New records of some saprophytic and pathogenic fungi isolated from declining grapevine in Salahaldin province , middle Iraq

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Abstract

During a survey on mycobiota associated with grapevine plants exhibited decline in several vineyards in Salahaldin province , several interesting pathogenic and saprophytic fungi were encountered . These include Cadophora spp. Clonostachys rosea , Doratomyces microspores , Lasiodiplodia theobromae , Melanospora pascuensis and Neocytalidium dimidiatum . Cadophora Lagerb.& Melin and Clonostachys Corda are newly recorded genera in Iraq . Species such as C.rosea , D.microsporus and M.pascuensis are new addition to Iraqi mycobiota , whereas , L.theohromae and N.dimidiatum are newly reported pathogens on grapevine in middle Iraq. Brief descriptions along with photographic illustrations are provided for the newly recorded taxa .

Introduction

Grapevine (*Vitis vinifera* L.) is widely cultivated in north and middle of Iraq . The cultivated area in Iraq is about 48000 hectares with production of about 265000 tons [1]. Salahaldin province is a major area in the middle of Iraq for cultivation and production of grapevine and the production is established to be 125 000 tons grape [2] . During a recent survey (2012-2013) on grapevine nurseries in different districts of Salahaldin province, several plants exhibited decline were found .

The declined plants showed external symptoms with chloratic leaves, cankers and stunted shoots. Internal symptoms included wood decay, black spots in cross section and irregular central necrosis .

Several pathogenic, saprophytic and endophytic fungi have been reported as a mycobiota associated with grapevine plants in several parts of the world [3,4,5,6,7,8] .

In Iraq , however , studies on the mycobiota associated with grapevine crop were mainly came from north Iraq [9,10,11,12,13] . A part from north Iraq, recently, *L. theobromae* and *N. dimidiatum* have been reported as the causal agents for grapevine dieback in Basrah , South Iraq [14] .

We are reporting here some newly recorded pathogenic and saprophytic fungi associated with grapevine in Salahaldin province, middle of Iraq .

Materials and methods

Samples (shoots and roots) from declining vines showing yellowing , reduced growth , cankers , different internal symptoms in wood including wood decay , black spots and irregular central necrosis were collected from eleven vineyards in Salahaldin province and brought to laboratory.

Transverse sections in shoots and roots were made. These disc-like sections (2-3 cm thick) were flame sterilized by holding wood by sterile forceps and immersing in 70% ethanol and then passing through a flame [15] .

The wood sections were blotted on moisten sterilized filter papers in moist chamber boxes and incubated at 25°C until fungal propagules were observed .

Sporulating fungi were isolated by transferring reproductive structures (conidia or ascospores) with a sterilized fine syringe needle and plated on to plates of fresh Potato Dextrose Agar medium (PDA) (Himedia laboratories . India), Malt Extract Agar (MEA) (LAB.U.K) and Oat Meal Agar (OTA) (35g oat, 15g agar,1L distilled water) and incubated at 25°C . All types of media were amended with 0.25 mg / ml chloramphenicol . Isolated fungi were identified based on microscopical characters in culture and on natural habitat according to [16 , 17 , 18 , 19 , 20 , 21 , 22 , 23 , 24] .

Results and discussion

1- Cadophora spp. Fig . 1(A-C)

Colonies on MEA , PDA , OTA media are flat reaching a diameter of 20mm , 40mm and 76 mm respectively after 8 days at 25°C , with red pigmentation on MEA and violet pigmentation on OTA . Aerial mycelium consisted of branched septate hyphae . Conidiophores are mostly short branched or unbranched arising from aerial or submerged mycelium .

Phialides are smooth, hyaline , subcylindrical , 6-14 × 2.5-3 *u*m . Conidia are hyaline ovoid to cylindrical 5-7 ×1.5 – 2 *u*m . Our isolates are obtained from vineyards from Dhuluaia and Balad on black local cultivar and Halwany CV. and represent the first report for the genus in Iraq .

Our *Cadophra* isolates differ from *C.luteo-olivacea* , a common fungus on a grapevine in their conidial size and shape as well as in colony growth rate on MEA , PDA and OTA media [23] . For accurate identification to species level for our isolates, molecular identification is needed .

