Characterization of *Lasiodiplodia* isolates obtained from acid lime and Valencia orange in Egypt.

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ABSTRACT

A total of six *Lasiodiplodia* isolates were obtained from acid lime and Valencia orange orchards located in Markaz Bader region. Isolates identification were performed using phylogenetic analysis based on partial sequences of internal transcribed spacer (ITS) sequences, in combination with cultural and microscopic characteristics. Based on ITS sequence data, two species were identified. Only one isolate identified as *L. pseudotheobromae* and the other five isolates were identified as *L. theobromae*. All isolates were pathogenic on shoots of acid lime and Valencia orange. Nine ISSR primers were screened with the DNA of the fungal isolates to detect of polymorphism among these isolates. The estimated genetic similarities ranged from 71.7% to 89.1% with *Lasiodiplodia* isolates. *L. pseudotheobromae* was previously recorded on mango trees in Egypt, but this is the first time it has been recorded on Egyptian citrus trees.

Keywords: *Lasiodiplodia*, Characterization, acid lime, Valencia orange.

INTRODUCTION

Citrus crops are one of the most important fruit crops worldwide. The genus Citrus belongs to the family Rutaceae which is comprising of 140 genera and 1300 species distributed throughout the world.

Gummosis is one of the most important citrus diseases. It potentially cause the financial loses for farmers due to the reduction of citrus production in both quantity and quality. The disease can affect both seedlings and adult citrus plant causing the total loss of citrus production and reduce the current expansion of citrus cultivation in many growing areas in the world Julinda et al., 2017. Gummosis disease was mostly caused by *Phythoptora*, such as *P. nicotianae*, *P. palmivora*, *P. citrophthora*, but the latest findings showed that another fungal species, i.e. *Botryodiplodia theobromae* and/or *Diplodia natalensis* can also induce gummosis Wang et al., 2011.

The plant pathogens can be conventionally identified based on morphological characteristics followed by molecular method such as polymerase chain reaction (PCR) for further confirmation. The comprehensive reports related to the identification of fungal pathogens associated with gummosis are, however, still limited. Therefore, an accurate identification of fungus for early detection of plant pathogen is very important. This can also provide basic and accurate information, which is required for a successful plant disease control management Ma and Themis 2006.

PCR is a powerful tool which has been successfully reported in the identification and detection of different fungal plant pathogens. This tool has high specificity, sensitivity, rapidity and can be operated automatically Ippolito et al., 2002. PCR method has been used in many applications, including detection of plant pathogens and is generally regarded as the most sensitive test available.

The molecular markers such as random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) have been extensively used to investigate the genetic diversity of plant pathogens at strain and population level Liu et al. 2014 ; Chen et al. 2015.

Genetic cluster dendrogram based on the results of molecular markers often keeps a relationship with the geographical region, host, pathogenicity, vegetative compatibility group and other aspects of the tested isolates, and the genetic diversity and difference of intra- and inter-species from different sources could be understood by analyzing the dendrogram Shafagh et al. 2008; Baysal et al. 2010.

Sabalpara et al. 1991 described morphological variation among *Botryodiplodia theobromae* isolates. They reported that size of the immature and mature pycnidia varied greatly with the substrate. The pycnidia were smallest in naturally infected twigs and biggest in nutritionally rich medium such as oat meal agar.

The objectives of this study were to identify some isolates of *Lasiodiplodia* associated with dieback and gummosis of acid lime and Valencia orange in Egypt and confirm their pathogenicity on acid lime and Valencia orange tress.

MATERIALS AND METHODS

Sampling and pathogens isolation:

Sample collection.

Symptomatic samples were collected from affected acid lime (*Citrus aurantifolia*) and Valencia orange (*Citrus sinensis*) trees grown in orchards located in Markz badr region (Al-Buhayrah Governorate) during the late winter and early spring of 2018 growing season. The trees were carefully investigated and the plant materials (tender and wooden branches) showed symptoms of gummosis, stem cankers, and dieback were collected and kept in plastic bags, then labeled and transported to the laboratory and kept at 5 °C until processed.

Isolation

The collected samples were washed thoroughly by tap water then left until completely dried. In regard to the tender samples, small pieces (2 to 3 mm) were cut from the margin between necrotic and healthy tissues. In regard to the wooden samples, the affected bark and a portion of the healthy surrounded tissues were skinned. These prepared materials were surfaces sterilized using 5% sodium hypochlorite for 2 min then washed 3 times with sterile distilled water and left in the laminar flow cabin until dried. The dried plant materials were subsequently transferred to Petri plates containing PDA medium and incubated at 25°C. The incubated Petri plates were investigated daily and any fungal growth resulted from plant material were picked up and subcultured on PDA medium. The obtained isolates were purified on PDA medium using hyphal tip technique and coded then kept in PDA slant for further studies.

Identification of the obtained isolates

Macro and micromorphological characteristics

Preliminary identifications to the test genus and tentative species level were based on colony and conidial morphology according to Barnett and Hunter, 1998.

