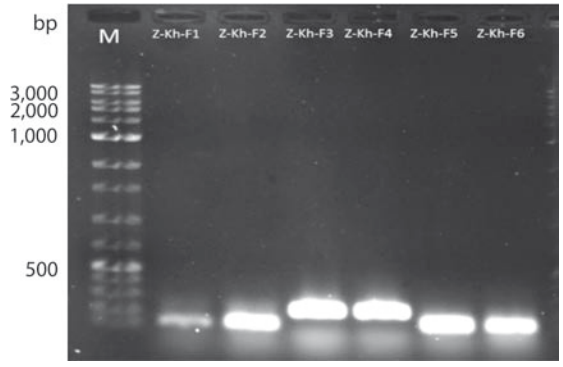

Fig. 4. Photograph of ITS-DNA amplified band for six fungal pathogenic strains (Z-Kh-F1, Z-Kh-F2, Z-Kh-F3, Z-Kh-F4, Z-Kh-F5 and Z-Kh-F6) isolated from cucumber fruit from greenhouse cultivation (lanes: 2, 3, 4, 5) using ITS1 and ITS2 primers against 100 bp ladder DNA marker (lane M)

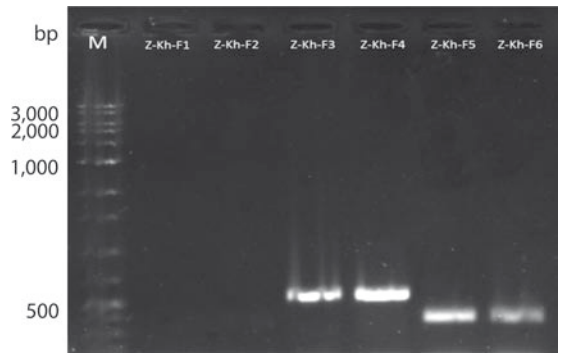

Fig. 5. Photograph of ITS-DNA amplified band for six fungal pathogenic strains (Z-Kh-F1, Z-Kh-F2, Z-Kh-F3, Z-Kh-F4, Z-Kh-F5 and Z-Kh-F6) isolated from cucumber fruit from greenhouse cultivation (lanes: 2, 3, 4, 5, 6, 7) using ITS1 and ITS4 primers against 100 bp ladder DNA marker (lane M)

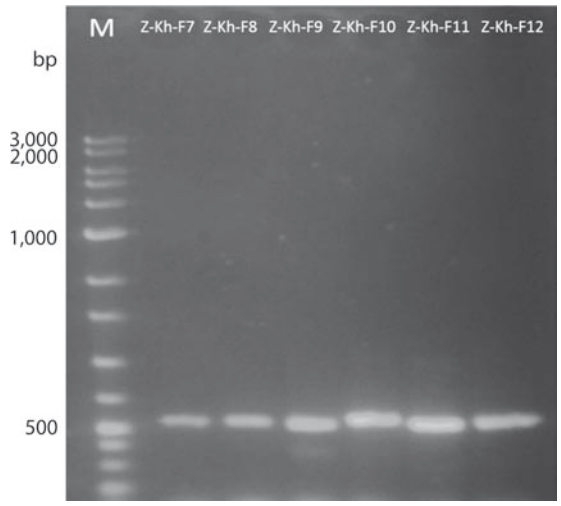

Fig. 6. Photograph of ITS-DNA amplified band for six fungal pathogenic strains (Z-Kh-F7, Z-Kh-F8, Z-Kh-F9, Z-Kh-F10, Z-Kh-F11 and Z-Kh-F12) isolated from cucumber fruit from open field cultivation (lanes: 2, 3, 4, 5, 6, 7) using ITS1 and ITS4 primers against 100 bp ladder DNA marker (lane M)