2- Clonostachys rosea (link: Fr.) Schroers, Samuels , Seifert & W.Gams . Mycologia 91:369 (1999) . Fig. 2 (A-B)

Colonies are white , yellowish white to light yellow reaching a diameter 75mm, 72 mm and 80 mm on MEA , PDA and OTA media, respectively, after 8 days at 25 C° in dark . Conidiophores are dimorphic, primary conidiophores (verticillium – like) and secondary conidiophores (penicillate – like) . Primary conidiophores are generally formed first, arising from the agar surface or aerial mycelium, monoverticillate, 1-3 level verticillate or with short side branched .

Phialides are generally in whorls of 2-5, slightly tapering towards the tip, with or without visible collaret, 20 -30 ×2-2.5 *u*m. Conidia are hyaline slightly curved 6-9 × 2.5-3.5 *u*m.

Secondary conidiophores are generally developed in old colonies mainly from aerial mycelium bi-to terverticillate. Phialides are in tighted whorles of 4-8 per metula, slightly flask – shaped, without visible collaret, 10-15 × 2-3 *u*m. Conidia are arranged in white or orange – white columns forming slimy masses. Conidia are hyaline, smooth, slightly curved 4-7 ×2-3 *u*m. This is the first report for the species from Iraq. The species has been reported among the most frequently isolated fungi from grapevine cuttings in south Africa [5] and from decaying grapevine cuttings in Poland [7].

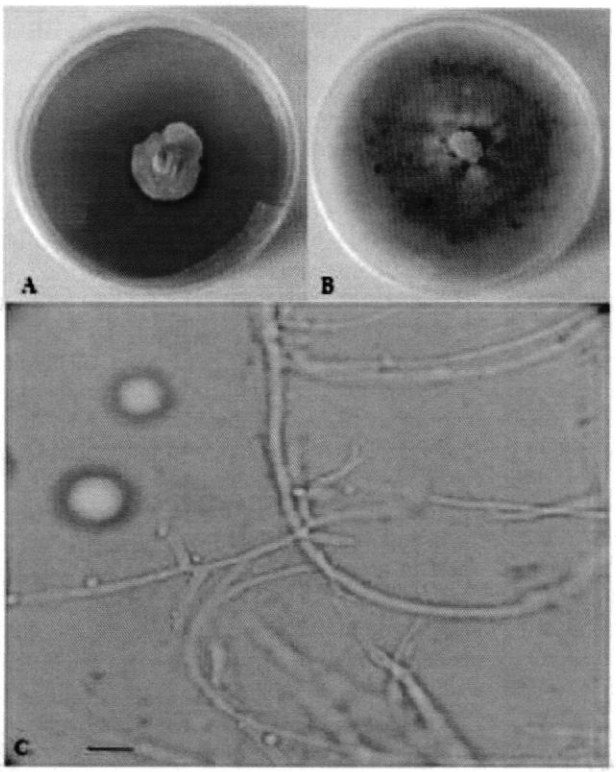

Fig. 1 (A-C). Cadophora sp. A: Colony with red pigmentation on MEA medium, B; Colony with violet pigmentation on OTA medium. C: Hyphae with phialide. Scale bar C=5um

