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*.Verticillium dahliae* Kleb

( RVD FVD )

*V.dahliae*

Polymerase Chain Reaction (PCR)

*V.dahliae*

330 bp

0.21

.0.64 ( )

## Study on Verticillium Wilt of Pistachio and its Control

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### ABSTRACT

The present study was conducted on pistachio wilt, which recently spread in Iraq. Results of isolation and diagnosis showed that the pistachio wilt disease caused by *Verticillium dahliae* Kleb. Identification was confirmed by the Polymerase Chain Reaction (PCR) to diagnose the pathogen using a specific pair of primers (FVD and RVD). The PCR results showed that *V.dahliae* Kleb is the causative agent of pistachio wilt disease in Iraq. The PCR produced two fragments bands of 330 bp as was expected for these two specific primers.

The results of chemical control indicated that the fungicide Beltanol had a surprising effect in reducing disease severity to 0.26n compared to 0.64 in the untreated samples, in addition to its significant effect on studied characteristics.

15                    *Pistacia*

.(Hadj-Hassan and Padulosi, 1998) *Pistacia vera* L  
( Anacardiaceae)

1998                    )

.( 2002

*Septoria*

*Verticillium dahliae* Kleb.

.(2001                    ) *Cladosporium* sp.

*pistacina*

*V.albo-atrum    V.dahliae*

, 1981 ; Issac, 1967 ; Engelhard , 1957 )

2004) 1950 *V.dahliae* (Schnathorst

(Mohammadi *et al.*,

( Epstein *et al.*, 2004 Michailides *et al.*, 1995 )

*V. dahliae*

*V. dahliae.* ( )

/

Banihashemi1, (2008) Mohammad and ( Mohammadi *et al.*, 2007)

Polymerase

DNA

.Chain Reaction (PCR)

:

%1

0.5

2 3 5 7.5 (Selective media)

0.01

1

0.5

20

1 Penta Chloro Nitro Benzene (PCNB) 0.05

( Aushor *et al.* ,1975)

2 ± 20

/ 100

Smith, 1965)

(Hyphal tip method )

. (Isaac, 1967;

*Verticillium dahliae*

/

## DNA

*V. dahliae* (6)

(Rogers and Bendich , 1985) CTAB DNA

0.2

( Extraction buffer) <sup>3</sup> 1.5

65

50 mM Tris-HCl [pH<sub>8</sub>], 700mM NaCl, 10mM EDTA [Ethylene Diamine Tetra Acetic acid, (Disodium salt)], 2% CTAB [Cetyl Trimethyl Ammonium Bromide], 0.2% [v/v] 2-Mercaptoethanol, and 0.4% [w/v] polyvinylpolypyrrolidone ).

15

70-65

(24 : 1)

Chloroform : Isoamylalcohol

600

/ 14000

Vortex

( )

Isopropanol

600

DNA

°20-

/ 14000

%70

( 1)

1000

( )

10mM Tris ,1mM ) TE buffer

50

DNA ( )

3

70

( EDTA , pH<sub>8</sub>

37

RNase

20-

### Polymerase Chain Reaction ( PCR)

V.albo-atrum      *V.dahliae*      PCR

(1)      (      )

PCR

:      25      (Sambrook *et al.*, 1989)

PCR buffer**	1X
dNTPs *	0.2mM
MgCl <sub>2</sub>	25mM
FVD Primer	0.25mM
RVD Primer	0.25mM
Taq Polymerase	1 unit
Formide	5%
DNA Template	50ng
Water	12.6 µL
<b>Total</b>	<b>25 µL</b>

\* (dTTP, dCTP, dGTP, dATP).

\*\* (500mM KCl, 15mM MgCl<sub>2</sub>, 100mM Tris-HCl [pH9 at 25°C] and 1% Triton X-100).

*.Verticillium spp*

: 1

		5' to 3'		(bp)	
FVD	59.5	GGTCCATCAGTCTCTCTG	Nuclear ITS1	330	<i>V.dahliae</i>
RVD	59.5	TCCGATGCGAGCTGTAAC	Nuclear ITS1		
FVA	53	GGTACATCAGTCTCTTTA	Nuclear ITS2	337	<i>V.albo-atrum</i>
RVU	66.7	GCGGGACTCCGATGCGAG	Nuclear ITS2		

DNA (Madison, WI, USA) Promega

PCR

DNA

DNA



20

*V.dahliae*

/  $10^6 \times 1$

%70

5

Haemocytometer

( )

( )

100

$3^3$

10

10

3

/ 100

$3^3$

/

$10^6 \times 1$

*V.dahliae*

:

/ 3-2

/ 1

$1.5^3$

*V.dahliae*

*V.dahliae*

:

( )	0
% 50-1	1
% 100-51	2
	3
	4

: MicKenny, 1923

( × )

=

×

*Verticillium dahliae*

:

Kleb

× 35-15

4 – 3

× 3.5 – 2.00

3-1

11 × 35 – 11

3.5 – 1.2

(1967) Smith , (1965)

190 –

*V.dahliae*

Issac,



**Polymerase Chain Reaction (PCR)**

(RVD,FVD)

( Sambrook *et al .*, 1989) %2

(1 ) 330 bp

*.V .dahliae*

*Fusicoccum* sp.

