J. Agric. Sci. Mansoura Univ., 33(5): 3427 - 3442, 2008

USE OF PATHOGENICITY AND ELECTROPHORETIC PROTEIN AND ALCHOL DEHYDROGENASE PATTERNS TO DIFFERENTIATE AMONG ISOLATES WITHIN *Fusarium* SPECIES

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ABSTRACT

Twenty isolates belonging to nine *Fusarium* spp. were tested for levels of pathogenicity on two cotton cultivars under greenhouse conditions. Preemergence damping-off, postemergence damping-off, survival and dry weight were used as criteria to evaluate pathogenicity of the isolates. Proteins and alcohol dehydrogenase (AD) isozymes of the isolates were separated by SDS-PAGE, and PAGE, respectively. Virulence patterns, protein banding patterns, and AD isozymic patterns patterns were used to differentiate among *Fusarium* isolates belonging to the same species. In the three methods, cluster analysis was used to differentiate among the isolates, which showed low similarity levels-that is, the isolates, which belonged to remotely related or unrelated subclusters. On the other hand, cluster analysis was not a reliable method to differentiate among the isolates belonging to closely related subclusters because such isolates had relatively high similarity levels. The results of the present study suggest that isolates belonging to the same *Fusarium* species could be differentiated by their differential pathogenicity, combined with their specific protein and AD banding patterns separated by electrophoresis.
Keywords: *Fusarium,* cotton, pathogenicity, electrophoresis, proteins, isozymes.

INTRODUCTION

Species of *Fusarium* have been associated with cotton seedling disease and are major cause of seedling death in some countries involving Egypt (Watkins, 1981; Minton and Garber, 1983; Aly *et al*., 1996, and El-Samawaty, 2004). *Fusarium oxysporum, F. solani* and *F. moniliforme* are commonly isolated from cotton seedlings infected with damping-off in Egypt (Jakob, 1969; Aly *et al.*, 1998; El-Samawaty, 1999, and Abd-El-Salam, 2006). *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).

Electrophoresis of proteins has been widely used for studying variation among isolates within *Fusarium* species. For example, Lo and Sun (1986) found that protein pattern in vertical slab electrophoresis of *F. oxysporum* from radish different from that of *F. oxysporum* from mustard and *F. oxysporum* from Kale. Lodwing *et al.* (1999) used electrophoretic prpfiles of total proteins to distinguish isolates 27 and 30 of *F. oxysporum* f.sp. *cubense* and *F. oxysporum* from *Triticum* sp. Aly *et al.* (2000) reported that electrophoretic banding patterns of dissociated proteins separated four Egyptian races of *F. oxysporum* f.sp. *ciceris*.

Isozymes (isoenzymes) are defined as genetically determined multiple molecular forms of an enzyme. There are three main causes of formation of multiple molecular forms of enzymes. These are (1) the presence of more than one gene locus coding for the enzyme, (2) the presence of more than one allele at a single gene locus coding for the enzyme, and (3) the post translation modifications of the formed enzymatic polypeptide resulting in formation of nongenetic or so-called "secondary" isozymes. The term isozymes is usually used to denote multiple molecular forms deriving from different genetic loci, where the term "allozymes" is used to denote multiple molecular forms deriving from different alleles of the sane genetic locus. The term "allelic isozymes" is also used by isozymologists (Manchenko, 1994). Isozymes have similar, if not identical enzymatic properties but slightly different amino acid sequences. Only those isozymes with amino acid compositions of different net charge, or those that result in large differences in the shape of an enzyme, can be differentiated by electrophoresis (Bonde *et al.*, 1993).

Isozymes analysis is a powerful biochemical technique that has numerous applications in plant pathology. It was being used routeinly to settle taxonomic disputes, identify cultures "fingerprint" patentable fungal lines, analysis genetic variability, trace pathogen spread, follow the segregation of genetic loci, and identify ploidy levels of fungi and other plant pathogens (Bonde *et al.*, 1993; Some and Tivoli, 1993 and YliMattila *et al.*, 1995).

The objectives of this investigation were to differentiate among isolates within *Fusarium* species by using virulence patterns, protein banding patterns, and isozymic patterns of alcohol dehydrogenase.

MATERIALS AND METHODS

Isolates of *Fusarium* spp.

Isolates of *Fusarium* spp. 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 Res. Dept., Plant Pathology Res. Inst., Agric. Res. Center, 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 a 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 isolate means within cultivars. Percentage data were transformed into arc sine angles before carrying out the ANOVA, to produce approximately constant variance.