Cultural characteristics of the obtained isolates were verified by transferring hyphal tips from the colony margin onto fresh PDA and incubated at 25°C in the dark. The incubated cultures were being checked daily and the grows criteria were recorded. These criteria included colony color, colony growth pattern, formation of aerial mycelia, formation of pycnidia and the rate of pycnidia production during growth stages and.

Based on microscopic observations, the micromorphological characteristics of the obtained isolates were recorded. The investigated characteristics were conidial size, shape, cooler, striation, septation, conidiogenous cells, and presence or absence of septa in the immature and mature conidia.

Testing pathogenicity of the isolates

Pathogenicity of the obtained isolates was tested on acid lime and Valencia orange trees during March and April 2019. Apparently healthy young trees (13–15-month old and 70–90 cm tall) of both citrus types were used. For inoculation, the bark of the stems and branches of test plants were wounded using a sterile scalpel, and a PDA disc (5-mm diameter) taken from the edge of actively growing a 4-day-old culture was placed on a wounded site. The inoculation site was wrapped with parafilm to prevent desiccation.

Two plants (the stem and two branches per plant) were inoculated with each tested isolate. Six plants were inoculated by the same manner with non-colonized PDA discs and served as a control. To maintain humidity around the inoculated sites, immediately after inoculation, each plant was covered with a semitransparent plastic bag for 72 h. All plants were maintained in a wire greenhouse under natural conditions.

Test plants were monitored for the development of gum, dieback or death on a weekly basis for 3-4 months. Re-isolation of the tested isolates was performed from the margins of the necrotic lesions on the seedlings developing any of the above symptoms in order to confirm association of the regarded isolate. The isolated fungi were tested for pathogenicity on healthy acid lime seedlings.

Molecular identification:

DNA extraction

Aerial mycelium was scraped directly from 4-day-old pure culture grown on PDA using a sterile spatula and placed in 2-ml microtubes. Total genomic DNA of the fungal isolates was extracted using a i-genomic BYF DNA extraction Mini Kit (iNtRON Biotechnology Inc., South Korea) following the manufacturer´s instructions Sambrook et al.

1989. The concentrations of DNA were quantified using a NanoDrop Lite Spectrophotometer and the samples were diluted to 100 ng/µl for polymerase chain reaction (PCR).

Polymerase chain reaction amplification and sequencing.

Molecular identification of the fungal isolates was based on partial amplification and sequencing of internal transcribed spacer (ITS). Partial sequences of the 18S rDNA were obtained following the method of Boekhout et al., 1994. The ITS region of ribosomal DNA was amplified using the primers ITS1 (sequence: 5' TCCGTAGGTGAACCTGCGG-3';) and ITS4 (sequence: 5' TCCTCCGCTTATTGATATGC-3'.). All primers were supplied by Operon Technologies Company, Netherlands. A volume of 12 ng of each 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 4oC till analysis. The PCR products were separated by electrophoresis in 1% agarose gel at a constant 100 V for about 2 h. The different band sizes were determined against 100 bp ladder (iNtRON Biotechnology Inc., South Korea).and the separated bands were stained with 0.5 µg/ml^{-1} ethidium bromide and photographed using the gel documentation system with UV trans eliminator.

The amplified PCR products were purified using the GeneJET™ PCR Purifcation Kit (Termo K0701) and sequenced by ABI 3730xl DNA sequencer (GATC Company, Germany) in both directions with the same primers that were used for the PCR reactions.

Phylogenetic analysis

To verification of the most closely related sequence, the DNA sequences of the fungal isolate were compared to the sequences available by Gene Bank data using the BLAST tools available in the National Center for Biotechnology Information the website (NCBI, http://www.ncbi.nlm.nih.gov/blast/blast.cgi. The sequence was aligned together with those of reference taxa retrieved from public databases. The evolutionary distance was generated based on parameter model Jukes and Cantor 1969) and phylogenetic tree was constructed by using the neighbor-joining method Saitou and Nei 1987.

Comparison between isolates using Inter Sample Sequence Repeat (ISSR)

ISSR-PCR Reactions

To detection the polymorphism between the isolates, 9 ISSR primers (table 1) were used to amplify the DNA of 6 isolates (Table 1). ISSR PCR amplification accomplished according to Hong-hui Xie et al, (2016). The amplification was carried out in a 25-µl reaction volume containing 1-µl template DNA (approximately 30 ng/µl), 1 µl primer (10 µmol/l), 0.5 µl dNTP (10 mM /µL), 2.5 µl Taq Buffer, 2 µl 25 mM MgCl2, 0.2 µl Taq polymerase and 17.8 µl ddH2O.

Thermocycling Profile PCR:

PCR amplification was performed in a Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems) with initial denaturation for 5 min at 94°C, followed by 40 cycles at denatured temperature 94°C for 1 min; annealing temperature 45ºC for 1 min, elongation temperature 72 for 1.5 min and the primer extension segment was extended to 7min at 72ºC in the final cycle the final extension at 72 for 15 min.

Detection of the PCR Products:

The amplification products of ISSR PCR were size separated by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5ug/ml) in1 × TAE buffer (40 mM Tris, 20 mM acetic acid and 1 mM EDTA) at 80 V for 120 min and 100 V for 180–200 min at room temperature, respectively Hong-hui Xie et al., 2016.

