Pathological study on *Monosporascus cannonballus* the causal of cucumber root rot and sudden wilt disease

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Abstract

Cucumber (*Cucumis sativus* L.) is considered one of the most important vegetable crops in the world. In the last twenty-five years, the international production of cucurbits was affected by fungal root rot diseases. Isolation trials from rotted of cucurbits plants yielded four genera belonging to six species of pathogenic fungi which identified as *Fusarium oxysporum, F. prolifiratum, F. semitectum, Monosporascus cannonballus, Rhizoctonia solani* and *Verticillium tricorpus*. All the tested isolates were pathogenic to cucumber since they significant increased root rot disease parameters. Among the tested fungi *F. oxysporum*-2, *F. prolifiratum*-1, *F. semitectum*-1, *M. cannonballus*-1, *R. solani*-1 and *V. tricorpus*-1 were the most aggressive than the others. The six virulent isolates were evaluated for their potentialities in causing cucumber sudden wilt disease. *M. cannonballus*-1 was the highest virulent in this respect. The main symptoms were root shrinkage, stunting and cankers on the main root as well as brown area on the lateral roots due to the infested roots with *F. oxysporum*, *F. prolifiratum*, *F. semitectum*, *V. tricorpus* and *R. solani*, while canker on the main roots, brown and black areas on the lateral roots on the sites attached with main roots were always due to the infection by *M. cannonballus*.

Using RAPD-PCR technique, the similarity between two isolates of *M. cannonballus* was 80%. These results confirm that the two isolates are genetically different and this might be affect in the variability pathogenicity and morphology of these fungi.

Keywords: Cucumber; Sudden wilt; *Monosporascus*; RAPD-PCR

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is one of the most a vegetable crops having significant economic importance in several countries. Soil borne diseases became the yield-limiting factor of cucurbits production in some countries of the world during the last twenty – five years **(Bruton, 1998).**

Monosporascus cannonballus (Pollack and Uecker) is one of the most important soil borne pathogens causing vine decline of muskmelon (*Cucumis melo* L.) and watermelon (*Citrullus lanatus*). In this respect, **Mertely *et al.*, (1991)** found that *M. cannonballus* was pathogenic of a broad range of cucurbits where *M. cannonballus* was recorded in United States, Libya, Iran, Japan, Israel, Spain, India, Pakistan, Tunisia, Taiwan, Guatemala, Honduras, Mexico, Saudi Arabia **(Bruton, 1998)**, Italy **(Infantino *et al.*, 2002),** Korea **(Kwon *et al.*, 2001)** and Egypt **(El Desouky and El-Wakil, 2003).** Generally the disease complex symptoms of *M. cannonballus* pathogen appeared primary as vine decline, *Monosporascus* wilt, collapse, root rot or sudden wilt to cucurbits. Cantaloupe sudden wilt disease, appeared in form of root rot, vine decline, crown blight, collapse, quick decline and canopy collapse, as a world-wide problem particularly in the arid and semi-arid regions under warm and hot weather conditions. High temperatures play a major role in the incidence of vine decline caused by *M. cannonballus*. *M. cannonballus* caused damage as vine decline including yellowing and death of crown leaves prior to harvest, gradual decline of the vine as the plant approaches maturity, necrosis of a root system and absence of most of the secondary and tertiary feeder roots. Finally, a rapid collapse of the vine typically occurs just before harvest **(Bruton *et al.*, 1999 and Abou El-Yazied *et al.*, 2012). Tsay and Tung (1995)** studied the pathogenicity of 10 fungal isolates among them *M. cannonballus* isolate on cantaloupe seedlings (cv. Magnum45) in the greenhouse after 28 days of incubation, the fungus produced symptoms similar to those produced by Texas isolate of *M. cannonballus*. Also, the apparently-healthy plants were wilted or declined within a period of 5 to 10 days as the plants approached maturity**. Bruton *et al.*, (1995)** conducted pathogenicity tests under field conditions in artificially infested microplots, and observed the first wilt symptoms at various stages of fruit maturation. The highest mortality levels (73.97%) were recorded with the combined inoculations of *Monosporascus* sp. **Heo *et al.*, (2001)** described vine

