

Verticillium dahliae Kleb

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Verticillium dahliae Kleb

2005	0.26 % 30.14	2005 2004	0.29 % 48.72
(RVD FVD) <i>V.dahliae</i> DNA Nested-PCR NESF 18S) (RVD FVD) <i>V.dahliae</i>		Polymerase Chain Reaction (PCR) <i>V.dahliae</i> 330 bp (NESR 28S PCR	
365 (FAO , 2000) 1999	12550		

<i>Verticillium dahliae</i> Kleb. <i>Cyloconium</i>	<i>oleaginum</i>	<i>Olea europea</i> L. 30 35) (1988	Oleaceae <i>Olea</i> 5,340,000 3,373,531 (FAO, 2005) 2,100,000
Mamluk et Qasem,1970) (2001 <i>V.dahliae</i>	al.,1984 <i>V.albo-atrum</i>		
(Engelhard, Issac ,1967	Woolliams ,1966 1957 (Schnathorst ,1981		

.2009/7/2 2008/7/28

:
 $.100 \times =$

(1991) Tjamos
 :(1)

Microsclerotia
 Collins et Tjamos,1993) 13

. (al., 2003

:1

Tjamos

%20-1	1
%40-21	2
%60-41	3
%80-61	4
%100-81	5

DNA
 .Polymerase Chain Reaction (PCR)

McKinney

: (1923)

: 2005 2004

(×)

=

×

:

=

:

=

(Isaac ,1967 Smith ,1965)

Verticillium dahliae

/
/NCARTT

DNA

V. dahliae

(44)

0.5

%1

DNA

(Bendich and Rogers ,1985) CTAB

DNA

V.dahliae

DNA

Selective)

5

7.5

(medium

0.5

2

0.01

1

)

1 PCNB 0.05

(

(Aushor et al.,1975)

20

DNA

/ 100

0.2

2 ± 20

65

Hyphal tip method

1.5

(Masoud,2002 1995 . 15 70-65

PTC-200 DNA) PCR 480bp DNA
 .(MJ-Research , USA) (thermocycler DNA Gel Electrophoresis
 () Templet DNA %0.7 (Bio-RAD, USA)
 (3) (Tris-) 0.5X TBE buffer
 (FVD,RVD) Size marker Borate-EDTA
V. dahliae (Masoud,2002) (Promega) 1Kb
 (4) DNA 10
 4 DNA
 UV Transilluminator
 DNA (Vilbertourmat,France)
 260 Spectrophotometer
 280
 Hand Polaroid Camera
 . 667 Polaroid
 2005 – 2004 **Polymerase (PCR)**
Chain Reaction
 PCR
 (4) .*V.dahliae*
 PCR
 PCR
 .Sambrook et al. (1989)
 (Promega) PCR
 DNA Madison,WI,USA)
 DNA
 .DNA
 4
Nested-PCR
 DNA
 Nested – PCR
 (Volossiouk et al., (NESF18s,NESF28s)

V.dahliae

V.dahliae

. (2003 2001)

0.25 % 35.2

0.15 % 27.45

Levin et al. (2003a,b)

Koike et al.,)

.(2003)

2001

Regan and Heffer, 1996 1994

.(Marcone and Camele, 2005

(3)

0.42 %75.6

0.12 %25

0.24 % 30

.(Agrios , 2005)

Al-Ahmad and Mosli,1993)

(2)

.(Serrhini and Zeroual, 1995

0.19 % 39.16

48.72

0.29 %

(1988)

(1993) Thanassoulopoulos

0.20 % 28.43

(1993) Al-Ahmad and Mosli

0.34 % 46.29

Mercado-Blanco (1993) Thanassoulopoulos
(2005) Nigro (2003) et al.