The gels were visualized under UV light and photographed using a Gel Documentation System (BIO-RAD 2000). The sizes of amplified DNA fragments were estimated by comparison with 10 kb DNA ladder markers.

Data analysis

For ISSR analysis, only the clear and unambiguous amplified bands of ISSR were converted and normalized into a data pattern as binary matrix with "1" for presence and "0" for absence. Polymorphic and monomorphic bands were scored to determine the similarity among the isolates. Hierarchical clusters were analyzed to construct dendrograms, using Dice's coefficient and unweighted pair group method with arithmetic mean (UPGMA)

methods with the Euclidean similarity index using the PAST software Version 1.91 Hammer et al., 2001.

RESULTS

Isolation of the fungal pathogen:

Isolation from symptomatic materials of citrus plants on PDA medium yielded a lot of fungal growth with various cultural characteristics. After purification processes, the fungal isolates suspected to be *Lasiodiplodia* spp. were selected and non-regarded isolates were discarded. Generally, 22 fungal isolates were selected. The selected isolates were kept at 5 °C on PDA slant to accomplish the study.

Identification of the fungal pathogens.

Cultural and microscopic characteristics

The *Lasiodiplodia* isolates were characterized by colony morphology and conidial characteristics. Based on the combination between macromorphological and micromorphological characteristics, only 9 isolates from 22 tested isolates were found to be closely related to the genus *Lasiodiplodia*. These isolates were very similar to each other in their characteristics with a few differences in colony morphology. All isolates grew rapidly on PDA, covering the entire surface of the Petri plate within 4 days. The aerial mycelium was initially white, turning dark greenish-grey or greyish after 4–5 days at 25°C in the dark.

The initially appearance of the colonies of these isolates were white accompanied with fluffy and aerial mycelia. The aerial mycelium was initially white, turning dark greenish-grey or greyish after 4 to 5 days. The reverse of the Petri plates showed black color (Fig. 1).

The conidia of all isolates were initially unicellular, hyaline ellipsoid to sub ovoid. Mature conidia were dark brown, bicelled, and thick walled and ellipsoid (Fig.2, 3).

Pathogenicity test.

The pathogenicity of *Lasiodiplodia* isolates was confirmed by artificially inoculation of stem and branches of acid lime and Valencia orange plants using a mycelial disc inoculation method. Generally, all tested isolates produced typical symptoms of Lasiodiplodia gummosis disease on both hosts. After 10 to 12 days from inoculation date, the symptoms of the disease on the inoculated trees begin to appear on the sites of infection. The sites initially appeared as watery brown lesion then produced a heavy gum exudation from these sites (Fig 4). The bark was longitudinally cracked and accompanied by profuse gumming that extends up and down the inoculation sites producing canker lesions. The canker lesions extended in the inner stem and the xylem tissue gradually turned brown. Eventually, the twigs wilted and showed di-back, and then the leaves on the twigs began to defoliated (Fig. 5). Advanced stages of infection resulted in yellow, sparse foliage.

No disease symptoms were observed on the control plants. Tested isolates were consistently recovered from the diseased branches, whereas no *Lasiodiplodia* spp. was isolated from healthy control plants.

Molecular identification of fungal isolates using ITS rDNA gene.

The isolates of *Lasiodiplodia* spp were identified based on phylogenetic analyses of the ITS region. After isolation of DNA from the test fungal isolates, the region of the rDNA repeat unit that includes the ITS from the genomic DNA were amplified using the ITS1 and ITS4 primers. After amplification, fragments of approximately 500 bp were determined (Fig.6). After the DNA sequencing of the purified PCR products, the amplified sequences were used as BLAST queries against the NCBI database for the sequence similarity searching. The BLAST searches in GenBank showed 99–100% identity with reference sequences of representative isolates *Lasiodiplodia theobromae* or *Lasiodiplodia pseudotheobromae*. Based on ITS sequence data two species were identified. Only one isolate identified as *L. pseudotheobromae* and the other five isolates were identified as *L. theobromae*.

The obtained DNA sequences with the identified fungal isolates were conserved in the GenBank under the following accession number: MW965745, MW965746, MW965747, MW965748, MW965749 and MW965750.

ISSR:

Nine ISSR primers were screened out to detection the polymorphism among the tested isolates. The primers constantly amplified DNA and the bands between 450 and 7200 bp in length (500–4000 bp in most instances) were generated. There was a high level of genetic diversity among those isolates according to gene diversity information index based on ISSR molecular markers. Dendrograms produced from UPGMA analysis based on Dice's similarity coefficient ISSR molecular markers showed that, the similarity coefficient ranged from 0.775 to 0.975. The dendrogram divided the 6 isolates into 3 branches. The

isolate 1 was independently clustered in a branch and the isolates, 2 and 5 were clustered together while the isolates, 4, 6 and 7 were clustered together.

DISCUSSION

In the present study, Lemon and sweet orange trees showed dieback, branch cankers and gummosis symptoms that located in Markz badr locality (Al-Buhayrah - Egyptian Governorate) were sampled for isolation the of the associated pathogens during the winter of 2018. Isolation process from these collected samples resulted in obtain many fungal isolates suspected to be *Lasiodiplodia* spp.

