Plant Archives Vol. 19, Supplement 1, 2019 pp. 1301-1307 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

MOLECULAR IDENTIFICATION AND PATHOGENICITY OF *FUSARIUM* FUNGI CAUSING ROOT ROT DISEASE OF CUCUMBER UNDER PLASTIC GREENHOUSES IN EGYPT

Ashraf Gamil Attallah[1], Ahmed Farahat Sahab[2] and El Sayed Hussein Ziedan[2]
1- Microbial Genetics Department., National Research Centre, Cairo, Egypt
2- Plant Pathology Department, National Research Centre, Cairo, Egypt
*Correspondence author email : ziedanehe@yahoo.com

Abstract

A survey of cucumber root diseases on plants was carried out in protective plastic houses in four Governorates in Egypt during winter season (2016-2017). Root rot disease was observed only of cucumber plants associated with chlorosis, yellowish, wilt and death of cucumber plants. Root rot disease progress increased by increasing plants age during growing season. High incidence of root rot disease on cucumber plants was recorded in Kafr El-Sheickh Governorate, moderately in El-Giza and lower incidence in El-Beheira and El-Gharbeia Governorates. Isolation trails from root rot of cucumber samples yielded four fungi. *Fusarium oxysporum* and *Fusarium solani* were the common fungi in four Governorates, meanwhile less frequency of *Alternaria alternata* and *Macrophomina phaseolina* which recorded only in El-Gharbeia and El-Beheira Governorates respectively. Pathogenicity tests indicated that *Fusarium solani* (isolate No. 6) and *Fusarium oxysporum* (isolate No. 5) from El Giza Governorate were the most pathogenic responsible for root-rot disease on cucumber plants followed by *F. oxysporum* (isolate No. 4 and 2) in Kafr El-Sheichk Governorate respectively. Fungal isolates were identified according to cultural, morphological and molecular characterizations based on sequencing of internal transcribed spacer (ITS) then the nucleotides sequences were registries in Gene Bank under accession No. MG01877, MG018778, MG018779, MG018780, and MG018781. Data obtained will be helpful to study the pathogenesis, rapid detection and management root rot of cucumber**.**

Keywords**:** Cucumber, root rot, *Fusarium* spp. Molecular characterization

Introduction

Cucumber plants (*Cucumis sativus* L.) is an important economic vegetable crop cultivation in open field and protected houses worldwide for both local consumption and exportation. In Egypt, root rot disease of cucumber affected seriously of cucumber production due to pathogenic soil borne fungi causing root rot disease of cucumber were *Fusarium oxysporum*, *Fusarium solani*, *Rhizoctonia solani*, *Sclerotium rolfsii*, *Macrophomina phaseolina,* and *Sclerotinia* spp. (Farrag (Eman) *et al*., 2013; Sabet *et al.,* 2013; Elwakil *et al*., 2015; Yousef (Safaa) *et al*., 2016 and Ziedan and Saad (Moataza) 2016). In Greece, root and stem rots are the most destructive diseases of cucumber under greenhouse (Vakalounakis, 1996; Vakalounakis and Fragkiadakis 1999 and Dik *et al*., 2004). In addition, *Fusarium* wilt of cucumbers caused by *F. oxysporum* f. sp. radices *cumerinum* had been reported from British, Culombia and Spain. (Punja and Parker 2000 and Moreno *et al*., 2001). The most molecular protocols used in the identification of fungal species are based on the variability of the ribosomal genes 5.8S, 18S and 26S rRNA (Cai *et al.,* 1996 and 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 (Cheng *et al*., 2016). Previous results have demonstrated that the complex ITS 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 and Cai *et al*., 1996). The internal transcribed spacer (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 locate between genes encoding the 18, 5.8 and 28S nuclear ribosomal RNA (nrRNA) subunits. In addition, the 5.8s nrRNA are referred as nrDNA ITS region (Baldwin, 1992). The present study was conducted to survey of root rot disease, isolation and identification of causal organisms by molecular methods based on sequencing of internal transcribed spacer (ITS) regions.

