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Fungi associated with grapevine (*Vitis vinifera* L) decline in middle of Iraq

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ABSTRACT

Fungal species associated with three (*Vitis vinifera* L.)cultivars exhibited decline grown in the main grapevine production area in Salahaldin province, middle Iraq were surveyed during 2012 – 2013. Based on microscopical and cultural characteristics, a total of 24 species in addition to non-sporulating mycelia were identified. The most frequently isolated fungi from shoots were *Aspergillus niger*, *Cladosporium cladosporoides*, *Cadophora* spp., *Clonostachys rosea*, *Penicillium* spp. *Phaeoacremonium* sp. I, *Neocytalidium dimidiatum* and *Stachybotrys atra*, whereas, *Fusarium* spp., *Acremonium* sp., *Cylindrocladiella viticola*, *Cylindrocarpon* spp., and *Phaeoacremonium* sp. 2, were the most frequently isolated fungi from roots. *C. viticola* is recorded for the first time from Iraq.

INTRODUCTION

Grapevine (*Vitis vinifera* L.) is widely cultivated in middle and north (Kurdistan region) of Iraq. Salahaldin province is the most important production area for table grape in middle of Iraq. The estimated number of vine trees is about 6.5 million and the production is established to be 125 000 tons grape (AAS, 2011).

In a recent survey (2012–2013) on some well established grapevine plantations in different districts of Salahaldin province, several plants were found suffering from decline symptoms. The external symptoms included cankers, chloratic leaves reducing vigor and stunted shoots. Wood internal symptoms including wood decay, black spots in cross section and irregular central necrosis.

In Iraq , however , there were few reports on fungi associated with grapevine decline. Haleem *et al.* (2011a,b) isolated and identified several species associated with grapevine cuttings in Duhok nurseries (Kurdistan region) Iraq. The reported species included pathogenic species *Pheaoacremonium aleophilum* and *Cylindrocarpon destructans*. In a further study, Haleem *et al.* (2012a) isolated and identified *Botryosphaerea parva* from decline grapevine and tested its pathogenicity. *B. parva* and *P. aleophilum* were also isolated from grapevine wounds during pruning and 4 months after pruning (Haleem *et al*. 2012b). Al-Saadoon *et al.* (2012) reported grapevine dieback caused by *Lasiodiplodea theobromae* and *Neocytalidium dimidiatum* in Basrah, southern Iraq. A more recent survey on the occurrence and distribution of fungi associated with grapevine decline in Kurdistan region–Iraq, revealed the detection of *B .parva*, *P. aleophilum*, *C. destructans*, *N. dimidiatum*, *Fusarium* spp., *Phoma* sp., *Macrophomina phaseolina* in addition to other saprophytic species (Haleem *et al*. 2013a,b).

The purpose of this study was to identify fungi associated with declining grapevine plants from middle Iraq for better understanding their distribution in Iraq.

MATERIALS AND METHODS

Samples collection and symptoms

A survey on 11 vineyards in different districts of Salahaldin governorate, middle Iraq were conducted .Samples from declining vines showing yellowing, reduced growth, cankers, different internal symptoms in wood, including, wood decay, black spots and irregular central necrosis were brought to the laboratory. Transverse cuts from symptomatic shoots and roots were made to observe the internal symptoms that included wood decay, black spots and irregular central necrosis (Fig. 1).

Isolation of fungi

Two surface sterilization techniques were used to ensure fungal isolation from infected materials. Two sections from each sample showing various symptoms (Lugue *et al*. 2009) were taken ,.one section was flame sterilized by holding wood by sterile forceps and immersing it in 70 % of ethanol and then passing the wood through a flame (White *et al*. 2011). The wood sections were blotted on moisten sterilized filter papers in Petri dishes or plastic boxes and incubated at 25 Cº for approximately 4 weeks . Fungal growth was monitored daily.

Small pieces (5×5×5mm) from the other section were cut from sites showing symptoms and then surface disinfected for 1 min in 1.5 % sodium hypochlorite solution, washed twice with distilled water and then were placed on malt extract agar medium (MEA) (Himedia laboratories, India), amended with 0.250 mg/L chloramphenicol. Plates were incubated at 25Cº until fungal growth was observed.