Morphological characteristics combined with analyses of DNA sequences allowed us to identify and characterize L. theobromae and *L. pseudotheobromae* obtained from acid lime and Valencia orange. Depending on the identification schema, only 6 isolates belongs to the genus *Lasiodiplodia spp.* were found to be pathogenic. These isolates were identified depending on DNA sequencing to their species level as *L. theobromae* and *L. pseudotheobromae*.

Several Botryosphaeriaceae species are known to occur worldwide, causing dieback, canker and fruit rot on various hosts. *L. theobromae* is a plant pathogen that causes different plant diseases and has become an enormous economically important problem in almost fruit crops all over the world. It is distributed worldwide within tropical and subtropical regions and considered a serious threat to the cultivation of citrus crops and its postharvest quality. Members of the Botryosphaeriaceae family are known to cause gummosis on many woody plants worldwide. *Lasiodiplodia theobromae* was the most frequented pathogen isolated from citrus species showed these symptoms Sakalidis et al., 2011b.

Adesemoye et al., 2014 reported that, from various fungal species isolated from necrotic tissues of branch canker and rootstock samples of citrus species, 74 isolates were belonging to the Botryosphaeriaceae family, with *Neofusicoccum* spp., *Dothiorella* spp., *Diplodia* spp., (teleomorph *Botryosphaeria*), *Lasiodiplodia* spp., and *Neoscytalidium dimidiatum* (teleomorphs unknown) accounting for 39, 25, 23, 10, and 3% of the total, respectively.

Surveys conducted by Akila Berraf-Tebbal et al., 2020 in 10 citrus orchards in t Algeria revealed five species of Botryosphaeriaceae belonging to three genera associated with diseased trees. Thy reported that, morphological and cultural characteristics as well as phylogenetic analyses the ITS region and the tef1-α identified *Diplodia mutila, Diplodia seriata*, *Dothiorella viticola, Lasiodiplodia mediterranea* and *Lasiodiplodia mithidjana* sp. *nov*.

Colonies of *L. pseudotheobromae* were initially white, with fluffy and aerial mycelia, then, the colonies turn grew to black and spreading with superficial dark-branched septate mycelia. Black color of mycelia on reverse sides of Petri plates was visible. The conidia of the pathogen were initially unicellular, hyaline ellipsoid to subovoid. Mature conidia were dark brown, bicelled, and thick walled and ellipsoid described by Hong-hui Xie et al. 2016.

Pathogenicity tests were conducted to verify the ability of the fungal isolates to cause dieback and gummosis symptoms on artificially inoculated citrus plant. all tested isolates produced were pathogenic to acid lime and Valencia orange. The symptoms begin to appear on inoculated trees after 10 to 12 days from inoculation date. The symptom gradually devolved causing stem canker, browning of the xylem tissue and eventually twigs di-back and leaves defoliation resulting in yellowing and sparse foliage. Shahbaz et al. 2009 studied pathogenicity of *L. theobromae* by stem inoculation method after 30 days, lesion development was measured distal to the point of inoculation.

However, the morphological variability within species was established, morphology alone is not reliable for distinguishing different *Lasiodiplodia* species. The ITS universal barcode marker was used to identify the fungal isolates. The isolates of *Lasiodiplodia* spp were identified based on phylogenetic analyses of the ITS region. BLAST analysis of the nucleotide sequence of the ITS region of the isolates showed 99–100% similarity with reference sequences of representative isolates of *Lasiodiplodia theobromae* or *Lasiodiplodia pseudotheobromae.* Based on ITS sequence data two species were identified. Only one isolate identified as *L. pseudotheobromae* and the other five isolates were identified as *L. theobromae*. The obtained DNA sequences with the identified fungal isolates were conserved in the GenBank under the following accession number: MW965745, MW965746, MW965747, MW965748, MW965749 and MW965750. Phillips et al., 2019 suggest that combined LSU and ITS provide reliable resolution for phylogeny of Botryosphaeriales. In previous studies,

phylogenetic analyses were solely based on ITS nucleotide sequences to identify *Lasiodiplodia* species Abdollah Zadeh, 2010.

Nine ISSR primers were screened out to detection the polymorphism among the tested isolates. The primers constantly amplified DNA and the bands between 450 and 7200 bp in length (500–4000 bp in most instances) were generated. There was a high level of genetic diversity among those isolates according to gene diversity information index based on ISSR molecular markers. Dendrograms produced from UPGMA analysis based on Dice's similarity coefficient ISSR molecular markers showed that, the similarity coefficient ranged from 0.775 to 0.975. The dendrogram divided the 6 isolates into 3 branches. The isolate 1 was independently clustered in a branch and the isolates, 2 and 5 were clustered together while the isolates, 4, 6 and 7 were clustered together.

