In Basrah,grapevines suffer from dieback.Lasiodiplodia theobromae and Neoscytalidium dimidiatum were isolated from diseased grapevinesVitis vinifera L.and identified based on morphological characteristics and DNA sequence data of the rDNA internal transcribed spacer (ITS)region . The results of the pathogenicity test conducted under greenhouse conditions for L.theobromae and N.dimidiatum revealed that both species were the causal agents of grapevines diebacks in Basrah, Southern Iraq.A brief description is provided for the isolated species.Histopathological changes of 2-years old grapevine cv.Abbassi inoculated with Lasiodiplodia theobromae(syn.Botryodiplodia theobromae)were studied. Samples were taken at 7 and 25 days after artificial inoculation.At 7 days,cross and longitudinal sections revealed the presence of disorganized cells,degenerated areas and vascular bundles.The intra–and intercellular invasion of the inoculated tissues by the fungus were evident with dark brown color.At 25days,the fungus caused necrosis in xylem parenchyma and xylem vessels,the hyphae colonized the tissues.Dark deposits appeared in vessels and other tissues as well as abundant production of ty.

**Mohanad Khalaf Mohammed Ameen**

Dr. Mohanad Khalaf Mohammed Ameen Msc degree in entomopathogenic fungi in 1999from Basrah University, PhD degree in soil fungi in 2007 from Basrh University .He has worked in different projects of mycology field including phytopathology and biological control of many diseases and pest.

978-3-659-34851-8

Mohanad Khalaf Mohammed Ameen
Abdullah H.Al-Saadoon

# Study on grapevine die-back in Basrah southern Iraq

## Grapevine dieback in Iraq: pathogens,classification,genitic,and histopathology

LAP LAMBERT Academic Publishing

**Mohanad Khalaf Mohammed Ameen**
**Abdullah H.Al-Saadoon**

# Study on grapevine die-back in Basrah southern Iraq

Mohanad Khalaf Mohammed Ameen
Abdullah H.Al-Saadoon

# Study on grapevine die-back in Basrah southern Iraq

## Grapevine dieback in Iraq: pathogens,classification,genitic,and histopathology

LAP LAMBERT Academic Publishing

**Impressum / Imprint**
Bibliografische Information der Deutschen Nationalbibliothek: Die Deutsche Nationalbibliothek verzeichnet diese Publikation in der Deutschen Nationalbibliografie; detaillierte bibliografische Daten sind im Internet über http://dnb.d-nb.de abrufbar.


Bibliographic information published by the Deutsche Nationalbibliothek: The Deutsche Nationalbibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data are available in the Internet at http://dnb.d-nb.de.


Coverbild / Cover image: www.ingimage.com

Verlag / Publisher:
LAP LAMBERT Academic Publishing
ist ein Imprint der / is a trademark of
AV Akademikerverlag GmbH & Co. KG
Heinrich-Böcking-Str. 6-8, 66121 Saarbrücken, Deutschland / Germany
Email: info@lap-publishing.com

Herstellung: siehe letzte Seite /
Printed at: see last page
**ISBN: 978-3-659-34851-8**

# Study on grapevine dieback in Basrah southern Iraq

**Edited**

**By**

**Mohanad khalaf Mohammed Ameen**

**Departmet of Biology,College of Science,Basrah University**

**E mail:Mohanadkma@yahoo.com**

**TABLE OF CONTENTS**

## List of figures

**Chapter 1**





**Chapter 2**

# Chapter 1:

African Journal of Biotechnology Vol. 11(95), pp. 16165-16171, 27 November, 2012
DOI: 10.5897/AJB12.010
ISSN 1684–5315 ©2012 Academic Journals

# First report of grapevine dieback caused by *Lasiodiplodia theobromae* and *Neoscytalidium dimidiatum* in Basrah, Southern Iraq

Abdullah H.Al-Saadoon[(1)] Mohanad K.M. Ameen[(1)*] Muhammed A.Hameed[(2)] Adnan Al-Badran[(1)] and Zulfiqar Ali [(3)]

(1)Department of biology,College of science,Basrah university
(2) Date Palm Research Centre ,Basrah university
(3)Departments of Plant Breeding and Genetics, University of Agriculture, Faisalabad 38040, Pakistan

**Abstract :**

In Basrah , grapevines suffer from dieback .*Lasiodiplodia theobromae* and *Neoscytalidium dimidiatum* were isolated from diseased grapevines*Vitis vinifera L.*and identified based on morphological characteristics and DNA sequence data of the rDNA internal transcribed spacer (ITS) region . The results of the pathogenicity test conducted under greenhouse conditions for *L. theobromae* and *N.dimidiatum* revealed that both species were the causal agents of grapevines diebacks in Basrah, Southern Iraq.A brief description is provided for the isolated species .

Key words: grapevine,dieback,*Lasiodiplodia theobromae* and *Neoscytalidium dimidiatum,*ITS,rDNA,Iraq

## Introduction:

Grapevine *Vitis vinifera* L. is the most widely planted fruit crop worldwide and is cultivated on all continents except Antarctica (Mullins *et al*.,1992).The area under plantation in Iraq is 240,000 hectares, and generate an annual crop of 350,000 tons grape(FAO,1996).It is an economically important fruit crop globally. In Basrah

Province, Southern Iraq ,grapevines are mainly cultivated in the Abo-Alkasib area which is located near Shatt Al-Arab river and dominated by date palm plantations.

In a survey of grapevines grown in Basrah,several vineyards were found to suffer from dieback disease that killed the plants. The water salinity of the Shatt Al-Arab at Qarmatt Ali was found to be approximately 0.5 p.p.t but in recent years it was noted as being more than 2 p.p.t.Several of the contributing factors for the increase in salinity are likely to have been the great reduction in levels, continuous flushing of salts from irrigated land via drainage canals into the river and the linkage of the of the southern part of Haur Al-Hammar(at Qarmat Ali) to a new canal, the "Al-Basrah Canal", which run parallel to Shatt Al-Arab river into the Arabian Gulf(Bedair,2006).

Many environmental stress factors weaken plant hosts and make them more susceptible to infection by fungi. Out of these factors is the salinity which may contribute to reduce plant growth through the effect on physiological functions such as photosynthesis, respiration and absorption(Bernstein and Hayward, 1958;Leonova and Shevyalova,1970;Alsaidi,2000),and increase the vulnerability to organism.Reckhaus(1987) considered that stress factors enhanced the severity of dieback disease caused by Botryosphaeriaceae species.

Species of the Bortyosphaeriaceae are economically important opportunistic pathogens, as well as being saprophytes on decaying plant materials, whereas others are entirely endophytic(Barr,1987;Smith *et al.*1996;Denman *et al.*,2000).Many of these fungi cause canker and dieback diseases on numerous plant hosts throughout the world(von Arx,1987 ;Burgess *et al.*,2006;Slippers and Wingfield,2007).The taxonomy and identification of Botryosphaeriaceae is mostly based on anamorphic descriptions, because the teleomorphs are observed rarely in nature and seldom produced in culture(Jacobs and Rehner,1998;Denman *et al.*2000;Zhou and Stanosz,2001;Phillips,2002;Crous *et al.*,2006;Schoch *et al.,*2006).In the recent studies on the basis of 28s rDNA sequence data and multi-gene approach,16 genera

were related to Botryosphaeriaceae ( Crous *et al.*,2006;Damm *et al.*,2007;Phillips *et al.*2008).The anamorphic states of this group, including *Diplodia, Lasiodiplodia, Neofusicoccum* and *Neoscytalidium* have been reported from infected grapevines as a causal agents of cankers, dieback, bud mortality and bunch rot(Phillips,2002;van Niekerk *et al.*,2004;Luque *et al.*,2005;Taylor *et al.*,2005;Úrbez-Torres *et al.*,2006a,2008).Species in a Botryosphaeriaceae have been reported to cause various dieback symptoms in grapevines. These symptoms include wood streaking, shoot dieback, bud necrosis, cane bleaching and graft failure( Lehoczky,1974;Larignon *et al.*2001;Phillips,2002;Taylor *et al.*2005,Úrbez-Torres *et al.,*2006b,2008).The symptoms are characterized by the slow development and their severity increased with age of the grapevines that are 8 or more year old or that are subjected to stress(Boyer,1995;Larignon and Dubos,2001).Some of the symptoms,especialy brown wood streaking, are resemble those found in trunks and arms affected by Petri disease and esca complex caused by *Phoeomoniella chlamydospora*(Mugnai *et al.*,1999;Phillips ,2002).Various species in this family can be isolated from grapevine are only weakly pathogenic or entirely non-pathogenic(Phillips,2002).

