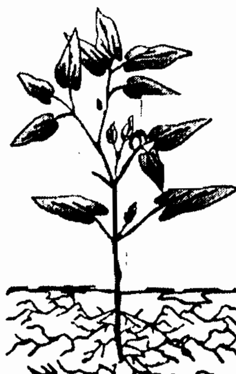

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CHARACTERIZATION OF EGYPTIAN ISOLATES OF *VERTICILLIUM DAHLIA* KLEB. FROM OLIVE TREES USING RAPD MARKERS

[35]

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ABSTRACT

Survey studies of *Verticillium dahliae* Kleb wilt disease of olive trees, covering the main nine districts in olive production, clarified the extensive spread of the disease in Egypt during 2003-2004 seasons. Matrouh and Beheira districts recorded the highest values of infection percentage and disease severity of wilted olive trees. Twenty two isolates of *V. dahliae*, isolated from diseased olive foliage among 1654 isolates yielded, exhibited clear variances in cultural growth criteria. Such isolates were grouped according to their pathogenicity in artificial inoculation tests in pot experiment. The highest pathogenic were 6 isolates from Matrouh (3 isolates), Beheira (2 isolates) and Alexandria (one isolate). The genetic diversity and pathotype analysis of the 22 isolates of *V. dahliae* were studied using Random Amplified Polymorphic DNA (RAPD) technique to determine the polymorphism associated with such isolates. Three random primers were used, produced 38 bands of DNA, 16 of them were polymorphed. The resulting UPGMA dendrogram showed 5 distinct clusters with the primer (no. 1), 3 groups with both primers no. 2 and no. 3. The ratios of similarity between isolates cluster groups were demonstrated in relation to their location origin and their capability to infect olive transplants under greenhouse conditions. However, such correlation between cluster grouping of *V. dahliae* isolates with RAPD and colony morphological characters or their pathogenicity to olive trees was not so tight.

INTRODUCTION

Verticillium wilt is one of the most important diseases occurring throughout the range of olive cultivation. The disease was first described by **Ruggeri (1946)** in Italy, and afterwards was reported in California (**Snyder *et al* 1950**), Greece (**Sarejanni *et al* 1952**), Turkey (**Saydam and Copcu, 1972**), France (**Vigouroux, 1975**), Syria (**Al-Ahmad and Mosli, 1993**), Egypt (**Radwan-Fatma and Hilal, 1994**) and Morocco (**Serrhini and Zeroual, 1995**). Verticillium wilt can substantially reduce the production of olive orchards and may cause tree death .Inspection in established olive plantation in Andalucia found that Verticillium wilt was present in 27% of orchards seen (**Sanchez-Hernandez *et al* 1998**). Studies in Egypt recorded incidence of Verticillium wilt in olive trees during 1992-1994 reached about 17% (**Radwan-Fatma and Hilal, 1994**). In Syria inspection in nine provinces of olive production indicated that the disease incidence over 6.5 mil-

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lion trees recorded about 4.5% **(Al-Ahmad and Mosli, 1993)**.

Verticillium wilt, caused by the soil fungus *Verticillium dahliae* **Kleb.**, the pathogen is a soil inhabiting fungus and inoculum consists of microsclerotia which form in the senescing tissues of the diseased plant, may survive in the soil for many years. *V. dahliae* exhibits high variability of important traits (e.g. pathogenicity, vegetative compatibility, morphology, etc.) and isolates can be genetically diversified also according to their ecological niches in which many factors may intervene **(Barbara *et al* 1998)**. During the last decades, molecular techniques have been largely used to characterize *Verticillium* species **(Carder and Barbara, 1991; Robb *et al* 1993** and **Typas, 2000)**. Several different types of molecular markers have been used in studies of *V. dahliae* RFLPs **(Carder and Barbara, 1991)**, RAPDs **(Koike *et al* 1996)**; and ITS sequencing **(Morton *et al* 1995)**. Overall, these studies have shown that molecular variation is readily detectable in *V. dahliae* and may resolve into more or less discrete groups; molecular groups may be correlated with other characters, such as pathogenicity group **(Horiuchi *et al* 1990)** in Japan, and defoliating pathotype **(Mercado-Blanco *et al* 2002)** in Spain.