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Table 1. ISSR primers used to amplify the DNA of 6 *Lasiodiplodia* isolates

Primer Name	Sequence
ISSR-1	5'-AGAGAGAGAGAGAGAGYC-3'
ISSR-2	5'-AGAGAGAGAGAGAGAGYG-3'
ISSR- 3	5'-ACACACACACACACACYT-3'
ISSR- 4	5'-ACACACACACACACACYG-3'
ISSR -5	5'-GTGTGTGTGTGTGTGTYG-3'
ISSR -6	5'-CGCGATAGATAGATAGAT-3'
ISSR- 8	5'-AGACAGACAGACAGACGC-3'
ISSR -9	5'-GATAGATAGATAGATAGC-3'
ISSR -16	5'-GACAGACAGACAGACAAT-3'

Table 2: Percent Identity Matrix - created by Clustal2.1

	TLI_1	TLI _2	TLI _4	TLI _5	TLI _6	TLI _7
TLI _1	100					
TLI _2	98.0	100				
TLI _4	99.0	98.2	100			
TLI _5	99.4	98.2	99.2	100		
TLI _6	99.4	98.6	99.6	99.6	100	
TLI _7	99.0	98.2	100	99.2	99.6	100

Table 3: Matrix statistical analysis of (ISSR) for six isolates of *Lasiodiplodia*.

	1	2	4	5	6	7
1	100					
2	71.7	100				
4	81.1	83.2	100			
5	75.4	84.3	79.4	100		
6	79.6	77.6	84.8	84.4	100	
7	81.9	79.4	85.2	80.0	89.1	100

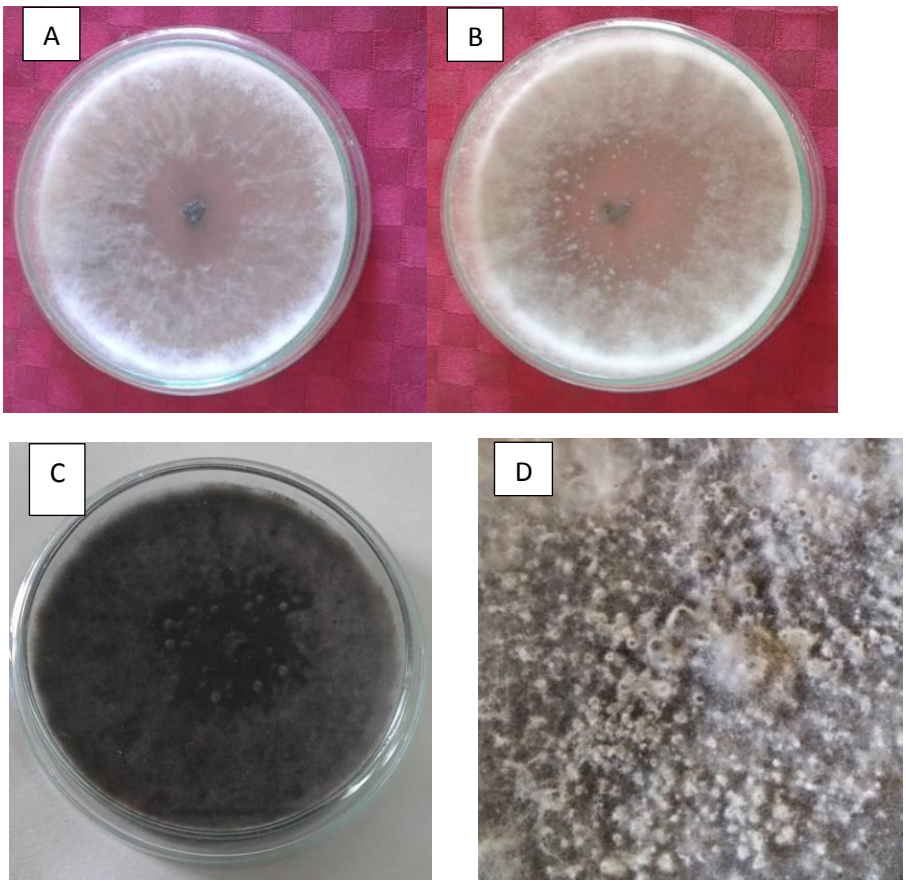

Figure 1: Colony characteristics of *Lasiodiplodia theobromae*:

a) 4-day-old white to light-cream colony.
b) 6-day-old dark gray colonies, fluffy aerial mycelium.
c) Pycnidia produced.
d) Liquid secretions may mark the open position of the conidia

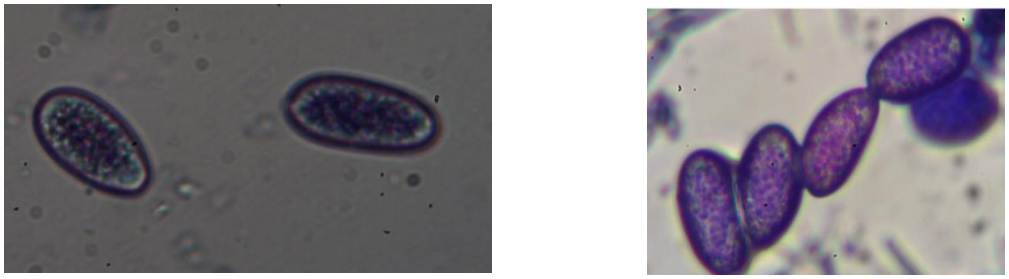

Figure 2: Conidia characteristics of *Lasiodiplodia theobromae.* Conidia are oval, both ends round and blunt, wider in the middle, initially transparent and aseptate or not partitioned.