*Lasiodiplodia theobromae*(Pat.) Griffon and Maubl.,is a common plant pathogen in the tropical and subtropical areas of the world. It is associated with various diseases including cankers, dieback and root rot on a wide range of hosts(Punithalingam,1980).Vascular cankers and grapevine dieback caused by *Lasiodiplodia theobromae* first were reported in Egypt in 1972(El-Goorani and El-Meleigi,1972).After fifteen years, a field study carried out in California showed *L.theobromae* to be an important grapevine pathogen(Laevitt and Munnecke,1987).Recent studies showed that the fungus has been a significant pathogen associated with declining of grapevine in south Africa and Australia(van Neikerk *et al.,*2004;Taylor *et al.*,2005;wood and wood,2005).A study conducted in vineyards of Sonora and Baja California revealed that perennial cankers and consequent grapevine dieback caused by *L.theobromae* and *Diplodia seriata* were a

major problem in the most important grape-production areas of Mexico(Úrbez-Torres *et al.*,2008).Canker of grapevine caused by a Botryosphaeriaceae species first was reported in Sicily, and the first reported of infections caused by *L.theobromae* on grapevine in Italy(Burruano *et al.,*2008).

*Neoscytalidium* was introduced by Crous and Slippers as a new genus in the Botryosphaeriaceae and the type species designated is *Neoscytalidium dimidiatum* (Penz.) Crous and Slippers,comb.nov.(Crous *et al.*,2006). *Neoscytalidium dimidiatum* has been reported on diverse woody plants, frequently as *Hendersonula toruloidea* (Punithalingam and Waterston ,1970;Sutton and Dyko,1989;Farr *et al*.,2004).Although reported to be cosmopolitan ,the disease caused by this fungus tend to occur in tropical countries as well as California (Farr *et al*.,2005).Symptoms include gummosis and dieback of stone fruit trees in Egypt (Nattrass,1933),branch wilt, decline and death on citrus in Iran (Alizadeh *et al*. 2000),leaf spot diseases in India (Chandra,1974) and a canker and a dieback of *Eucalyptus* in Arizona ( Matheron and Sigler,1993).So far little attention has been given to mycotic grapevine diseases in Iraq. Early research by Natour and Ahmed (1969) reported that *Hendersonula toruloida* caused branch wilt of grapevines, in the central region of Iraq.Recently Saido(2007) isolated some fungi associated with grapevine decline in Duhok Province, Northern Iraq, includes particularly *Botryosphaeria* spp.,*Phaeoacermonium aleophilum* and *Cylindrocarpon destructans.*these represented new records for Iraq.Therefore,this is the first report on fungi grapevine diseases in Basrah region, Southern Iraq.

**Material and Methods:**

Symptomatic grapevine parts (arms, canes and leaves) were collected from several vineyards in Abo-alkasib area,Basrah Province, Southern Iraq. Small pieces of infected tissue, taken from the margin between necrotic and apparently healthy

tissue, were surface sterilized with 5% sodium hypochlorite for 5 min.and,rinsed three times with sterile distilled water. The surface– sterilized pieces were placed in Petri dishes lined with sterilized, moist filter paper and 10 pieces were placed in Petri plates containing potato dextrose agar(PDA)(Himedia Laboratories Pvt.Ltd.-india) supplemented with chloramphenicol (50 µg/ml)to prevent bacterial growth. All plates were incubated at 25 $^{0}$C until fungal propagules were observed. Pure cultures of each isolate were obtained by excising a hyphal tip on to plates of potato dextrose agar. In order to enhance sporulation ,cultures were placed on 2% water agar bearing pieces of autoclaved grapevine canes and inoculated at 25 $^{0}$C.Isolates were examined weekly for the formation of pycnidia and conidia. Isolated fungi were identified based on the morphological characters in culture and on natural substrates.

**Molecular phylogenic characterization**

**DNA isolation**

Total genomic DNA was isolated from fresh mycelium according to a miniprep protocol described by Cenis (1992).Pure cultures of each isolate were inoculated onto 500 µl of liquid potato dextrose medium (Difco Laboratories Ltd.,Surrey,United Kingdom) and incubated at 25 ºC for72 h.The mycelial mat was pelleted by centrifugation at 13,000 rpm for 5 min and was washed with 500 µl of Tris-EDTA,then homogenized by hand in 300 µl of extraction buffer (200 mM Tris-HCl [pH 8.5],250 mM NaCl,25 mM EDTA, and 0.5% sodium dodecyl sulfate) for 5 min. 150 µl of 3 M sodium acetate (pH 5.2) was added, and the mixture was cooled to 20°C for 10 min. Fungal debris was pelleted by centrifugation at 13,000 rpm for 5 min, the supernatant transferred to a fresh tube, and an equal volume of isopropanol was added. DNA was then pelleted by centrifugation at 13,000 rpm for 10 min and washed with 70% ethanol, then air dried and resuspended in 100 µl TE (10 mM Tris-HCl [pH 8.0], 1 mM EDTA).10 mg/ml of RNase was added at 37ºC for 45 min.to digest the RNA. The DNA quantified using Eppendorf® BioPhotometer and was stored at -20ºC.

**PCR amplification of ribosomal DNA regions**

The universal primers ITS1-F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') were used to amplify the ITS/5.8S coding rDNA gene regions as described by White *et al*. (1990).Each PCR reaction mixture contained 5 ng of genomic DNA, 1µM of each primer, PCR reaction buffer (50 mM KCl,50 mM Tris-HCl;[pH 8.3] 0.1 mg/ml bovine serum albumen), 3 mM $MgCl_2$, 200 µM of each dNTP, 2.5 U of Taq DNA polymerase (Promega) and autoclaved $d_2H_2O$ to make-up the total volume of 50 µl. The PCR protocol consisted of an initial denaturation step at 95°C for 2 min,followed by 30 cycles of 94°C for 1 min, 54°C for 30 s, and 72°C for 1 min and a final extension at 72 °C for 10 min. The PCR products were separated on a 1.2% agarose gel, stained with ethidium bromide and viewed with ultraviolet light. PCR amplified fragments were purified using QIAquick PCR (Qiagen, Amersham, Piscataway, NJ, USA) columns following the manufacturer's instructions and negative control (PCR reagent mixture) was included in the amplification.

**DNA sequencing**

PCR products were purified using the Promega gel and PCR clean-up system. The instructions of the QIAquick Gel extraction kit (QIAGEN) were followed and directly cycle sequenced in both directions using the BigDye Ready Reaction Kit (PE Applied Biosystems, Foster City, California) on an ABI Prism automated DNA sequencer (model 377, version 2.1.1;Applied Biosystems Warrington,United Kingdom) with the same primers.

**Phylogenetic analysis**

The sequence results from all samples when compared to DNA database demonstrated that they were derived from the fungal ITS regions. To identify the

species, a sequence similarity search was performed using BLAST (Basic Local Alignment Search Tool) at NCBI website (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The most similar 100 sequences were downloaded from NCBI and aligned using ClustalX2.

**Pathogenicity test:**

Pathogenicity tests were made on 2 years old grapevine plants of the Abbassi cultivar. In each experiment, apparently healthy looking plants were selected and specimens were taken from their arm, cane and leaves to confirm the absence of the test pathogens. Plants found infected with the test pathogen(s) were not used in the study. A cut in the arm, cane and leaf was made using a sterile sharp blade and inoculated by spraying either 40 ml of conidial suspension (5 x $10^6$ conidia $ml^{-1}$)(as above) or sterile water(controls) using an atomizer .The conidia quantified microscopically using a Neuberger chamber and adjusted to $10^6$ conidia/ml with $d_2H_2O$ containing 0.02% Tween 20. Plants were monitored for one week to observe the development of disease symptoms. To satisfy Koch´s postulates, small pieces of necrotic tissue were cut from the edge of each lesion, surface–sterilized for 5 min. with 5% sodium hypochlorite, washed three times with sterile distilled water and placed on PDA Petri plates, then incubated at 25 °C. After 4 days of inoculation, small pieces/tissues were examined for lesions/disease symptoms.

**Results and discussion:**

**Morphology :**

Two organism were consistently isolated from plant parts showing dieback symptoms. Based on the morphological characters, isolates from the Basrah Region of Southern Iraq were identified as *L. theobromae* and *N. dimidiatum*. The identification of the isolates was performed using the relevant publications,Ellis(1971), Punithalingamm(1976,1980), Sutton and Dyko(1989), Crous *et al.*(2006) and Alves *et al.*(2008)Colonies of *Lasiodiplodia theobromae*

white,becomign dark olivaecious with dense aerial mycelium (Fig.1,A).Pycnidia were dark brown to black formed on grapevine canes after 20 days in culture(Fig.1,B).Conidiogenous cells hyaline with cyliderical hyaline and aseptate paraphyses(Fig.1,D).Conidia were initially hyaline becoming dark brown with one septa when mature and longitudinal striations,17-23x10-12 µm (Figs.1,C.D) .This is first record of this fungus as a pathogen of grapevines in Iraq. The climatic conditions of Basrah city is mostly humid with high temperatures. These conditions might be favorable for infection by this pathogen. This result is agreed with other studies (Punithalingam,1980; Leavitt, 1987;Hewitt,1988;Úrbez-Torres,2006a,2008; Burruano *et al.,*2008).This fungus has been isolated from grapevine showing decline and dieback symptoms in Egypt (El-Goorani and El-Meleigi,1972), Califonai (Leavitt,1987), Arezona,Mexico(Leavitt,1990), Portugal (Phillips, 1998, 2002), Australia(Castillo -Pando *et al.,*2001;Taylor *et al.,*2005), south Africa (van Niekerk *et al.,*2004), Spain(Úrbez-Torres,2006b;Aroca *et al.,*2008;Martin *et al.,*2009), Argentina (Alves *et al.,*2008) and Bolivia(Kaiser *et al.,*2009). It was also reported on other hosts in adjacent countries, including mango in Oman and Pakistan(Al-Adawi *et al.,*2003;Khanzada *et al.,*2004),coconut palms in India(Lakshman ana Jagadeesan,2004), apricot and peaches in China(Li *et al.,*1995).