2005 2004 : 2

2005		2004	
	%		%
0.29	48.72	0.19	39.16
0.15	24.32	0.13	26.66
0.21	30.57	0.14	24.89
0.26	30.14	0.19	22.23
0.23	31.16	0.17	23.32
0.34	46.29	0.20	28.43
0.05		*	

2005 2004 : 3

2005		2004	
	%		%
0.32	75.00	0.26	66.00
0.43	79.74	0.27	68.35
0.62	85.40	0.42	75.60
0.41	76.60	0.32	65.50
0.60	80.20	0.41	73.30
0.24	30.00	0.12	25.00
0.05		*	

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Verticillium dahliae Kleb

V.dahliae

Moukhamedov et al., 1994)
 Camele and Marcone Masoud, 2002 al., 1994
 .(2005

DNA

V.dahliae

FVA)

4 - 3

(4) *V. albo-atrum*

(RVU

3-1 × 35-15

337bp DNA

3.5 - 1.2 × 3.5 - 2.00

Nested-PCR

Nested- PCR

190 - 11 × 35 - 11

(2)

(NESF18s NESR28s)

(1967) Issac (1965) Smith

480 bp

Thanassoulopoulos et al.,1979) *V.dahliae*

Fravel, 1989 1988

Wells

Al-Ahmad and Duksi, Karajeh, 1997 1992

(3)

.(Stapleton and Duncan, 2000 1997

Nested- PCR

(RVD FVD)

V.dahliae

330bp

(50)

V.dahliae

(1982) Biris et al.

(1993) Tjamos and Botseas (1987) Tjamos

(1997) Karajeh (1996) Naser

V.dahliae

Polymerase (PCR)

Nested - PCR

Chain Reaction

330bp

(RVD,FVD)

()

(Sambrook et al.,1989) %2

V.dahliae

330 bp

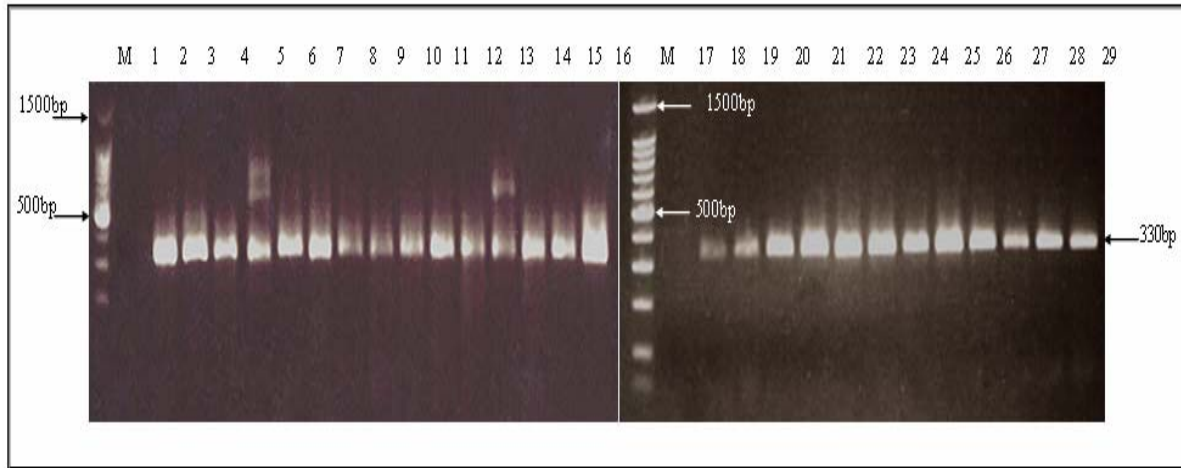
PCR

Mercado-Blanco et al., 2001, 2002, 2003)
 .(Masoud, 2002

Verticillium spp.

:4

5' to 3'			(bp)		
FVD	59.5	GGTCCATCAGTCTCTCTG	Nuclear ITS1	330	<i>V.dahliae</i>
RVD	59.5	TCCGATGCGAGCTGTAAC	Nuclear ITS2		
NESF18s	58.4	CTCATAACCCTTTGTGAACC	18s gene	480	<i>Verticillium</i>
NESR28s	66.6	CCGAGGTCAACCGTTGCCG	28s gene		
FVA	53	GGTACATCAGTCTCTTTA	Nuclear ITS1	337	<i>V.albo-atrum</i>