decline or collapse syndrome on cucurbits which caused by *Acremonium cucurbitacearum* and *M. cannonballus*. The pathogenicity of both fungi was confirmed by evaluating cultivars of muskmelon (*Cucumis melo*) and watermelon (*Citrullus lanatus*) cultivated in Brazil. All tested cultivars were highly susceptible to these pathogens. **El-Desouky and El-Wakil (2003)** studied the pathogenicity of *M. cannonballus*, the etiological agent causing the collapse of melon (*Cucumis melo*) plants under controlled conditions where the infected plants showed symptoms typical to those reported previously for the disease. **Sales *et al.*, (2004)** studied the genetic variation of 106 isolates of *F.oxysporum* isolated from cucumber with respect to pathogenicity using random amplified polymorphic DNA (RAPD) analysis. In this respect, isolates of *F. oxysporum* f.sp. *radicis-cucumerinum* were assigned to a single RAPD group(I)while, isolates of *F.oxysporum* f. sp. *cucumerinum* were assigned to two different RAPD groups(II, III). **El-Fadly *et al.*, (2008)** used large numbers of highly informative DNA markers for the identification of genetic polymorphism. In the last decade, the random amplified polymorphic DNA (RAPD) technique based on the polymerase chain reaction (PCR) is one of the most commonly used molecular techniques to develop DNA markers. RAPD markers are amplification products of anonymous DNA sequences using single, short and arbitrary oligonucleotide primers and thus do not require prior knowledge of a DNA sequence. **Motlagh and Anvari (2010)** used the RAPD-PCR techniques to determine the amount of Intraspecific genetic variability among *Biopolaris oryza*e isolates using seven primers. The resulted segments of DNA at size 200-300 bp revealed clustered groups of tested *B. oryzae* fungi. Levels of polymorphism among DNA of the different isolates were observed. However, the pattern of RAPD bands could not show any direct correlation between polymorphism and climates or geographical areas. **Singh *et al.*, (2011)** reported that *Fusarium oxysporum* f. sp. *ricini*, caused severe disease of castor bean (*Ricimus communis*). They identified three markers (RKC 231375, RKC 211080 and OPBE 18900) flanking the wilt resistance gene were identified. The developed markers segregated from the screened Fusarium wilt resistant progenies of F2 and F3 families confirmed their linkage with Fusarium wilt resistance and the resistant castor bean genotypes were successfully identified among thirteen cultivars screened. Linkage analysis was carried

out using the three markers on the 200 F2 individuals which showed that the genetic distance for the three markers at the wilt resistance gene was 5cM, 10.7cM and 7.6cM respectively. The predicted protein 3D model of the translated amino acid sequence from RKC 23 showed characteristic features of DNA binding protein (2BIN).

This study aimed to test the pathogenicity of 4 isolates of *Fusarium oxysporum*, 2 isolates of *F. prolifiratum*, 10 isolates of *F. semitectum*, 2 isolates of *Monosporascus cannonballus*, 2 isolates of *Rhizoctonia solani* and 2 isolates of *Verticillium tricorpus* isolated from cultivated cucumber in Egypt and showing symptoms of vine decline in Egypt. Also using RAPD-PCR technique to study the polymorphism among 2 isolates of *M. cannonballus*, causing vine decline and wilt/root rot of cucumber in Egypt.

MATERIALS AND METHODS

Isolation and identification of the associated fungi.

The rotted, wilted cucumber plants were collected from different open field in Behira Governorate during summer and autumn of seasons 2009. The diseased plants were uprooted and then the roots were washed under running tap water to remove the adhering soil particles. The tap and lateral roots were excised into small pieces (0.5-0.8 cm). The root pieces were disinfested in 0.5% sodium hypochlorite then 70% ethanol for two minutes, after that they were rinsed in sterilized distilled water and dried between two sterilized filter papers. The surface sterilized samples were plated onto (PDA) medium and incubated at 25°C until the recovery of the fungal colonies. The recovered fungi were microscopically examined. Counted and the frequency of each fungus was determined **(Habib, 2008)**.

$$\text{Frequency (\%)} = \frac{\text{Number of each isolated fungus}}{\text{Total number of all isolated fungi}} \times 100$$

The isolated fungi were purified and identified according to **Barnett (1960)**, **Pollack** and **Uecker (1974)**, **Domsch *et al.*, (1980)** and **Nelson *et al.*, (1983)** in Fungal Taxonomy Department, Plant Pathology Research Institute, Giza, Egypt. The isolated fungi were sub-cultured on PDA slants and kept at 5°C for further studies.