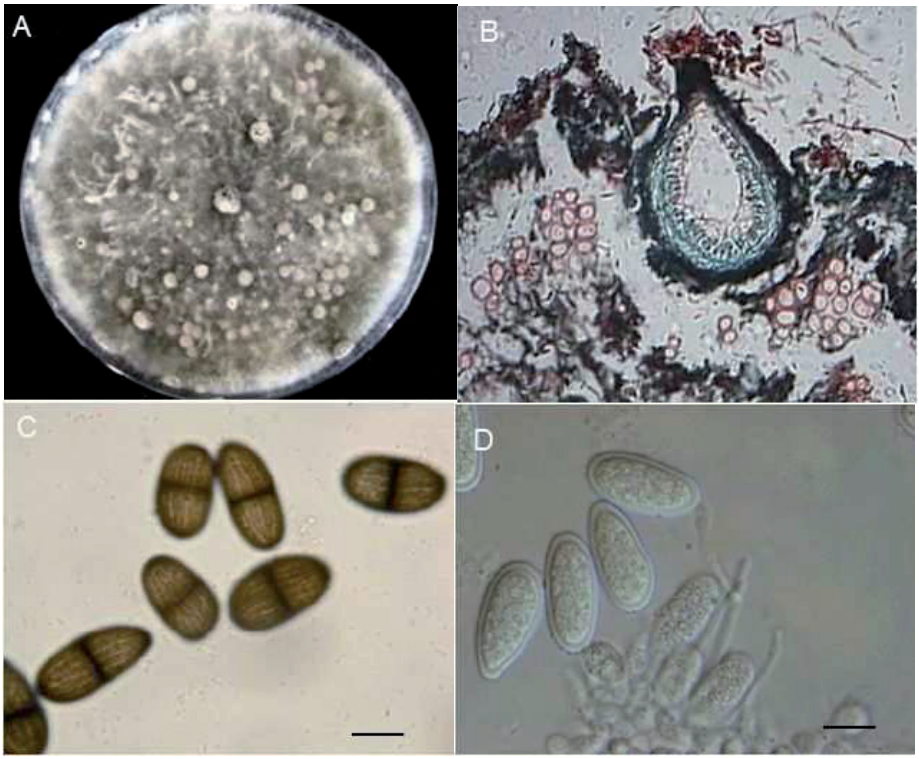



Fig.1.*Lasiodiplodia theobromae*.A.Growth on potato dextrose agar(PDA)after 10 days. B.Longitudinal section of infected shoot of grapevine showing pycnidium with 2-celled dark pycnidiospores C. Conidia mature dark brown with one septum D . Paraphyses cylindrical, aseptate, hyaline. Conidia produced initially hyaline and aseptatate, subovoid to ellipsoid-ovoid bar= 10µm.

The hyphae of *Neoscytaliduim dimidiatum* mid to dark brown,pycnidia were dark brown to black formed on infected grapevine canes. Chains of arthroconidia were developed from unidentified, brown hyphae,conidia were cylinderical,brown,0-2 septate at maturity,4-6x7-12µm(Fig.2,B). *N. dimidiatum* has been reported to cause branch wilt of grape in Baghdad, central Iraq (Natour & Ahmed, 1969).This is the

first time to record of this fungus as pathogen of grapevine in Basrah province southern Iraq.*N.dimidiatum* has been reported from *Andansonia perrieri* in Australia(Sakalidis *et al.,*2011), citrus in Iran and Italy(Alizadeh *et al.,*2000;Polizzi *et al*.,2009), almond in California(Inderbitzin *et al.,*2010) and *Prunus* spp.in Egypt(Farr *et al.,*2005), mango in Niger( Pandey *et al.,*1981;Reckhaus and Adamous,1987).

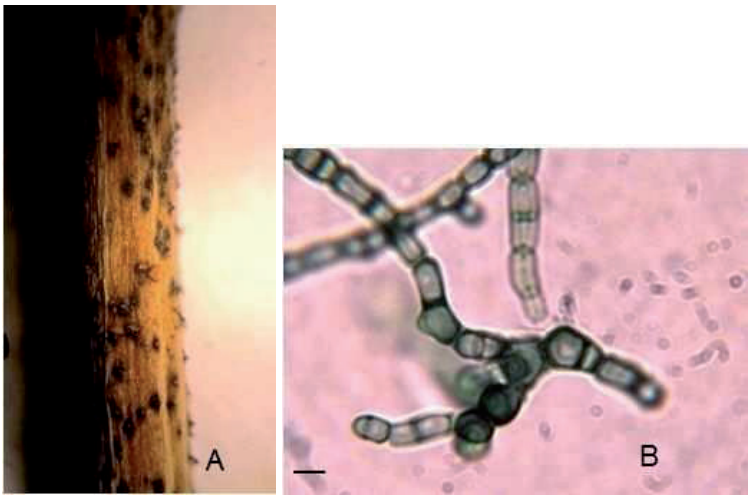



Fig.2. A. *Lasiodiplodia theobromae*.Pycnidia dark brown to black formed on grapevine canes.B. *Neoscytalidium dimidiatum*.Hyphae and chains of orthroconidia.Bar= 4µm

**Phylogenetic analyses**

The PCR-based method is considered a quicker and more sensitive technique compared to morphological identification, with high discriminative ability between morphologically similar species.

The ITS (the universal transcribed spacer) regions are typically variable and quite informative (Diaz and Fell, 2004). The universal fungal primers (ITS1/ITS4)

successfully amplified the genomic DNA for all isolates that we tested.

The sequence results demonstrated that they were derived from the fungal ITS regions when compared to the database of sequences on GenBank. BLAST results showed the most identical sequence was ITS/5.8S rDNA region of *L. theobromae* and *N. dimidiatum* showing 100% identity. The results of sequence similarity indicated that the isolated strains are *L. theobromae* and *N. dimidiatum*.

**Pathogenicity tests**

Grapevine plants that were inoculated with spore suspensions of *L. theobromae* and *N. dimidiatum* developed clear dieback symptoms after approximately 2 weeks (fig.3 A, C, D). No symptoms were observed on uninoculated plants (control) (fig.3,B).

The most obvious symptoms on plants inoculated with *L. theobromae* and *N. dimidiatum* was the presence of dark wood or discoloration observed in the green arms and canes.

During the trial period, there were no obvious symptoms of *L. theobromae* observed on leaves of inoculated plants. This agrees with previous studies conducted in California, Portugal and Mexico, that found that there were no foliar symptoms associated with this pathogen (Phillips, 1998; Urbez-Torres *et al*. 2006, 2008). The present study showed that *L. theobromae* was more virulent than *N. dimidiatum*. This result is in agreement with a previous studies conducted in California,south Africa and Mexico in which *L.theobromae* was shown to be one of the most virulent species(Leavitt,1990;van Niekerk *et al.,*2004;Úrbez-Torres,2008).However, in western Australia *L.theobromae* isolates were avirulent or only weakly pathogenic in inoculated Red Globe mature canes(Taylor *et al.,*2005).

*L.*theobromae and *N.dimidiatum* may create a serious dieback disease to vineyards in Iraq, particularly with the relationship to the environmental conditions.Pycnidia of *L. theobromae* started developing on inoculated canes and trunk about 2 weeks after

inoculation (fig.3 A. C. D). Plants inoculated with *N. dimidiatum* began to desiccate and lost their green color from the apex toward the base 7 days after inoculation .

After 4 weeks the entire seedling turned dark brown (fig.3, A, C, D). *Lasiodiplodia theobromae* and *N. dimidiatum* were reisolated from the artificially inoculated plants. Whereas no fungi were reisolated from the control treatments.

It was worth mentioning that accurate identification of botryosphaeriaceae species is necessary to monitor global distribution of these pathogens, contribute to appropriate application of quarantine decisions and develop an effective disease management strategies, because these species differ considerably in their interactions with different hosts and environmental conditions(Britton and Hendrix,1986;Denman *et al.,*2003).

This work represent the first molecular detection and pathogenicity of *L.theobromae* and *N.dimidiatum* on grapevine in this part of the world and is still preliminary which may be useful for a further surveys to correlate botryosphariaceae species with the different disease in vineyards of Iraq.