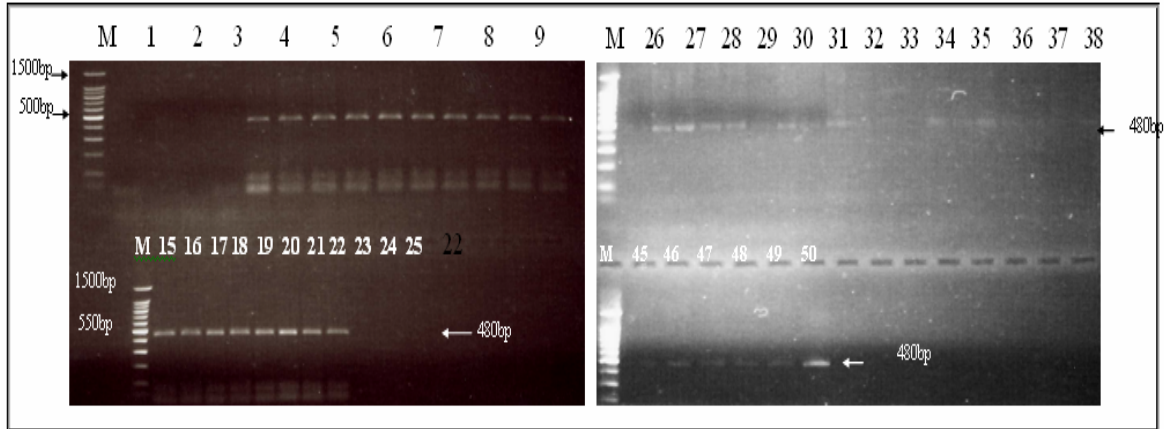


(FVD , RVD) *Verticillium dahliae*

Single PCR :1

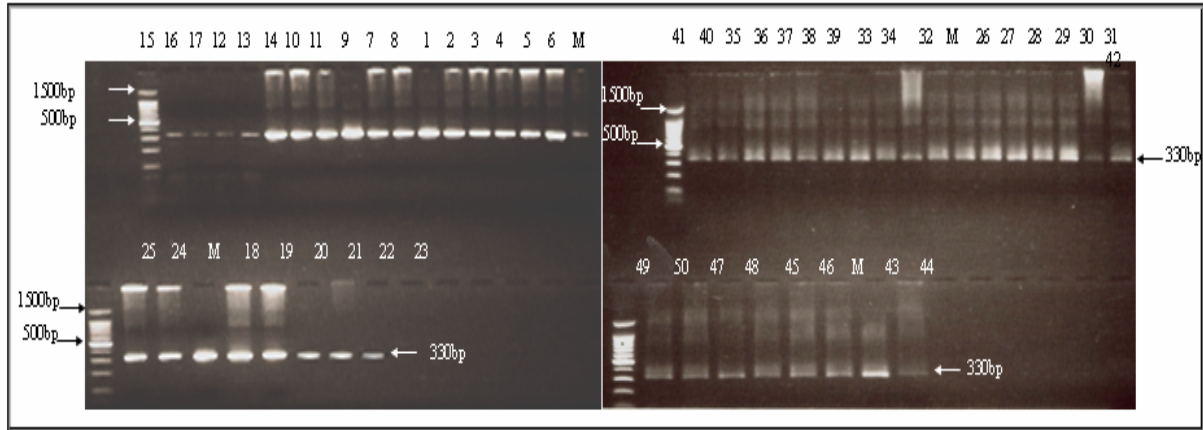
.%2

						100 bp DNA ladder :M		
2	/	-21	2	/	-11			-1
2	/	-22	2	/	-12	1	/	-2
	/	-23	1	/	-13	2	/	-3
	/	-24			-14			-4
3	/	-25		/	-15		/	-5
1	/	-26		/	-16		/	-6
3	/	-27			-17		/	-7
3	/	-28			-18		/	-8
3	/	-29		/	-19			-9
					-20			-10