Pure cultures of each isolate was obtained by excising hyphal tip on to plates of potato dextrose agar (PDA) (200 g potato, 20 g dextrose, 20 g agar, 1L D.W), Oat meal agar OTA (35g Oat, 15 g agar and 1L D.W) and MEA media for identification. Isolated fungi were identified based on microscopical characters in culture and on natural habitat according to (Ellis, 1971; Domsch *et al*., 1980; Van Coller, *et al*. 2005; Crous *et al*., 2006; Alves *et al*., 2008; Urbez-Torres *et al*. 2008; Grameje *et al*., 2011).

RESULTS

A total of 24 species in addition to non-sporulating mycelia were isolated and identified from grapevine plants showing decline collected from eleven well established vineyards distributed in five districts of Salahaldin province, middle Iraq . The isolation frequency percentage of these fungi is presented in Table 1. The highest isolation frequency from grapevine shoots was displayed by *Aspergillus niger* (72.76%), *Cladosporium cladosporoides* (54.55%), *Clonostachys rosea* (54.55%) and *Neocytalidium dimidiatum* (45.55%), whereas, *Fusarium* spp. (72.7 %) followed by *Acremonium* spp. and *Phaeoacremonium* sp.2 (27.27% each) were the most frequent species isolated from roots.

Twenty two taxa were isolated from shoots, whereas,ten species were detected from roots. *Cylindrocladiella viticola, Pheaoacrmonium* sp.2 , were detected only from roots, whereas, *Alternaria alternata, C. cladosporoides, Cadophora* spp., *Phoma* sp., *Penicillium* spp., *Stachybotrys arta, Trichotheccium roseum, Trichurus spiralis, Phaeocremonium* sp. I., *Lasiodiplodia theobromae, Melanospora pascuensis, Neocytalidium dimidiatum, Chaetomium* sp., *Trichoderma* sp. and *Doratomyces microsporus* were isolated from shoots. Species were found common to both shoots and roots included *Acremonium* spp., *Fusarium* spp., *Clonostachys rosea, Rhizopus stolonifer* and non-sporulating mycelia.

The five pathogenic fungi *Cylindrocarpon* sp., *Cadophora* sp., *L. theobromae, N. dimidiatum* and *Phaeoacremonium* sp.1 and sp.2 were detected from vineyards in Dhulluae district. With respect to vineyard location, Dhuluae district showed the highest number (6 species) of detected pathogens (Table 2). *Cylindrocladella viticola* was isolated from vineyard in Balad and Ishaqi sites. *C. viticola* is newly recorded for Iraq. *N. dimidiatum* was found common to all sites. With respect to grapevine cultivars, black local cv showed the highest spectrum of pathogenic fungi (6 species), whereas , Halawani cv and Black French cv harboring 4 pathogenic fungi each .

Table 1: Percentage occurrence of fungi on grapevine shoots and roots.

Species Fungal	Shoots	Roots
1-*Alternaria alternate* (Fr.) Keissler	27 . 27	
2- *Acremonium* sp.	18 . 2	27 . 27
3- *Aspergillus niger* Tiegh.	72 . 7	
4- *Cladosporium cladosporocdes* (Fres.) devries	54 . 55	
5- *Cadophora* sp.	36 . 36	
6- *Cylindrocarpon* sp.		18 . 2
7- *Fusarium* spp.	27 . 27	72 . 7
8- *Cylindrocladiella viticola* Crous & G. J. Van Coller		18 . 2
9-*Clonastachys rosea* (Link: Fr.) Schroers, Samuels, Seifert & W. Gams	54 . 55	18 .2
10- *Phoma* sp.		
11- *Penicillium* spp.	9 . 1	
12- *Stachybotrys atra* Corda	36 . 36	
13- *Trichothecium roseum* (Pers.) Link	36 . 36	
14- *Trichurus spirale* Hasselbr.	36 . 36	
15- *Phaeoacremonium* sp.1	18 . 2	
16- *Phaeoacremonium* sp.2	36 . 36	27 . 27
17- *Lasiodiplodia theobromae* (Pat.) Griffen & Maubl.	18 . 2	
18- *Melanospora pascuensis* Stchigel & Guarro	18 . 2	
19- Black mycelium	18 . 2	9 . 1
20- White mycelium	27 . 27	9 . 1
21- *Neocytalidium dimidiatum* (Penz.) Crous & Slippers	45 . 45	
22- *Verticillium* sp.		9 . 1
23- *Rhzopus stolonifer* (Ehrenb.) Vuill.	36 . 36	
24- *Chaetomium* sp.	9 . 1	18 . 2
25- *Trichoderm*a sp.	18 . 2	
26-*Doratomyces microsporus* (Sacc.) F.J. Morton & G. Sm	18 . 2	