Among the different techniques, Random Amplified Polymorphic DNA **(RAPD)** has been used for the characterization of *V. dahliae* isolates from different host species. RAPD markers have been used to study genetic diversity in 38 isolates of olive *Verticillium dahliae* from Morocco **(Cherrab *et al* 2000)** and also 50 isolates of olive *Verticillium dahliae* from Algeria **(Bellahcene *et al* 2000)**. However , in Spain , RAPD markers used as effective technique for detection of olive *V. dahliae* pathotype to identify the defoliating (D) and non-defoliating (ND) isolates of olive *V. dahliae* **(Mercado-Blanco *et al* 2002)**. Recently, **Nigro *et al* (2005)** used the same technique to analyse more than 60 *V. dahliae* isolates from olive trees in Apulia (southern Italy) RAPD-PCR technique indicated a low level of genetic diversity in the Apulian population of pathogen from infected olive trees. To date, no information are available about the characterization of *V. dahliae* isolates pathotype on olive trees in Egypt.

The objective of this study was to determine the degree of the genetic variability of the *V. dahliae* populations in Egyptian olive orchards, through the study of RAPD markers. The polymorphism associated with all isolates, the correlation between the profiles of the genetic variability with morphological characteristics of *V. dahliae* isolates as well as their geographic origins were also studied.

MATERIALS AND METHODS

Disease Quantitative survey

Quantitative, extensive survey of *Verticillium* wilt disease was carried out during 2003 and 2004 growing seasons on commercial cultivars of olive trees in different orchards in thirty locations of nine distinct growing governorates in Egypt. In each location **120** trees were selected to determine seasonal differences in incidence of infection as well as disease severity, during **2003/2004** seasons.

Disease assessment

The disease assessment was determined as percentage of infection by calculating the total number of infected and non-infected trees in each location of different distinct. Disease severity **(D.S.)** was determined by visual estimation method of **Tjamos *et al* (1991)** based on foliar visible symptoms, as follows: the **0** = healthy tree, **1** = up to **25%** (mild leaf rolling and yellowing), **2** = up to **50%** (intermediate symptoms, defoliation of the twigs and browning), **3** = up to **75%** (severe symptoms, partial dead of branches) and **4** = more than **80%** diseased foliage (tree nearly dead. The overall assessment of disease severity was calculated for diseases trees only by the following equation:

$$\text{Disease severity} = \frac{\sum (\text{Class rating X Class frequency})}{\text{Total numbers of diseased trees X highest rating}} \text{ X } 100$$

Isolation, purification and identification of V. dahliae isolates

Individual infected samples of olive trees which exhibited typical symptoms of wilt disease and representing each location, were collected through the survey studies. The presence of V. dahliae in the wood was assessed by isolating the fungus on 1.5% potato dextrose agar (PDA), according to the standard methods **(Pegg and Brady, 2002)**. Pure fungal colonies isolated from the xylem were identified according to their morphological and microscopical characteristics ac-

cording to **Clement and Shear (1957), Kendrick (1971), and Pegg and Brady, (2002).**

Determination of pathogenic diversity of* V. dahliae *isolates

V. dahliae isolates consistently isolated from diseased trees of olive were tested for potential pathogenicity during a greenhouse experiment, One year old olive transplants (cvs. Agizi Shami) were inoculated as follows: Inocula of 22 isolates *V. dahliae* were separately used, preparation of inoculua have been done on autoclaved Barley grain medium (barley - dried sand – water at 2:1:2 v/v). Plastic pots containing autoclaved sandy-clay (1:1W/W) were infested separately with the tested *V. dahliae* isolates using the inoculum grown in barley grain medium. The barley inoculua were added and thoroughly mixed with the autoclaved soil mixture at the rate of 5 % (W/W) singly of the air dried soil, then 1 olive transplant was planted in each plastic pot (25 cm diameter) containing soil mixture infested with the tested *V. dahliae* isolates. Five pots were used for each treatment. A set of five pots filled with uninfested soil sand mixture was used for control. Pots were kept for 120 days under greenhouse condition at (18°C to 28°C) and irrigated regularly with tap water as needed. At the end of experiment data was recorded as percentage of disease severity 120 days after inoculation **(Radwan and Hilal, 1994).**