Inocula preparations and pathogenicity tests.

All tested fungi under study were grown individually on autoclaved cornmeal-sand medium. Cornmeal-sand medium were inoculated with fungal disc (4mm ϕ) of 7 days PDA old cultures of each fungus and incubated at 25°C for15 days. All cultured fungi were tested for their pathogenic abilities to select to highly pathogenic fungi. In this respect, sterilized sandy loam and clay soil (1:1 w.w) and 40 cm in diameter pots (5kg) were used. The prepared fungal Inocula of each fungus were added to pots at rate 2%. Inocula were mixed thoroughly with the soil in each pot, watered regular for one week to ensure the distribution and growth of added inocula. Control pots were filled with the same soil mixed with the same amount of sterilized and not inoculated corn meal-sand medium. A set of four pots with ten surface sterilized cucumber seeds per pot were sown. Pots were irrigated every 3 days and infected seedlings were collected to re-isolate the causal pathogens.

Disease assessment.

Pathogenicity was assessed after 2 and 4 weeks of sowing as percentages of pre and post-emergence damping off disease respectively. Sudden wilt disease incidence and healthy survival plants were recorded at the fruit setting stage (after 70 days from sowing) of cucumber plants. Plants were uprooted washed carefully, and the disease incidence was determined. Wilt infection was expressed as a percentage (%) according to **Hassanin (2007)**:

$$\text{Wilt infection (\%)} = \frac{A}{B} \times 100$$

A= number of wilted plants.

B= Total number of control.

RAPD-PCR analysis.

Fungal cultures:

In this trail, 2 *M.cannonballus* isolates which isolated from infected cucumber grown in Abo-hommos, Behira governorate, Egypt, were cultured and incubated under conditioned growth chamber supplemented with florescent light for 6-7 days at 24-26°C.

Extraction and purification of genomic DNA:

A modified CTAB (Cetyl trimethyl ammonium bromide) procedure based on the protocol of Karthinkeyan *et al.*, (2010) was adopted for obtaining good quality of total DNA. Fifty mg of fungal mycelia was scraped from 10day old PDA cultures, manually ground in 1.5 ml of microfuge tube with micro pestle adding 500ul of pre-warmed (60°C) TES lysis buffer (100mM Tris pH 8.0; 10mM EDTA pH 8.0; 2% SDS). Then 50ug of proteinase K were added to the ground material, incubated at 60°C for 60min. 140ul of 5M of Nacl and 64ul of 10% of CTAB were added to the suspension incubated at 65°C for 10min. DNAs were extracted by adding equal volume of chloroform : isoamyl-alcohol(24:1) centrifuged at 14000xg /10min. DNA was precipitated by adding 0.6 volume of cold isopropanol and maintained at-20°C, centrifuged and washed twice with 70% ethanol suspended in 100ul of TE (10mM Tris pH 8.0; 1mM EDTA pH 8.0). RNA was digested by adding 10mg / ml of RNase A and incubating at 37°C for 45min and stored at -20°C for further use.

Estimation of DNA concentration:

DNA concentration was determined by diluting the DNA 1:5 in dH_2O. The DNA samples were electrophoresed in 1% agarose gel against 10ug of a DNA size marker. This marker covers a range of concentration between 95 ng and 11 ng. Thus, estimation of the DNA concentration in a given sample was achieved by comparing the degree of fluorescence of the unknown DNA band with the different bands in the DNA size marker.

RAPD-PCR reactions;

A set of ten random 10-mer primers (**Table 1**) was used in the detection of polymorphism among the two tested fungi. The amplification reaction was carried out in 25 µl reaction volume containing 1X PCR buffer, 1.5 mM MgCl2, 0.2 mM dNTPs, 1 µM primer, 1 U *Taq* DNA polymerase and 25ng template DNA.

Thermocycling Profile and Detection of the PCR Products:

PCR amplification was performed in a Perkin-Elmer/Gene Amp® PCR System 9700 (PE Applied Biosystems) programmed to fulfill 40 cycles after an initial denaturation cycle for 5 min at 94°C. Each cycle consisted of a denaturation step at 94°C for 1 min, an annealing step at

36°C for 1 min, and an elongation step at 72°C for 1.5 min. The primer extension segment was extended to 7 min at 72°C in the final cycle. The amplification products were resolved by electrophoresis in a 1, 5 % agarose gel containing ethidium bromide (0.5ug/ml) in 1X TBE buffer at 95 volts. PCR products were visualized on UV light and photographed using a camera. Amplified products were visually examined and the presence or absence of each size class was scored as 1 or 0, respectively.