Table 2: Distribution of pathogenic fungi with respect to grapevine cultivar and location.

Cultivar	pathogen	Location
Black local	*Cylindrocarpon* sp.	Dhuluia
	Neocytalidium dimidiatum	Dhuluia
	Cadophora sp.	Dhuluia
	Phaeoacremonium sp.1	Muatassim
	Lasiodiplodia theobromae	Dhuluia
	Cylindrocladiella viticola	Balad
Halwany	*Cadophora* sp.	Balad , Dhuluia
	Phaeoacremonium sp.1	Dhuluia , Muatassim
	Neocytalidium dimidiatum	Balad , Muatassim
	Lasiodiplodia theobromae	Balad
Black French	*Cylindrocladiella viticola*	Ishaqi
	Phaeoacremonium sp.1	Ishaqi
	Phaeoacremonium sp.2	Dhuluia
	Neocytalidium dimidiatum	Dhuluia

Phenotypic characterization of *Cylindro-cladiella viticola.*

Cylindrocladiella viticola Crous & G. J. Van Coller Australasian Plant Pathology, 34:493 (2005). Figure (2). Conidiophores are hyaline, comprising a stipe, a penicillate arrangement of fertile branches, or a stipe extension and a terminal vesicle. Stipes are hyaline, septate, smooth, straight, up to 120 um long, with one basal septum and terminating with irregularly ellipsoidal to clavate vesicle, 4-7 um wide. Primary branches of penicillate conidiophores

