USE OF RANDOM AMPLIFIDE POLYMORPHIC DNA (RAPD) TO DIFFERENTIATE AMONG ISOLATES OF *FUSARIUM* SPP. PATHOGENIC ON COTTON

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ABSTRACT

Twenty Isolates of nine *Fusarium* spp. were used in this study they were obtained from roots of cotton seedlings infected with damping-off disease. Pre-emergence damping-off was recorded 15 days after planting for the cotton cultivars of Giza 80, and Giza 90. Post-emergence damping-off, survival and dry weight (mg/plant) were recorded 45 days after planting. Isolate, cultivar, and isolate X cultivar interaction were significant sources of variation in all the tested parameters except the cultivar which was a nonsignificant source of variation in pre-emergence damping-off. Random Amplified Polymorphic DNA (RAPD) Technique has been used as a molecular technique for typing and genetic characterization of *Fusarium* spp. using three different decamer random primers (3, 4, and 5). DNA was extracted from 9 different *Fusarium* spp. and used for polymerase chain reaction (PCR). The similarity levels were determined by Cluster analysis for RAPD-PCR profiles via phylogeny tree. Primer 3 was successful in separating the isolates of *F. solani*, *F. moniliforme*, *F. oxysporum*, and *F. semitectum*. Isolates of *F. oxysporum* gave high DNA similarity (99.65%) with this primer. Primer 4 was successful in the separation of *F. solani*, *F. oxysporum*, *F. moniliforme*, *F. semitectum*, and *F. sporotrichioides*. This primer is suggested to be the best of all primers in separating *F. sporotrichioides* since it showed the highest DNA similarity degree (98.87%). Primer 5 was successful only in the separation of *F. solani*, *F. oxysporum*, *F. moniliforme*, and *F. semitectum* isolates. This primer was the best of

all the primers in the separation of *F. solani*, and *F. semitectum* since it showed high DNA similarity degrees among these isolates (99.71%, and 99.78% respectively). The results in this study evoke that isolates of *Fusarium* spp. exhibited diversity in pathogenicity on cotton cultivar and demonstrate that the use of RAPD analysis is useful in classification of *Fusarium* spp.

Key words: Fusarium, Cotton, Pathgenicity, RAPD analysis, DNA similarity.

INTRODUCTION

Fusarium species occur frequently among the fungal microflora associated with cotton seedling diseases and considers among the major causes of seedling death in some countries including Egypt (Watkins, 1981; Minton & Garber 1983; Aly *et al.* 1996; and El-Samawaty 2004). *Fusarium oxysporum*, *F. solani*, and *F. moniliforme* are commonly isolated from infected cotton seedlings in Egypt (Jakob 1969; Aly *et al.* 1996; El-Samawaty 1999; and Abd-Elsalam 2006). Seven *Fusarium* species including *Fusarium semitectum*, *F. tabacinum, F. sambucinum*, *F. avenaceum*, *F. poae*, *F. fusarioides*, *F. subglutinans*, and *F. sporotrichioides* were recorded as new pathogens to seedling of the Egyptian cotton (El-Samawaty 1999 and 2004).Thirty –seven genera representing 58 species of fungi were isolated from upland cotton, including 9 species of *Fusarium*. *Fusarium oxysporum*, *F. solani* and *F. equiseti* are the most common members of this genus (Plamater *et al.*, 2004). Identification of *Fusarium* spp. associated with cotton seedling disease is important to improve our understanding of their ecology and the epidemiology of the disease.

The present system of *Fusarium* classification is based mostly on morphological characterizations, especially the shape of macro conidia (Toussoun & Nelson, 1976). However, morphological designation of fusaria is a difficult and tedious process. It is further complicated in that the morphology of spores is influenced to a greet extent by cultural and environmental factors. In certain instances, some isolates from "mycelial types" without production of macro conidia. Also, classification based on morphology could be influenced by a personal bias (Snyder & Toussoun, 1965).

Random Amplified Polymorphic DNA (RAPD) is a useful technique to evaluate taxonomic identity and kinship (Hadrys *et al.*,

1992). RAPD markers have been applied widely in the detection and genetic characterization of *Fusarium* spp. (Garjal-Martin *et al.*, 1993; Bentley *et al.*, 1995; Jimenez-Gasco *et al.*, 1998; and Moller *et al.*, 1999). The objectives of this investigation were to evaluate the pathogenicity of *Fusarium* spp. isolates on some cotton cultivars under greenhouse conditions and to differentiate among these isolates using RAPD techniques.

MATERIALS AND METHODS

Isolates of *Fusarium* Spp.:

Isolates of *Fusarium* spp. (Table 1) used in this study were obtained from roots of cotton seedlings infected with damping-off disease. Isolation, purification, and identification of these isolates were carried out at Cotton Pathology Lab, Plant Path. Res. Inst, Agric. Res. Cent., Giza, Egypt.