Table (1): Sequence of the 10 decamer arbitrary primers assayed in RAPD-PCR.

Primer	Sequence
OP A03	**5'-AGTCAGCCAC-3'**
OP A04	**5'-AATCGGGCTG-3'**
OP A06	5'-GGTCCCTGAC-3'
OP A11	5'-CAATCGCCGT-3'
OP A17	5'-GACCGCTTGT-3
OP C01	5'-TTCGAGCCAG-3'
OP C07	5'-GTCCCGACGA-3'
OP C12	5'-TGTCATCCCC-3'
OP G03	5'-GAGCCCTCCA-3'
OP Z17	5'-CCTTCCCACT-3'

Data Analysis

The banding patterns generated by RAPD-PCR marker analyses were compared to determine the genetic relatedness of the two fungal. Clear and distinct amplification products were scored as '1' for presence and '0' for absence of bands. Bands of the same mobility were scored as identical. The genetic similarity coefficient (GS) between two genotypes was estimated according to Dice coefficient **(Sneath and Sokal, 1973).**

Dice formula: $GS_{ij} = 2a/(2a+b+c)$

Where GS_{ij} is the measure of genetic similarity between individuals ***i*** , ***j*** and **a** is the number of bands shared by ***i*** , ***j*** and **b** is the number of bands

present in ***i*** and absent in ***j***, and **c** is the number of bands present in ***j*** and absent in ***i*.** **Statistical analysis LSD at 5%.**

Data were subjected to ANOVA by using Costat program (1988) and significant difference among the treatments was portioned by LSD test multiple range test at probability levels of P = 0.05.

Results

Isolation and identification of the associated fungi:

Data in **Table (2)** show that four genera belong to six species of pathogenic fungi were isolated from diseased roots of Cantaloupe, Cucumber, Squash, Snake cucumber and Watermelon and identified as *Fusarium oxysporum, Fusarium prolifiratum, Fusarium semitectum, Monosporascus cannonballus, Rhizoctonia solani and Verticillium tricorpus*. Data indicated that the highly number of isolated fungi were found with cucumber roots which give 138 isolates, followed by watermelon 75 isolates, snake cucumber 55 isolates, squash 39 isolates and cantaloupe 37 isolates of fungi. On the other hand, *Fusarium oxysporum* recorded the highly number of isolates which give 131 isolates equal 38% of all isolated fungi while, *Verticillium tricorpus* gave the low number which recorded 18 isolates only from all plants under experiment.

Table (2): Frequency of and numbers of isolated fungi from naturally infected roots of some cucurbits with sudden wilt disease.

Fungi	Wilted Plants/No. of isolates/frequency (%)										Total of isolates	Freq. (%)
	Cantaloupe		Cucumber		Squash		Snake cucumber		Watermelon			
	No.	Freq. (%)	No.	Freq. (%)	No.	Freq. (%)	No.	Freq. (%)	No.	Freq. (%)		
F. oxysporum	14	37.84	44	31.88	19	48.77	21	38.18	33	44.0	131	38.00
F. prolifiratum	0.0	0.00	22	15.94	8	20.51	0.0	0.00	5	6.70	35	10.20
F. semitectum	7	18.99	23	16.67	0.0	0.00	11	20.00	20	26.60	61	17.70
M. cannonballus	9	24.32	19	13.80	0.0	0.00	8	14.55	6	8.00	42	12.20
R. solani	7	18.99	19	13.80	12	30.77	11	20.00	8	10.70	57	16.70
V. tricorpus	0.0	0.00	11	7.97	0.0	0.00	4	7.27	3	4.00	18	5.20
Total	37	100	138	100	39	100	55	100	75	100	344	100

Pathogenicity tests:

The pathogenicity of different fungal isolates from cucumber was conducted in order to confirm their virulence and to define the most aggressive fungal isolates causing serious damage on cucumber plants. Twenty two fungal isolates of those selected among hundred thirty eight fungal isolates were tested as follows; **(Table 3)** four isolates of *F. oxysporum*, ten isolates of *F. semitectum*, two isolates of *F. prolifiratum, M. cannonballus, R. solani* and *V. tricorpus*.