- Seq3 [organism = *Fusarium fujikuroi*] Z-Kh-F3, ITS1, partial sequence; 5.8S ribosomal RNA gene, and ITS2, complete sequence; and 28S ribosomal RNA gene, partial sequence (GenBank accession umber MF373435),
- Seq4 [organism = *Fusarium verticillioides*] Z-Kh-F4, ITS1, partial sequence; 5.8S ribosomal RNA gene, and ITS2, complete sequence; and 28S ribosomal RNA gene, partial sequence (GenBank accession number MF373436),
- Seq5 [organism = *Galactomyces candidum*] Z-Kh-F5, ITS1, partial sequence; 5.8S ribosomal RNA gene, and ITS2, complete sequence; and 28S ribosomal RNA gene, partial sequence (GenBank accession number MF373437),
- Seq6 [organism = *Geotrichum* sp.] Z-Kh-F6, ITS 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and ITS2, partial sequence (GenBank accession number MF373438),
- Seq7 [organism = *Alternaria tenuissima*] Z-Kh-F7, 18S ribosomal RNA gene, partial sequence; ITS1, 5.8S ribosomal RNA gene, and ITS2, complete sequence; and 28S ribosomal RNA gene, partial sequence (GenBank accession number MF373439),
- Seq8 [organism = *Alternaria tenuissima*] Z-Kh-F8, 18S ribosomal RNA gene, partial sequence; ITS1, 5.8S ribosomal RNA gene, and ITS2, complete sequence; and 28S ribosomal RNA gene, partial sequence (GenBank accession number MF373440),
- Seq9 [organism = *Fusarium graminearum*] Z-Kh-F9, ITS1, partial sequence; 5.8S ribosomal RNA gene, and ITS2, complete sequence; and 28S ribosomal RNA gene, partial sequence (GenBank accession number MF373441),
- Seq10 [organism = *Pleospora allii*] Z-Kh-F10, 18S ribosomal RNA gene, partial sequence; ITS1, 5.8S ribosomal RNA gene, and ITS2, complete sequence; and 28S ribosomal RNA gene, partial sequence (GenBank accession number MF373442),
- Seq11 [organism = *Fusarium solani*] Z-Kh-F11, ITS1, partial sequence; 5.8S ribosomal RNA gene, and ITS2, complete sequence; and 28S ribosomal RNA gene, partial sequence (GenBank accession number MF373443),
- Seq12 [organism = *Fusarium incarnatum*] Z-Kh-F12, ITS1, partial sequence; 5.8S ribosomal RNA gene, and ITS2, complete sequence; and 28S ribosomal RNA gene, partial sequence (GenBank accession number MF373444).