Pathogenicity test of *Fusarium* spp. on cotton cultivars:

Substrate for growth of each isolate was prepared in 500 ml glass bottles, each bottle contained 50 g of sorghum grains and 40 ml of tap water. Contents of bottles were autoclaved for 30 min. Isolate inoculum, taken from one-week old culture on PDA, was aseptically introduced into the bottle and allowed to colonize sorghum for 3 weeks. The present test was carried out by using autoclaved clay loam soil. Batches of soil were infested separately with inoculum of each isolate at the rate of 50 g/kg of soil. Infested soil was dispensed in 10 cm diameter clay pots and these were planted with seeds (10 seeds /pot) of tow cotton cultivars (Giza 80, and Giza 90). In control treatment, sterilized sorghum grains were mixed thoroughly with soil at the rate of 50 g/kg of soil. Pots were randomly distributed on greenhouse benches. Prevailing temperatures during pathogenicity tests were 24± 3°C to 33± 2.5°C. Percentage of pre-emergence damping-off was recorded 15 days after planting. Post-emergence damping-off, survival and dry weight (mg/plant) were recorded 45 days after planting.

Statistical analysis of data:

The experimental design of pathogenicity test was a randomized complete block with four replicates. Analysis of variance (ANOVA) of the data was performed with the MSTAT-C statistical package (A micro-computer program for the design, management, and analysis of

agronomic research experiments, Michigan State Univ., USA). Least significant difference (LSD) was used to compare treatment means. Percentage data were transformed into arc sine angles before carrying out the ANOVA, to produce approximately constant variance.

Table (1): Geographic origin, Host genotype, and previous crop of *Fusarium* Isolates:

Isolate no.	*Fusarium* spp.	Geographic origin	Cotton cultivar	Previous crop
1	*F. solani*	Sohag	Giza 83	Broad bean
2	*F. solani*	Assiut	Giza 83	Clover
3	*F. solani*	Assiut	Giza 83	Clover
4	*F. moniliforme*	Minia	Giza 80	Onion
5	*F. moniliforme*	Minia	Giza 80	Coriander
6	*F. moniliforme*	Assiut	Giza 83	Clover
7	*F. oxysporum*	Sohag	Giza 83	Clover
8	*F. oxysporum*	Minia	Giza 80	Onion
9	*F. oxysporum*	Assiut	Giza 83	Clover
10	*F. sporotrichioides*	Sohag	Giza 83	Clover
11	*F. sporotrichioides*	Assiut	Giza 83	Clover
12	*F. subglutinans*	Minia	Giza 80	Onion
13	*F. sambucinum*	Assiut	Giza 83	Cumin
14	*F. sambucinum*	Minia	Giza 80	Clover
15	*F. sambucinum*	Minia	Giza 80	Onion
16	*F. poae*	Minia	Giza 80	Clover
17	*F. poae*	Asisut	Giza 83	Broad bean
18	*F. semitectum*	Minia	Giza 80	Clover
19	*F. semitectum*	Sohag	Giza 83	Clover
20	*F. fusarioides*	Sohag	Giza 83	Clover

DNA Extraction:

DNA was extracted from the 9 different *Fusarium* spp. (50 mg each) using Qiagen kit (Cat. No. 69104) for DNA extraction as described in the manufacturer manual. The extracted DNA was dissolved in 100 µl of eluting buffer. Concentration and purity of the obtained DNA was measured using "Gen qunts" system-pharmacia Biotec. DNA purity of all samples ranged between 1.7-1.8. Concentration was adjusted to 6ng/µl for all samples using TE buffer (PH8.0).

Random amplified polymorphism DNA (RAPD) technique:

Thirty ng from the extracted DNA were used for polymerase chain reaction (PCR) or DNA amplification reaction. PCR mixture contained PCR beads tablet (Amre sham pharmacia Biotec.) containing all the necessary reagents except the primers and the DNA templates. Five µl of the following primers (10-mer) were added to each PCR mixture.

Sequences of the used primers (Amresham pharmcia Biotec kit) were as follows:

RAPD analysis primer 3: d (GTAGACCCGT)-3

RAPD analysis primer 4: d (AAGAGCCCGT)-3

RAPD analysis primer 5: d (AACGCGCAAC)-3

The total volume of the PCR mixture was raised up to 25 µl using sterile distilled water. The amplification protocol was carried out as follows using PCR unit II biometar:

a) Denaturation at 95°C for 5 min.

b) 45 cycles each cycle consists of three different steps:

1) Denaturation at 95°C for 1 min.