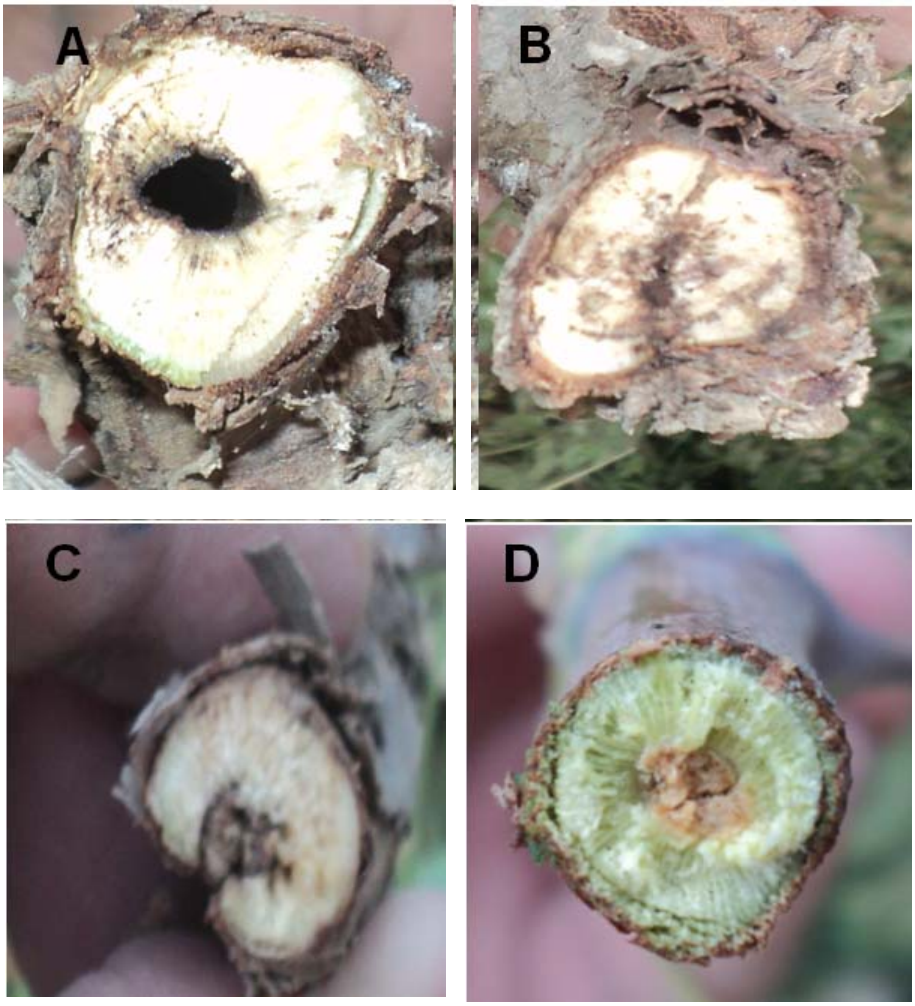

Fig. 1: Internal symptoms in cross section: A. Co-occurrence of necrosis and black spots, B. Wood decay, C. V-shaped necrosis, D.Black spots.

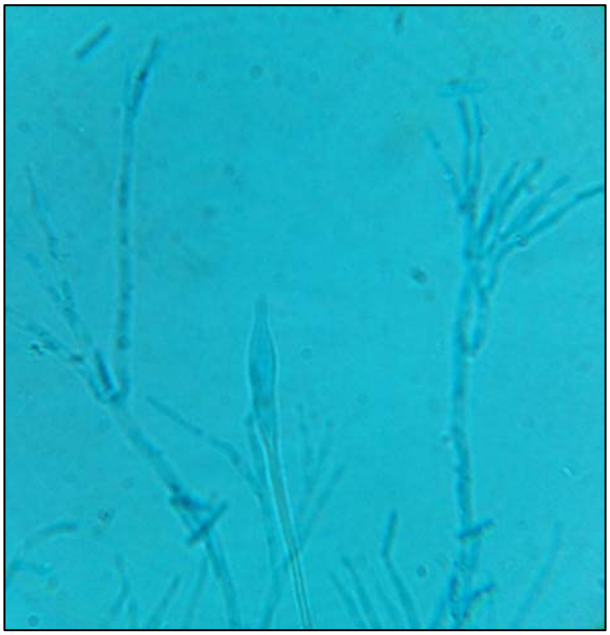

Fig. 2: *Cylindrocladiella viticola*, Penicillate conidiophore, stipe with vesicle and conidia. (scale bar= 10µ).

apparatus are 12-16 X2.5-3.0 um, aseptate, hyaline. Secondary branches, aseptate, 9-12 X 2.5-3.0 um, each terminating with 2-4 hyaline doliform to renform phialides, 10-12X2.5-3.0 um with collarette. Conidia hyaline, cylindrical, straight, rounded at both ends,1-septate,8-12 x2-2.5 um size.

DISCUSSION

The study described the isolation and identification of fungi associated with shoots and roots of three different *Vitis vinefera* L. cultivars widely grown in Salahaldin province, middle Iraq. All fungi were isolated after surface disinfection of wood tissues obtained from cross section in shoots and roots, showing disease symptoms. Among the fungal genera identified in this study, *Cadophora*, *Cylindrocladiella*, *Cylindrocarpon. Lasiodiplodia, Neocytalidium* and *Phaeoacrmonium* are of a particular interest. Species from these genera have been repeatedly isolated from apparently healthy grapevine as well as from plants showing decline symptoms (Armengol *et al*. 2001; Halleen *et al*. 2003; Van Coller *et al*. 2005; Halleen *et al.* 2007; Casieri *et al*. 2009; Haleem *et al*. 2013a; Mohammadi *et al*. 2013). Several isolates assigned to *Phaeoacremonium* as *Phaeoacremonium* sp.I and *Ph*. sp.2 have been detected from grapevine shoots and roots with internal wood discoloration respectively. It has been well documented that species in *Phaeoacremonium* (particularly *P. aleophilum*) has been isolated from declining vines and causing trunk disease in most grape vine production areas in the world (Armengol *et al*., 2001 ; Auger *et al* . 2005 ; Aroca *et al*. 2009; Lungue *et al.* 2009; Haleem *et al.* 2013b; Mohammadi *et al*. 2013).