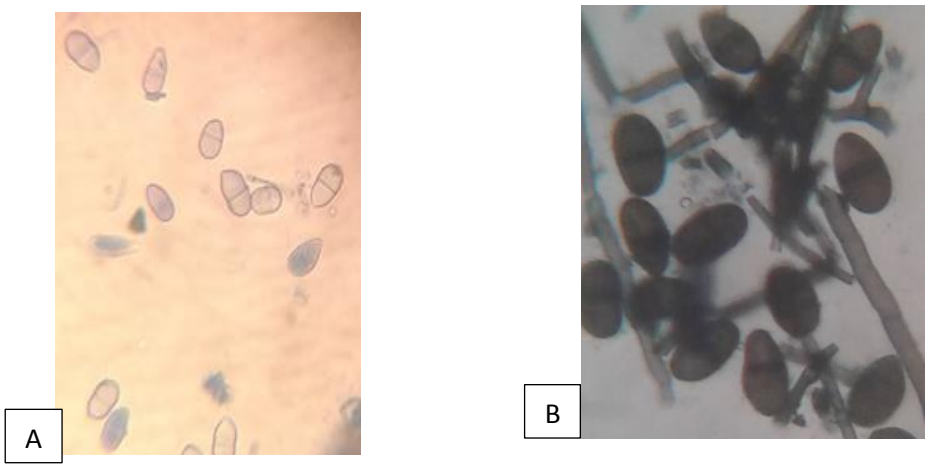

Figure 3. (A) Pre-maturity bi-celled conidia, (B) After maturity; melanin appeared in the conidia and appeared as a dark brown, bi-celled, thick walled.

Figure. 4: Initially appearance of infection on stem of Valencia orange after artificially inoculation by *Lasiodiplodia* isolate No. 3/10 (The inoculation site appeared as watery brown lesion).

Figure.5: Gum exudation as advanced symptoms on the sites of infection.

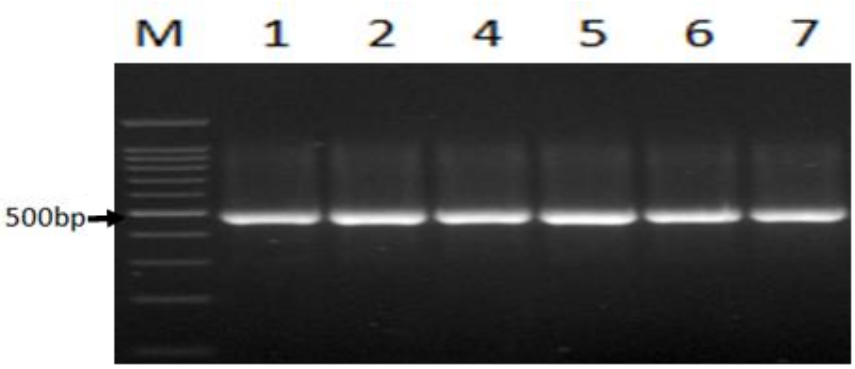

Figure 6: Photograph of ITS-DNA amplified bands for six isolates belongs to *Lasiodiplodia spp*. using ITS1 and ITS4 primers against 100 bp ladder DNA marker (lane M).

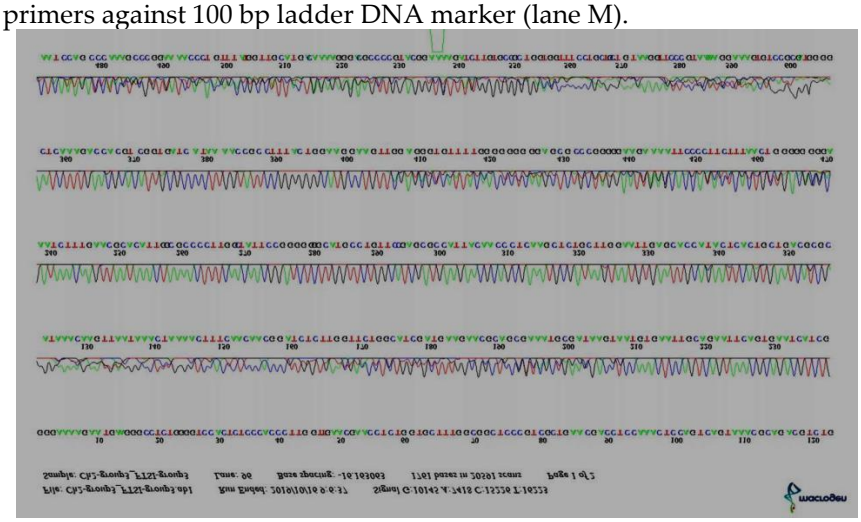

Figure 7: Partial chromatogram of DNA sequencing of ITS-DNA for *Lasiodiplodia pseudotheobromae* (strain TLI01) isolated from open field lemon trunk.