(NESF18s,NESR28s) Nested- PCR :2

		<i>Verticillium dahliae</i>			DNA %2			100 bp DNA ladder : M						
		-41	/	-31	/	-21	2	/	-11	1	/	-1		
/		-42	1	/	-32	/	-22	1	/	-12	2	/	-2	
		-43	2	/	-33	3	/	-23		-13			-3	
1	/	-44	3	/	-34	1	/	-24	/	-14	/		-4	
		-45	4	/	-35	3	/	-25	/	-15	/		-5	
		-46	5	/	-36	3	/	-26		-16	/		-6	
		-47	/		-37	3	/	-27	/	-17	/		-7	
		-48	3	/	-38	2	/	-28		-18			-8	
		-49	/		-39	3	/	-29	2	/	-19		-9	
		-50	2	/	-40	1	/	-30	2	/	-20	2	/	-10



(FVD , RVD)

Nested- PCR

:3

DNA %2

100bp DNA ladder : M													
	-41	/		-31	/		-21	2	/	-11	1	/	-1
/	-42	1	/	-32		/	-22	1	/	-12	2	/	-2
	-43	2	/	-33	3	/	-23			-13			-3
1	-44	3	/	-34	1	/	-24	/		-14		/	-4
	-45	4	/	-35	3	/	-25	/		-15		/	-5
	-46	5	/	-36	3	/	-26			-16		/	-6
	-47	/		-37	3	/	-27		/	-17		/	-7
	-48	3	/	-38	2	/	-28			-18			-8
	-49	/		-39	3	/	-29	2	/	-19			-9
	-50	2	/	-40	1	/	-30	2	/	-20	2	/	-10

- 456
2003
- 1988
- 138 -130 :8
1998
- 32-27: 6
- 1992
- 1998 / 58 / / .() . 139-131 :10
- 2001
- Agrios,G.N. 2005. Plant Pathology. 5th ed. Academic Press.New York. 922 pp.
- Al-Ahmad, M.A.and Mosli, M.N. 1993. *Verticillium* wilt of olive in Syria. *OEPP/EPPO Bulletin*, 23: 521-529.
- Al-Ahmad, M. A.and A.Duksi. 1997. Solar chamber as an inclusive method to control *Verticillium* wilt of olive trees.Second international conference on soil solarization and integrated management of soilborn. Aleppo (Syria). ICARDA. 45-46.
- Aushor, K., J.Katan and S.Ovadias. 1975. Improved selective medium for the isolation of *Verticillium dahliae*.*Phytoparasitica*, 3: 133-137.
- Biris, D., C.C.Thanassoulopoulos and E.C.Tjamos. 1982. Ways of infection of olive tree by *Verticillium dahliae* and dissemination of the fungus in field by irrigation water. *Georgiki-Ervenal (Greece)*, 6: 289-296.
- Camele, I.and Marcone, C. 2005. *Verticillium* wilt of *Xanthium italicum* caused by *Verticillium dahliae* in Italy. *Plant Dis.*, 89: 908.
- Carder, J. H., A.Morton, A.M. Tabrett and D.T.Barbara. 1994. Detection and differentiation by PCR of specific groups within two *Verticillium* species causing vascular wilts in herbaceous hosts .Pages 91-97, in: Modern Assays for Plant Pathogenic Fungi: Identification, Detection and Quantification .A.Shots , F.M.Dewey and R.Oliver (eds.), CAB International, Oxford .
- Collins, A., C.A.N.Okoli, A.Morton, D.Parry, S.G. Edwards and D.J.Barbara. 2003. Isolates of *Verticillium dahliae* pathogenic to crucifers are of at least three distinct molecular types. *Phytopathology*, 93:364-376.
- Engelhard, A. W. 1957. Host index of *Verticillium albo-atrum*.
- FAO. Production Yearbook. 2000. Food and Agriculture Organization of the United Nations, Rome, Italy.
- FAO. Production Yearbook. 2005. Food and Agriculture Organization of the United Nations, Rome, Italy.
- Fravel, D.R. 1989. Biocontrol of *Verticillium* wilt of eggplant and potato.Pages 457-492 in: Vascular Wilt Diseases of Plants. Vol. H28. E.C. Tjamos and C.H. Beckman (eds.), NATO ASI Series. Springer-Verlag, Berlin, Germany.
- Heffer, V. J.and R.P.Regan. 1996. First report of *Verticillium* wilt caused by *Verticillium dahliae* of ash trees in Pacific Northwest nurseries. *Plant Dis.*, 80: 342.
- Isaac, I. 1967. Speciation in *Verticillium*. *Annu. Rev. Phytopathol.*, 5:201-222.
- Karajeh, M. 1997. Control of *Verticillium* wilt of olive trees in Jordan. M.Sc. Thesis. Agric. Faculty. Jordan Univ., Amman. Jordan.
- Koike, S. T., Subbarao, K. V., Davis, R. M., Gordon, T.R.and Hubbard, J.C. 1994. *Verticillium* wilt of cauliflower in California. *Plant Dis.*, 78: 1116-1121.