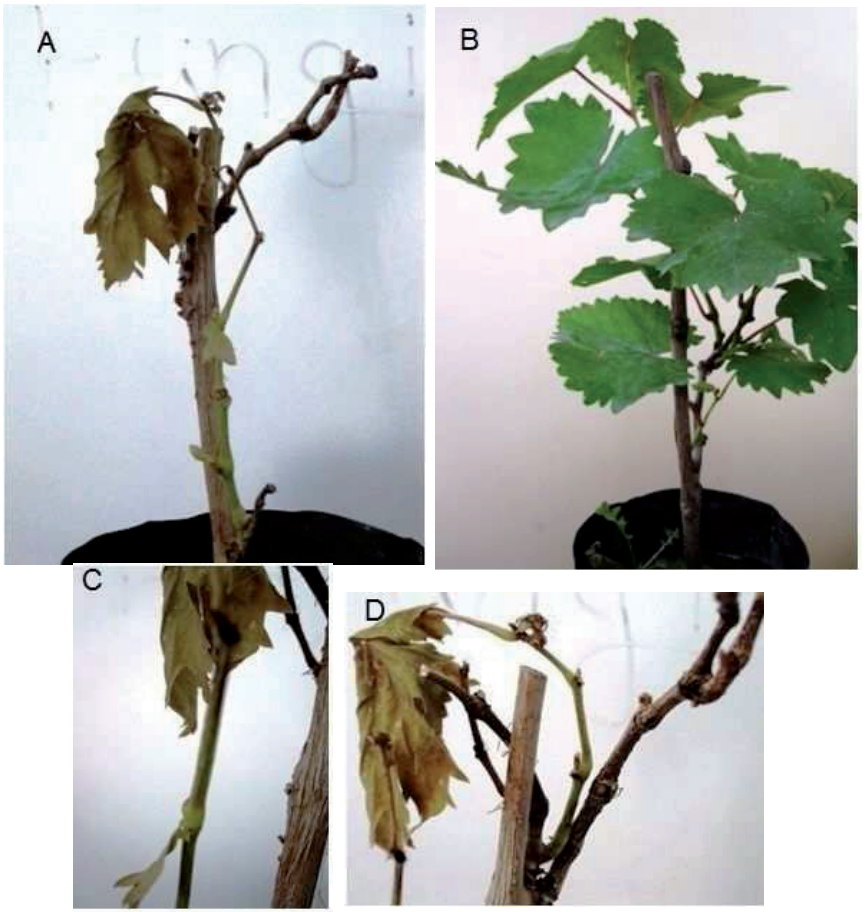



Fig. 3 .Grapevine plant inoculated with spore suspensions of [A.D. *Lasiodiplodia theobromae* C. *Neoscytalidium dimidiatum*] developed clear dieback symptoms approximately 2 weeks after inoculation. Darkened wood or discoloration was observed in the green arms and canes B. Grapevine plant uninoculated with spore suspensions of *L. theobromae* and *N. dimidiatum*

**References**

Al-Adawi AO,Deadman ML,Al-Rawahi AK,Kha AJ,Al-Maqbali YM(2003).*Diplodia theobromae* associated with sudden decline of mango in Sultanate of Oman.Pl.pathol.52:419.

Alizadeh A,Heidarian A, Farrokhi- Nejad R(2000).Citrus branch wilt ,decline and death caused by *Nattrassia mangiferae* and its other hosts in Khuzestan Province. Iran. J.PL.Pathol.36:21–24.

Alsaidi IH(2000).Viticul ture.Part 1.Mousel.Iraq.pp595.

Alves A,Crous PW,Correia A,Phillips AJL(2008).Morphology and molecular data reveal cryptic species in *Lasiodiplodia theobromae*.Fung.Diver. 28:1–13.

Aroca A,Raposo R,Gramaje D,Armengol J,Martos S,Luque J(2008).First report of *Lasiodiplodia theobromae* associated and decline of grapevine rootstock Mother plants in Spain.Pl.Dis.92:832.

Barr ME(1987).Prodromus to class Loculoascomycetes,Amherst, Massachuseees:Published by the author.

Bedair HM,Al-Saad HT,Salman NA(2006).Iraq´s southern marshes something special to be conserved; a cause study. Mar.Bul. 2:99-126.

Bernstein L, Hayward HE(1958).Physiology of salt tolerance.Ann.Rev.Plant Physiol.9:25-46.

Boyer JS(1995).Biochemical and biophysical aspects of water deficits and the predisposition to disease .Ann.Rev.Phytopathol.33:251-274.

Britton KO,Hendrix FF(1986).Population dynamics of *Botryosphaeria* spp. In peach gummosis canckers.Pl.Dis.70:134-136.

Burgess,TreenaT,Barber,Paul A,Mohali Sari Pegg,Geoff,deBeer,Wilhelm, Wing field,Michacl J(2006).Three new *Lasiodiplodia* spp.From the tropics, recoguized

based on DNA sequence comparisons and morphology. Mycol. 98:423–435.

Burruano S,Mondello V,Conigliaro G,Alfonzo A,Spagnolo A, Mugnai L(2008).Grapevine decline in Italy caused by *Lasdiodiplodia theobromae.*Phytopathol.Mediterr.47:132-136.

Castillo-Pando M,Somer A,Green CD,Priest M,Seriskanthades M(2001).Fungi associated with decline of semillon grapevines in the humter Valley of new south Wales.Aust.Plant Pathol.30:59-63.

Cenis JL( 1992). Rapid extraction of fungal DNA for PCR amplification. Nucl. Acids Res. 20: 238.

Chandra S(1974).Some new leaf–spot diseases from allahbad (India).Nova Hedw.Beih 47:35–102.

CrousP W,Slippers B,Wing field MJ,Rheeder J,Marasas WFO,Phillips AJL, Alves A,BurgessT,Barber P,Groene wald JZ(2006).Phylogenetic lineages in the Botryosphaeriaceae .Stud. in Mycol. 55:235–253.

Damm U,Crous PW,Fourie PH(2007).Botryosphaeriaceae as potential pathogen of *Prunus* in south Africa,with descriptions of *Diplodia africana* and *Lasiodiplodia plurivora* sp.nov.Mycol.99:664-680.

Denman S,Crous PW,Taulor JE,Kang JC,Pascoe I,Wing field MJ(2000).An over view of the taxonomic history of *Botryosphaeria*,and a re–evaluation of its anamorphs based on morohology and ITS,DNA phylogeny.Stud. in Mycol. 45:129-140.

Denman S,Crous PW,Groenewald JZ,Slippers B,Wingfield BD,Winfield MJ(2003). Circumscription of *Botryosphaeria* species associated with Proteaceae based on morphology and DNA sequence data.Mycol.94:294-307.

Diaz MR,Fell JW(2004).High-throughput detection of pathogenic yeast of the genus

*Tricosporon* .J.Clin. Micr.42(8):3696-3706.

El–Goorani MA,EL–Meleigi MA(1972).Dieback of Grapevine by *Botryodiplodia theobromae* Pat.InEgypt.Phyto.Medi.11:210–211 .

Ellis MB(1971).Dematiacous Hyphomycetes.Commonwealth Mycological Institute,Kew,Surry,England.608 p .

Farr DF,Elliott M,Rossman AY,Edmonds RL(2005).*Fusicoccum arbuti* sp . nov.causing cankers on Pacific madrone in western North America with notes on *Fusicoccum dimidiatum*,the correctname for *Scytalidium dimidiatum* and *Nattrassia mangiferae*.Mycol.97:730–741 .

Farr DF,Rossman AY,Palm ME,Mc Cray EB(n.d.).Fungal databases ,Systematic Botany and Mycology Laboratory.ARS.USDA.Retrieved 12 Nov.2004,from http://nt.Ars–grin.gov/fungaldatabases.

Food and Agriculture Organizatio(FAO).The united nations.(1996).Year book production vol.50,Rome.

Hewitt WB(1988).Diplodia cane die–back;bunchrot-In:Pearson,R.C. A.C.Goheen(eds,):Compendium of grape diseases,pp.26- 28.St.Paul ,Minnesota (U.S.A),Am.Phytopath.Soc .

Inderbitzin P,Bostock RM,Trouillas FP,Michailides TJ(2010).A six locus phylogeny reveals high species divrsity in Botryosphaeriaceae from Califiornia almond.Mycol.102:1350-1368.

Jacobs KA,Rehner SA(1998).Comparison of cultural and morphological characters and ITS sequences in anamorphs of *Botryosphaeria* and related taxa.Mycol.90:601–610.

Jan M van Niekerk,Crous pw,Fourie PH,Halleen F(2004).DNA phylogeny,morphology and pathogenicity of *Botryosphaeria* species on grapevines.

Mycol.96(4):781-798.

Kaiser WJ,Rivero GM,Valverade BE(2009).First report of *Diplodia* cane dieback of grapevine in Bolivia.Pl.Dis.93:320.

Khanzada MA,Lodhi AM,Shahzad S(2004).Pathogenicity of *Lasiodiplodia theobromae* and *Fusarium solani* on mango. Pak.J.Bot.36:181-189.

Lakshman P,Jagadeesan R(2004).Malformation and cracking of nuts on coconut palms (*Cocos nucifera*) due to the interaction of eriophyid mite *Aceria guerreronis* and *Botryodiplodia theobromae* in tamil Nadu,India.J.Pl.Dis.Prot. 111:206-207.

Larignon P,Dubos B(2001).The villainy of black dead arm,wines and vines.82:86-89.