Materials and Methods

Root rot disease incidence survey of cucumber plants

Survey of root rot disease incidence of cucumber in various famous plastic greenhouse was carried out in different location in Egypt i.e., El-Giza, Kafr El-Sheickh, El-Beheira and El-Gharbeia Governorates, Egypt. Root rot disease incidence and their severity were determined during winter season 2016 and 2017 as follows:

$$\text{Disease incidence}\,\% = \frac{\text{No of inf ected plants}}{\text{Total no. of plant assessed}} \times 100$$

Disease Assessment

Percentage of root rot disease incidence of cucumber plants were recorded 1, 2 and 3 months after cultivation of transplanting cucumber plants. Disease severity on shoot system were determined according Carver *et al*. (1996) as follows: 0= healthy plant, 1= initial signs of yellowing on aerial parts, 2= wilt up to 25% of plant, 3= wilt up to 50% of plant and 4=100 % plant dead.

Isolation and identification of fungi associated with root rot disease of cucumber

Isolation of fungi associated with root rot of cucumber plants was performed using samples of diseased roots. The roots were washed thoroughly with tap water to remove the attached soil particles and cut into small pieces then surface sterilized with 5% sodium hypochlorite for 3 minutes. The specimens were rinsed twice in sterile distilled water and dried between sterile filter papers. Five specimens of diseased plants tissue were plated on plan agar, potato dextrose agar (PDA) and Martin's media. amended with streptomycin sulphate (120mg/L). Plates were incubated at 27±2°C up to five days. Different fungal colonies were purified using the single spore and hyphal tip techniques according to (Booth, 1971). Frequency occurrence of isolated fungi were recorded using the following formula:

$$\text{Frequency}\% = \frac{\text{Total fungal colonies of each sample}}{\text{Total fungal colonies of each sample}} \times 100$$

Fungal colonies were identified based on cultural and morphological characteristics according Booth (1971); Nelson *et al*. (1983) and Barnett and Hunter (1998) confirmed by polymerase chain reaction (PCR) using the ITS primers (Abd Murad *et al*., 2016)

Fungal cultivation for DNA extraction

Mycelial mat of *Fusarium* isolates were prepared by cultured each isolates on 100ml of potato dextrose broth (PDB) in a flask (250 ml) for 7 days at 25°C then inoculated with 5-mm of mycelial disk 10 days old on potato dextrose agar (PDA). Flasks were incubated at 25°C for 7 days. Mycelial mate were harvested by filtration under sterilized conditions, frozen and stored at -40 °C until used.

Isolation of DNA from *Fusarium* spp. strains.

DNA was isolated from 25 mg mycelial mats conidia spores were harvested using the standard extraction procedures according to the protocol recommended for the DNA tissue purification mini kit QIAamp DNA mini kit (Qiagene Hiden Germany). The total genomic DNA was checked by agarose gel electrophoresis and the concentrations of the purified total genomic DNA were determined with a NanoDrop 1000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and stored at -20°C for further use.

Molecular characterization using ITS

Molecular identification of *Fusarium* spp. cultures were carried out based on conserved ribosomal internal transcribed spacer (ITS) region. We amplified the ITS regions between the small nuclear 18S rDNA and large nuclear 28S rDNA, including 5.8S rDNA using universal primer pairs ITS1 (5'-TCCGTAGGTGAACCTGCGG-3'), ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). The ITS primers ITS 5 (5′-GGAAGTAAAAGTCGTAACAAGG-3') and ITS 2 (5′-TCCTCCGCTTATTGATATGC-3'). Amplification was performed on a Thermal Cycler (Bio-Rad T100) with 25 µl reaction mixtures containing 2.5 µl of 10X buffer (10 mM Tris– HCl, pH 8.8); 2.5 mM $MgCl_2$; 2 mM each of dNTP; 25 pmol $ml^{-}1$ primer (each of ITS-1, ITS-4, ITS-5 and ITS-2); 1U of Taq DNA Polymerase; 60–100 ng genomic DNA. The amplification cycle consists of an initial denaturation at 95°C for 2 min followed by 35 cycles at 94 °C for 30 s, 50°C for 1 min, and 72°C for 2 min and a final extension at 72°C for 10 min. Amplified PCR products were separated on an agarose gel (2% w/v) in 1X TAE buffer at 75V for 150 min and photographed under Gel Doc™ XR+ Gel Documentation System Gene ruler ladder 1oobp Thermo Scientific GeneRuler 100bp DNA Ladder (Fermentas) was used as a size standard. They were then eluted by MinElute PCR purification kit (Qiagen). DNA from All isolates was subjected to automated DNA sequencing and subsequently used for sequencing.