Genetic diversity and pathotype analysis of *V. dahliae* isolates

a. Preparation of fungal growth isolates

Twenty two isolates were chosen according to their morphology and pathogen city from 1654 total isolates yielded throughout samples collection, grown in 100ml. of potato dextrose (PD) broth medium in 250ml. conical flasks, were inoculated with uniform discs 4mm. baring 7days old obtained from the desired *V. dahliae* isolates colonies grown in PDA medium, for 15 days at 20°C. Mycelial growth was harvested by filtered through a double layer of sterile muslin, and the mycelium was washed with sterile water, dried and ground to a fine powder in liquid nitrogen freeze – dried by using a mortar and pestle.

b. Extraction of fungal DNA

According to **(Dellaporta *et al* 1983)** from each sample, 50mg of fungal mats were prepared. Then, 15ml.extraction buffer (100mM Tris – Hcl, pH 8.0; 50mM EDTA, pH 8.0, 100mM NaCl and 10mM Mercapto-ethanol) was added and mixed. The mixture was poured in a 30ml. oak Rindge tube. One ml. of 20% SDS (Sodium dodecyl sulfate) was added and vortexed. The suspension was then incubated at 65°C for 20 min., then five ml. of 5 M potassium acetate was added, the samples were shaken vigorously, then incubated at 0°C for 20 min. and centrifuged in a microfuge at 25.000rpm for 20 min.

The supernatants were poured through Mira cloth filter into 30 ml. tubes containing 10 ml. isopropanol then, mixed and incubated at -20°C over night. The tubes were centrifuged at 20.000rpm for 20min. the supernatants were poured off gently and tubes were inverted on paper towels for 5-10min. DNA pellets appeared on the bottom of the tubes. The DNA pellets were redissolved in 1.0 ml. TE buffer (Tris EDTA; 10mM Tris – HCl pH 8.0 and 0.1mM Na – EDTA pH 8.0) and transferred to Eppendorf tubes. The concentration and purity of the obtained DNA was determined by using Gen Qunta system farmacia Bio-tech. The purity of the DNA for all samples was between 90-97% and the ratio ranged between 1.7 – 1.8. Concentration was adjusted at 6ng/µl for all samples using TE buffer, pH 8.0.

c. Amplification of DNA and primer selection

The primers used in this study were listed in **Table (1)** Arbitrary random amplification of DNA sequences was performed with a set of three primers obtained from (Kit of Amessham Pharmacia Biotech) each primer was a 10-mer of arbitrary sequence that was specifically designed and tested for use in RAPD. Three RAPD primers were tested with twenty two of *V. dahliae* isolates, based on the number of polymorphic markers. The primers No.1, 2 and 3 were selected.

Five µl from the previous genetical material containing 30mg of the extracted DNA were used for amplification reaction by the PCR (polymerase chain reaction) mixture which consists of one taplet PCR beads containing all of the necessary reagents except the primer which added to the mixture at the rate of 5µl/ reaction from each of three decamer oligonucleotide primers (10mer).

Table 1. Code and nucleotide sequence of primers used in the random amplified polymorphic DNA (RAPD) reactions, G+C content, and number of polymorphic bands produced in the *V.dahliae* isolates

Primer number	**Sequence**	G+C (%)	Polymorphic bands
1	5-GGTGCGGGAA-3	60	5
2	5-GTTTCGCTCC-3	60	2
3	5-GTAGACCCGT-3	60	3

This mixture contains a Taq. DNA polymerase and d NTP (a mixture of nitrogen bases necessary to synthesis these chains). The total volume was completed to 25µl using sterilized distilled water and three drops of mineral oil were added.

PCR amplified was performed by using the following PCR program in a Biometric thermocycler for 45 cycles after initial denaturation at 95°C for 5 min before electrophoresis, 7µC of 6 × tracking buffer were added to 25µl of the amplification product, (Manufactured by Qiagen kit). 15µl of PCR product were electrophorised in 1% agarose containing ethidium bromide at the rate of 0.5µg/ml using electrophoresis unit (WIDE mini-sub-cell GT Bio – RAD). The run was performed at 75 constant volts. Bands were detected on UV-transilluminatar and photographed