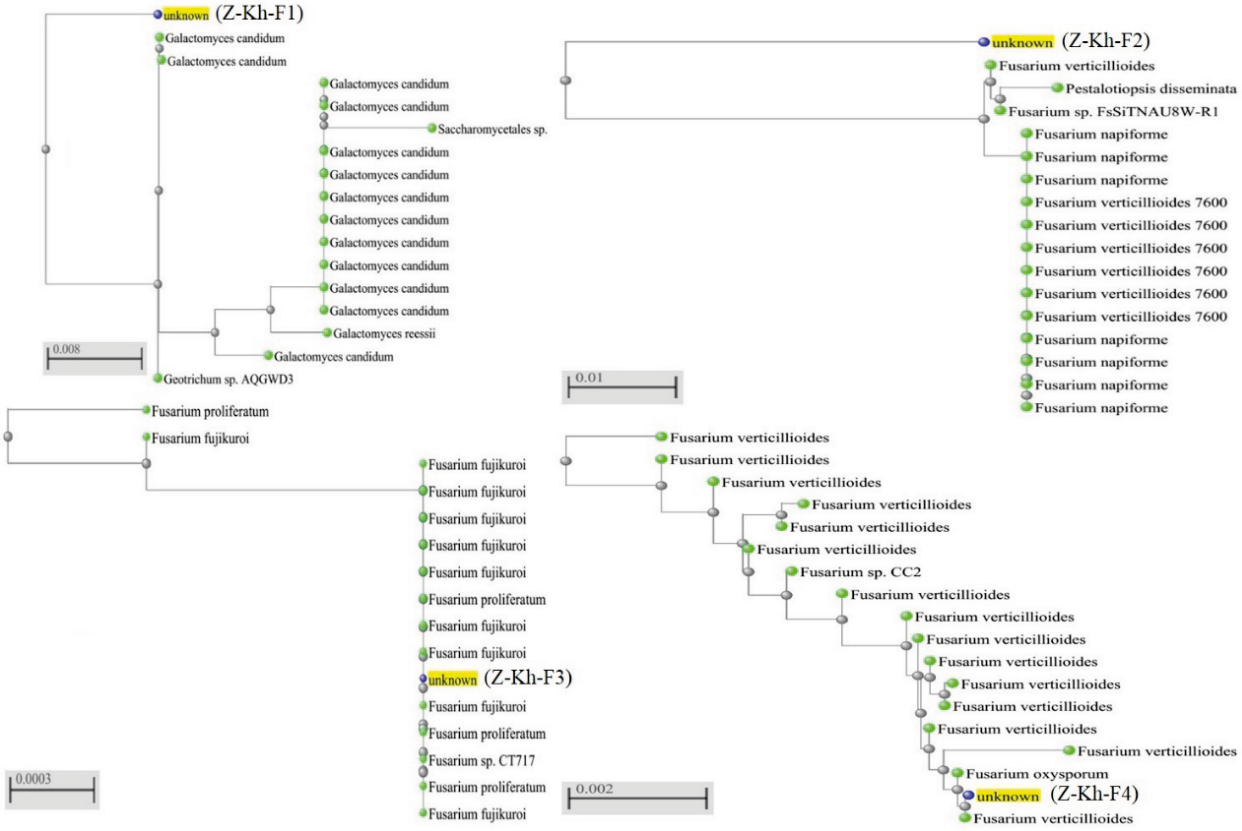

Fig. 7. Phylogenetic dendrogram showing the taxonomic positions of different fungal strains (Z-Kh-F1, Z-Kh-F2, Z-Kh-F3 and Z-Kh-F4) isolated from greenhouse cultivation, based on the ITS sequences and other closely related species available from NCBI

DNA sequencing of the ITS1, 5.8S, ITS2 and 28S regions was conducted for the differentiation of fungal pathogenic strains in comparison with the reference strains from GenBank. In the amplified sequences from fungal pathogenic strains with other sequences from GenBank no significant size variation could be detected between strains after alignment. Moreover, according to ITS sequences most of the fungi we isolated had 97–100% similarity with the related fungi recorded in the GenBank. Furthermore, the ITS regions of the fungal pathogenic strains have many nucleotide substations in comparison with the strains from Gen -Bank. From phylogenetic analysis of the obtained sequences in comparison with the related sequences from the GenBank, the phylogenetic trees showed the taxonomic positions of six fungal strains isolated from greenhouse cultivation (Figs 7 and 8). Also, the phylogenetic trees of six fungal strains isolated from open field cultivation are presented in Figures 9 and 10.

The amplification gene and DNA sequencing have led to the detection of new pathogens as agents of disease and have enabled us to better classify microorganisms isolated from samples. DNA sequencing has greatly improved the ability to accurately and reproducibly identify plant pathogenic fungi. Fungal taxonomists have been using DNA sequences for many

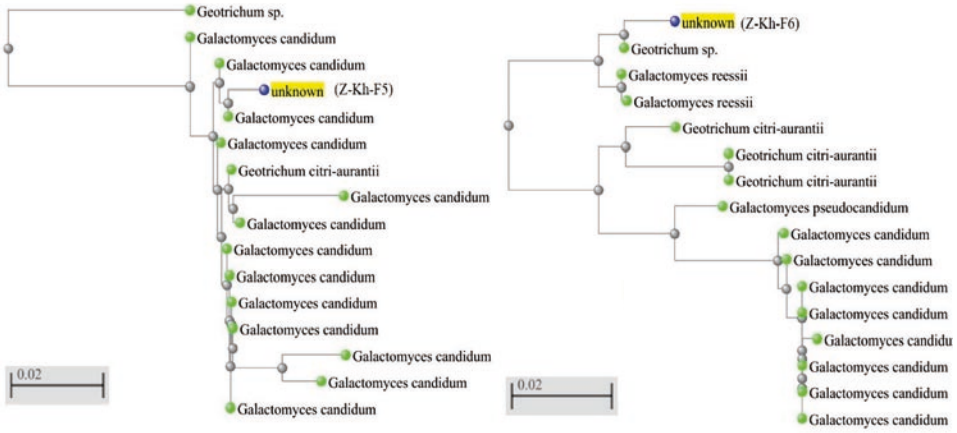

Fig. 8. Phylogenetic dendrogram showing the taxonomic positions of different fungal strains (Z-Kh-F5 and Z-Kh-F6) isolated from greenhouse cultivation, based on the ITS sequences and other closely related species available from NCBI