Data in **Table (3)** indicate that all the tested isolates were pathogenic on cucumber cv. Faris f1 since they significantly increased root diseases parameters.*F.oxysporum* isolates number 2 and 4, *F.semitectum* isolates number (10, 1, 4, 8 and 9) gave the highest percentage of pre- emergence damping off followed by *F. semitectum* isolates No.(2, 5 and 6), *F. oxysporum* isolate No.(3), *F.prolifiratum* isolates No.(1) and *Monosporascus cannonballus* isolate No.(1) while the another isolates recorded the lowest percentage of pre-emergence damping off. On the other

hand, *F. oxysporum* No. (3 and 1), *F. semitectum* No. (4, 3, 6, 7, 8 and 5), *F.prolifiratum* No. (2), *R. solani* No. (1 and 2) and *V. tricorpus* No. (1) recorded the highly percentage of post-emergence damping off. The highly percentage of Vine decline was recorded with *M. cannonballus* (1) 45% followed by *F. semitectum* (1) 40%, *F. oxysporum* (2) and *V. tricorpus* (1) 37.5% and *F. semitectum* (9) 35% while *F. prolifiratum*, *F. semitectum* (2 and 5), *M. cannonballus* (2) and *F. oxysporum* (4) caused moderate vine decline symptoms where they gave 32.5, 32.5, 30, 30, 30 % of vine decline respectively.

Generally, data of Table (3) reveal that *F. oxysporum* (isolate No.2), *F. prolifiratum* (isolate No. I), *F.semitectum* (isolate No.1), *M. cannonballus* (isolate No.1), *R. solani* (isolate No.1), and *V. tricorpus* (isolate No.1), were the most aggressive isolates than the others with significant differences between them.

The six virulent isolates of *F. oxysporum* (isolate No. 2), *F. prolifiratum* (isolate No. 1), *F. semitectum* (isolate No.1), *M. cannonballus* (isolate No.1), *R. solani* (isolate No.1) and *V. tricorpus* (isolate No.1), were used in pathogenicity test to evaluate their potentialities in causing sudden wilt disease symptoms (Fig 1).

Table (3): Virulence of some fungal isolates attacking cucumber cv. Faris f1 under field conditions.

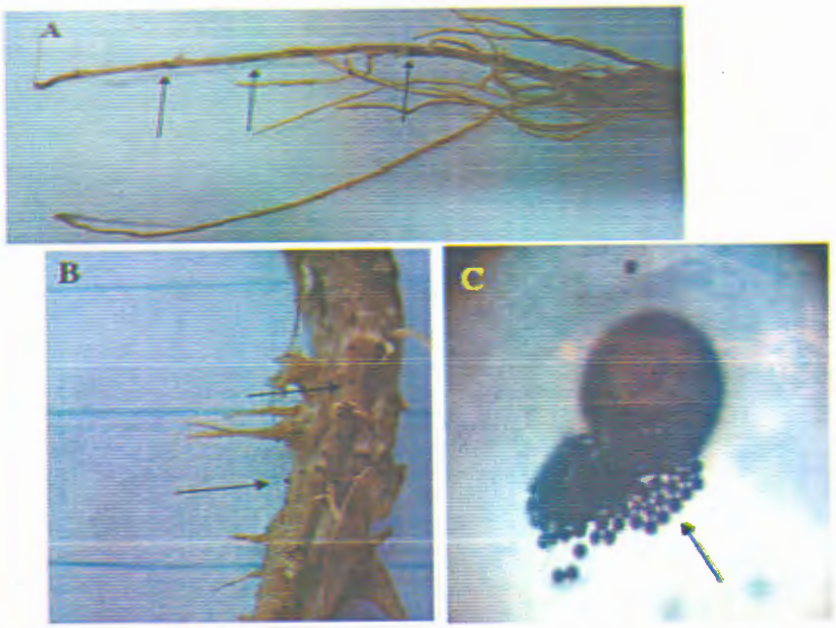
Fungi	No.	Disease parameters			Survival plants (%)
		Pre- emergence (%)	Post-emergence (%)	Wilted plantes (%)	
Fusarium oxysporum	1	17.50	22.50	22.50	32.50
	2	30.00	15.00	37.50	17.50
	3	20.00	27.50	27.50	25.00
	4	22.50	7.50	30.00	32.50
L.S.D.at 5%		1.7	2.1	1.7	2.4
Fusarium prolifiratum	1	20.00	17.50	32.50	30.00
	2	12.50	22.50	25.00	40.00
L.S.D.at 5%		2.1	2.4	6.6	6.6
Fusarium semitectum	1	25.00	12.50	40.00	22.50
	2	20.00	12.50	32.50	35.00
	3	17.50	22.50	17.50	42.50
	4	22.50	25.00	17.50	35.00
	5	20.00	20.00	30.00	30.00
	6	20.00	22.50	22.50	35.00
	7	17.50	22.50	25.00	35.00
	8	22.50	22.50	25.00	30.00
	9	22.50	15.00	35.00	27.50
	10	37.50	10.00	20.00	32.50
L.S.D.at 5%		0.38	0.63	0.77	0.83
Monosporascus cannonballus	1	20.00	12.50	45.00	22.50
	2	12.50	17.50	30.00	40.00
L.S.D.at 5%		3.5	2.4	3.6	2.4
Rhizoctonia solani	1	12.50	22.50	22.50	42.50
	2	10.00	20.00	20.00	50.00
L.S.D.at 5%		1.3	4.3	4.3	5.2
Verticillium tricorpus	1	5.00	22.50	37.50	35.00
	2	12.50	15.00	17.50	55.00
L.S.D.at 5%		1.3	3.03	5.6	4.6