d. Cluster analysis

The banding patterns generated by RAPD – PCR were analyzed using gel documentation program (scanned for bands of restriction fragment). The different molecular weight (MW) of bands was determined against PCR marker promega G317 A by outweighed pair – group method based on arithmetic mean. Image data were automatically collected and simultaneously recorded during electrophoresis. RAPD fragments were scored, and converted into numerical data using Gel Documentation System, the fingerprint patterns were analyzed by using the software AAB Program (Advanced American Biotechnology and Imaging, Fullerton CA 92831, USA). The fragments were scored for presence or absence of bands showing the same mobility in the gel, regardless of their optical density. RAPD fragments were treated as biallelic marker loci with two alleles encoding presence or absence of a band. The fragment data were coded as a binary matrix where "1" designated presence, and "0" absence of a particular band. Genetic similarities (GS) among all the isolates were calculated according to the definition of **NEI and LI (1979)**. Mathematically, $S_{ij} = 2a / (2a + b + c)$, where S_{ij} is the similarity between the two individuals *i* and *j*; *b* is the number present in *i* and absent in *j*; and *c* is the number of bands present in *j* and absent in *i*. The matrix of similarity was analyzed by the unweighted pair group method using the arithmetic average (UPGMA), as suggested by **Sneath** and **Sokal (1973)**.

RESULTS AND DISCUSSION

1. Disease quantitative survey

Data in **Table (2)** reveal that, general natural infection with wilt of olive trees was observed during season 2004 as compared with season 2003. This was true with all disease inspection districts surveyed. However, Matrouh followed by Beheira districts recorded the higher values of averages percentage of diseases severity as well as the average percentage number of naturally infection, compared with the other districts surveyed and this was the same at 2003 as 2004. Menoufyia districts, on the other hand, recorded the least percentage of olive trees wilt during the two seasons.

2. Determination of pathogenic diversity of *V. dahliae* isolates

Twenty two isolates *V. dahliae* among 1654 isolates obtained from nine surveyed different geographic location as mentioned before, were coded based on colony characteristics, and used to test its pathogenic capabilities on Cv. Agizi Shami under green house condition. Data in **Table (3)** show that all tested *V. dahliae* isolates were able

Table 2. Percentage of naturally infection and diseases severity of *V. dahliae* wilt of olive trees, determination was carried out at nine districts during the survey of two successive seasons 2003 and 2004

Districts*	Percentage (%) of naturally infection of wilted olive trees		Diseases severity (%) of naturally infection of wilted olive trees	
	Seasons 2003	Seasons 2004	Seasons 2003	Seasons 2004
Matrouh	15.62	16.46	26.91	36.59
Beheira	14.37	15.00	24.53	27.97
Fayoum	10.42	11.87	21.71	24.38
North Sinai	11.87	13.54	21.25	23.04
Ismailia	13.54	14.17	22.40	24.74
Giza	11.94	13.33	21.31	23.19
Menofyia	5.00	6.67	16.65	20.41
New Valley	9.17	10.83	20.71	19.30
Alexandria	9.44	12.50	17.99	19.83
Mean	11.26	12.71	21.40	24.38

*. Average number of naturally infection of wilted olive trees calculated from thirty locations of nine districts

to infect olive transplants developed from cuttings (TDC) one year old causing different degree of (TDC) severity.

According to the severity of the symptoms (D.S%) data in **Table (3)** show that, *V. dahliae* isolates were classified in 4 groups: group (I) with severity > 80% (isolates coded 1 & 3 & 5 & 6 & 7), group (II) with severity > 60 to 80 % (isolates coded 2 & 8 & 9 & 11 &12 & 24 & 26), group (III) with severity > 40 to 60% (isolates coded 4 & 16 & 17 &18 & 19), and group (IV) with severity <40 % (isolates coded 13 &14 & 20 & 21& 34).

The same data indicate that there are correlation between disease severity groups and geographic location isolation. The most aggressive isolates (group I) was isolated from Matrouh & Beheira and Alexandria districts, however, the less pathogenic isolates (group IV) was located mainly in Fayoum, Giza, Menoufyia, and New Valley

The *V. dahliae* isolates exhibited high variability in cultural characteristics on PDA. Significant differences were recorded in the growth of isolates. The range of mean growth varied between 22 mm diameter of latent isolate colony up to 90 mm for the faster ones on the 14th day of incubation at 20°C. The growth behavior of mycelium was either different in colors and the density of microsclerotia formation. Twelve different growth behaviors were observed for the 22 isolates of *V. dahliae*. Meanwhile, data in **Table (3)** clear that there is no correlation between disease severity groups and colony characteristics of isolates (growth ratio or morphological characters) was recorded.