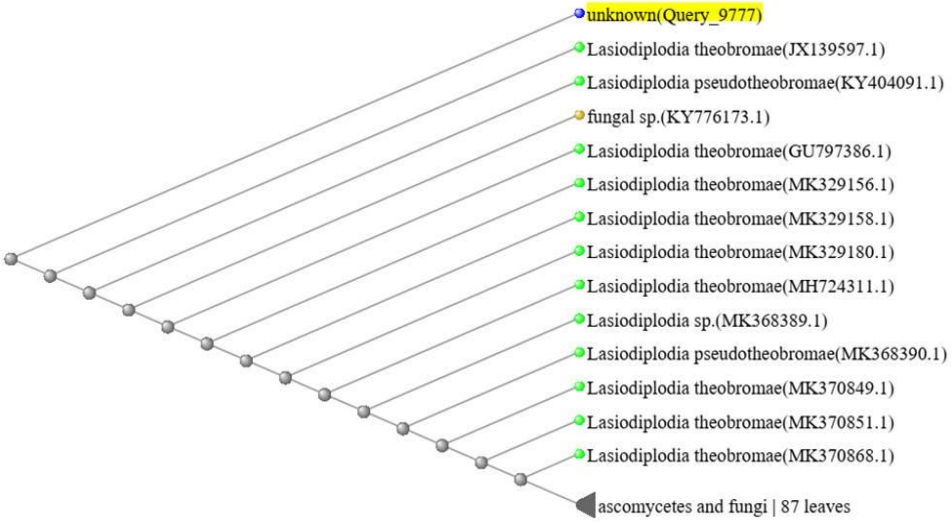

Figure 8: Phylogenetic dendrogram showing the taxonomic position of *Lasiodiplodia pseudotheobromae* (strain TLI01) isolated from open field lemon trunk, based on the ITS sequences and other closely related species available from NCBI

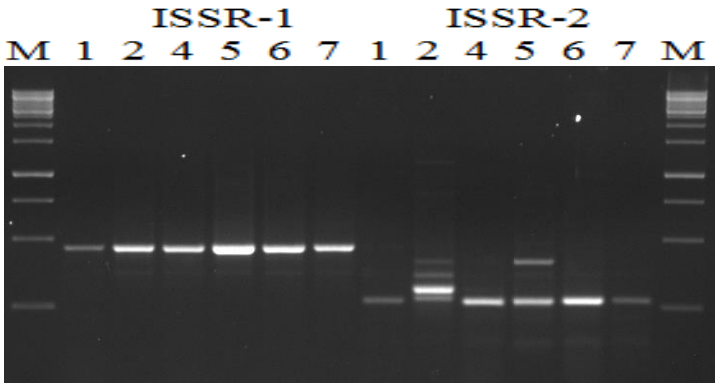

Figure 9: Gel electrophoresis of ISSR PCR products of 6 *Lasiodiplodia* isolates obtained by primers ISSR-1and ISSR-2.

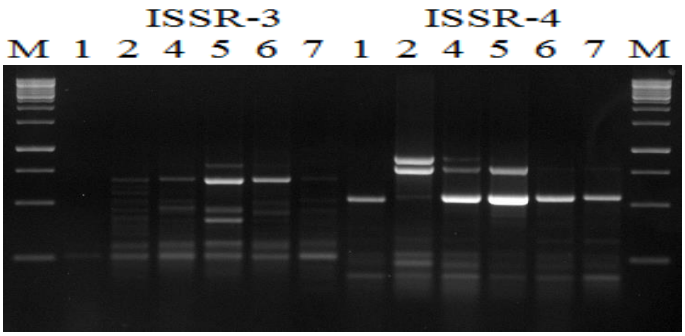

Figure 10: Gel electrophoresis of ISSR PCR products of 6 *Lasiodiplodia* isolates obtained by primers ISSR-3and ISSR-4.

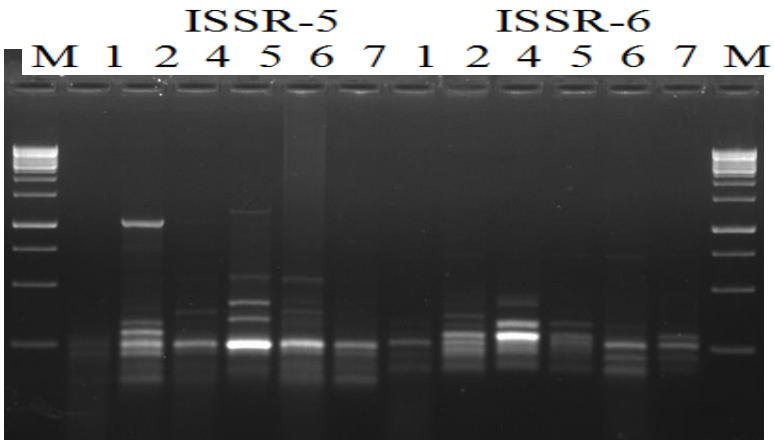

Figure 11: Gel electrophoresis of ISSR PCR products of 6 *Lasiodiplodia* isolates obtained by primers ISSR-5 and ISSR-6.

