FIRST RECORD OF THE WILT AND DEATH DISEASE ON DATE PALM TISSUE CULTURE CLONES OFFSHOOTS IN BASRAH PROVINCE –IRAQ

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ABSTRACT

Wilt death disease on date palm tissue culture offshoots is a new interested phenomenon observed in Iraq since a several years, represented by fungal disease infecting plants in pots and/or soil, causing graduate wilt starts from down fronds to the top, ending with death. The survey results revealed that infection percentage ranged from 4% in Abulkhaseeb district to 4.5% in Al-Seeba area with no significant differences among infected orchards (P-0.369), while the death percentage was 6.2% in Al-Seeba area comparing with 2.4% Abulkhaseeb with no significant differences too (P=0.368). The isolation results confirmed that *Fusarium solani* was the main isolated fungus from all root samples. Five isolates of *F.solani* (MLA5, MLA6, MLA7, MLA9 and MLA10) were identified at morphological and molecular levels then deposited in NCBI gene bank under the registration numbers (MG932641.1-MG932645.1). The present study recorded *F.solani* as a causal fungus of wilt and death disease on date palm tissue culture offshoots for the first time in Iraq.

Keywords: wilt death, date palm, tissue culture, *Fusarium solani*, yellow death.

تسجيل أول لمرض ذبول وموت فسائل النخيل النسيجية في محافظة البصرة . العراق

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المستخلص

تعد ظاهرة ذبول وموت فسائل النخيل النسيجي من الظواهر المرضية المثيرة للاهتمام والتي لوحظت على فسائل النخيل النسيجي في العراق خلال السنوات القليلة الماضية تمثلت بإصابة فطرية على الفسائل النسيجية في الأصص أو بعد زراعتها في التربة حيث تبدأ بذبول الدوار السفلي من السعف وتيبسه ثم يتطور المرض إلى الدوارات العليا ثم القمة النامية وتنتهي بموت النبات. أوضحت نتائج الدراسة المسحية التي أجريت جنوب محافظة البصرة في بساتين مزروعة بالنخيل النسيجي أن نسبة الإصابة تراوحت بين 4% في قضاء ابي الخصيب إلى 4.5% في ناحية السيبة وبدون فروق معنوية بينها (P=0.368)، بينما كانت نسبة موت للفسائل المصابة في ناحية السيبة 6.2% مقارنة بابي الخصيب الذي بلغت فيه نسبة الموت 2.4% وبدون فروق معنوية بينها أيضا (P=0.368). أظهرت نتائج العزل المختبري للمسبب أن الفطر *Fusarium solani* هو الفطر الرئيسي المعزول من جميع عينات الجذور للفسائل المصابة حيث تم تشخيص خمسة عزلات (MLA5 و MLA6 و MLA7 و MLA9 و MLA10) حيث شخصت مظهريا وجزيئيا واودعت نسخ من تتابعات الحامض النووي للعزلات في المركز الأمريكي لمعلومات التقانات الأحيائية NCBI حسب ارقام التسجيل (MG932641.1–MG932645.1). سجلت الدراسة الحالية الفطر *F.solani* كمسبب لمرض ذبول وموت فسائل نخيل التمر النسيجية لأول مرة في العراق.

الكلمات المفتاحية: الذبول والموت، نخيل التمر، الزراعة النسيجية، *Fusarium solani*، الموت الأصفر

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INTRODUCTION

Iraq is one of the famed countries in date palm production. Indeed, date palm cultivation distributed along several governorates including Basrah, Karbala, Babil, Diyala and Thi-qar. Basrah, considered as the most important area of date palm production in Iraq. The date palm orchards spread from the Faw at the deep South to the Qurna and Mudaina, the northern parts of governorate. Fortunately, Basrah is renowned of its abundance date palm cultivars, which is associated with high quality of these cultivars like Barhi, Breim, and Shewaithi in addition to the well-known commercial cultivars like Sayer, Hillawi, and Zahdi (2, 12). Date palm trees generally infected by many agricultural pests including fungi, bacteria, nematodes, phytoplasma, insects, mites, rodents and birds (2, 7). Fungal diseases like terminal bud rot or (fool disease or Head bending), which is caused by *Thielaviopsis paradoxa,* Inflorescence rot disease that caused by *Mauginella scaettae,* Rachis blight on leaves that caused by *Seronomyces phoenicis* and leaf spot, which is caused by several species of fungi, are currently considered as the most important diseases affecting date palm production in Iraq (1, 8, 9, 10, 11, 12, 16). Generally, date palm production in Iraq and especially in Basrah, exposed to a marked deterioration in the last decades that represented by clear reduction in the number of date palm trees (10 million trees at 1968 to 3 million tree at 2000), low productivity and low quality (9). Rehabilitation trails were performed in last few years to remediate this important agricultural field using several strategies like providing farmers with offshoots or initiation of plant nurseries to produce cultivar origins for future mass production, but these strategies faced several problems like offshoots death after short period of planting (11). Alternatively, cultivation of date palm tissue culture offshoots, which are mostly imported from neighbor countries, increased gradually since a few years ago especially in Basrah, which is associated with appearance of new pathogenic phenomenon represented by wilt symptoms followed by death of tissue culture offshoots after a short period of plantation. Actually, there are no current studies about the mentioned disease on tissue culture offshoots in Iraq; however, several studies were performed in some Gulf States, Iran and Italy referred to isolation of some fungal species accompanied by yellowish and deterioration symptoms on the infected plants. The isolated fungi were *Fusarium solani, F.proliferatum, F.branchygibbosum, F.oxysporum, and F.verticillioides* (14, 17). The present study aimed to isolating and identifying of the fungal species that associated with date palm tissue culture offshoots death phenomenon.