3. Genetic diversity and pathotype analysis of *V. dahliae* isolates

The three random primers used in this study **Table (1)** produced reproducible ampilicons for all 22 *V. dahliae* isolates **Table (3)**. Thirty eight bands were obtained, and 16 were polymorphic. The amplilicons ranged from 1.0 to 0.2 kbc, representative banding patterns from primers 1, 2 and 3 are illustrated in **Figs. 1, 2 and 3**, respectively.

The resulting UPGMA dendrogram of the first random primers (1) showed 5 distinct clusters **(Fig. 1)**. 16 isolates from olive trees from various districts grouped into four significantly distinct clusters with a similarity coefficient of 85.56 %, Three isolates (coded 24, 26 and 34) two from New valley and one from Menoufyia formed one distinct clusters showing a moderate value of genetic similarity coefficient (82.3%) in comparison

Table 3. View of *V. dahliae* isolates location, growth ratio, disease severity (D.S.) group and morphological characters

Isolate code	Location	Growth ratio*	% D.S**	D.S*** group	Morphological characters
1	Matrouh	54	86.66	I	Colonies are totally black due to presence of microsclerotia spread over the whole surface.
2	Matrouh	42	69.32	II	Colonies brownish colour
3	Matrouh	56	86.66	I	Colonies black and white colour, of an aspect more cottony, with high density of microsclerotia
4	Alexandria	27	53.33	III	Colonies of brown whitish colour
5	Alexandria	22	86.66	I	Grayish growth surface. With low density of microsclerotia.
6	Beheira	19	80.00	I	Colonies whitish cottony colour and flocked tufts without scattered
7	Beheira	38	86.66	I	Colonies faint white (transparent) in center with whitish colour in the border
8	Ismailia	50	66.66	II	The mycelium appears strongly pigment with high density of microsclerotia
9	Ismailia	80	64.00	II	The mycelium appears strongly pigment with high density of microsclerotia
11	Ismailia	45	74.66	II	Colony orange in colour and microsclerotia arranged in sectors
12	Ismailia	47	73.33	II	Colonies of black whitish colour with cottoned and flocked tufts with weekly pigmented microsclerotia
13	Fayoum	90	26.66	IV	Colonies whitish colour with cottoned and flocked tufts scattered irregularly over the surface
14	Fayoum	30	28.00	IV	The mycelium appears strongly pigment with high density of microsclerotia
16	North Sinai	30	41.33	III	The mycelium appears strongly pigment with high density of microsclerotia
17	North Sinai	37	46.66	III	Sectors transparency, mycelium with low density of microsclerotia
18	Giza	90	57.33	III	The mycelium appears strongly pigment with high density of microsclerotia
19	Giza	90	60.00	III	Colony and microsclerotia of an dark orange colour and powdery aspect
20	Giza	38	34.66	IV	Peachy, abundant floccose mycelia.
21	Menoufyia	90	36.00	IV	The mycelium appears strongly pigment with high density of microsclerotia
24	Menoufyia	42	66.66	II	Colony and microsclerotia of an dark orange colour and powdery aspect
26	New Valley	90	69.32	II	Colony and microsclerotia of an dark orange colour and powdery aspect
34	New Valley	90	40.00	IV	Colony and microsclerotia of an dark orange colour and powdery aspect

* Growth ratio= average of colony growth diameter (mm) 14 days after of incubation at 20°c on PDA.
**%D.S = disease severity (%) on olive transplants 120 days after of artificial inoculation
*** D.S. group = group severity classification (I : IV)

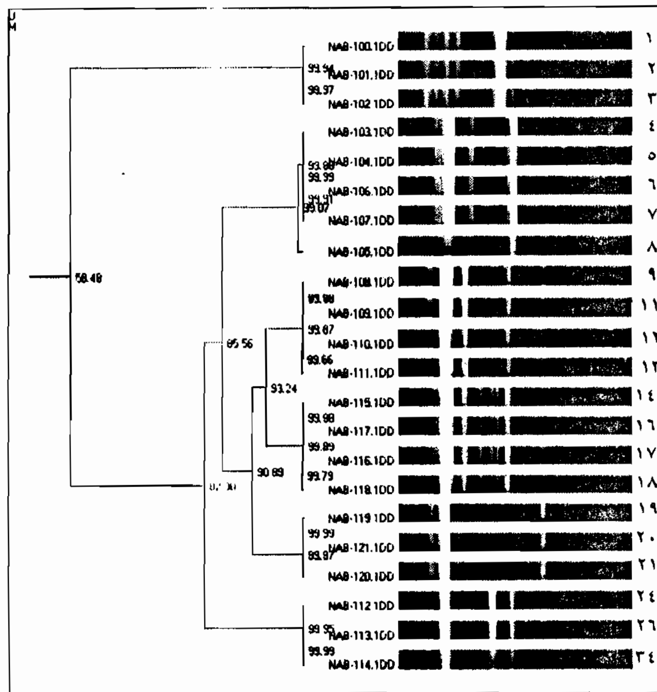

Figure 1. Banding patterns of random amplified polymorphic DNA (RAPD) of *V. dahliae* isolates obtained with the primer (1) listed in **Table (1)**. The list of isolates was reported in **Table (3)**