2) Anealing at 36°C for 1 min.

3) Extension at 72°C for 2 min.

c) Final extension at °C for 5 min.

d) Hold at 4°C.

Seven µl of 6X tracking buffer (Qiagen kit) were added to 25 µl of the PCR product.

Amplification product analysis:

The amplified DNA fragments for all samples were immediately loaded in 1% agarose gel contaning ethedium bromide (0.5mg/ml), the electrophoresis was carried out in the presence of 1x TE gel-running buffer at 75 constant volt using electrophoresis WIDE mini-sub-cell GT Bio-RAD unit DNA bands were determined with UV-transilluminator (Williams *et al.*, 1990).

Gel analysis:

A gel documentation system was used (AAB advanced American Biotechnology 1166E.Valencia Dr.Unit 6C, Fullerton CA 92631) for gel analysis. The similarity levels were determined by Pearson's Correlation Coefficient and the dendrogram were constructed according to the unweighted pair- group method based on arithmetic mean (UPGMA).

RESULTS AND DISCUSSION

ANOVA (Table 2) showed that isolates, cultivars, and isolate X cultivar interaction were very high significant sources of variation in all the tested parameters except the cultivar, which was a nonsignificant source of variation in pre-emergence damping-off.

Table (2): Analysis of variance of effect of *Fusarium* isolates, cotton cultivar, and seedling disease parameters under greenhouse conditions:

Parameter and source of variation*	D.F	M.S	F. value	P > F
Preemergence damping-off				
Replication	3	117.949	2.306	0.080
Isolate (I)	20	568.950	11.123	0.000
Cultivar (C)	1	33.760	0.660	
IXC	20	272.149	5.320	0.000
Error	123	51.152		
Postemergence damping-off				
Replication	3	92.029	1.815	0.148
Isolate (I)	20	701.703	13.841	0.000
Cultivar (C)	1	467.167	9.215	0.003
IXC	20	342.244	6.750	0.000
Error	123	50.699		
Survival				
Replication	3	196.943	1.171	0.324
Isolate (I)	20	1618.970	9.624	0.000
Cultivar (C)	1	1325.084	7.877	0.006
IXC	20	531.786	3.161	0.000
Error	123	168.214		
Dry weight				
Replication	3	25117.609	1.624	0.187
Isolate (I)	20	171139.518	11.068	0.000
Cultivar (C)	1	211225.292	13.661	0.000
IXC	20	27124.679	1.754	0.033
Error	123	15461.918		

* Replication is random, while each of isolate and cultivar is fixed.

Isolate was the first in importance as a source of variation in all tested parameters, while isolate X cultivar interaction was the second in importance, and cultivar was the least important source of variation in all the tested parameters (Table 3).

Due to the significance of isolate X cultivar interaction, (LSD) was calculated to compare means of isolates within each cultivar. This comparison showed that the differences between isolates and the control were not the same for each cultivar. Similarly, the differences among isolates differed from one cultivar to another e.g. Isolates of both *F. solani* 3 and *F. oxysporum* 8 were pathogenic on Giza 80 and non pathogenic on Giza 90. The differences between *F. sporotrichioides* isolates 10 and11 was significant on cultivar Giza 80, while it was nonsignificant on cultivar Giza 90. Also the difference between *F. Sambucinum* isolates 14 and 15 was significant on cultivar Giza 90 and non significant on Giza 80 (Table 4). The same conclusion held true for the data of post-emergence damping-off, survival, and dry weight (Tables 5, 6, and 7). The results in this study suggest that isolates of *Fusarium* spp. exhibited diversity of pathogenicity on cotton cultivars. This result is in agreement with that of Batson & Borazjani (1984), Aly *et al.* (1996), El-Samawaty (1999), and Abd-Elsalam (2007).

Table (3): Relative Contribution of cotton cultivar, *Fusarium* isolate, and their interactions to variation in seedling disease parameters:

Source of Variation	Preemregence Damping-off	Postemergence Damping-off	Survival	Dry weight
Isolate	66.12	64.91	72.07	80.50
Cultivar	0.20	2.16	2.95	4.97
IXC	31.63	31.66	23.67	12.76

* Calculated as percentage of sum squares of the explained (model) variation.