ITS Data Analysis

The ITS nucleotide sequences for each isolate were determined on both strands for each of the isolates and were aligned for comparison. Most sequence comparisons are carried out using BLASTn (http://www.ncbi.nlm.nih.gov/BLAST) analysis which aligns two or more homologues to detect for presence of one or more ambiguous region within the segments under comparison. Phylogenetic tree was created the online version of MAFFT (https://mafft.cbrc.jp/alignment/ server) based on UPGMA (un weighted pair group method for arithmetic analysis). The alignments were further edited manually in MEGA v. 6.0 and deposited in Tree BASE (http://treebase.org).

Pathogenicity test of *Fusarium* isolates

Pathogenic potential of *Fusarium* isolates for induce root rot disease symptoms on cucumber plants was carried out under greenhouse conditions at National Research Centre (NRC) Egypt. Five seeds of cucumber (Cv. Golden) were sown in sterilized plastic pots (25 cm-diameter) filled with sterile loamy sand soil artificially infested by the rate of 5% (w/w), inoculum of each isolate which previously grown for 20 days at 27±2°C on sterilized sand corn meal medium 1:3 (w/w) and 100ml of water. Five pots were used as replicates for each isolate as well as control. Percentage of root rot on cucumber plants was recorded one month after seed sowing as mentioned before. Fungal isolates were re-isolated from root of cucumber plants

Statistical Analysis

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

Results and Discussion

Root Rot Disease Survey

Root rot disease incidence occurred on cucumber plants grown under protected cultivation in plastic houses during winter season of 2016 and 2017 at El Giza, Kafr El-Sheickh, El-Beheira and El-Gharbeia, Governorates in Egypt were performed. As shown in (Fig. 1) chlorosis of lower to apical leaves, turned to yellowish and wilting, soften root tissues and stem base were observed. Finally, leaves become chloritic ,necrotic, and stem become girdled then died. Also, root rot of root syndromes have been observed on cucumber plants. It could be noticed from the Data illustrated in Table (1) that root rot disease syndrome in cucumber shoot system and their severity were progress increased with increasing cucumber growth, as maximum occurrence of the root rot disease percentage and their severity were observed after 3 months from sowing. The highest values of epidemic incidence of root rot disease on cucumber plants were detected in Kafr El-Sheickh followed by El-Giza due to continous cultivation cucumber before 10-12 years during winter seasons . On the other hand, the lowest root rot disease incidence was observed in El-Beheira followed by El-Gharbeia Governorates as the result of the location in this study recently cultivated with cucumber since 1-2 years ago. Similar survey for pre, post emergence damping-off and root rot on cucumber plants caused by many fungi isolated from seeds, plants and soil, i.e., *Fusarium oxysporum*, *Trichoderma* spp., *Alternaria alternata, Aspergillus niger, Macrophomina phaseolina,* and *Rhizoctonia solani* have been reported on cucumber plants by Sabet *et al.* (2013); Al-Ameiri (2014); Al-Tuwaijri (2015); Elwakil *et al.* (2015); Yousef (Safaa) *et al.* (2016); Ziedan and Saad (Moataza) (2016).

Frequency and Isolation of Fungi

Isolation from root rot disease of cucumber plants revealed the *Fusarium oxysporum*, *Fusarium solani, Alternaria alternata* and *Macrophomina phaseolina* as shown in Table (2). Data also showed that *Fusarium* spp. in particular more frequent than any other fungi, moreover, *Fusarium oxysporum* and *Fusarium solani* were the most common fungi associated with root rot diseased plants in all locations. High frequency occurrence of *Fusarium oxysporum* from El-Beheira (75%), followed El-Giza (60%). Meanwhile, high frequency of *Fusarium solani* was recorded in Kafr El-Sheickh (84.2%) followed by El-Giza (40%), then El-Gharbeia (25%). Colonization percentage of the *Macrophomina phaseolina* was recorded only in El-Gharbeia (25%). These fungi were previously reported to be associated with seeds and soil of cucumber plants (Vakalounakis, 1996; Dik *et al.* 2004; Farrag (Eman) *et al.*, 2013; Sabet *et al.*, 2013; Al-Ameiri, 2014; Al-Tuwaijri, 2015; Elwakil *et al.*, 2015 ; Ziedan and Saad (Moataza) 2016).