- Levin, A. G., Lavee, S. and Tsrur (Lahkim), L. 2003a. Epidemiology of *Verticillium dahliae* on olive (cv. Picual) and its effect on yield under saline conditions. *Plant Pathol.*, 52: 212-218.
- Levin, A. G., Lavee, S. and Tsrur (Lahkim), L. 2003b. Epidemiology and effects of *Verticillium* wilt on yield of olive trees (cvs. Barnea and Souri) irrigated with saline water in Palastine. *Phytoparasitica*, 31:333-343.
- Mamluk, O., Abu-Gharbieh, W. and Shaw, G. 1984. A checklist of Plant Diseases in Jordan. 1st Edition. Al-Dustour Press, Amman, 107pp .
- Masoud, S. A. 2002. Specificity of different PCR primers for *Verticillium dahliae* isolated from olive trees in Jordan. *Mu'tah Lil-Buhuth wad-Dirasat*, 17: 89-104.
- Mckinney, H.H. 1923. Influence of soil temperature and moisture on infection of wheat seedling by *Helminthosporium sativum*. *J.Agric. Res.*, 26: 195-217.
- Mercado-Blanco, J., D.Rodriguez-Jurado, E.Perez-Artes and M.Jimenez-Diaz. 2001. Detection of the nondefoliating pathotype of *Verticillium dahliae* in infected olive plants by nested PCR. *Plant Pathol.*, 50: 609-619.
- Mercado-Blanco, J., D.Rodriguez-Jurado, E.Perez-Artes and M.Jimenez-Diaz. 2002. Detection of the defoliating pathotype of *Verticillium dahliae* in infected olive plants by nested PCR. *Eur. J. Plant Pathol.*, 108:1-13.
- Mercado-Blanco, J., Rodriguez-Jurado, D., Parrilla-Araujo, S. and Jimenez-Diaz, R. M. 2003. Simultaneous detection of defoliating and nondefoliating *Verticillium dahliae* pathotypes in infected olive plants by duplex, nested polymerase chain reaction. *Plant Dis.*, 87:1487- 1494.
- Miller, P. M. and Stoddard, E. M. 1965. Hot water treatment of fungi infecting strawberry roots. *Phytopathology*, 55: 694-696.
- Naser, Z. 1996. Epidemiology of *Verticillium* wilt of olive trees in Jordan. M.Sc. thesis, University of Jordan, Amman, Jordan .
- Nigro, F., P.Gallone, G. Romanazzi, L. Schena, A. Ippolito and M.G.Salerno. 2005. Incidence of *Verticillium* wilt on olive in Apulia and genetic diversity of *Verticillium dahliae* isolates from infected trees. *Plant Pathol.*, 87: 13-23.
- Pullman, G. S. and DeVay, J. E. 1982. Epidemiology of *Verticillium* wilt of cotton: A relationship between inoculum density and disease progression. *Phytopathology*, 72: 549-554.
- Pullman, G. S., DeVay, J. E., Garber, R. H. and Weinhold, A. R. 1981. Soil solarization: effects on *Verticillium* wilt of cotton and soil-borne populations of *Verticillium dahliae*, *Pythium* spp., *Rhizoctonia solani* and *Thielaviopsis basicola*. *Phytopathology*, 71: 954-959.
- Qasem, S. 1970. Occurrence and distribution of plant diseases in Jordan. *Research Bulletin*, 28pp.
- Rogers, S. O. and A.J.Bendich. 1985. Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues. *Plant Molec. Biol.*, 5: 69-76.
- Schnathorst, W. C. 1981. Life cycle and epidemiology of *Verticillium*. Pages 81-111 in: Fungal Wilt Diseases of Plants. M. E. Mace, A. A. Bell and C. Beckman, eds. Academic Press, New York, USA.
- Serrhini, M.N. and Zeroual, A. 1995. *Verticillium* wilt of olive trees in Morocco. *Olivae*, 58: 58-61.
- Smith, H. C. 1965. The morphology of *Verticillium albo-atrum*, *V.dahliae* and *V. tricorpus*. *Newzealand J. Agric. Res.*, 8:450-478.
- Stapleton, J. J. and Duncan, R. A. 2000. Biology and management of *Verticillium* wilt of *Prunus* spp. in the Central Valley of California. *KAC Plant Protection, Quarterly*, 10:9.
- Thanassoulopoulos, C. C. 1993. Spread of *Verticillium* wilt by nursery plants in olive groves in the Halkidiki area (Greece). *OEPP/EPPA Bulletin*, 23, 517-520.
- Thanassoulopoulos, C. C., Biris, D. A. and Tjamos, E. C. 1979. Survey of *Verticillium* wilt of olive trees in Greece. *Pl. Dis. Repr.*, 63: 936 -940.