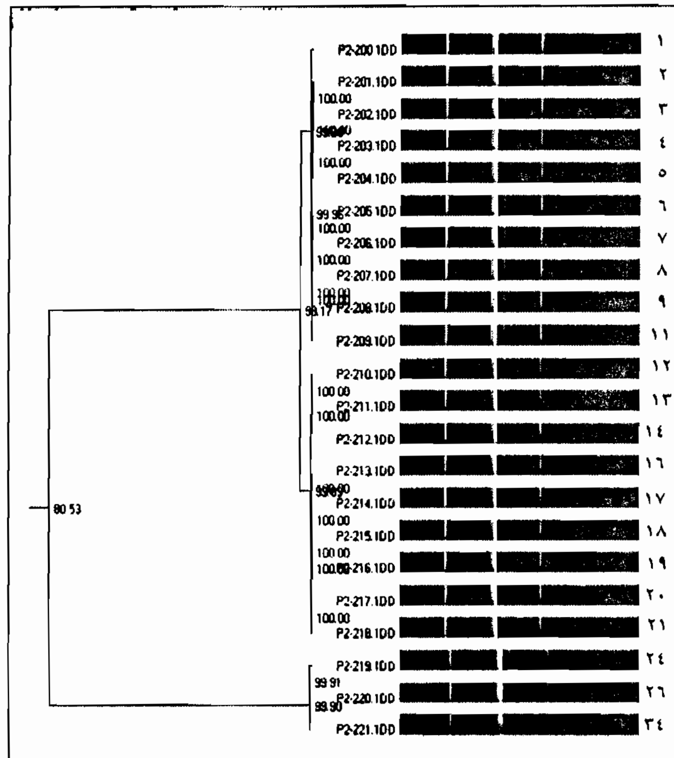

Figure 2. Banding patterns of random amplified polymorphic DNA (RAPD) of *V. dahliae* isolates obtained with the primer (2) listed in **Table (1)**. The list of isolates was reported in **Table (3)**

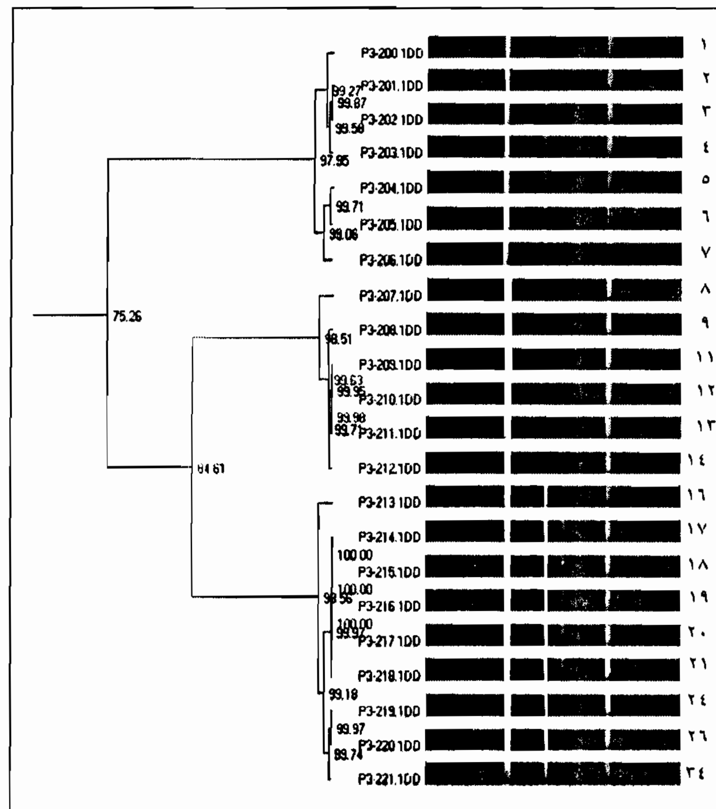

Figure 3. Banding patterns of random amplified polymorphic DNA (RAPD) of *V. dahliae* isolates obtained with the primer (3) listed in **Table (1)**. The list of isolates was reported in **Table (3)**