Michailides ,) PCR

*Xanthomonas translucens*

.(Facelli *et al .*, 2005 1995

DNA

Reverse Verticillium Universal Forward Verticillium alb-atrum

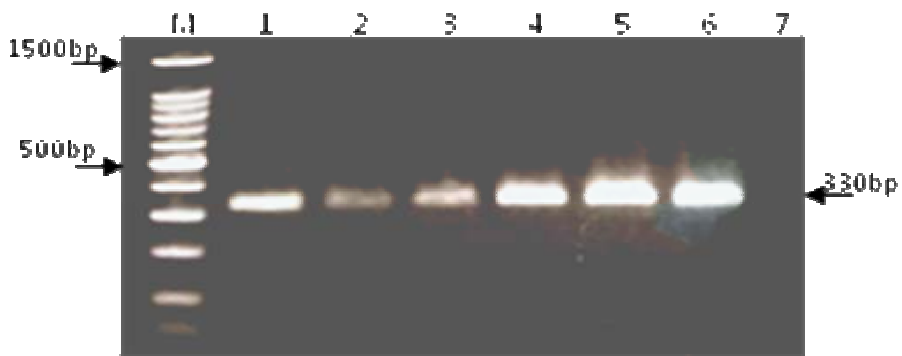
(1 )

*V. albo-atrum*

(RVU FVA)

.(2 )

337bp DNA



*Verticillium dahliae*

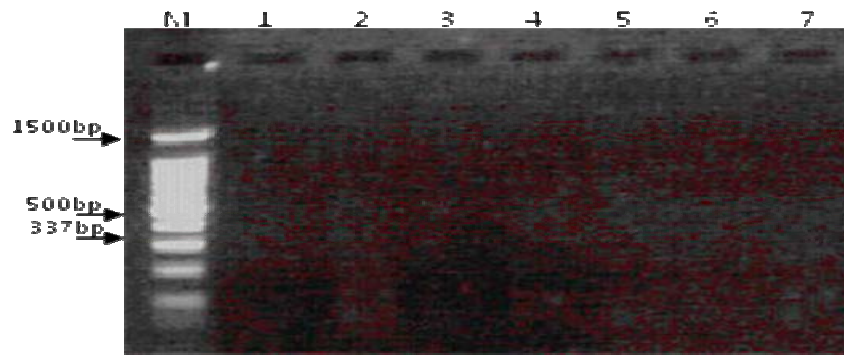
PCR :1

M= DNA ladder %2

(FVD , RVD)

-7 *Verticillium dahliae*

6-1



*Verticillium albo-atrum* PCR :2  
 M= DNA ladder %2 (FVA , RVU)  
 -7 *Verticillium dahliae* 6-1

*Verticillium dahliae* (Mohammadi *et al.*, 2004)

0.21  
 ( )  
 ( ) 0.64  
 (2007) (2006)  
 0.32  
 .( 2 ) 0.35

0.21	0.26	0.16	
0.32	0.38	0.25	
0.35	0.45	0.24	
0.64	0.79	0.49	
0.00	0.00	0.00	
	0.28	0.23	

0.05

\*

(2)

0.26 0.16

0.79 0.49 ( )

0.38

0.25

.(3 )

; Karajeh , 1997 ; 1992 )

.(2007

Al-)

.(2007 Hudelson and Rouse ,2001 Karajeh , 1997 1993 Ahmad

: 3

	%		( )	
90.0	100	80.0	10.14	
92.5	100	85.0	6.67	
90.0	100	80.0	7.00	
92.5	100	85.0	5.72	
0.00	0.00	0.00	10.07	
	80.0	66.0		

0.05

\*

10.14

(3)

( )

6.67 7.0

5.72

.(2002 )

- (1998).  
 1998 /95 /  
 Cultar (2002)  
*Verticillium dahliae* Kleb (2007)  
 (1992)  
 10  
 147-140  
*Fusarium* (2006)  
*solani* (Mart.)  
 (2002)
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