Larignon P,Fulchic R,Cere L,Dubos B(2001).Observations of black dead arm in French vineyards.Phytopathol.Mediterr.40:336-342.

Leavitt GM(1990).The occurrence,distribution,effects and control of *Botryodiplodia theobromae* on *Vitis ninifera* in California,Africa and northern Mexico.Ph.D.dissertation,university of California,River side.

Leavitt GM,Munnecke DZ(1987).The occurrence distribution of *Botryodiplodia theobroma*e on grapes (*Vitis vinifera* L .)In California.Phytopathol. 77,1690(Abstr.).

Lehoczky J(1974).Black dead arm disease of grapevine caused by *Botryosphaeria stevensii* infection.Acta Phytopathol.Hung.9:319-327.

Leonova TC,Shevyalova(1970).Incorporation of $s^{35}$ methionine in to free s-aminoacids in plant leaves under salinization of the medium.Sov.plant Physiol.37:689-693.

Li HY,Cao RB,Mu YT(1995).In-vitro inhibition of *Botryosphaeria dothidea* and *Lasiodiplodia theobromae* and chemical control of gummosis disease of Japanese

apricot and peach tree in Zhejiang province,China.Crop Prot.14:187-191.

Luque J,Martos S,Phillips AJL(2005)*Botryosphaeria viticicola* sp.nov. on grapevine:a new species with a *Dothiorella* anamorph.Mycol. 97:1111-1121.

Martin MT,Martin L,de Francisco MT,Cobos R(2009).First report of *Lasiodiplodia theobromae* and *Cryptovalsa ampelina* associated with grapevine decline from Castilla Yleon,Spain.Pl.Dis.93:545.

Matheron ME,Sigler L(1993).First report of Eucalyptus die-back cause by *Nattrassia mangiferae* in North America.Plant Dis.78:432.

Mugnai L,Graniti A, Surico G(1999).Esca(black measles)and brown wood streaking:two old and elusive diseases of grapevines.Plant dis,83:404-418.

Mullins MG,Bouquet A,Williams LE(1992).Biology of the grapevine.press Syndicate of University of Cambridge.Cambridge.239.

Nattrass RM(1933).A new species of *Hendersonula* (H.toruloidea)on deciduous trees in Egypt.Trans.Brit Mycol.Soc.18:189 –198.

Natour RM,Ahmed JM(1969).control of branch wilt disease of grape. Dis.Rep.53:152–153.

Pandey RS,Bhargava SN,Shukala DN,Khati DVS(1981).A new leaf spot disease of mango.Pl.Dis.65:441-442.

Phillips AJL(1998).*Botryosphaeria dothidea* and other fungi associaled with Excoriose and dieback of grapevines in Partugal.J.Phytopathol.146:327–332

Phillips AJL(2002).*Botryosphaeria* species associated with diseases of grapevines in Portugal.Phytopathol.Mediterr.41:3–18.

Phillips AJL,Alves A,Pennycook SR,Johnson PR,Ramaley A,Akulov A,Crous PW(2008).Resolving the phylogenetic and taxonomic status of dark-spored

teleomorph genera in the Botryosphaeriaceae.Persoonia 21:29-55.

Polizzi G,Ajello D,Vitale A,Giuffrida FF,Groenewald JZ,Crous PE(2009).First report of shoot blight,canker and gummosis caused by *Neoscytalidium dimidiatum* on citrus in Italy.Pl.Dis.93:1215.

Punithalingam E(1976).*Botryodiplodia theobromae*.CMI descriptions of pathogenic fungi and bacteria No.519.Kew,Surrey,England:Commonwealth Mycol.Instit.

Punithalingam E(1980) Plant diseases attributed to *Botryodiplodia theobromae*.In:Biblioteca Mycologica .J.Cramer.Berlin,113pp.

Punithalingam E, Waterston JM(1970).*Hendersonula toruloidea* C.M.I.Descrpt Pathog Fungi Bact 274 :1–2.

Reckhaus P(1987).*Hendersonula* dieback of mango in niger.Plant Dis.71:1045.

Reckhaus P,Adamous I(1987).*Hendersonula* dieback of mango in Niger.Pl.Dis.71:1045.

Saido KA(2007).Fungal decline of grapevines in Duhok province.M.Sc. thesis. College of Agriculture , Duhok University .

Sakalidis ML,Hardy GES,Burgess TI(2011).Endophytes as potential pathogens of baobab species *Andansonia gregorii* :a fous on Botryosphaeriaceae.Fung.Ecol.4:1-14.

Schoch CJ,Shoemaker RA,Seifert KA, Hambleton S,Spatafora J,Crous PW(2006).A multigene phylogeny of the Dothideomycetes using four nuclear loci.Mycol.98:1041-1052.

Slippers B,Winfield MJ(2007).Botryosphaeriaceae as endophytes and latent pathogens of woody plants:diversity.ecology and impact.Fung.Biol.Rev.21:90-106.

Smith H,Wingfield MJ,Crous PW,Coutinho TA(1996).*Sphaeropsis spainea* and

*Botryosphaeria dothidea* in *Pinus* spp.in south Africa.Sou.Afri.J.Bota. 62:86-88.

Sutton BC,Dyko BJ(1989).Revision of *Hendersounla*.Mycol.Res.93:466– 488 .

Taylor A,Hardy GEStJ,Wood P,Burgess,T(2005).Identification and pathogenicity of *Botryosphaeria* species associated With grapevine decline in western Ausralia.Aust.Plant Pathol.34:187-195.

Úrbez–Torres JR,Leavitt GM,Guerrero JC,Guevara J,Gubler WD(2008). Identification and pathogenicity of *Lasiodiplodia theobromae* and *Diplodia seriata* ,the causal agents of bot canker disease of grapevines in Mexico.Plant Dis.92:519–529.

Úrbez–Torres JR,Leavitt GM,Voegel T,Gubler WD(2006).Identification and distribution of *Botryosphaeria* species associated with grapevines cankers in California.Plant Dis.90:1490–1503.

Úrbez–Torres JR,Pelaez H,Santigo Y,Martin C,Moreno C,Gubler WD(2006b).Occurrence of *Botryosphaeria obtusa,B.dothidea* and *B.parva* associated with grapevine trunk diseases in Castillay León region,Spain.Plant Dis.,90:835.

Van Niekerk JanM van,Crous Pedro W,Groenewald J,Fourie PaulH,Halleen Francois (2004).DNA phylogeny,morphology andPathogenicity of *Botryosphaeria* species on grapevines.Mycol.96:781–798.

Von Arx JA(1987).Plant pathogenic fungi .Berlin ,Germany:J.Carmer. 288 p .

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: a guide to methods and applications. (Innis MA, Gelfand DH,

Sninsky JJ, White TJ, eds). Academic Press, New York, USA: 315–322.

Wood PM,Wood CE(2005).Cane dieback of Dawn seedlessTable grapevines (*Vitis*

*vinifera*) in western Australia caused by *Botryosphaeria rhodina* .Aust.Plant Pathol.34:393–395.

Zhou S,Stanosez GR(2001).Primers for amplification of mt SSU,DNA ,and a phylogenetic study of *Botryosphaeria* and associated anamorphic fungi, Mycol.Rese.105:1033–1044.

.

## Chapter 2:

**Basrah J.Agricu.Sci.2012,25(1):1-12**

# Histopathology of grapevine inoculated with *Lasiodiplodia theobromae*

Abdullah H.Al- Saadoon [1] , Mohanad K.M.Ameen[1*] and Eman M.A.Al-Rubaie[1]

[1] Department of Biology, College of Science, Basrah University,Iraq

---

**ABSTRACT**

Histopathological changes of 2-years old grapevine cv. Abbassi inoculated with *Lasiodiplodia theobromae*(syn.*Botryodiplodia theobromae*)were studied. Samples were taken at 7 and 25 days after artificial inoculation.At 7 days,cross and longitudinal sections revealed the presence of disorganized cells,degenerated areas and vascular bundles.The intra–and intercellular invasion of the inoculated tissues by the fungus were evident with dark brown color.At 25days,the fungus caused necrosis in xylem parenchyma and xylem vessels, the hyphae colonized the tissues.Dark deposits appeared in vessels and other tissues as well as abundant production of tyloses. *L. theobromae* has also been found to produce pycnidia on the shoots and petioles of grapevine which yielded 2-celled dark pycindiospores.After more than 4-weeks,the inoculated grapevine seedlings look flaccid, brown in color and wilting symptoms began to appear and finally the seedlings were dead.

***Keywords:*** grapevine,die-back,histopathology,*Lasiodiplodia theobromae.*

## INTRODUCTION

*Lasiodiplodia theobromae* ( Pat.) Griffon and Mauble is recognized as an important wood pathogen and has been reported to cause cankers,die–back,and fruit

as well as root rot in over 500 different hosts.It is geographically wide spread but is most common in the tropical and sub-tropical regions worledwide (1).

Die–back of vitis ( *Vitis vinifera* L.)caused by *L. theobromae* is an important disease affecting grapevine all over the world (2).