Extraction of fungal proteins

Protein extracts from *Fusarium* spp. isolates were prepared according to Hussein (1992) in the following way: Fungal isolates were grown for 22 days at 22-30°C on liquid Czapek medium. The mycelium was harvested by filtration through cheesecloth, washed with distilled water several times, and freeze-dried. This frozen mycelium was suspended in phosphate buffer pH 8.3 (1-3 ml/g mycelium), mixed thoroughly with glass beads, and ground in liquid nitrogen to a fine powder. The ground mycelium was centrifuged at 19,000 rpm for 30 minutes at 0°C. The protein content in the supernatant was estimated according to Bradford (1976) by using bovine serum albumin as a standard protein. If protein concentration was low, protein was precipitated from the clarified supernatant by adding ammonium sulphate at 70% of saturation (60g/100 ml) then kept in the refrigerator for 30 hr. Pellets, collected by centrifugation at 11,000 rpm for 30 minutes, were resuspended in phosphate buffer pH 8.3 and subjected to dialysis for 24 hr against the buffer and centrifugation at 11,000 rpm for 30 minutes. Protein was estimated in the obtained supernatant.

Electrophoresis of dissociated protein (SDS-PAGE)

Each supernatant was mixed with an equal volume of a solution consisting of (by volume) 64% buffer (0.15 M Tris HCl, pH 6.8), 20% glycerol, 6% SDS, 10% 2-6-mercaptoethanol, and 0.1% bromophenol blue, before boiling in a water bath for three minutes. Twenty-microliters samples (40 μg of protein) were subjected to electrophoresis in a 7.5% polycarylamid gel prepared 0.1% SDS with a 3.5% stacking gel (Laemmli, 1970). Electrophoresis was conducted at 10°C for 4 hr at 15 and 30 mA for the stacking and the separating gels, respectively, until dye reached the bottom of the separating gel. Electrophoresis was performed in vertical slab mold (16x18x0.15 cm). Gels were stained with silver nitrate for the detection of protein bands (Sammons *et al.*, 1981).

Electrophoresis of isozymes

Protein-extract supernatant was mixed with an equal volume of a solution containing 20% glycerol (vol/vol) and 0.1 bromophenol blue (vol/vol) in 0.15 M Tris-HCl, pH 6.8. Twenty microliters of the resulting suspension (40 to 60 μg of protein) was subjected to electrophoresis in 25 mM Tris buffer containing 192 mM glycin at pH 8.3. Electrophoresis was conducted at 10°C for 4 hr on a 7.5% polyacrylamide gel with a 3.5% stacking gel, at 30 and 15 mA, respectively, until the dye reached the bottom of the separating gel (Laemmli, 1970). Electrophoresis was performed in a vertical slab mold (16x18x0.15 cm). Gels were stained according to Manchenko (1994) for the detection of isozymes of alcohol dehydrogenase.

Gel analysis

A gel documentation system (Advanced American Biotechnology 1166 E. Valencia Dr. Unit 6C, Fullerton CA 92631) was used to document the results of PAGE and to cluster the electrophoretic patterns by the unweighted pair-group method based on arithmetic mean (UPGMA).

RESULTS AND DISCUSSION

ANOVA (Table 1) showed that isolate, cultivar, 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.

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 importance source of variation in all the tested parameters (Table 2).

Due to the significance of isolates 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 differ 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 3). The same conclusion held true for the data of post-emergence damping-off, survival, and dry weight (Tables 4, 5, and 6). The results in this study suggest that isolates of *Fusarium spp*. exhibited diversity in 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 1. Analysis of variance of effect of *Fusarium* isolate, cotton cultivar and their interaction on seedling diseases 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.**

Table 2. Relative Contribution of cotton cultivar, *Fusarium* spp. isolate, and their interaction to variation in seedling diseases parameters:

Source of Variation	Relative contribution[a] to variation in			
	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

[a] Calculated as percentage of sum squares of the explained (model) variation.

In the present study, virulence patterns, protein banding patterns, and isozymic patterns of 20 isolates of *Fusarium* spp. were used, basically, to differentiate among *Fusarium* isolates belonging to the same species. In the three methods, cluster analysis was used to differentiate among the isolates, which showed low similarity levels- that is, the isolates, which belonged to remotely related or unrelated subclusters. On the other hand, cluster analysis was not a reliable method to differentiate among the isolates belonging to closely related subclusters because such isolates had relatively high similarity levels.

The phenogram of Fig. (1) was constructed based on pathogenicity test shown in Tables (3-6).

Table 3. Effect of cotton cultivar, *Fusarium* isolate, and their interaction on preemergence damping-off of cotton seedlings under greenhouse conditions.

Fungus isolates	Cultivar					
	Giza 80		Giza 90		Mean	
	%	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	32.5	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		

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

Isolates of *F. sambucinum* nos. 13, 14, and 15 were easily distinguished based on their virulence patterns (Fig. 1), which placed the isolates in three remotely related subclusters. It was not possible to differentiate between *F. solani* isolates nos. 1 and 3, while it was easy to differentiate between any of the two isolates and *F. solani* no. 2, which belonged to remotely related subcluster. Within *F. oxysporum* and *F. moniliforme*, it was difficult to differentiate among isolates.