with isolates from the other districts. The three isolates from Matrouh district formed a distinct cluster showing a lower value of genetic similarity coefficient (58.48%) in comparison with isolates from the other districts (**Fig. 1**). One from the five clusters of the first primer showed relationship with locality of isolation (the three isolates from Matrouh). However, none of the five clusters of the first primer showed relationship neither with D.S groups nor the growth characterization of the isolates.

The resulting UPGMA dendrogram of the second random primers (2) showed 3 distinct clusters (**Fig. 2**). 19 isolates from olive trees from various districts grouped into two significantly distinct clusters with a highly similarity coefficient of 98.17%, the same three isolates (coded 24, 26 and 34) two from New valley and one from Menoufyia formed one distinct clusters showing a lower value of genetic similarity coefficient (80.53%) in comparison with isolates from the other districts. None of the three clusters of the second primer showed relationship neither with symptoms expression nor with the locality of isolation nor the growth characterization of the isolates (**Fig. 2**).

The resulting UPGMA dendrogram of the third random primers (3) showed also 3 distinct clusters (**Fig. 3**). 15 isolates from olive trees from various districts grouped into two significantly distinct clusters with a similarity coefficient of 84.61%, Seven isolates (coded 1, 2, 3, 4, 5, 6 and 7) three from Martouh and two from Alexandria and two from Beheira formed one distinct clusters showing a lower value of genetic similarity coefficient (75.26%) in comparison with isolates from the other districts. One from the three clusters of the third primer showed relationship with locality of isolation (the seven isolates from Matrouh, Alexandria and Beheira considered northern west part of Egypt districts). However, none of the three clusters of the third primer showed relationship neither with D.S groups nor the growth characterization of the isolates.

DISCUSSION

The results of the present study revealed an influence of geographic origin on genetic variability among the populations of *V. dahliae* of olive in Egypt. Disease survey studies during 2003-2004 seasons, update the little comforting picture about the disease incidence in Egypt. The disease is widespread and causes serious losses in surveyed fields, i.e., Matrouh districts which had the highest average number of naturally infected trees in two successive seasons, followed by Beheira and Ismailia while Menoufyia districts the least. In all the surveyed districts the pathogen was recovered from old and young olive- trees These results are in agreement with other studies which indicated that *V. dahliae* isolates are mainly responsible for olive wilt diseases of trees and cause severe damage and reduction in yield and expanding in the Mediterranean olive- growing area (**Radwan and Hillal, 1994; Levin and Tsror, 2002 and Nigro *et al* 2005**).

Morphology of *V. dahliae* colonies were variable on media depending on shape of microsclorotia and abundance of dark hyphae and on the distribution of microsclorotia covering the colony. So, this morphological characterization does not seem to be useful for practice. In conclusion, the morphological characters used to identify pure cultures of *V. dahliae* can not be used uncritically for distinguishing the isolates, (**Goud *et al* 2003**).

The pathogenicity tests demonstrated that all tested isolates were clearly pathogenic to olive and reproduced typical symptoms of *Verticillium* wilt at different disease severity grad on cv. Agizi Shami under green house condition. Variation in pathogenicity of different isolates of *V. dahliae* from olive trees have also been reported (**Pegg and Brady, 2002**).

In the present study, the polymorphism of RAPD profiles has allowed the characterization of isolates obtained from olive trees according to their original locations in Egypt. Cluster analysis of RAPD band resulted in the classification of different number of clusters with a coefficient similarity depending on the type of primer, i.e. the first random primers (1) showed 5 distinct clusters for total 22 *V. dahliae* isolates but the second random primers (2) showed 3 distinct clusters for the same isolates, however; the third random primers (3) showed also 3 distinct clusters for this isolates of *V. dahliae* . In general, no correlation could be established among the isolates including into the all type of clusters obtained neither based on the presence of symptoms, nor the growth characterization of fungal isolates. These results agree with work demonstrating high genetic homogeneity among population of *V. dahliae* wilting olive tree from some Mediterranean countries (**Bellahcene *et al* 2001; Tsror and Levin, 2003 and Nigro *et al* 2005**). However, the first and third primer showed relationship with locality of isolation of some *V. dahliae* isolates, i.e. the seven isolates from Matrouh, Alexandria and Beheira considered northern west part of Egypt districts.