Table (4): Effect of cotton cultivar, *Fusarium* isolate, and their interaction on preemergence damping-off of cotton seedlings under greenhouse conditions:

	Cultivar					
	Giza 80		Giza 90		Mean	
Fungus Isolates	%	Trans*	%	Trans	%	Trans
1-*F. solani*	35	36.22	15	22.50	25	29.36
2-*F. solani*	15	22.50	35	36.00	25	29.25
3-*F. solani*	55	47.88	25	29.36	40	38.62
4-*F. moniliforme*	50	45.00	42.5	40.61	46.25	42.81
5-*F. moniliforme*	37.5	37.44	27.5	31.02	31.5	34.23
6-*F. moniliforme*	30	33.05	60	52.56	45	42.81
7-*F. oxysporum*	55	47.88	50	45.00	52.5	46.44
8-*F. oxysporum*	30	33.05	20	28.22	25	30.64
9-*F. oxysporum*	37.5	37.72	27.5	31.02	32.5	34.37
10-*F. sporotrichioides*	65	54.06	45	42.05	55	48.06
11-*F. sporotrichioides*	40	39.17	40	39.10	40	39.14
12-*F. subglutinans*	17.5	24.53	30	32.53	23.75	28.53
13-*F. sambucinum*	45	42.11	22.5	28.22	33.75	35.17
14-*F. sambucinum*	30	33.05	42.5	40.39	36.25	36.72
15-*F. sambucinum*	45	42.05	85	70.45	65	56.25
16-*F. poae*	40	39.17	47.5	43.56	43.75	41.36
17-*F. poae*	55	47.88	57.5	49.39	56.25	48.64
18-*F. semitectum*	37.5	37.50	22.5	28.22	30	32.86
19-*F. semitectum*	30	33.05	17.5	21.58	23.75	27.32
20-*F. fusarioides*	40	38.95	40	39.40	40	39.03
Control	15	19.92	15	22.50	15	21.21
Mean	38.3	37.72	36.6	36.83		

*: % data were transformed into arc sine angles before the analysis of variance.
LSD (transformed data) for cultivar X isolates interaction = 10.01 ($P \leq 0.05$) or 13.23 ($P \leq 0.01$).

Table (5): Effect of cotton cultivar, *Fusarium* isolate, and their interaction on postemergence damping-off of cotton seedlings under greenhouse conditions:

	Cultivar					
	Giza 80		Giza 90		Mean	
Fungus Isolates	%	Trans*	%	Trans	%	Trans
1-*F. solani*	65	53.78	37.5	37.44	51.25	45.61
2-*F. solani*	17.5	24.53	30	32.31	23.75	28.42
3-*F. solani*	37.5	37.51	57.5	49.39	47.50	43.45
4-*F. moniliforme*	35	36.22	35	26.22	35.00	36.22
5-*F. moniliforme*	12.5	20.47	35	36.22	47.50	28.34
6-*F. moniliforme*	65	53.78	32.5	34.56	48.75	44.17
7-*F. oxysporum*	30	32.90	37.5	37.66	33.75	35.28
8-*F. oxysporum*	60	50.83	15	22.50	37.50	36.67
9-*F. oxysporum*	35	36.22	45	42.05	40.00	39.14
10-*F. sporotrichioides*	22.5	27.85	17.5	24.53	20.00	26.19
11-*F. sporotrichioides*	55	47.89	40	39.10	47.50	43.49
12-*F. subglutinans*	35	35.47	42.5	40.61	38.75	38.04
13-*F. sambucinum*	45	42.11	57.5	49.39	51.25	45.75
14-*F. sambucinum*	57.5	49.39	42.5	40.61	50.55	45.00
15-*F. sambucinum*	42.5	40.61	15	19.55	28.75	30.08
16-*F. poae*	42.5	40.61	27.5	31.39	35.00	36.00
17-*F. poae*	35	36.22	32.5	34.56	33.75	35.39
18-*F. semitectum*	37.5	37.66	60	50.90	48.75	44.28
19-*F. semitectum*	30	33.05	40	39.16	35.00	36.11
20-*F. fusarioides*	37.5	37.72	7.5	11.25	22.50	24.49
Control	5	9.22	2.5	4.61	3.75	6.91
Mean	38.21	37.34	33.81	34.00		

*: % data were transformed into arc sine angles before the analysis of variance. LSD (transformed data) for cultivar X isolates interaction = 9.97 ($P \leq 0.05$) or 13.17 ($P \leq 0.01$).

Table (6): Effect of cotton cultivar, *Fusarium* isolate and their interaction on survival of cotton seedling under greenhouse conditions:

	Cultivar					
	Giza 80		**Giza 90**		Mean	
Fungus Isolates	%	Trans*	%	Trans	%	Trans
1-*F. solani*	0.00	0.00	47.50	43.56	23.75	21.78
2-*F. solani*	67.50	55.44	35.00	34.60	51.25	45.02
3-*F. solani*	7.50	8.30	17.50	17.89	46.25	13.10
4-*F. moniliforme*	15.00	19.55	22.50	28.22	18.75	23.89
5-*F. moniliforme*	50.00	45.00	37.50	37.44	43.75	41.22
6-*F. moniliforme*	5.00	6.64	5.00	9.22	5.00	7.93
7-*F. oxysporum*	15.00	16.60	12.50	14.94	13.75	15.77
8-*F. oxysporum*	10.00	13.28	62.50	52.34	36.25	32.81
9-*F. oxysporum*	27.50	31.55	27.50	27.68	27.50	29.62
10-*F. sporotrichioides*	12.50	11.25	37.50	37.50	25.00	24.38
11-*F. sporotrichioides*	5.00	9.22	20.00	26.56	12.50	17.89
12-*F. subglutinans*	47.50	43.56	30.00	32.31	38.75	37.93
13-*F. sambucinum*	10.00	18.44	20.00	25.08	15.00	21.76
14-*F. sambucinum*	12.50	11.25	15.00	15.86	13.75	13.56
15-*F. sambucinum*	15.50	14.42	0.00	0.00	6.25	7.21
16-*F. poae*	17.50	21.58	25.00	29.73	21.25	25.65
17-*F. poae*	10.00	15.86	10.00	12.91	10.00	14.39
18-*F. semitectum*	25.00	29.14	17.50	23.64	21.25	26.39
19-*F. semitectum*	40.00	39.10	42.50	40.55	41.25	39.83
20-*F. fusarioides*	22.50	27.11	52.50	46.50	37.50	36.81
Control	80.00	66.75	85.00	65.45	82.50	66.10
Mean	26.67	24.00	29.64	29.62		

*: % data were transformed into arc sine angles before the analysis of variance.
LSD (transformed data) for cultivar X isolates interaction = 18.15 ($P \leq 0.05$) or 23.99 ($P \leq 0.01$).

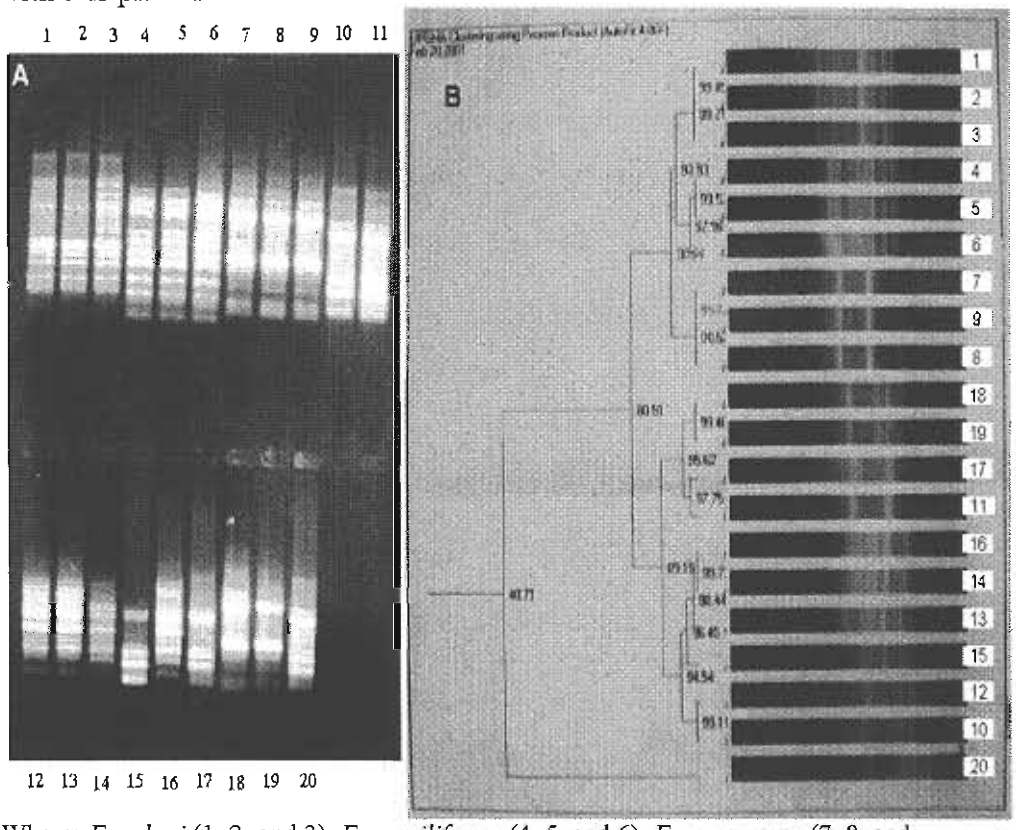
Table (7): Effect of cotton cultivar, *Fusarium* isolate and their interaction on dry weight of cotton seedlings (mg/ plant) under greenhouse conditions:

	Cultivar		
Fungus Isolates	**Giza 80**	**Giza 90**	Mean
1-*F. solani*	389.0	477.3	433.1
2-*F. solani*	208.8	346.0	277.4
3-*F. solani*	238.5	255.0	246.8
4-*F. moniliforme*	95.75	131.8	113.8
5-*F. moniliforme*	241.5	212.0	226.8
6-*F. moniliforme*	171.5	197.3	184.4
7-*F. oxysporum*	195.8	190.5	193.1
8-*F. oxysporum*	216.3	354.8	285.5
9-*F. oxysporum*	177.0	183.8	180.4
10-*F. sporotrichioides*	496.3	434.0	465.1
11-*F. sporotrichioides*	95.0	231.3	163.1
12-*F. subglutinans*	136.0	0.00	68.0
13-*F. sambucinum*	438.5	424.3	431.4
14-*F. sambucinum*	226.3	391.5	308.9
15-*F. sambucinum*	97.0	450.5	273.8
16-*F. poae*	101.5	287.5	194.5
17-*F. poae*	0.00	248.0	124.0
18-*F. semitectum*	363.5	290.8	327.1
19-*F. semitectum*	57.50	177.3	117.4
20-*F. fusarioides*	318.3	454.3	386.3
Control	672.0	687.5	679.8
Mean	235.0	306.0	

LSD for cultivar X isolates interaction = 174 ($P \leq 0.05$) or 230 ($P \leq 0.01$).

RAPD analysis has been used in molecular typing and genetic characterization of *Fusarium* spp. (Donaljons, *et al.*, 1995; Feng *et al.*, 2000; and Smith *et al.*, 2001). RAPD-PCR profiles were established in Figure 1A presented in phenogram (phylogeny tree) shown in Figure 1Bwas generated based on cluster analysis of RAPD-PCR profiles. This phenogram illustrated that primer 3 was successful in separation

the isolates of *F. solani*, *F. moniliforme*, *F. oxysporum*, and *F. semitectum*. Also it was successful in separation the isolates of *F. fusarioides* from other *Fusarium* spp. However, it failed in separation of the isolates of *F. sporotrichioides* and *F. poae* from other *Fusarium* spp. Isolates of *F. oxysporum* gave high DNA similarity (99.65%) with this primer.