Pathogenicity Potential of *Fusarium* Isolates

Upon testing the pathogenicity of fungal isolates belonging to *F. oxysporum* and *F. solani* were found more or less able to attack cucumber plants caused visible damping off or root rot diseases. No symptoms of root-rot disease occurred in un-infected control (Table 3). Means of root rot disease incidence ranged from 0.0 to 44.0% . Based on the obtained results, there were significant difference in disease severity among the *Fusarium* spp. isolates. *Fusarium solani* isolate (No. 6) was the most aggressive causing root-rot on cucumber plants (44%), followed by *Fusarium oxysporum* (isolate No. 5) (18%) from El- Giza Governorate, then *Fusarium oxysporum* (isolates Nos. 4 and 2) fungal isolates from Kafr El-Sheickh. Whereas, the least aggressive isolates were *Fusarium oxysporum* (isolates Nos, 1 and 3) which isolated from Kafr El-Sheickh Governorate. These results almost similar that obtained on cucumber plant by (Farrag (Eman) *et al.*, 2013; Sabet *et al.*, 2013; Al-Ameiri, 2014; Elwakil *et al.*, 2015; Yousef (Safaa) *et al.*, 2016; Ziedan and Saad (Moataza) 2016).

Molecular identification of Fusarium isolates causing root rot diseases of cucumber plants

Fungal diagnostics using molecular techniques can be used to confirm cultural and morphological characterization and classification (Lenc *et al.*, 2008). It is based on the nucleotide sequencing where variation in the DNA sequences is used to distinguish between species, or even between individuals (O'Donnell *et al.*, 1998). Identification of *Fusarium* spp. using PCR amplification of ITS region of the rDNA using the right primer pairs is quick, accurate and reliable. Duggal *et al*. (1997) stated that the ITS region shows polymorphisms between and within *Fusarium* spp. Nevertheless, the presence of non-orthologous copies of the ITS 2 is observed in *Fusarium* genus, which may result in wrong identification (O'Donnell *et al.*, 1998). We used the two primer pairs ITS 1, ITS 4 and ITS 5, ITS2 with all *Fusarium* isolates. DNA extracted from *Fusarium* species was amplified with ITS primer pairs ITS 1/4, and ITS 5/2, (Fig. 2). PCR amplification of *Fusarium* with ITS primers 1/4, and ITS 5/2, yielded an estimated 600-bp product, and about 300bp product, respectively. PCR amplification of *Fusarium* with ITS primers 1/4 which amplify the highly variable sequences surrounding the 5.8S-coding sequence and situated between the smal subunit-coding sequence (SSU) and the large subunite-coding sequence (LSU) of the rhibosomal operon yielded slightly larger products than did amplification with ITS 5/2 (White *et al.*, 1990). Most sequence comparisons are carried out using BLASTn (http://www.ncbi.nlm.nih.gov/BLAST) analysis which aligns two or more homologues to detect for presence of one or more ambiguous region within the segments under comparison. Using seven isolates from *Fusarium*, we amplified about 600 bp DNA fragment of the ITS region (Njambere *et al.,* 2008). Five sequences of the isolates were deposited in the GenBank and assigned with accession numbers MG01877, MG018778, MG018779, MG018780, and MG018781. BLASTn analysis of the ITS locus of isolate 1, isolate 2, isolate 3 and isolate 4 and isolate 5 displayed 100%, 84%, 88%, 96% and 99% homology to ITS locus of *F. oxysporum* in the GenBank , whereas the ITS region of the other isolates (including isolates 6 and 7) were identical to GenBank *F. solani* isolates. The phylogenetic tree of all isolates showed in Fig. (3). multiple alignments of the nucleotide sequences of the 7 isolates according to the highest sequences in GenBank were performed and their homology tree was constructed using the online version of MAFFT (https://mafft.cbrc.jp/alignment/server) with the interactive refinement method (FFT-NS-i) setting. The alignments were further edited manually in MEGA v. 6.0 and deposited in Tree BASE (http://treebase.org). The Phylogenetic tree (cladogram) based on the ITS sequences showed in (Fig. 3). The seven isolates were initially split into two groups based on ITS region similarities. The results show that the ITS regions were relatively similar with a range of 88-100% within the two groups. The larger group contain isolate 1,2,3,4 and 5 with similarities ranging from 88 to 100% to *F. oxysporum* and The second subgroup were placed into groups contains two isolates 6 and 7 and composed of *F. solani* with high ITS region similarities ranging from 99 to 100% according to the data obtained from NBCI BLAST n (http://www.ncbi.nlm.nih.gov/BLAST). In conclusion, the results demonstrate that root-rot disease is a common disease in Egypt, especially in greenhouses, mainly caused by fungal isolates of *Fusarium oxysporum* and *F. solani*. The obtained results also will be helpful to furtherly study the pathogenesis, rapid detection and molecular evolutionary of the fungal pathogenic strains.