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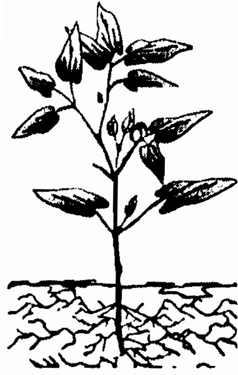

حوليات العلوم الزراعية
جامعة عين شمس، القاهرة
مجلد(٥١)، عدد (٢)، ٤٩٩-٥١٠، ٢٠٠٦

تحديد الصفات المزرعية والمرضية والتركيب الجينى بأستخدام الإكثار العشوائى للحمض النووى لعزلات الفطر *Verticillium dahliae* المسببة لمرض ذبول اشجار الزيتون فى مصر

[٣٥]

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أجريت هذه الدراسة فى موسمى ٢٠٠٣ و ٢٠٠٤ على أشجار الزيتون المنزرعة فى تسعة محافظات هى : مطروح ، البحيرة ، الفيوم ، شمال سيناء ، الأسماعيلية ، الجيزة ، المنوفية ، الوادى الجديد ومحافظة الأسكندرية بهدف عمل حصر لمرض ذبول اشجار الزيتون المتسبب عن فطر *Verticillium dahliae*. وقد أمكن الحصول على ١٦٥٤ عزلة مختلفة من مختلف مواقع الدراسة والتى بلغ عددها ٣٠ موقع من التسع محافظات التى خضعت الحصر .

تم اختيار ٢٢ عزلة منها فقط تمثل المحافظات التسعة لأجراء الدرسات عليها من حيث الصفات المزرعية المختلفة وقدرتها على احداث المرض نتيجة العدوى الصناعية فى الصوبة وكذلك تم اجراء تقييم للأختلافات الوراثية المتواجدة فى هذه العزلات بأستخدام الأكثار العشوائى للحمض النووى (RAPD) من خلال عدد ٣ بادئات عشوائية بغرض عمل تصنيف هذه العزلات الى مجموعات متشابه او مختلفة من حيث عدد الحزم من الحمض النووى DNA الناتجة مع كل بادئ على حدى. ولقد استهدفت الدراسة محاولة ايجاد ارتباط بين هذه المجاميع التصنيفية من نتائج RAPD والأختلافات الظاهرة فى المواصفات نمو العزلات او قدرتها المرضية او أماكن عزلها الجغرافية وتلخصت النتائج فى التالى :

١- أظهرت نتائج حصر انتشارمرض ذبول اشجار الزيتون ان محافظة مطروح والبحيرة ينتشر بها المرض بنسب اكبر من باقى المحافظات خلال موسمى ٢٠٠٣ و٢٠٠٤ .
٢- أظهرت نتائج العدوى الصناعية للعزلات المختلفة (٢٢ عزلة) أن هناك تباين واضح فى القدرة المرضية لهذه العزلات وتم تصنيف العزلات الى اربع مجاميع أحتوت المجموعة الأولى الأكثر قدرة على العدوى على ٥ عزلات (عزلتين من مطروح عزلتين من البحيرة وواحدة من الأسكندرية).
٣-كانت هناك اختلافات واضحة بين هذه العزلات من حيث مظهر النمو واللون وتكون الأجسام

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الحجرية الدقيقة وتوزيعها على سطح النمو العزلات المختلفة .

٤-أثبت تحليل الأكثار العشوائى للحمض النووى (RAPD) بإستخدام ثلاث بادئات عشوائية ظهور ٥ مجموعات من حزم الحمض النووى DNA مع البادئ الأول (رقم ١) و ٣ مجموعات مع كل من بادئ رقم ٢ والبادئ رقم ٣ واظهر تصنيف هذه المجاميع درجات تقارب مختلفة بين العزلات تراوحت من ٥٨,٥% الى ٩٢,٢% حسب لمجاميع العزلات والبادئ المستخدم.

٥- لم يكن هناك معامل ارتباط بدرجة كبيرة واضحة بين الصفات المزرعية والقدرة المرضية والتباين الوراثى للحمض النووى DNA للعزلات المختبرة.