Amino acid sequence of polypeptides (components of proteins and isozymes) are dependent on nucleotide sequence of their coding genes; therefore, an analysis of protein and isozymic variation among *Fusariumi* isolates within species by electrophoresis, approximates on analysis of their genetic variation (Markert and Faulhaber, 1965). The phenogram shown in Fig. 3 was constructed based on cluster analysis of protein banding patterns of the tested isolates (Fig. 2).

F1

f2

F3

In this phenogram, it was difficult to differentiate between isolates nos. 7 and 8 of *F. oxysporum* due to their high similarity level; however, it was possible, to some extent, to differentiate between any of them and isolate no. 9. It was difficult to differentiate between isolates nos. 4 and 5 of *F. moniliforme* based on their protein profiles; however, these profiles distinguish each of them from isolate no. 6 of *F. moniliforme*. The same conclusion held true for isolates nos. 1 and 2 of *F. solani* and isolate no. 3. Protein profiles were highly successful in differentiating between isolate no. 14 of *F. samucinum* and each of isolates nos. 13 and 15 of the same fungus. Similarly, isolates nos. 19 and 18 of *F. semitectum* were easily distinguished by their protein profiles.

Electrophoresis patterns of isozymes can be obtained rapidly and the growing conditions have no influence on these patterns (Koberhel and Gautier, 1974). Fig. 5 showed a phenogram based on cluster analysis of alcohol dehydrogenase (AD) isozymes (Fig. 4).

Table 4. Effect of cotton cultivar, *Fusarium* isolate, and their interaction on postemergence damping-off of cotton seedlings under greenhouse conditions.

Fungus Isolates	Cultivar					
	Giza 80		Giza 90		Mean	
	%	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		

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

F4

F5

It was clear that the intraspecific differentiatiability of AD isozymes was much better than that of protein profiles due to low similarity levels among isolates based on AD isozymes. Thus it was easy to differentiate among isolates within species. For example, the two isolates of *F. semitectum* no$_s$. 18 and 19, the two isolates of *F. sporotrichoides no$_s$.* 10 and 11, and the three isolates of *F. samucinum*. Isolates no$_s$. 7 of *F. oxysporum*, no. 6 of *F. moniliforme*, and no. 1 of *F. solani* were easily distinguished from the other isolates belonging to the same species.

Therefore, it seems reasonable to conclude that the observed isolate specific protein and isozymic patterns may be useful as biochemical markers in ecological studies in soil where "marked" isolates are needed. The virulence patterns of *Fusarium* isolates combined with their protein and isozymic patterns would facilitate their identification after reisolation from soil.

Table 5. Effect of cotton cultivar, *Fusarium* isolate, and their interaction on survival of cotton seedlings under green house conditions.

Fungus Isolates	Cultivar					
	Giza 80		Giza 90		Mean	
	%	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*	12.50	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		

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

Table 6. Effect of cotton cultivar, *Fusarium* isolate, and their interaction on dry weight (mg/ plant) of cotton seedlings under green house conditions.

Fungus Isolates	Cultivar		
	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< 0.05) or 230 (P< 0.01).

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إستعمال القدرة المرضية وأنماط البروتين والمشابهات الإنزيمية للكحول ديهيدروجينيز الناتجة من التفريد الكهربى للتفرقة بين العزلات داخل أنواع الفيوزاريوم

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قيمت ٢٠ عزلة تتبع ٩ أنواع من الفيوزاريوم وذلك من حيث القدرة على إصابة صنفين من القطن تحت ظروف الصوبة. إستعملت النسب المئوية للبادرات الميتة قبل وبعد ظهور البادرات فوق سطح التربة والنسبة المئوية للبادرات الباقية على قيد الحياة والوزن الجاف كمعايير لتقييم القدرة المرضية للعزلات. إستعمل التفريد الكهربى لفصل بروتينات العزلات ومشابهات إنزيم الكحول ديهيدروجينيز. إستعملت أنماط القدرة المرضية والبروتينات ومشابهات الإنزيم للتفرقة بين العزلات. فى كل طريقة من هذه الطرق ، نجح التحليل العنقودى فى التفرقة بين العزلات المتباعدة فى أنماطها ومن ثم المنتمية إلى مجموعات متباعدة ، أما العزلات ذات الأنماط المتقاربة والتى تنتمى إلى مجموعات متقاربة فلم يمكن التفرقة بينها باستعمال التحليل العنقودى. تدل نتائج الدراسة الحالية أنه من الممكن التفرقة بين عزلات الفيوزاريوم التابعة للنوع الواحد بتقييم الإختلافات فيما بينها من حيث القدرة المرضية مع استخدام تقنية التفريد الكهربى لفصل بروتينات العزلات ومشابهات إنزيم الكحول ديهيدروجينيز.