In Egypt, according to the available literatures,a report concerning the host parasite–interactions of *Botryodiplodia theobromae* (syn.*L.theobromae*) involved in die–back disease of grapevine has been confined to the work of (3),who detected that necrotic xylem parenchyma and xylem vessels , dark inclusions bodies as well as abundant production of tyloses were caused by hyphae of *B. theobromae.* Histopathological studies of die–back disease incited by *L. theobromae* have been reported in other hosts ,including mango(4; 5;6) and peach (7).

So far,little attention has been given to mycotic gravevine diseases in Iraq.Early research by (8) reported of *Hendersonula toruloidea* to cause branch wilt of grapevine,in central region of Iraq,The aim of this investigation was to study the histopatholoy of grapevine(cv.Abbassi) artificially inoculated with *L.theobromae*.

**MATERIA AND METHODS**

The artificially infection by *Lasiodiplodia theobromae* was done using two years old grapevine seedlings Abbassi cultivars.The isolate of *L.theobromae* was obtained from grapevine shoots suffering from die-back disease (9).A cut in shoots , petioles and leaves was made using sterilized sharp blade and inoculated by sprayed with 40 ml of conidial suspension ($5x10^6$conidia $ml^{-1}$)of *L.theobromae* or water to used as a control using atomizer(10), Inoculated seedlings were covered with plastic bags to provide high moisture contents for two days in green house at 25 $C^o$.reisolation were done from the inoculated seedlings exhibited typical symptoms of die-back.Small pieces of necrotic tissue from the edge of each lesion were cut,surface–sterilized for 5 min,in 5% sodium hypochlorite,washed three times with sterile distilled water and transferred into surface of PDA in Petri plates,then incubated at 25 $C^o$ .

After 10 and 25 days after inoculation,samples of shoots,petioles and leaves were cut, washed three with sterilized distilled water and dried between folds of sterilized filter papers.The artificially inoculated and non inoculated tissues were cut into small portions (5–10 mm long) and fixed in a solution of formalin,acetic acid and ethyl alcohol 5% (5:5:90% ) for 24 hr.,washed in ethyl alcohol 50 %,then dehydrated by passage through a series of ethyl alcohol,cleared in xylol and embedded in paraffin (11).Sections of 10–15 µm thick were cut using a rotary microtome,then stained with safranin–light green and mounted in Canada balsam (12).The stained sections were examined by a light microscope and photographed.

**RESULTS AND DISCUSSION**

Microscopical examinations revealed several changes in the tissues of grapevine cv.Abbassi seedlings,artificially inoculated with *L.theobormae*.Transverse and Longitudinal sections showed the systemic nature of infection.

A 7 days post inoculation with *L. theobromae* , the transverse sections showed the site of the initial infection in the shoots (Fig.1) and the degeneration of the epidermis and parenchyma cells in the cortex ( Figs.1 and 2).

The intra–and intercellular invasion of the parenchymatous cells by fungal mycelia were evident in shoots with dark color (Figs.3).Fig.4 showed that the infection with *L.theobromae* damaged tissues especially cortical layer and the fungal hyphae of the pathogen were observed in the cortical cells.Under infection with *L.theobromae* histological examination of cross sections of the petioles of grapevine cv.Abbassi showed the sites of the initial infection (Fig.5).Transverse sections of the shoots of the grapevine infected with *L. theobromae* showed seriously damaged in the vascular bundles (Fig.6)in comparison with control(fig.10).

Also degenerated zones with protolysis symptoms were observed in different tissues,i.e. epidermis,cortex and vascular tissues (Fig.7).In addition , the longitudinal

sections in shoots of grapevine after 7 days of inoculation with *L. theobromae* revealed disorganized epidermal and cortical cells,xylem vessels colonized by hyphae and the formation of dark inclusions were observed (Figs.8).It is also noticed that,the xylem rays were a suitable path for rapid and easy spreading of the hyphae.

Microscopical examination of the sections of the non–inoculated samples control showed normal and intact tissues without any visible changes in their histological structures compared to the infected tissues ( Figs.9 and10).

Sample taken at 25 days old,post inoculation with *L.theobromae* exhibited spread of the pathogen through the infected tissues and the sections figured out several histological changes in both shoots and petioles due to *L.theobromae* infection.The fungus spread intra-and inter-cellular in all tissues particularly in the vascular tissues causing clear destruction of phloem and cambial cells (Fig.11). Necrotic xylem parenchyma and xylem vessels, colonized by hyphae, and dark deposits appeared in vessels and other tissues as well as a abundant production of tyloses (Figs.12,13,14and15).No hyphae or tyloses were observed in uninfected controls(Fig16).The heavy establishment of the fungal mycelium in the various tissues of shoots and petioles led to complete degeneration were observed in(Figs.17,,18,19and20).Dense agglomerations of hyphae and pycnidia were found in the infected shoots and petioles of grapevine,which yielded 2–celled dark pycnidiospores (Fig.21).

Cross sections 7 days after artificial inoculation leaves of grapevine cv.Abbassi showed that, the infection occurred only in the midvein.The epidermal and cortical cells showed plasmolysis and xylem vessel ,were blocked by gummosis (Fig.22).While the leaf lamina sections appeared without any visible change in their histological structure compared with the control (Fig.23 and24 ).This result agrees with previous studies conducted in California,Portugal and Mexico,which found that there were no foliar symptoms associated with *L.theobromae*.(13;14;15).

After more than four weeks the whole inoculated grapevine cv.Abbassi seedlings became dark brown,flaccid and wilting began to appear and finally the seedlings were dead.

These results are consistent with those reported by (3)who found that,7 days after artificially inoculated grapevine shoots with *B. theobromae* induced disorganized epidermal cells with dark brown color consisting of plasmolysis cells and tissues.The fungal hyphae were clearly noticed 21 days after inoculation with *B.theobromae* in both xylem parenchyma and xylem vessels causing necrosis at these tissues , Also they noticed the xylem tissues were colonized by hyphae and dark inclusions bodies as well as a abundant productions of tyloses.No disorders were noticed in case of *Phomopsis viticola* and *Fusarium solani* after 7 days of inoculation,while,the above mentioned pathogenic fungal hyphae were clearly noticed 21 days after inoculationin both xylem parenchyma and xylem vessele causing necrosis at these tissues.The fungal hyphae spread intra-and intercellularly in all tissues and the vessels were plugged with gum as well as abundant prodution of tyloses.

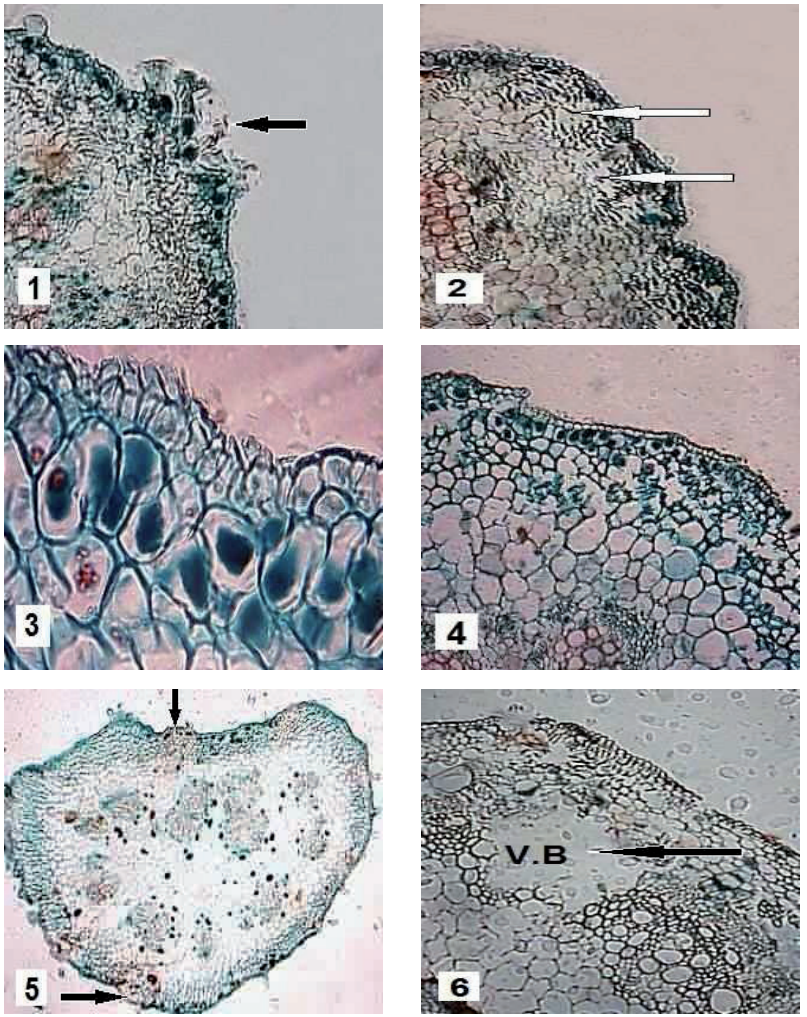
Similar findings were obtained by (16) who found that the fungus *Phaeoacremonium chlamydosporum* infects the xylem parenchuma cells of vine shoots as intercellular hyphae and these cells produce tyloses in adjacent xylem vessels.The hyphae also pentrate the vessels,often by way of tyloses.Brown deposits are seen in vessels and cells assumed to be accumulation of phenolic compounds.