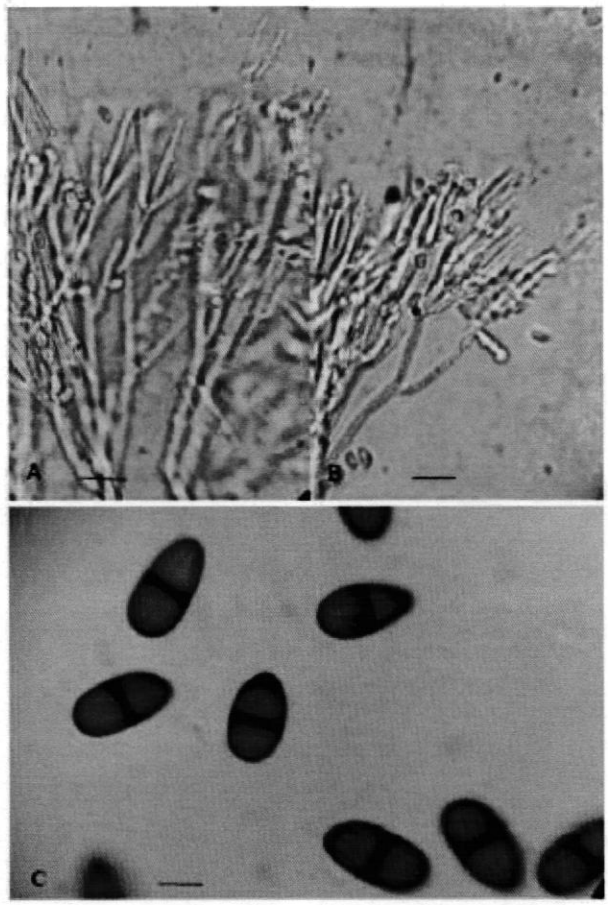

Fig.2 (A-B).Clonostachys rosea A:Verticillate conidiophores, B: Penicillate conidiophores

C: *Lasiodiplodia theobromae* conidia. Scale bar A=B =C= 10*u*m.

C.rosea is a common soil fungus and decomposer of decaying plant materials worldwide [17]. The fungus is also know as a destructive mycoparasite and can kill numerous fungal Species by hyphal penetration [25] . Therefore, the fungus was tested successfully as a biological control agent against several plant pathogens [26] .

3- Doratomyces microsporus (Sacc.) F J. Morton & G . Sm . Mycol . Pap 86: 77-80 (1963) .Fig.3 A

Colonies on natural substrate are black brown or black. Synnemata are characterized by cylindrical or elliptical head up to 500 *u*m long and 15-17 *u*m wide at base. Fertile portion is in the upper two thirds, composed of a parallel hyphae .

Annellides 4-7× 2-2.5 *u*m with swollen base. Conidia are slightly pigmented, ovate to truncate at the base and acute at the apex , thick –walled , smooth 1-celled produced in basipetal succession 3.6 – 5.4 × 2.7 – 3.2 *u*m .

This is the first report for the species from Iraq .

4- *Lasiodiplodia theobromae* (Pat.) Griffen & Maubl . Bull . trimmest . Soc . Mycol .Fr . 25 : 57 (1909) Fig.2 C

Conidiomata developed on grape vine shoot discs incubated in moist chamber after three weeks , dark brown to black. Conidiogenous cells are hyaline, holoblastic , cylindrical , proliferating percurently to form one or two annellations . Conidia are subovoid to ellipsoidal – ovoid with rounded apex and truncate base which are hyaline and aseptate but finally becoming dark – brown and one septum. Conidia discharged from the pycnidia with melanin deposit on the surface of the wall giving longitudinal striations in appearance 18 – 25 × 12 – 13 *u*m in size.

The fungus has been isolated from grapevine yards in Dhuluia and Balad on black local CV. *L.theobromae* has been know as the causal agent of dieback and canker formation on grapevine worldwide [27 , 21 , 22] . The fungus has been recently reported as the causal agent of grapevine dieback in Basrah , south Iraq [14] . The fungus has been also known associated with decline of grapevine in grape production areas in the world [27 , 28 , 29] .

5- Melanospora pascuensis Stchigel & Guarro . Mycol . Res 103: 1305 (1999) . Figs.3(C-D)

Colonies are growing rapidly on MEA medium reaching a diameter of 70 mm in 7 days at 25 C°. Aerial mycelium is white and scarce.

Ascomata developed abundantly mostly superficially , translucent , pale yellow to reddish brown appearing dark brown to black at maturity due to spore mass , 100 – 300 *u*m in diameter with a short neck and cetose .

Asci are clavate 42 – 50 × 18 – 22 *um* and their walles lacking any distinct apical apparatus, evanescent. Paraphysis are absent. Ascospores are one-celled, irregularly biseriate, dark brown, ellipsoidal 19 – 21 × 9 – 10 *um* with germ pore at each end surrounded by a dark ring – like structure . This is the first report for the species from Iraq . The species was originally isolated and described from soil samples from Easters Island, Chile [30].