- Tjamos, E. C. 1993. Prospects and strategies in controlling *Verticillium* wilt of olive. *OEPP/EPPO Bulletin*, 23: 505-512.
- Tjamos, E. C. and D. Botseas. 1987. Occurrence of *Verticillium dahliae* in leaves of *Verticillium* wilted olive-trees (Abstr.). *Can. J. Plant Pathol.*, 9:86.
- Tjamos, E. C. and Paplomatas, E. J. 1988. Long-term effect of soil solarization in controlling *Verticillium* wilt of globe artichokes in Greece. *Plant Pathol.*, 37, 507-515.
- Volossiuk, T., Robb E.J. and Nazar, R.N. 1995. Direct DNA extraction for PCR-mediated assays of soil organisms. *Appl. Environ. Microbiol.*, 61:3972-3976.
- Wheeler, T. A., Madden, L. V., Rowe, R. C. and Riedel, R. M. 2000. Effects of quadrat size and time of year for sampling of *Verticillium dahliae* and lesion nematodes in potato fields. *Plant Dis.*, 84: 961-966.
- Woolliams, G.E. 1966. Host range and symptomatology of *Verticillium dahliae* in economic, weed and native plants in interior British Columbia. *Can. J. Pl. Sci.*, 46:661-669.

Molecular Diagnosis of *Verticillium dahliae* Kleb; the Causal Agent of *Verticillium* Wilt of Olive

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ABSTRACT

The present study was conducted on olive wilt disease caused by *Verticillium dahliae* Kleb, which has recently distributed in Iraq and is considered as one of the most important and devastating diseases on olive trees in Iraq. The results of the field survey, carried out in certain regions of Ninevah province during the years 2004 and 2005, showed that the highest disease incidence and severity were found in Alsada region in 2005 and reached 48.72% and 0.29; while the lowest values were recorded in Khorsybad and reached 30.14% and 0.26, respectively.

For the first time in Iraq, the Polymerase Chain Reaction (PCR) was used to diagnose the different isolates of the pathogen using two specific pairs of primers (FVD and RVD). The PCR results showed that *V.dahliae* Kleb is the prevalent disease in Iraq which produced DNA fragment bands of 330 bp which was expected for these primers. The samples were also processed for detection using (NESF18s and NESR28s) primers in Nested PCR where these primers are used in common detection of all *Verticillium* spp. and the products were subjected to another PCR reaction using specific primers for *V.dahliae* (FVD and RVD). The results of these tests confirmed that *V.dahliae* is the common species and it was detected in healthy trees (unclear symptoms), indicating that this test is to be used to detect the disease in its early stages.

KEYWORDS: Olive, *Verticillium* wilt, Polymerase Chain Reaction (PCR).

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