The presence of necrotic vessels,hyphae and reduction in the size or collapse of vessels due to infection development of tyloses in the vessels release of larg molecule compounds in the vessels as result of cell wall breakdown by pathogenic enzymes might bring about dysfunction of the xylem elements with a restriction in the flow of water and minerals(6;17).The cell walls consist not only of polysaccharides material but also of condensed tannins and phenols.It appears that these last two substansces also accumulate within the tyloses(18),It is known that following attack by micro-

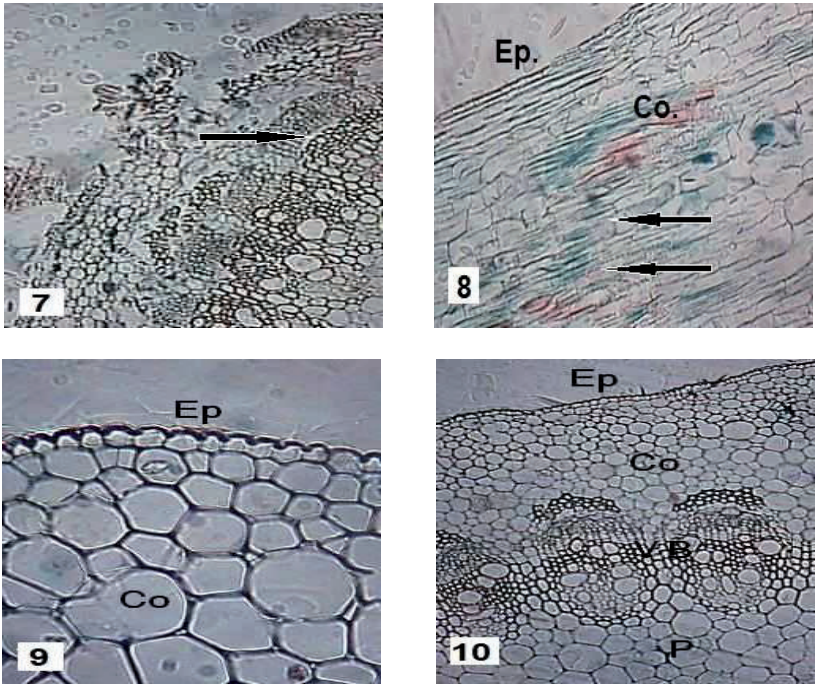
organisms, plants sometimes have higher levels of condensed tannins because of an increased probuction of new tannins or the mobilisation of pre-existing tannins towards infection sites,in order for example to inhibit fingal enzymes(19), to reinfofce the structural components of the cell walls(20)or to form a chemical barrier to infections(21).

The results of this study are also in agreement with those observed by (5) and (6)for mango , (7)for peach and (22)for apple .

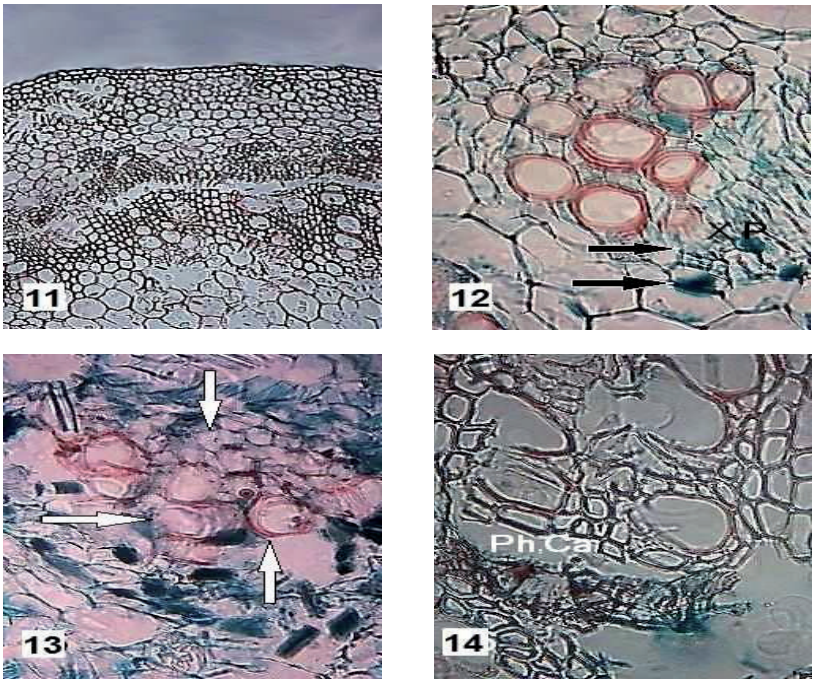
Mature pycnidia with pycnidiospores were produced with in 25 days after artificially inoculated grapevine shoots and petioles with *L. theobromae*.While the cross sections in shoots inoculated with *B.theobromae* after 21 days showed that pycnidia were embedded in epidermal layer and pycnidiospores in the pycnidial cavity( 3 ).



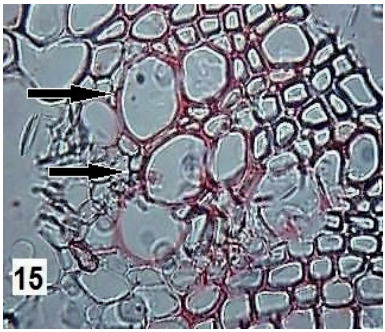

**Figures 1– 6.Light micrograghs of grapevine cv. Abbassi tissues 7 days after inoculation with *Lasiodiplodia theobromae*.1(X10),transverse section in the inoculated shoots showing initial infection in the epidermal cells (arrows).2(X10),showing degenerated epidermal and cortical cells(arrows).3(X40),infected cortex showing the presence of fungal hyphae colonizing the cortical cells .4(X10),development of infection in the cortex .note the degeneration of the parenchyma cells in the cortex and the presence of the fungal mycelia in the cells.5(X4.5),transverse section in the infected petiole showing the initial infection in the epidermis (arrows).6(X10),transverse sections in the inoculated shoots showing severely damaged of vascular bundle.**

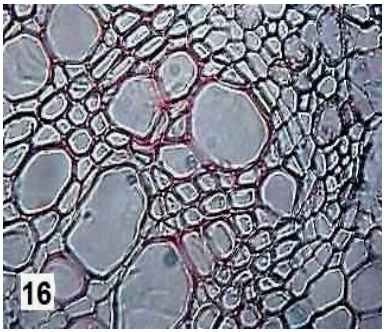


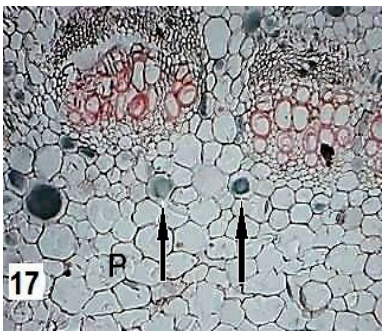

**Figures 7 – 10, Light micrographs of grapevine cv.Abbassi shoots 7 days after inoculation with *Lasiodiplodia theobromae* and non – inoculated shoots.7(X10),showing apparent break down of epidermal and cortical cells,extending the infection to the vascular tissues.note craks in the vascular bundle(arrows).8(X10),Longitudinal section of shoots7 days after inoculation showing disorganized epidermal and cortical cells,xylem(X) necrosis,fungal hyphae in the tissues.9(X40),transverse sections in the non-inoculated shoots showing normal epidermis and cortex.10(X10),non–inoculated shoots showing intact epidermis(E P),cortex (CO),vascular bundles(V B),pith(P).**

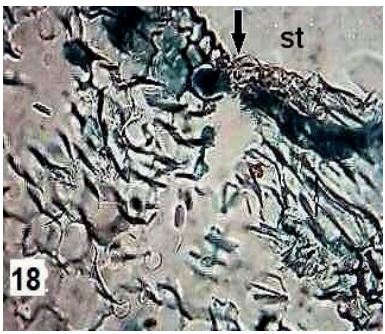



**Figures 11-14,Light micrographs of grapevine cv,Abbassi shoots 25 days after inoculation with *Lasiodiplodia theobromae*.11(X10),transverse sections in the inoculated shoots showing the infection in the cortex and vascular tissues.note the presence of clear cracks in the vascular bundles,12(X40),showing infected vascular bundle.note xylem parenchyma(x.p) colonized by hyphae (arrows).13(X40),showing vascular bundle completely destroyed and colonization of the tissues by fungal hyphae.14(X40),showing destroyed phloem and cambium (Ph,Ca) cells.**