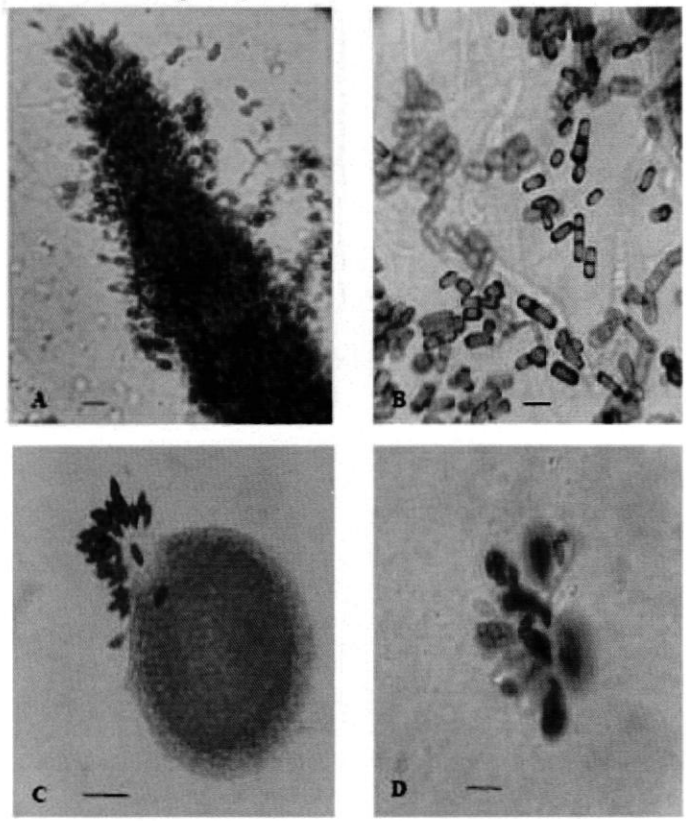

Fig.3. .A- Doratomyces microspores Conidiophore and conidia. B- Neocytalidium dimidiatum arthroconidia..C.-Melanospora pascuensis ascomata with ascospores.D- M.pascuensis asci and ascospores. Scale bar A=5um, B=5um,C=50um, D=20um

6- Neocytalidium dimidiatum (Penz.) Crous& Slippers. Studies in Mycology 55:444 (2006) Fig.3B
Colonies are blackish brown, effuse reaching a diameter of 80 mm on PDA medium after10 days at 25 C°. Hyphae are branched septate differentiated into chains of arthroconidia. Arthroconidia are 0 – 1 septate , thick walled , cylindrical to ellipsoidal , 3.5 – 5 × 7 – 10 *um*.
The fungus is very common and was isolated from shoots of the three cultivars under study (Black local cv., Halawany cv. And Black French cv.) . The fungus was reported previously from Iraq in Baghdad [31] . The fungus is also very common on grapevine cuttings in Duhok nurseries [9]. And is also reported as a part of the mycobiota associated with grapevine decline in Kurdistan region of Iraq [13] and as a member of the mycobiota associated with dried seeds of sumac (*Rhus coriaria* L) growing in Iraq [32]. Moreover, the fungus was isolated from diseased grapevine exhibiting dieback in Basrah, Southern Iraq [14].

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تسجيل جديد لبعض الفطريات الرميه و المرضية المعزولة من نباتات العنب المتصفة بالتدهور في محافظة صلاح الدين – وسط العراق

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الملخص

من خلال مسح للمجموعة الفطرية المصاحبة لنباتات العنب التي تعاني من ظاهرة التدهور فى العديد من بساتين الأعناب في محافظة صلاح الدين تم عزل العديد من الفطريات الممرضة والرمية والفطريات المعزولة شملت كل من الأنواع *Cadophora* spp , *Clonostachys rosea* , *Doratomyces microsporus, Lasiodiplodia theobromae Melanospora pascuensis , Neocytalidium dimidiatum* . الجنسان *Cadophora* Lagerb & Melin and *Clonostachys* corda يسجلان لأول مرة في العراق الانواع المتمثلة ب *C.rosa* , *D.Microsporum* , *M.pascuensis* تمثل إضافة جديدة للمجموعة الفطرية العراقية بينما النوعين *N.dimidiatum* , *L.theohromae* شخصت لأول مرة كفطريات ممرضة على الأعناب في وسط العراق كما تم وصف الأنواع المسجلة حديثا مع التوضيح بالصور .