Acknowledgment

The authors extend their appreciation to the National Research Centre, Egypt for funding this work through research project No. (11030135) during 2016-2019.

Table 1: Survey of root rot disease incidence of cucumber plants grown under plastic greenhouses conditions

Governorate	**Root rot(%) and severity(DS) month after cultivation**					
	1		**2**		**3**	
	%	**D.S**	**%**	**D.S**	**%**	**D.S**
El- Giza	13.1 a	1.0 a	25.0 a	2.2 a	40.0 b	2.8 b
Kafr El-Sheickh	4.5 b	1.0 a	23.1 ab	1.6 b	81.4 a	3.3 a
El- Beheira	0.0 c	0.0 b	0.0 c	0.0 c	0.5 c	1.5 c
El- Gharbeia	0.0 c	0.0 b	0.0 c	0.0 c	0.2 c	1.2 c

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

Table 2: Frequency occurrence of different fungi isolated from diseased cucumber plants by root rot disease under plastic greenhouse conditions

Governorate	**Fungal isolate**	**Frequency occurrence% (DS)**
Kafr El-Sheickh	*Fusarium oxysporum*	15.8 h
	Fusarium solani	84.2 a
El- Giza	*Fusarium oxysporum*	60.0 c
	Fusarium solani	40.0 e
El- Beheira	*Fusarium oxysporum*	75.0 b
	Alternaria alternata	25.0 f
El- Gharbeia	*Fusarium solani*	25.0 f
	Fusarium oxysporum	50.0 d
	Macrophomina phaseolina	25.0 g

-Value followed by the same letter are not significantly different at $P \leq 0.05$ according to Duncan's multiple range.

Table 3: Pathogenicity tests of *Fusarium* isolates on cucumber plants under plastic greenhouse conditions.

Location	**Fungi isolates**		**Root rot %**
	No	**Name**	
Kafr El-Sheichk	1	*F. oxysporum*	00.0 e
	2	*F. oxysporum*	12.0cd
	3	*F. oxysporum*	00.0 e
	4	*F. oxysporum*	14.0 c
El- Giza	5	*F. oxysporum*	18.0 b
	6	*F. solani*	44.0 a
	7	*F. solani*	10.0 d
Control	0	---------	00.0 e

-Value followed by the same letter are not significantly different at $P \leq 0.05$ according to Duncan's multiple range.

Fig. 1: Root rot and symptoms of cucumber plants under plastic greenhouse at different growth stages of shoot system shown yellowish , wilting and death plants

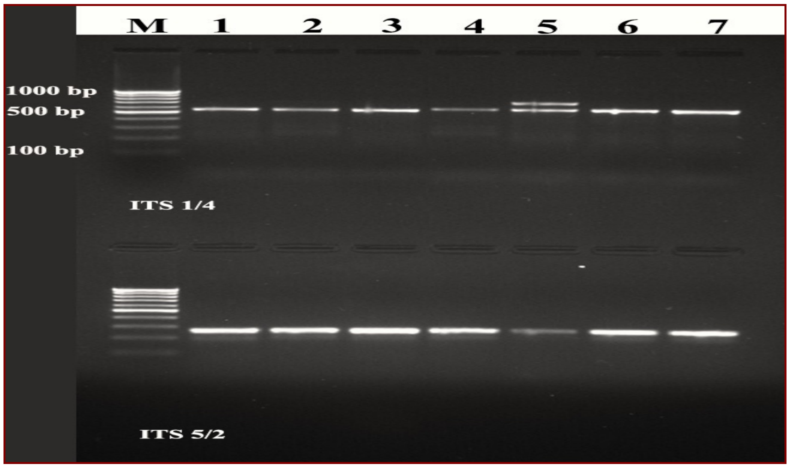

Fig. 2: Extracted DNA of *Fusarium* was amplified with primer pairs ITS 1 and 4 , ITS 5 and 2 ,DNA amplified with the same primer pairs is shown in the intervening lanes. and 100-bp DNA ladder are also shown.