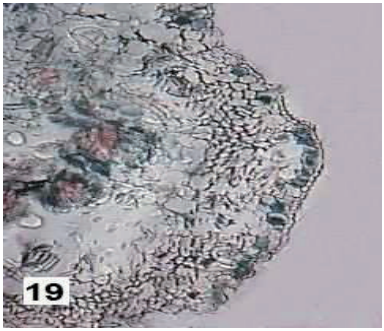


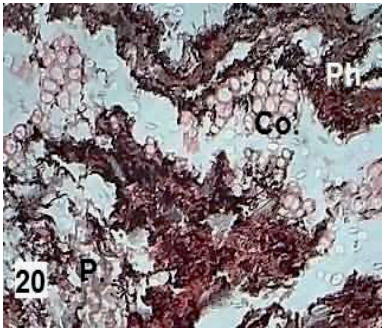



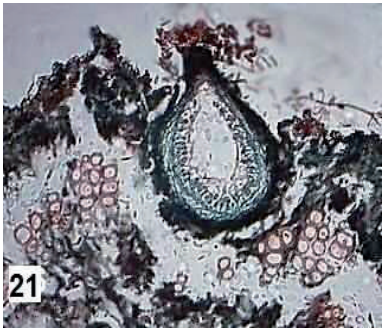



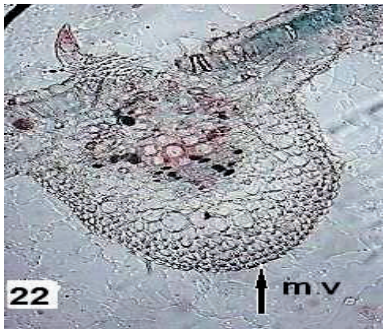

**Figures 15– 18,Light micrograhs of grapevine cv.Abbassi 25 days after inoculated with *Lasiodiplodia theobromae*.15(X40),transverse sections in the inoculated shoots showing fungus mycelium spread through intercellular spaces(arrows),Xylem vessels blocked with tylosses.16(x40),transverse section in the non–inoculated shoots showing normal vascular bundle.17(X40),showing fungus mycelium spread through intra–and intercellular spaces,pith tissues colonized by fungal hyphae(arrows).18(X40),substomatal(st)infection in the shoots.note the hyphal invasion of the tissues and destroyed cortex cells.**

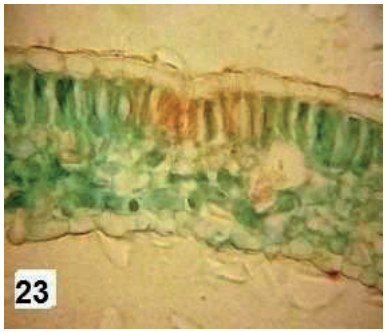


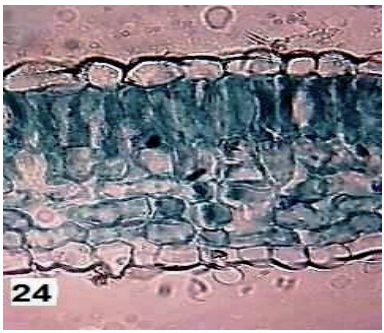




**Figures 19- 21,Light micrographs of grapevine cv.Abbassi 25 days after inoculated with *Lasiodiplodia theobromae*.19(X10),transverse section in the inoculated shoots showing severely damaged and disorganized tissues.20(X10),seriously damaged and degenerated tissues cortex(Co.),phloem(Ph.) and pith(P.) of the inoculated shoots.21(X10),Longitudinal sections of shoots showing pycnidium of *Lasiodiplodia theobromae* and 2–celled dark pycnidiospores.**




**Figures 22–24,Light micrographs of grapevine cv.Abbassi Leaves 7 days after inoculated with *Lasiodiplodia theobromae* and non – inoculated ones.22(X10), transverse sections in the inoculated Leaves showing infected midvein(m.v), destroyed epidermal and cortical cells,xylem vessels blocked by gummosis. .23(X10),transverse sections in the inoculated Leaves showing no signs of infection in the blade (not affected).24(X10),transverse sections in non – inoculated Leaves showing normal mesophyll .**

## CONCLUSION

There is still little data available about die-back disease of grapevine in Iraq,especially histopathology,however,our findings from the present study revealed the systemic nature of infection.Xylem rays were a suitable path for rapid and easy spreading of the hyphae.The heavy establishment of the fungal mycelium,dark deposits as well as abundant production of tyloses led to complete degeneration of various tissues.

## ACKNOWLEDGMENT

We are deeply indebted to Professor Dr.Samir K.Abdullah University of Zakho,for his continuous support and reviewing this paper.

**REFERENSES**

1-Punith-alingam ,E.(1980).Plant disease attributed to *Botryodiplodia theobromae*. In : Biblioteca Mycologica . J . Cramer . Berlin .

2-Leavitt , G.M.and Munnecke,D.E.(1987).The occurrence distribution of *Botryodiplodia theobromae* on grapes( *Vitis vinifera* L. ) in California . Phytopathology 77 : 1690 .

3-Atia ,M.M.M.Aly,A.Z;Tohamy,M.R.A.; EL–Shimy,H.and Kamhawy,M.A .(2003).Histopathological studies on grapevine die – back. Journal of Plant Disease and Protection 110:131–142.

4-El – Adawy ,A.A.(1969). Studies on the dieback of mango- Ph.D.Thesis / Fac. Agric.,Cairo Unvi.

5-Ogundana ,S.K.(1970).The control of soft rot in Nigeria . International Bio – Deterioration Bulletin 8 : 75 .

6-Ramos , L.J.; Lara,S.P. ;Mc Millan, R.T.J. and Narayanan,K.R.(1991).Tip die – back of mango ( *Mangifera indica* ) caused by *Botryosphaeria ribis*.Plant Disease 75 : 315 – 318 .

7-Biggs, A.R. and Britton, K.O. (1988).Pre – symptoms histopathology of peach trees inoculated with *Botryodiplodia obtusa* and *Botryodiplodia dothidae*. Phytopathology 78 : 1109 – 1118 .

8-Natour, R.M. and Ahmed ,J .M.(1969).Control of branch will disease of grape. Plant Dis . Rep. 53: 152 – 153 .

9-AL- Saadoon, A.H;Ameen M.K.;Hmeed,M.A;Al-badran A.I.;Ali,Z. (2012).First report of grapevine dieback caused by *Lasiodiplodia theobromae* and *Neoscytalidium dimidiatum* in Basrah-Southern-Iraq.African J.Biotechnology 11(95): 16165-16171.

10-Abdullah,S.K;Asension,L.;Monfort,E.;Gomez-Vidal,S.;Palma-Guerrero,J;Salinas,J .;Llorca,L.V.; Jansson,H.B.and Guarro,J.(2005) .Occurrence in Elx,SE spain of inflorescence rot disease of date palms caused by *Mauginiella stcattae.*J.Phytopathology 153:1-6.

11-Hassan, Z. M. and Littlefield , L.J.(1979).Ontogeny of the Uredium of *Melampsora Lini.* Can.J. Bot. 57 : 639 – 649

12-Johansen , D. A.(1940).Plant microtechnique. Mc Graw – Hill. Pubishing Company. New York . 523 pp.

13-Phillips , A.T.L.(1998).*Botryosphaeria dothidea* and other fungi associated with Excoriate and die back of grapevines in PortugalJ.Phytopathol.146:327–332.

14-Úrbez – Torres,J.R.;Leavitt,G.M.; Voegel and Gulber, W,D. (2006) .Identification and distribution of *Botryosphaeria* species associated with grapevines cankers in California .Plant Dis . 90 : 1490 – 1503 .

15-Úrbez – Torres,J.R.;Leavitt,G.M.;Guerrero,J.C.;Guevara,J.and Gubler,W.D.(2008). Identification and pathogenicity of *Lasiodiplodia theobromae* and *Diplodia seriata* , the caused agents of bot canker disease of grapevines in Mexieo . Plant Disease 92 P 519 – 529 .

16-Pascoe,I.and Cottral (2000).Developments in grapevine trunk diseases research in Australia. Phytopathol. Mediterr. 39:68-75.

17-Agrios,G.N.(2005).Plant pathology($5^{th}$ edition). San Diego, USA: Academic Press, 922 pp..

18-Lorena,T.;Calamassi,R.;Mori,B.;Mugnai,L.and Surico,G.(2001) .*Phaeomoniella chlamydospora* grapevine interaction:histochemical reactions to fungal infection.Phytopathol.Mediterr. 40:400-406.

19-Byrde,R.J.W.(1963).Natural inhibtors of fungal enzymes and toxins in disease resistance. In:Prespectives in biochemical plant pathology .(S.Rich,ed.),Connecticut Agricultural Experimental Station Bulletin 663.

20-Bell,A.A.(1981).Biochemical mechanisms of disease resistance.Annual Review of Plant Pathology 32:21-81.

21-Dai,G.H.;Andary,C.;Mondolot-Cosson,L.andBoublas(1995).Histochemical studies on the interaction between three species of grapevine,*Vitis vinifera,V.rupestis* and *V.rotundifolia* and the downy mildew fungus, *Plasmopara viticola.*Physiological and Molecular Plant Pathology 46:177-188

22-Brown,E.A.and Hendrix,F.F.(1981).Pthogenicity and histopathology of *Botryosphaeria dothidea* on apple stems. Phytopathology 71:375-379.