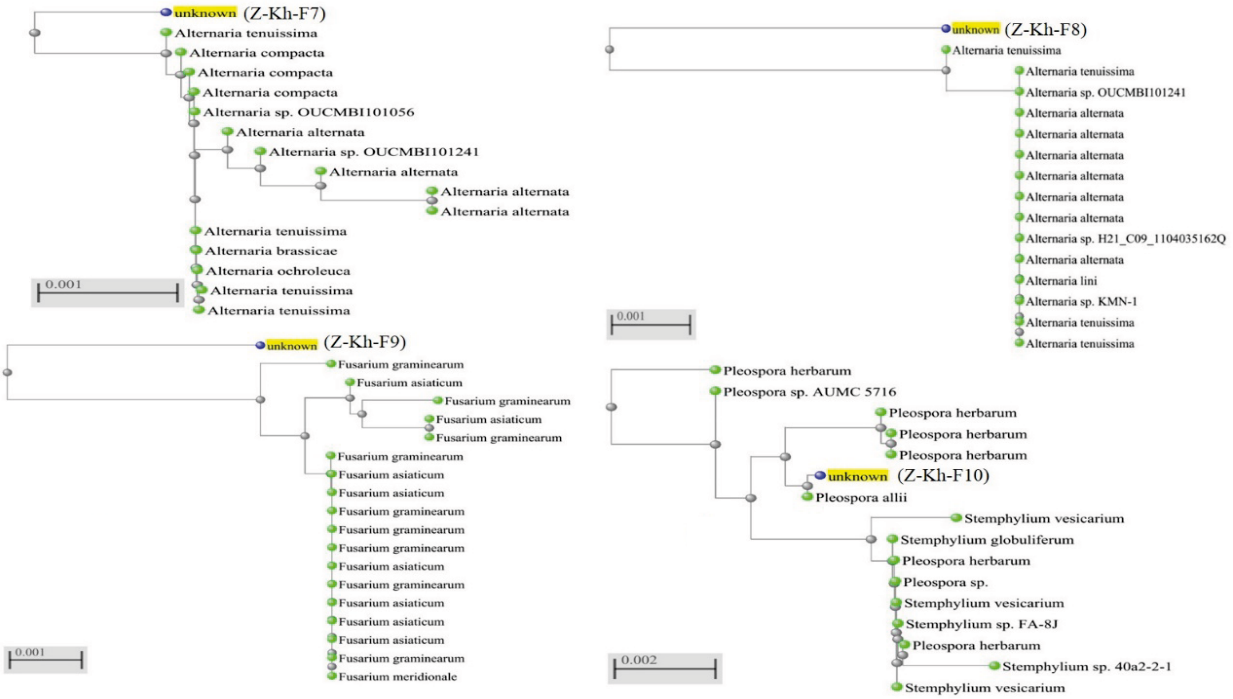

Fig. 9. Phylogenetic dendrogram showing the taxonomic positions of different fungal strains (Z-Kh-F7, Z-Kh-F8, Z-Kh-F9 and Z-Kh-F10) isolated from open field cultivation, based on the ITS sequences and other closely related species available from NCBI