MATERIALS AND METHODS

Field survey study

Three orchards of imported date palm tissue culture offshoots in A-Seeba and Abulkhaseeb areas (Figure 1) were surveyed to estimate disease incidence, each orchard involved 1000-3000 offshoots within ages ranged between 1-2 year in addition to several thousand date palm tissue culture still in the pots. Each studied orchards divided into four parts 50 × 50 m. The yellowish, wilt and died plants were counted and the disease incidence was calculated according to the following formula:

$$\textit{Disease incidence}\ \% = \frac{\textit{Number of infected plants}}{\textit{Total number of plants within studied area}} \times 100$$

Fig. 1. The locations of the orchards that have been covered by the survey study

Isolation of associated fungi

Live and/or wilt plants were completely taken off and brought to the laboratory. The roots were washed carefully with tap water to clean them from soil and clay. The root samples 0.5 – 1 cm/piece were surface sterilized with 10% of commercial sodium hypochlorite solution (Clorox, 5.25%). A Petri plates filled with 20 ml of (PDA) Potato Dextrose Agar (Oxoid, LTD) were inoculated with three pieces of sterilized roots for each then incubated for 7 days at 25ºC±2. The isolates were purified by sub-culturing on PDA.

Morphological identification

The fungal isolates MLA5, MLA6, MLA7, MLA9, MLA10 were identified morphologically according to Leslie and Summerell (13).

Molecular identification

The molecular identification was performed using 4 days old culture of each mentioned isolate that were incubated on PDA medium at 25ºC±2, the mycelium was scraped then flash frozen with liquid nitrogen and ground with mortar and pistil to a fine powder (3). Up to 25 mg of powder was transferred to 1.5 Eppendorf tube to extract the DNA using (Promega Genomic DNA Purification kit A1120, USA) according to manufacturer instructions. The quality and quantity of extracted DNA was confirmed using Nanodrop (Termo-Scientific™, NanoDrop 2000) under 260/280 nm wavelength. A PCR amplification was performed to the ITS1-ITS4 rRNA region to identify the fungus molecularly. The primers used for PCR were, For: 5'TCCGTAGGTGAACCTGCGG3' and Rev: 5'TCCTCCGCTTATTGATATGC3' (5). The PCR conditions were 5 min of initiation at 90ºC followed by 35 cycles of: denaturation at 94ºC for 1 min, annealing at 58ºC for 1 min and 2 min of extension at 72ºC, after that the reaction ended with final extension at 72ºC for 5 min. The product quality and quantity were confirmed using Nanodrop device (Termo-Scientific™, Nano Drop 2000) under 260/280 nm wavelength. The PCR products of all the mentioned samples were send to Macrogen Inc. (Macrogen Korea: 10F, 254 Beotkkot-ro, Geumcheon-qu, Seoul, 08511, Rep. of Korea) for sequencing. The molecular identification was confirmed through processing of gotten sequences with Chromas 2.6.5 software (3), then multiple alignment was performed for each sample

sequence separately with database of NCBI (15) using BLAST software (6). The processed sequences of the identified isolates were submitted to the NCBI for registration.

Statistical analysis

The survey results were analyzed statistically according to Kruskal-Wallis 1-way ANOVA (k samples) analysis using IBM® SPSS® statistics package, Version 24 (IBM Corp.).

RESULTS AND DISCUSSION

Field survey

The percentage of the shoots with the yellowish and wilt symptoms in the three orchards that covered by scanning of this study (Table 1) ranged from 4–4.5%. The highest disease incidence recorded in Al-Seeba area (4.5%), and the lowest percentage was in Abu-Alkhaseeb district (4%) with no significant differences (P=0.368), while the highest death percentage was at Al-Seeba area (6.2%) and the lowest one was at Abu-Alkhaseeb district (2.4%) with no significant differences (P=0.368). The symptoms represented by transforming of fronds of lower row from green to umber (brawny-yellow), these symptoms develops towards the center of the head then ended with death (Figure 2).