In Iraq , however, *P. aleophilum* has been reported in several occasions associated with grape vine plants exhibiting decline symptoms as well as from apparently healthy grapevine cuttings in Duhok nurseries, North Iraq (Haleem *et al*. 2011 a b, 2012b, 2013a). The present finding of *Phaeoacremonium* species in middle Iraq indicating that this genus has a wide distribution in grapevine production areas in Iraq.

Cylindrocladiella viticola Crous & G. J. Van Coller isolated from root samples collected from grapevine nurseries in Balad and Ishaqi

districts is reported for the first time in Iraq . The fungus was originally isolated and described from grapevine cuttings showing rot from Western Cape Province South Africa (Van Coller *et al*. 2005). Species of *Cylindrocladiella*, however, reported as pathogens or saprobes on various hosts and also isolated from soils (Boesewinkel, 1982; Crous and Wingfield, 1993; Lombard *et al*. 2012). Four species of *Cylindrocladiella* have been reported from *Vitis vinifera* viz *C. laginiformis, C. peruviana, C. pseudoparva* and *C. viticola* (Van Coller *et al*. 2005; Lombard *et al*. 2012).

Cylindrocarpon sp. isolated from roots of black local CV from Dhuluia district. In a previous survey in Iraq, *C. destructans* was isolated from roots and rooted cuttings of three cultivars (Kamali, Rhashmew and Taefi) commonly growing in Kurdistan region – North Iraq (Haleem *et al*. 2011a, 2013a). Species of *Cylindrocarpon* wollenw are well known as root colonizers, weak pathogens or pathogens on various plants (Brayford, 1993). Among them *C. destructans* has frequently been described as the agent of black foot disease of grapevine (Halleen *et al.* 2004).

Lasiodiplodia theobromae and *N. dimidiatum*, two well known pathogens were isolated from grapevine shoots with a percentage frequency 18.2 % and 45.45% respectively. The former species was isolated from black local cv. in Dhuluai district, whereas, *N. dimidiatum* was found common to the three cultivars under study and showed a wider distribution within Salahaldin province . The two species have been recently reported from Basrah, southern Iraq as the causal pathogens of grapevine dieback (Al-Saadoon *et al*., 2012). *N.dimidiatum* (=*Hendersonula toruloidea*) was reported earlier in central Iraq causing branch wilt of grapevine (Natour and Ahmed, 1969). *N. dinidiatum* was also reported from Kurdistan region, North Iraq associated with grapevine exhibited decline (Haleem *et al*. 2013a) and causing sooty canker on a variety of thin bark forest trees (Hassan *et al*. 2009). The fungus was also commonly detected from seeds of sumac (*Rhus coriaria* L.) growing in North Iraq (Abdullah & Abdullah, 2013). *N. dimidiatum* was reported (as *Nattrassia* sp.) among the fungal pathogens associated with grapevine trunk disease in Iran (Mohammadi *et al*. 2013).

Several isolates of *Cadophora* spp. were isolated from black local cv and Halawany cv during this study. Halleen *et al.* (2003) reported the frequent association of *C. luteo-olivaceae* with apparently healthy rooted grapevines. A

further study by Halleen *et al*., (2007) has proven the pathogenicity of *C.leuto-olivaceae*, when pruning wounds were artificially inoculated with the fungus and stated that the inoculated plants displayed vascular discoloration similar to that seen in Petri diseased grapevine.

Acremonium spp. and *Fusarium* spp. were detected from both shoots and roots. Our result is in line with other studies (Halleen *et al*. 2003; Krol, 2006; Casieri *et al* . 2009; Haleem *et al.*2011, 2013; Mohammadi *et al*. 2013). *Fusarium* displayed high percentage occurrence (72. 72%) on roots compared to other isolated fungi and this result suggests that infection comes from soil. The work of Highet and Nair (1995) and Omer (1999) showed that *F. oxysporum* can cause decay of grapevine cuttings. Marais (1979) stated that although *Fusarium* species were found associated in high number with root rot in South Africa vineyard, but he suggested that they were of less importance.

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