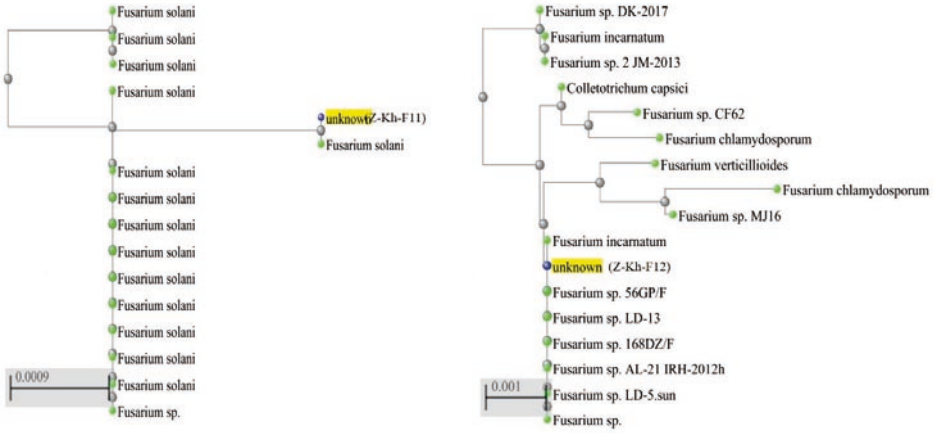

Fig. 10. Phylogenetic dendrogram showing the taxonomic positions of different fungal strains (Z-Kh-F11 and Z-Kh-F12) isolated from open field cultivation, based on the ITS sequences and other closely related species available from NCBI

years as a basis for the re-classification of all fungal taxa and have more recently moved to ITS sequencing as the "Gold Standard" (Hall *et al.* 2003). The obtained results are in harmony with those obtained by Barry *et al.* (2000); Maiko (2013); and Jeewon (2013). Alwakeel (2013) demonstrated that sequence analysis of the ITS regions of the nuclear encoded rDNA showed significant alignments for *P. chrysogenum*, *P. adametzii* and *A. oryzae*. Jeewon (2013) showed that the most commonly isolated fungi were related to *Aspergillus*, *Guignardia*, *Fusarium*, *Penicillium*, *Pestalotiopsis*, and *Trichoderma*. Phylogenetic analyses revealed that the recovered fungi belong to five different fungal lineages (Hypocreaceae, Trichocomaceae, Nectriaceae, Xylariaceae, and Botryosphaeriaceae). DNA data from the ITS regions were reliable in the classification of all recovered isolates up to the genus level, but identification to an exact species name was not possible at this stage. Agwanande *et al.* (2016) isolated nine fungal strains which were identified through 18S rDNA sequencing. It was found that *Rhizopus oryzae*, *Aspergillus flavus, A. oryzae and Cunninghamella polymorpha* were common in both maize and groundnuts. *Aspergillus tamari, Talaromyces purpureogenus* and *Penicillium citrinum* were present only in maize, while *A. parasiticus* and *Rhizopus stolonifer* were identified only from groundnuts.

Conclusions

This was the first report of postharvest disease of cucumber fruits caused by several fungal genera i.e., *Galactomyces candidium, Geotrichum* sp., *Alternaria tenusinium*, *Plerospora alli* and *Fusarium* spp. (*F. fujikuroi*, *F. verticiolides, F. solani, F. geraminearium* and *F. incarnatum*) in El Gharbeia and El Giza Governorates, Egypt. Fungal isolates were identified according to cultural and morphological characterization, PCR amplification and sequencing of ITS regions. Several new nucleotide sequences were conserved in GenBank. We can detect and classify the isolated fungal precisely and rapidly using the DNA-based technology. The usefulness of ITS sequencing has already been proved in phylogenic analysis of the fungal pathogenic strains isolated from the surface of cucumber fruits according to the present study. Our results will be helpful for rapid detection and to further study the pathogenesis and molecular evolution of the fungal pathogenic strains.

Acknowledgements

The authors extend their appreciation to the National Research Centre, Egypt for funding this work through research project No. P11030135 (2016-2019).

References

Agwanande A.W., Leopold T.N., Michel J.D.P., Priya P., Mariya A., Manilal V.B., Krishnakumar B., Henri A.Z.P. 2016. Isolation and molecular identification of fungi in stored maize (*Zea mays* L.) and groundnuts (*Arachis hypogaea* L.) in Ngaoundere, Cameroon. American Journal of Microbiological Research 4 (3): 85–89. DOI: 10.12691/ajmr-4-3-4

Alam M.W., Rehman A., Malik A.U., Iqbal Z., Amin M., Ali S., Hameed A., Sarfraz S. 2017. First report of *Geotrichum candidum* causing postharvest sour rot of peach in Punjab. Pakistan Plant Disease 101 (8): 1543.

Alice L.A., Campbell C.S. 1999. Phylogeny of *Rubus* (Rosaceae) based on nuclear ribosomal DNA internal transcribed spacer region sequences. American Journal of Botany 86 (1): 81–97. DOI: 10.2307/2656957

Al-Sadi A.M., Al-Said F.A., Al-Kaabi S.M., Mohammed S., Al-quraini S.M., Al-Mazroui S.S., Al-Mahmooli I.H., Deadman L.D. 2011. Occurrence, characterization and management of fruit rot of immature cucumbers under greenhouse conditions in Oman. Phytopathologia Mediterranea 50 (3): 421–429.