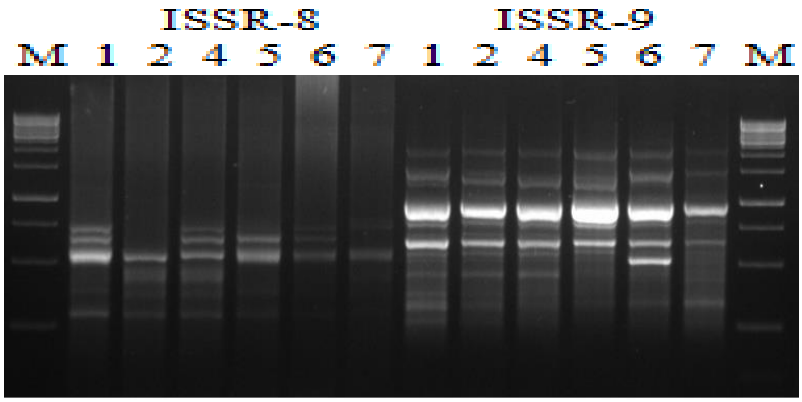

Figure 12: Gel electrophoresis of ISSR PCR products of 6 *Lasiodiplodia* isolates obtained by primers ISSR-8 and ISSR-9.

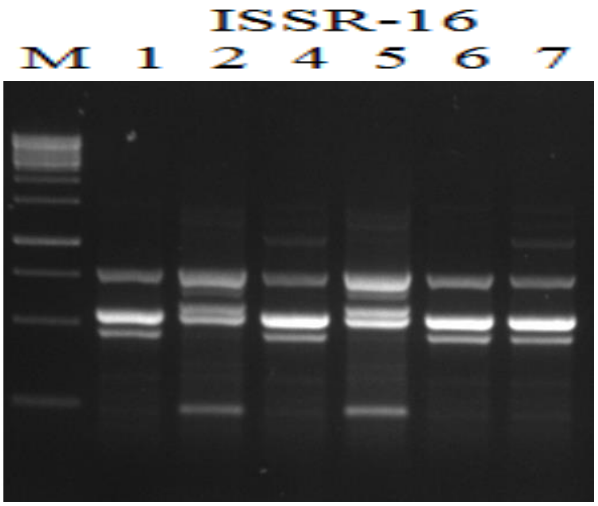

Figure 13: Gel electrophoresis of ISSR PCR products of 6 *Lasiodiplodia* isolates obtained by primer ISSR-16.

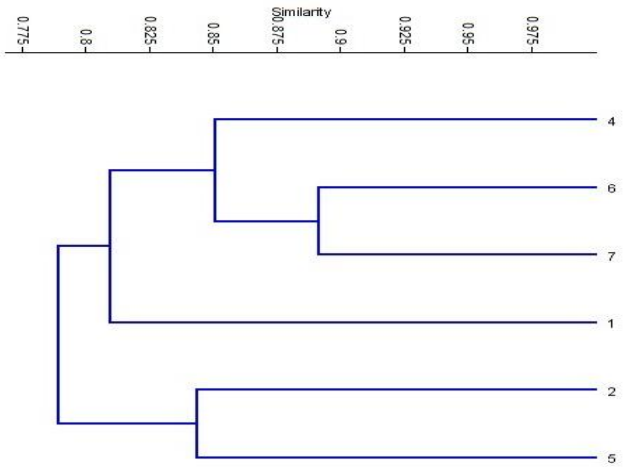

Figure 14: Dendrogram for the six isolates of constructed from the ISSR analysis using arithmetic averages (UPGMA) and phylogenetic tree was performed according to Euclidean similarity index using the PAST software Version 1.91.

توصيف عزلات من الفطر *Lasiodiplodia* المعزولة من الليمون الحامض والبرتقال فالنسيا في مصر.

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الملخص العربى:

تم الحصول على ست عزلات من الفطر *Lasiodiplodia* معزولة من الليمون الحامض والبرتقال فالنسيا من منطقة مركز بدر التابعة لمحافظة البحيرة- مصر. تم تعريف العزلات المتحصل عليها إعتماداً على الصفات المورفولوجية و الميكروسكوبية وكذلك باستخدام تقنية (Internal transcribed spacer (ITS - PCR. تم تحديد نوعين من الفطر منهم عزلة واحدة فقط على أنها *L. pseudotheobromae*. وتم تحديد الخمس عزلات الأخرى على أنها *L. theobromae* جميع عزلات *Lasiodiplodia* المتحصل عليها كانت ممرضة على أفرع الليمون الحامض والبرتقال فالنسيا. تم الكشف عن الإختلافات بين العزلات بإستخدام تسع بريمرات فى اختبار ISSR مع الحمض النووى للعزلات وتبين أن نسبة التشابهات الجينية بين العزلات تراوحت ما بين 71,7% إلى 89,1%. تم تقييم الإختلافات في الشراسة المرضية بين العزلات ، أظهرت العزلات اختلافات كثيرة فيما بينها في الشراسة المرضية.

الكلمات الاسترشادية: توصيف, الليمون الحامض, البرتقال فالنسيا.