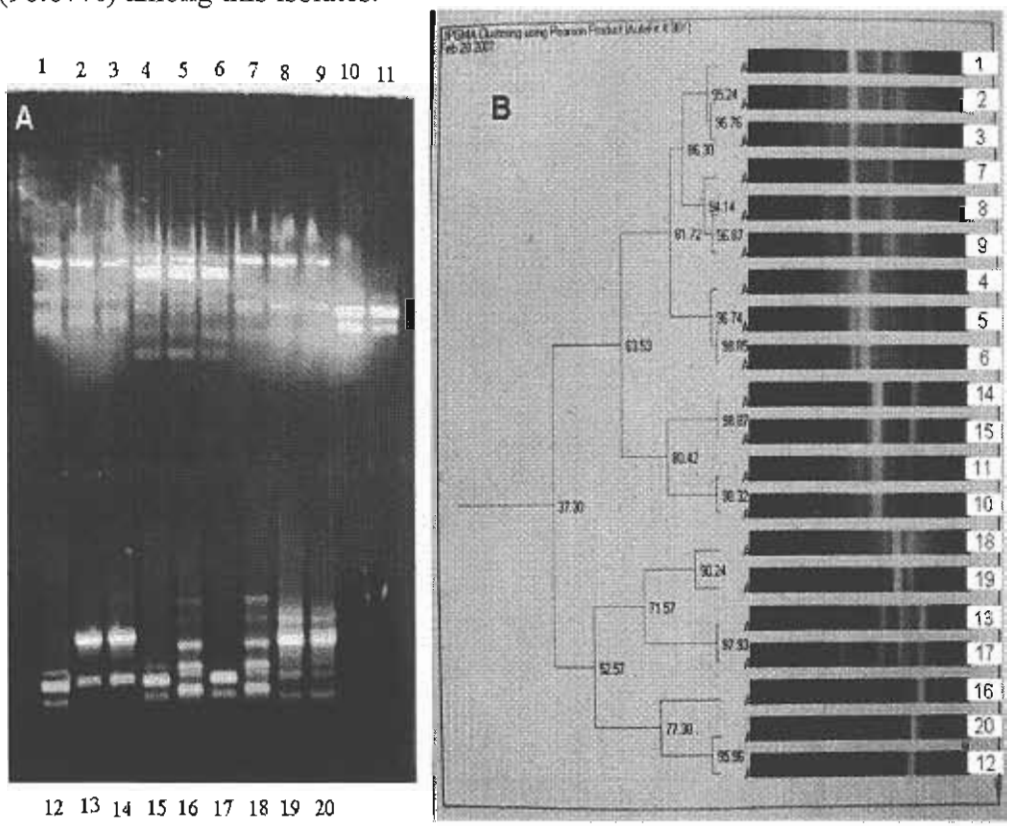


Where: *F. solani* (1, 2, and 3), *F. moniliforme* (4, 5, and 6), *F. oxysporum* (7, 8, and 9), *F. semitectum* (18, and 19), *F. poae* (17), *F. sporotrichioides* (11), *F. poae* (16), *F. sambucinum* (14, 13, and 15), *F. subglutinans* (12), *F. sporotrichioides* (10), and *F. fusarioides* (20) in order.

Figure (1) A: RAPD-PCR profiles of *Fusarium* spp., **B**: Phylogeny tree (Phenogram) based on cluster analysis of RAPD banding patterns of isolates of *Fusarium* spp. obtained by primer number 3.

The phylogeny tree in Figure 2B illustrated that primer 4 was successful in separating of *F. solani*, *F. oxysporum*, *F. moniliforme*, *F. semitectum*, and *F. sporotrichioides*. Primer 4 also showed a partial

separation of *F. sambucinum* isolates and it failed in separation of *F. poae* isolates. However primer 4 showed a successful separation between *F. solani* isolates from one side and both *F. semitectum* and *F. sporotrichioides* isolates on the other side. This primer is suggested to be the best of the three primers in the separation of *F. sporotrichioides* since it showed the highest DNA similarity level (98.87%) among this isolates.



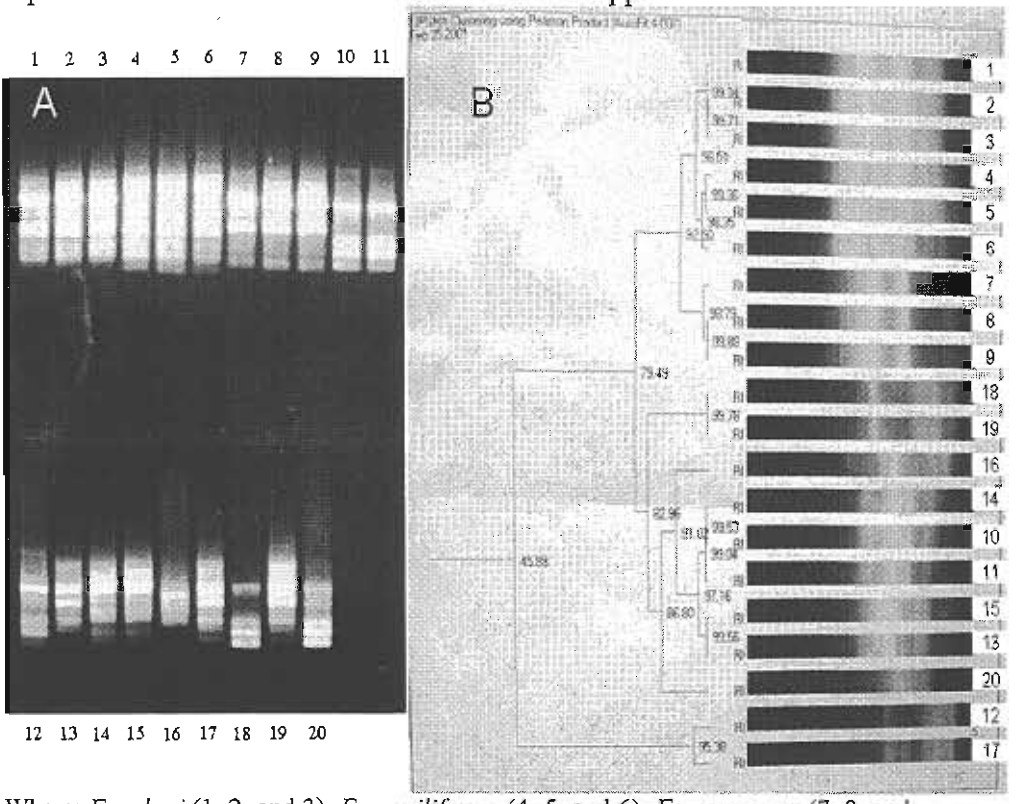
Where: *F. solani* (1, 2, and 3), *F. oxysporum* (7, 8, and 9), *F. moniliforme* (4, 5, and 6), *F. sambucinum* (14, and 15), *F. sporotrichioides* (11, and 10), *F. semitectum* (18, and 19), *F. sambucinum* (13), *F. poae* (17, and16), *F. fusarioides* (20), and *F. subglutinans* (12) in order.