Alwakeel S.S. 2013. Molecular identification of isolated fungi from stored apples in Riyadh, Saudi Arabia. Saudi Journal of Biological Sciences 20 (4): 311–317. DOI: https://doi.org/10.1016/j.sjbs.2013.05.002

An R.P., Ma Q. 2005–2006. Control of cucumber grey mold by endophytic bacteria. Cucurbit Genetics Cooperative Report 28–29: 1–6.

Baldwin B.G. 1992. Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: An example from the Compositae. Molecular Phylogenetics and Evolution 1 (1): 3–16. DOI: https://doi.org/10.1016/1055-7903(92)90030-K

Barnett H.L., Hunter B.B. 1998. Illustrated Genera of Imperfect Fungi. 4th ed. APS Press, St. Paul, Minnesota, USA, 218 pp.

Barry C.S., Llop-Tous M.I., Grierson D. 2000. The regulation of 1-aminocyclopropane-1-carboxylic acid synthase gene expression during the transition from system-1 to system-2 ethylene synthesis in tomato. Plant Physiology 123 (3): 979–986. DOI: https://doi.org/10.1104/pp.123.3.979

Blancard D., Lecoq H., Pitrat M. 2005. A color atlas of cucurbit diseases observation, identification and control Manson Publishing Ltd. London, United Kingdom, 304 pp.

Boekhout T., Kurtzman C.P., O'Donnell K., Smith M.T. 1994. Phylogeny of the yeast genera *Hanseniaspora* (anamorph Kloeckera), *Dekkera* (anamorph Brettanomyces), and *Eeniella* as inferred from partial 26s ribosomal DNA nucleotide sequences. International Journal of Systematic Bacteriology 44 (4): 781–786. DOI: 10.1099/00207713-44-4-781

Booth C. 1971. The Genus *Fusarium*. Commonwealth Mycological Institute, Kew Surrey, England, 237 pp.

Cai J., Roberts I.N., Collins, M.D. 1996. Phylogenetic relationships among members of the ascomycetous yeasts genera *Brettanomyces*, *Debaryomyces*, *Dekkera* and *Kluyveromyces* deduced by small subunit rRNA gene sequences. International Journal of Systematic Bacteriology 46 (2): 542–549.

Cohen Y., Crisi U., Mosinger E. 1991. Systemic resistance of potato plants against *Phytophthora infestans* induced by unsaturated fatty acids. Physiological and Molecular Plant Pathology 38 (4): 255–268. DOI: https://doi.org/10.1016/S0885-5765(05)80117-1

Di Francesco A., Fruk M., Martini C., Jemric T., Mari M. 2015. First report of Asiatic brown rot (*Monilinia polystroma*) on apple in Croatia. Plant Disease 99 (8): 1181. DOI: https://doi.org/10.1094/PDIS-12-14-1290-PDN

Effiuvwevwere B.J.O. 2000. Microbial Spoilage Agents of Tropical and Assorted fruits and Vegetables (An Illustrated References Book). Paragraphics Publishing Company, Port Harcourt, Nigeria, 39 pp.

Ellis M.B. 1971. Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, England, 608 pp.

Farrag E.S.H., Ziedan E.H., Mahmoud S.Y.M. 2007. Systemic acquired resistance induced in cucumber plants against powdery mildew disease by pre-inoculation with tobacco necrosis virus. Plant Pathology Journal 6 (1): 44–50. DOI: 10.3923/ppj.2007.44.50

Hall L., Wohlfiel S., Roberts G.D. 2003. Experience with the MicroSeq D2 large-subunit ribosomal DNA sequencing kit for identification of filamentous fungi encountered in the clinical laboratory. Journal of Clinical Microbiology 42 (2): 622–626. DOI: 10.1128/JCM.42.2.622-626.2004

James S.A., Collins M.D., Roberts I.N. 1996. Use of an rRNA internal transcribed spacer region to distinguish phylogenetically closely related species of the genera *Zygosaccharomyces* and *Torulaspora*. International Journal of Systematic Bacteriology 46 (1): 189–194. DOI: 10.1099/00207713-46-1-189