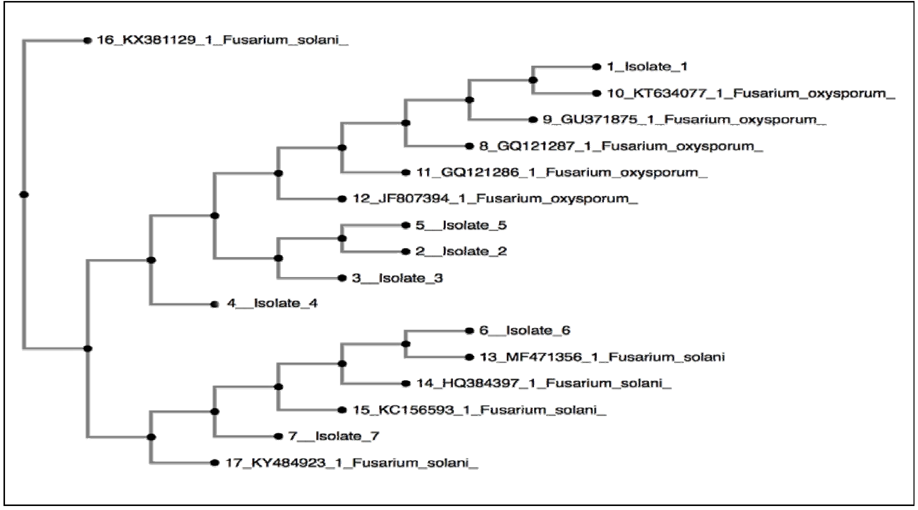

Fig. 3: Phylogenetic tree based on ITS region rDNA sequences of *Fusarium* isolates.

References

Abd Murad N.B.; Kusai, N.A. and Zainudin, I.M. (2016). Identification and diversity of *Fusarium* species isolated from tomato fruits. *Journal of Plant Protection Research*, 56(3): 232-236.

Al-Ameiri, N.S. (2014).Control of cucumber damping – off in the field by the bio-agent *Trichoderma harzianum*. International *J. of Agric. and Foresty,* 4 (2):112-117.

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

Al-Tuwaijri, M.M.Y. (2015). Studies on *Fusarium* wilt disease of cucumber. *J. of Applied Pharmaceutical Science,* 5(2): 110-119.

Baldwin, B.G. (1992) . Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: An example from the compositae. *Molec Phylogenet Evol.,* 1: 3–16.

Barnett H.L. and Hunter, B.B. (1998). Illustrated genera of imperfect fungi 4th edition APS Press st. Paul. Minnesota, USA, pp. 218.

Booth, C. (1971). The genus *Fusarium*. Kew Surrey, England: Commonwealth Mycological Inst.237 pp.

Cai, J.; Robertsand, I.N. and Collins, M.D. (1996). Phylogenetic relationships among members of the ascomycetous yeasts genera Brettanomyces, Debaromyces, Dekkera and Kluyveromyces deduced by smallsubunit rRNA gene sequences. *Int J. Syst. Bacteriol.*, 46: 542-549.

Carver, C.E.; Pittand, D. and Rhodes, D.J. (1996). Aetiology and biological control of *Fusarium* wilt of Pinks (*Dianthus caryophyllus*) using *Trichoderma aureoviride*. *Plant Pathol.,* 45: 618–630.

Cheng, T.; Xu, C.; Lei, L.; Li, C.; Zhang, Y. and Zhou, S. (2016). Barcoding the Kingdom Plantae: new PCR primers for ITS regions of plants with improved universality and specificity. *Molecular Ecology Resources*, 16(1): 138-149.

Dik, A.J.; Gaag, D.J.; Pijnakker, J. and Wubben, J.P. (2004). Integrated control strategies for all pests and diseases in several glasshouse crops and implementation in practice. *Bulletin OILB/SROP*, 27(8): 35-39.

Duggal, A.; Dumas, M.T.; Jeng, R.S. and Hubbes, M. (1997). Ribosomal variation in six species of *Fusarium*. *Mycopath.,* 140: 35-49.