Control without fungi recorded zero

Symptoms and effects:

The resulted symptoms of the tested fungi in pathogenicity test were recorded and described on affected plant roots (70-days-old). Roots were uprooted washed carefully and remarks were recorded for full description of the disease symptoms. Symptoms description show that all observations were external symptoms on root system but no internal symptoms were recorded on the inoculated cucumber plants.The main symptoms obtained were root shrinkage, stunting and cankers on the main root as well as brown areas on the lateral roots due to the infection by *F. oxysporum*, *Fig2 prolifiratum*, *F. semitectum, V. tricorpus* and *R. solani*, while cankers on the main root; brown and black areas on the lateral roots on the sites attached with the main root were always due to the infection by *M. cannonballus* (Fig 2)

(Fig 1) : Effect of six fungi on cucumber cv. Faris f1 by artificial inoculation with *R.solani* (A), *V. tricorpus* (B), *F. prolifiratum* (C), *F. semitectum* (D), *M. cannonballus*(E) and *F. oxysporum*(F).



(Fig 2): Symptoms on cucumber roots due to in lesion with *Monosporascus cannonballus*. Roots of infected plants discrete necrotic lesions and lacking secondary and tertiary feeder roots (a and b) caused by *M. cannonballus* and disrupted perithecium of *M.cannonballus* releasing ascospores (c).

DNA polymorphism among *M. cannonballus* isolates using RAPD-PCR technique.

Ten RAPD primers were screened with the DNA of the two fungal**.** These produced multiple band profiles with a number of amplified DNA fragments ranging from 9 to 18 **(Table 4 and Fig 3, 4).** In the present study, the total number of fragments produced by the ten primers was 130 with an average of 13 fragments / primer **(Table 4).** While, the number of polymorphic fragments ranged from 0 to 10. A maximum number of 18 amplicons were amplified with primer OPA-17, while the minimum number of fragments (9) was amplified with primer OPC-07. The highest number of polymorphic bands (10) was obtained with primers OPA-04, which exhibited the highest percentage (71%) of polymorphism. **Table (4)** also revealed that the total number of polymorphic amplicons obtained by the ten studied primers was 44. This corresponds to a level of

polymorphism of 33.9% and an average number of polymorphic fragments/primer of 4.4.

Table (4): Total number of amplicons, monomorphic amplicons, polymorphic amplicons and percentage of polymorphism as revealed by RAPD markers among the two fungal.

Primer	Total of amplicons	Monomorphic amplicons	Polymorphic amplicons	% of polymorphism
OP A-03	14	7	7	50
OP A-04	14	4	10	71
OP A-06	11	7	4	36
OP A-11	11	8	3	27
OP A-17	18	15	3	16
OP C-01	12	6	6	50
OP C-07	9	7	2	22
OP C-12	13	7	6	46
OP G-03	14	14	0	0
OP Z-17	14	11	3	21
Total	130	86	44	33.9
Average	13	8.6	4.4	.....