Fig. 2. Wilt and Death symptoms on date palm tissue culture offshoots after transferring to the soil

Similar symptoms were observed on the date palm tissue culture offshoots that still in the pots before they transferred to soil, which proposing that the plants might infected at their cultivation origin *i.e.* before they entered Iraq. Actually, there is no recorded studies about date palm tissue culture offshoots death (especially those that transferred to the soil) in Iraq, but some studies referred to similar phenomenon on offshoots, which recorded that *Fusarium solani* was one of the causing pathogens (4, 18, 19). Furthermore, Mansoori and Kord (14) recorded the same disease on date palm in Iran

Table 1. The wilted and died shoots percentage

Area	Wilted shoots %	Died shoots %
Al-Seeba	4.5	6.2
Abu-Alkhaseeb 1	4	2.4
Abu-Alkhaseeb 2	6	3.8
P value	0.368	0.368

1 and 2 refers to orchard 1 and 2

Morphological identification

The results of isolation (Figure 3) and identification revealed that ***F.solani*** was the main isolated fungus in all root samples that obtained from infected date palm tissue culture offshoots. The isolates

(MLA5, MLA6, MLA7, MLA9 and MLA10) identification was confirmed morphologically as ***F.solani*** according to Leslie and Summerell **(**13**)**. The fungal colonies were white to creamy with spare mycelium. The macroconidia were mostly straight or slightly curved includes 3-7 septate with rounded ends, and were enclosed in creamy sporodochia. The microconidia were oval to ellipsoidal or reniform shape, non-septate and sometimes includes one septum. The microconidia were formed on round false heads and the phialides were mostly single and prolonged. The chlamydospores were formed abundantly on medium, which were mostly in piers.

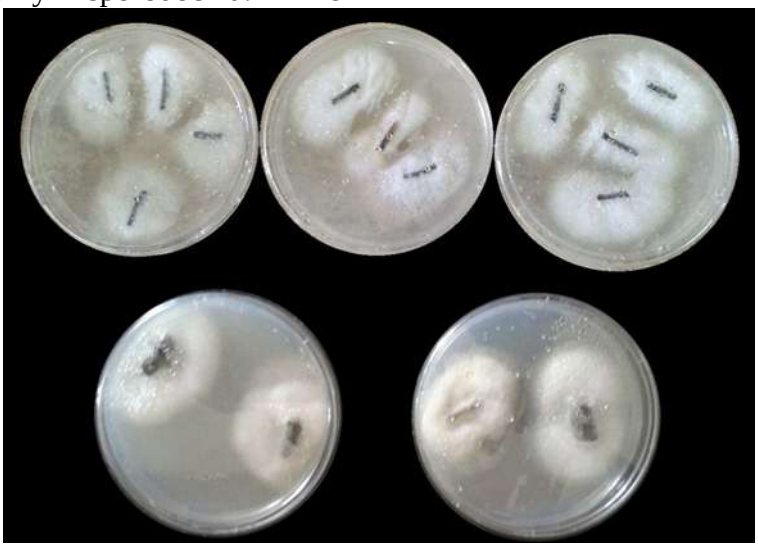

Fig 3. The primary isolation results represents that *F.solani* is the only isolated fungus

Molecular identification

The sequence multiple alignment results (Table 2) of the isolates (MLA5, MLA6, MLA7 MLA9 and MLA10) with NCBI database confirmed the identity of all mentioned isolates were identified as ***F.solani*** within identity percentage ranged from 98-99%. The sequences of ITS1-ITS4 region of the mentioned isolates were deposited in the NCBI gene bank under registration numbers (MG932641.1 - MG932645.1) respectively.

Table 2. Identification of *Fusarium solani* isolates

Isolate name	NCBI registration No.	Compatible with	Identity	score
MLA5	**MG932641.1**	**LC317619.1**	**99%**	**649**
MLA6	**MG932642.1**	**LC317619.1**	**98%**	**706**
MLA7	**MG932643.1**	**LC317619.1**	**99%**	**750**
MLA9	**MG932644.1**	**KX349467.1**	**99%**	**1046**
MLA10	**MG932645.1**	**KX349467.1**	**99%**	**1018**

The yellow death disease was not recorded as a date palm effective disease in Iraq before 2006. All the previous studies before 2006 were not approved *F.solani* as the main cause of disease. The first certain confirmation of *F.solani* as main pathogen of yellow death of date palm was by Mansoori & Kord (14) in Iran, which was followed by Salim et al. (18). This study can be considered as a first record of the wilt and death disease on tissue culture offshoots in Iraq with confirmation of *F.solani* as a causal pathogen of this disease.

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