Figure (2) A: RAPD-PCR profiles of *Fusarium* spp., **B**: Phylogeny tree (Phenogram) based on cluster analysis of RAPD banding patterns of isolates of *Fusarium* spp. obtained by primer number 4.

The phenogram in Figure 3B illustrated that primer 5 was successful in separation of *F. solani*, *F. oxysporum*, *F. moniliforme*, and *F.*

semitectum isolates, and failed in the separation of all other species. This primer is the best of the three primers in separation of *F. solani*, and *F. semitectum* since it showed high DNA similarity levels among these isolates (99.71%, and 99.78% respectively).

All three primers showed a monomorphism within the isolates of the same species as well as polymorphism within isolates belonged to different species. Results in this study suggested that isolates of *Fusarium* spp. exhibited diversity of pathogenicity on cotton cultivar and proved that random RAPD DNA-markers were successful in separation of the various isolates of *Fusarium* spp.



Where: *F. solani* (1, 2, and 3), *F. moniliforme* (4, 5, and 6), *F. oxysporum* (7, 8, and 9), *F. semitectum* (18, and 19), *F. poae* (16), *F. sambucinum* (14), *F. subglutinans* (12), *F. sporotrichioides* (11), *F. sambucinum* (15), *F. fusarioides* (20), *F. sambucinum* (13), *F. poae* (17), and *F. sporotrichioides* (10) in order.

Figure (3) A: RAPD-PCR profiles of *Fusarium* spp., **B**: Phylogeny tree (Phenogram) based on cluster analysis of RAPD banding patterns of isolates of *Fusarium* spp. obtained by primer number 5.

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أستخدام التضاعف العشوائي لمناطق متابينة من الحمض النووي DNA للتفرقه بين عزلات أنواع الفيوزاريوم الممرضة للقطن

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أستخدم في هذه الدراسة عشرون عزلة مثلت 9 أنواع من جنس الفيوزاريوم تم عزلها من بادرات قطن مصابة بمرض موت البادرات و تم تقييم قدرتها المرضية علي صنفي قطن (جيزة 80 و جيزة 90) تحت ظروف الصوبة. في هذه الدراسة تم استخدام تقنية التضاعف العشوائي لمناطق متابينة من الحمض النووي DNA (RAPD) للتفريق بين عزلات أنواع الفيوزاريوم بأستعمال ثلاثة بادئات عشوائية (أوليجونيكلوتيدات) لأتمام تفاعل البوليميريز المتسلسل (PCR) علي الحامض النووي DNA المستخلص من عزلات فطر الفيوزاريوم موضع الدراسة.كما إستعمل التحليل العنقودي لتصنيف هذه العزلات داخل الأنواع بناءً علي مابينها من تماثل في أنماط الحمض النووي DNA وتم التعبير عن النتائج في صورة شجرة نسب السلالات Phylogeny tree .

أهم نتائج البحث:

- أظهر تحليل التباين ان العزلات و الأصناف وتفاعل العزلات مع الأصناف كانت مصادر عالية المعنوية للتباين في جميع معايير تقييم القدرة المرضية بإستثناء الأصناف التي لم تكن مصدراً معنوياً للتباين في حدوث المرض قبل ظهور البادرات فوق سطح الأرض. كما تشير معنوية تفاعل العزلات مع الأصناف إلي تنوع القدرة المرضية للعزلات محل الدراسة.
- تمكنت بادئات الأوليجونيكلوتيدات المستعملة في هذه التجربة من أحداث تضاعف للحمض النووي DNA و كان البادىء رقم 3 أفضل البادئات لتصنيف عزلات النوع *F. oxysporum* حيث أعطي أعلي درجة تماثل في التتابع النيكليوتيدي للحامض النووي DNA قدرها 99.65% بين العزلات.
- في حين أن البادىء رقم 4 كان أفضل البادئات لتصنيف عزلات النوع *F. sporotrichioides* حيث أعطي درجة تماثل قدرها 98.87%.
- أما البادىء رقم 5 فكان ناجحاً في تصنيف عزلات أنواع مثل *F. solani*، *F. moniliforme* و *F. semitectum* حيث أعطي درجة تماثل في التتابع النيكليوتيدي للحامض النووي DNA قدرها 99.71%، 98.35% و 99.78% علي التوالي.

أثبتت هذه الدراسة أن عزلات الفيوزاريوم المختلفة أظهرت تباين في الأصابة المرضية لأصناف القطن المستخدمة في هذه الدراسة. كما أمكانية أستخدام تحليل ال RAPD في التعرف علي أو الفصل بين أصناف الفيوزاريوم المختلفة.

قام بتحكيم هذا البحث: أ. د محمد عبد العزيز أحمد الشافعي و أ. د. علي عبد الهادي علي