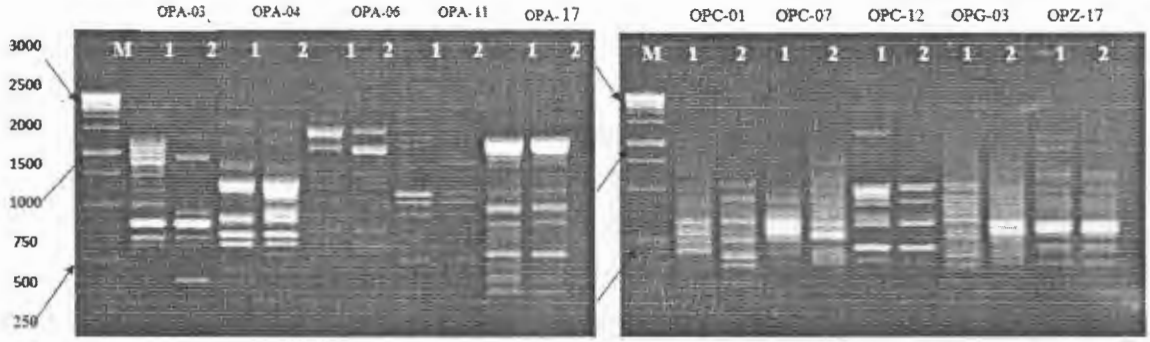


fig (4): RAPD profil7es of two tested fungi of *M. cannonballus* (1‹2) as detected with primers OPC-01, OPC-07, OPC-12, OPG- 03 and OPZ-17. M: 1 Kb ladder DNA marker. Lane 1 = *M. cannonballus* (1) and Lane 2 = *M. cannonballus* (2).

To examine the genetic relationships among the two fungi based on RAPD results, the scored data were analyzed using the Dice coefficient to compute the similarity matrices. The genetic similarity 80 % among two fungi **Table (5)**.

Table (5): Genetic Relationships among the two fungi

isolates of *M.cannonballus*	isolates of *M.cannonballus*	
	1	2
1	100	80
2	80	100

Discussion

F. oxysporum, *F. prolifiratum*, *F. semitectum*, *M. cannonballus*, *R. solani* and *V. tricorpus* isolates were isolated from infected cucurbits plants with root rot and sudden wilt diseases and collected from different localities in Egypt. Many investigators *i.e.* **Gonzalez-Torres *et al.*, (1988), Bruton and Miller (1997), Pivonia *et al.*, (1997) and Agerteer *et al.*, (2000)** recorded the occurrence of cucurbits root rot and sudden wilt disease and attributed its incidence to one or more of the previous identified fungi, except *Monosporascus cannonballus* which was not previously reported on cucumber in Egypt. All tested isolates were pathogenic of cucumber where they significantly increased root rot disease parameters. *F. oxysporum* (isolate 2), *F. prolifiratum* (isolate 1), *F. semitectum* (isolate 1), *M. cannonballus* (isolate 1), *R. solani* (isolate 1) and *V. tricorpus* (isolate 1) were the most aggressive isolates than the others tested. The most aggressive isolates, however, were used alone in pathogenicity tests to evaluate their potentialities in causing sudden wilt disease symptoms. Results indicated that most pathogens were synergistic, since they increased infection percentage and disease severity. These results are to some extent, in agreement with the previous reports. Where **Pivonia *et al.*, (1997)** found in artificial inoculations that *Monosporascus* sp. is the single virulent species causing the sudden wilt of melons in the Arava region in Israel producing 73 % mortality, to be significantly higher than *P. aphanidermatum*, *F. solani*, *Olpidium sp.*, and *F. prolifiratum*. Although, they were more frequently isolated, they were shown to be less important as single pathogens under Arava conditions. Their importance seems to be

in their ability to co-colonize the roots of melon plants and to induce wilt to a greater extent than their individual effects, thus contributing to the disease complex. In addition, a variety of organisms has been reported as causal agent of melon collapse in various regions is: *M. cannonballus* (**Uematsu *et al.*, 1992, Martyn *et al.*, 1994, Miller *et al.*, 1995 and Martyn and Miller, 1996**). It is possible that all of these organisms act similarly by suppressing the root system, and finally affecting the balance between sink and source, especially during the period of fruit maturation.