Elwakil, M.A.; El-Metwally, M.A.; Elsherbinyand, A. and Eisa, K.N.M. (2015). Enhancing systemic acquired resistance in cucumber to control root rot and wilt diseases with reference to yield and quality. *Plant Pathology J.,* 14(4): 223-233.

Farrag (Eman) S.H.; Moharam, M.H.A. and Ziedan, E.H. (2013). Effect of plant extracts on morphological and pathological potential of seed-borne fungi on cucumber seeds. *Inter. J. Agric Tech.,* 9(1): 141- 149.

Khot, P.D.; Koand, D.L. and Fredricks, D.N. (2009). Sequencing and analysis of fungal rrna operons for development of broad-range fungal PCR assays. *Applied and Environmental Microbiology,* 75: 1559–1565.

Kurtzman, C.P. (1992). rRNA sequence comparisons for assessing phylogenetic relationships among yeasts. *Int. J. Syst. Bacteriol.,* 42:1-6.

Lenc, L.; Lukanowskiand, A. and Sadowski, C.Z. (2008). The use of PCR amplification in determining the toxigenic potential of *Fusarium sambucinum* and *F. solani* isolated from potato. *Phytopathol. Pol.,* 48: 13–23

Moreno, A.; Alferez, A.; Aviles, M.; Dianez, F.; Blanco, R.; Santos, M. and Tello, J.C. (2001). First report of *Fusarium oxysporum* f. sp. radicis-*cucumerinum* on cucumber in Spain. *Plant Dis.,* 85:1206.

Nelson, P.E.; Toussoumand, T.A. and Marasas, W.F.O. (1983). *Fusarium* spp. An illustrated Manual for identification. The Pennsylvania Univ, USA, 189 pp.

Njambere, E.N.; Chen, W.; Frate, C.; Wu, B.M.; Templeand, S. and Muehlbauer, F.J. (2008). Stem and crown rot of chickpea in California caused by *Sclerotinia trifoliorum*. *Plant Dis.,* 92: 917–922.

O'Donnell, K.; Cigelnikand, E. and Nirenberg, H.I. (1998). Molecular systematics and phylogeography of the *Gibberella fujikuroi* species. *Complex,* 90(3): 465-493.

Punja, Z.K. and Parker, M. (2000). Development of *Fusarium* root and stem rot a new disease on greenhouse cucumber in British Columbia, caused by *Fusarium oxysporum* f. sp. *radicis-cucumerinum*. *Canadian Journal of Plant Pathology*, 22: 349-363.

Sabet, K.K.; Saber, M.M.; El-Naggar, M.A.; El-Mougy, N.S.; El-Deebnand, H.M.; El-Shahawy, I.E. (2013). Using commercial compost as control measures against cucumber root-rot disease. *J. of Mycology, Article* ID 324570, 13 pages.

Sang, T.; Crawford, D.J. and 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.

Snedecor, G.W. and Cochran, W.G. (1980). Statistical Methods. 7th Edn. Iowa State Univ. Press, Ames, 507.

Vakalounakis, D.J. (1996). Root and stem rot of cucumber caused by *Fusarium oxysporum* f. sp. *radicis-cucumerinum* f. sp. *nov. Plant Dis.,* 80: 313-316.

Vakalounakis, D.J. and Fragkiadakis, G.A. (1999). Genetic diversity of *Fusarium oxysporum* isolates from cucumber: Differentiation by pathogenicity, vegetative compatibility and RAPD fingerprinting. *Phytopathology,* 89:161-168.

White, T.J.; Bruns, T.; Lee, S. and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *in* PCR protocols: a guide to methods and applications. *eds* Innis M.A.; Gelfand, D.H.; Sninsky, J.J.; White, T.J. (Academic Press, Inc. New York, N.Y*),* pp. 315–322.

Yousef (Safaa) A.; El-Metwally, M.M.; Gabrand, S.A. and Al-Ghadir, A.H. (2016). New strategy for managing damping-off and root rot disease of cucumber caused by *Rhizoctonia solani* by seed soaking in formula of antioxidants with micronutrients. *J. Plant Pathol. Microb.,* 4 (9): 1000196.

Ziedan, E.H. and Saad (Moataza), M. (2016). Efficacy of nanoparticles on seed borne fungi and their pathological potential of cucumber. International J. Pharma Tech Research, 9 (10):16-24.