Jeewon R., Ittoo J., Mahadeb D., Jaufeerally-Fakim Y., Wang H.K., Liu A R. 2013. DNA Based identification and phylogenetic characterisation of endophytic and saprobic fungi from *Antidesma madagascariense*, a medicinal plant in Mauritius. Journal of Mycology 2013: 1–10. DOI: http://dx.doi.org/10.1155/2013/781914

Jukes T.H., Cantor C.R. 1969. Evolution of protein molecules. p. 21–132. In: "Mammalian Protein Metabolism" (H.N. Munro, ed.). Academic Press, New York.

Khot P.D., Ko D.L., Fredricks D.N. 2009. Sequencing and analysis of fungal rRNA operons for development of broad-range fungal PCR assays. Applied and Environmental Microbiology 75 (6): 1559–1565. DOI: 10.1128/AEM.02383-08

Kim Y.K., Kim T.S., Shim H.S., Park K.S., Yeh W.H., Hong, S.J., Shim C.K., Kim J.S., Park J.H., Han E.J., Lee M.H., Jee H.J. 2011. First report of sour rot on post-harvest oriental melon, tomato, cucumber, potato, pumpkin and carrot caused by *Geotrichum candidum*. Results Plant Disease 17 (2): 232–234. DOI: 10.5423/RPD.2011.17.2.232

Kurtzman C.P. 1992. rRNA sequence comparisons for assessing phylogenetic relationships among yeasts. International Journal of Systematic Bacteriology 42: 1–6. DOI: 10.1099/00207713-42-1-1

Li W.H. 1997. Molecular Evolution. Sinauer Associates, Inc., Publishers Sunderland, MA, 479 pp.

Maiko W. 2013. Molecular phylogeny and identification of *Fusarium* species based on nucleotide sequences. Mycotoxins 63 (2): 133–142. DOI: https://doi.org/10.2520/myco.63.133

Nelson P.E., Toussoum T.A., Marasas W.F.O. 1983. *Fusarium* Species. An Illustrated Manual for Identification. The Pennsylvania University, USA, 206 pp.

Saitou N., Nei M. 1987. The neighbor joining method: a new method for constructing phylogenetic trees. Molecular Biology and Evolution 4 (4): 406–425. DOI: 10.1093/oxfordjournals.molbev.a040454

Sambrook J., Fritsch E.F., Maniatis T. 1989. Molecular Cloning; A Laboratory Manual. 2nd ed. Cold Spring Harbor Laboratory Press, 1659 pp. DOI: https://doi.org/10.1016/0167-7799(91)90068-S

Sang T., Crawford D.J., Stuessy T.F. 1995. Documentation of reticulate evolution in *Peonies* (Paeonia) using internal transcribed spacer sequences of nuclear ribosomal DNA: implications for biogeography and concerted evolution. Proceedings of the National Academy of Sciences of the United States of America 92: 6813–6817.

Sani M.A., Usman N., Kabir F., Kutama A.S. 2015. The effect of three natural preservatives on the growth of some predominant fungi associated with the spoilage of fruits (Mango, Pineapple and Cucumber) Global Advanced Results Journal of Agricultural Sciences 4 (12): 923–928.

Snedecor G.W., Cochran W.G. 1980. Statistical Methods. 7th ed. Iowa State University Press, Ames, 705 pp.

Soliman H.M., El Metwall M.A., Elkahky M.T., Badawi W. 2015. Alternative to chemical control of grey mold disease on cucumber caused by *Botrytis cinerea* Pers. Asian Journal of Plant Pathology 9 (1): 1–15. DOI: 10.3923/ajppaj.2015.1.15

Suwannarach N., Kumla J., Nitiyon Limtong S.S., Lumyong S. 2016. First report of sour rot on tomato caused by *Galactomyces reessii* in Thailand. Journal of General Plant Pathology 82 (4): 228–231. DOI: 10.1007/s10327-016-0663-x

Ziedan E.H.E., Saad M.M. 2016. Efficacy of nanoparticles on seed borne fungi and their pathological potential of cucumber. International Journal of PharmTech Research 9 (10): 16–24.