Results of RAPD-PCR showed that there is a percentage of 80% similarity between the two isolates of *M. cannonballus*. Hereby, these results confirm that the two isolates are genetically different and that difference in the pathogenicity and morphology is due to the genetic diversity, according to (**Feng *et al.*, 1999**) who used RAPD technique to analyse the relative relationship of 3 races of *F. oxysporum* f. sp. *vasinfectum* from China and 3 isolates from elsewhere. They showed that race III in China is very close to race 3 of Foreign, and belongs to the same RAPD section. Race 7 in China is a special Chinese type and race 8 in China has a complex genetic background and is divided into 2 RAPD sections. Also, **Vakalounakis and Fragkiadakis, (1999)** characterized a total of 106 isolates of *F. oxysporum* using pathogenicity, vegetative compatibility groups and RAPD-PCR. They concluded that, pathogenicity, vegetative compatibility groups and RAPD-PCR were effective in distinguishing isolates of *F. oxysporum* f.sp. *radicis-cucumerinum* from those of *F. oxysporum* f.sp. *cucumerinum*. Similarly, **Carmer *et al.*, (2003)** characterized genetic diversity and pathogenicity of 166 isolates of *F. oxysporum* obtained from common bean and sugar beet plants using RAPD analysis. They concluded that RAPD markers had only limited usefulness in correlating pathogenicity among the isolates and races. In parallel, **Abd EL-Salam *et al.*, (2003)** reported that RAPD data were utilized to elucidate genetic relationships among 11 *Fusarium* species and one subspecies, *i.e. F. oxysporum* f.sp. *vasinfectum*. They suggested that RAPD markers can be a quick and reliable alternative for differentiating *Fusarium* spp. isolates. On the contrary, **Riveros *et al.*, (2001)** compared RAPD-PCR with classical taxonomy, morphological and pathogenicity of *Fusarium* strains isolated from melons and found that the obtained results were inconsistent.

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الملخص العربى

المسبب لمرض عفن الجذور *Monosporascus cannonballus* دراسة مرضية على فطر والذبول المفاجئ على الخيار.

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يعتبر الخيار من محاصيل الخضر الهامة عالمياً. وخلال ال٢٥ عاماً الأخيرة تأثر الإنتاج العالمي للقرعيات لإصابتها بأمراض أعفان الجذور الفطرية. عزلت ٦ أنواع تابعة لأربعة أجناس من فطريات التربة المسببة لأعفان الجذور من جذور نباتات العائلة القرعية المصابه بمرض عفن الجذور من منطقة ابوحمص بالبحيرة وهى فطريات فيوزاريوم أكسيسبورم، فيوزاريوم بروليفريتم، فيوزاريوم سيميتيكتم، مونوسبوراسكس كانونبالوس، رايزوكتونيا سولانى وفرتسيليوم ترايكوربس. أكدت اختبارات القدرة المرضية قدرة جميع الفطريات المختبرة على اصابة الخيار بعفن الجذور والذبول المفاجئ. كما أكدت الدراسات أيضاً أن العزلات الفطرية (العزلة رقم (٢) من الفطر فيوزاريوم أكسيسبورم والعزلة رقم (١) لكل من فيوزاريوم بروليفريتم، فيوزاريوم سيميتيكتم، مونوسبوراسكس كانونبالوس، رايزوكتونيا سولانى وفرتسيليوم ترايكوربس) هى الأكثر شراسة فى إحداث الإصابة عن باقى الفطريات المختبرة. كما أظهرت إختبارات المرضية أيضاً أن الفطر مونوسبوراسكس كانونبالوس هو الأكثر قدرة بين باقى الفطريات الأخرى إحداثا فى دراسة التباين RAPD-PCR لمرض الذبول المفاجئ على الخيار. كما تم إستخدام تقنية الوراثى بين عزلتين لفطر مونوسبوراسكس كانونبالوس المسبب لمرض الذبول المفاجئ فى الخيار % وربما قد يؤثر ذلك على درجة 80حيث أكدت الدراسة وجود درجة قرابة بين العزلتين تصل الإختلاف فيما بين العزلتين فى القدرة الإمراضية والتباين المورفولوجى.