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MOLECULAR CHANGES IN PROTEIN PATTERNS AS AN INDIRECT OF A MICROBIAL DNA CHANGES BY USING SDS-PAGE

By

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ABSTRACT: The total cell protein patterns of 16 strains of ***Fusarium equesti*** and ***Fusarium moniliforme*** which grown on media supplemented with or without different concentrations of Trifloxystrobin, Azoxystrobin or Flutolanil fungicides by SDS-PAGE on 12.5 % polyacrylamid resolving gels. Twenty – four protein bands were detected with molecular weights in the range of 20.77 to 145.42 KDa. Protein of 20.77, 25.51, 27.11, 31.46, 33.47, 36.60, 40.75, 45.47, 47.49, 50.02, 53.18, 62.23, 68.01, 72.52, 75.44, 80.02, 88.63, 91.56, 97.17, 101.16, 113.64, 125.36, 130.14 and 145.42 KDa were present but their frequencies varied among the strains of ***F. equesti***. The results also demonstrated that twenty – sex protein bands were detected with molecular weight in the range of 17.46 to 423.48 KDa.

Protein of 17.46, 18.18, 19.77, 20.48, 22.62, 23.37, 26.54, 33.23, 34.39, 36.00, 38.55, 40.39, 41.43, 49.64, 51.81, 53.22, 58.59, 76.66, 80.20, 92.47, 137.78, 304.95, 312.00, 368.20, 412.35 and 423.48 KDa were present but their frequencies varied among the strains of ***F.moniliforme***. Protein bands of 20.77, 31.46, 33.47, 36.60, 62.23, 91.56 and 113.64 KDa for strains of ***F. equesti*** & 18.18, 20.48, 33.23, 38.55, 41.43, 49.64,53.22 and 137.78 for strains of ***F.*** were com ***moniliforme*** mon among the strains and could be specific to recognize species deference. Protein analysis by SDS – PAGE could be considered a useful technique in identifying differences among the mentioned strains above.

INTRODUCTION

Resistance to fungicides in fungi populations is one of the most significant problems in the area of chemical disease management. There has been a gradual increase in the occurrence of fungicide resistance since the introduction of systemic fungicides in the early 1970s. Resistance to

some groups of fungicides has occurred more frequently than others. The fungal resistance to most fungicides was controlled by chromosomal genes. Major gene controlled resistance is qualitative, while polygenic resistance is quantitative **Yuan** and **Zhou (2004).** The study of proteins by electrophoresis for taxonomy has provided a valuable tool for the study of phylogenetic relationships. Homology of protein fractions by electrophoretic methods has been used in some studies previously **Ibrahim *et al.*, (1985)**.

Comparison of protein patterns may be regarded as an indirect method for comparison of microbial DNA, differences in genome being reflected in the structure of the encoded macromolecules **Zaini *et al.*, (1991)**.

Sparkes *et al.*, (1994) mentioned that water-soluble antigens liberated from the disrupted mycelium of nine dermatophytes (seven isolates of ***Microsporum canis***, one each of ***Microsporum gypseum*** and ***Trichophyton mentagrophytes)*** were compared by analytical slab SDS-PAGE. No substantial differences were observed between the protein bands of the ***M. Canis*** isolates, but certain distinctive bands were appearing in the other two species examined. Western immunoblotting using ***M. canis***-derived antigens separated by SDS-PAGE was used to investigate the humoral immune response in 79 cats with naturally-occurring dermatophytosis (72 with ***M. canis***, six with ***M. gypseum*** and one with ***T. mentagrophytes***) and this information was compared to results of immunoblots from 46 control (non-dermatophyte exposed) cats. Seven dominant bands (bands which occurred frequently and stained heavily) were identified in immunoblots from the dermatophyte-infected cats with appearing molecular weights varying between 39 and 120 kD. None of these bands were totally specific markers for dermatophytosis as a variable proportion of the control cats showed reactivity to all these proteins. However, most (73%) of the dermatophyte-infected cats showed reactivity to six or seven of the identified bands whereas most (80%) of the control cats showed reactivity to between zero and three of these bands ($p < 0.005$). Western immunoblotting could be used to select individual immunodominant antigens for further evaluation of protective (cell-mediated) immunity.

Lisette et al.*, (2000)** mentioned that five hundred spores from monospecific Arbuscular mycorrhizal fungi (AMF) cultures of ***Glomus

clarum, G. mosseae, G. versiforme, G. fasciculatum, G. etunicatum, and a ***Glomus sp.*** isolate BVB1 were crushed in a modified *sodium* dodecyl sulphate (SDS) reducing buffer and 0.5 µg spore protein aliquots were subjected to one dimensional SDS polyacrylamide gel electrophoresis (1D SDS-PAGE). Silver-stained gels revealed a unique and reproducible protein profile for each of the AMF species tested. The average similarity between any two AMF species ranged ca 10-51%, as determined using an unweighted pair-group average approach. Furthermore, each of the AMF species possessed signature protein bands that were reproducible and consistent. SDS-PAGE of AMF spores is a simple and sensitive technique capable of distinguishing between AMF species that could be used for the routine identification of unknown AMF isolates.

Mahmoudi Rad *et al.*, (2001) investigated total cell protein patterns of ten isolates of ***Trichophyton rubrum*** by SDS-PAGE on 12.5% polyacrylamide resolving gels. Twenty-two protein bands were detected with molecular weights in the range of 23.2 to 131.8 KD. Same of proteins bands were present but their frequencies varied among the isolates. Protein bands of 23.2, 38, 47.9, 52.5, and 84.1 KD were common among the isolates and could be specific to recognize species differences. Protein analysis by SDS-PAGE could be considered a useful technique in identifying differences among the dermatophyte isolates.

The present study was undertaken in order to:

(a)- Investigate the genetical and biochemical effects of some fungicides group on the behaviour of tolerant strains for two fungus.

(b)- Studding the molecular changes by SDS-PAG, and comparison of protein patterns as an indirect of a microbial DNA changes.

MATERIALS AND METHODS

1. Strains and Medium;

The isolates of ***Fusarium equesti*** and ***Fusarium moniliforme,*** which used in this study, were donated by **Sayed et al.,(2010).**

The potato Liquid dextrose broth PDB medium was used as a maintenance medium which contains; extract 200 gm potato + 20 gm dextrose.

2. Fungicides ;

A. strobilurin group.

(a)- Trifloxystrobin (Flint 50 % W.G).

(b)- Azoxystrobin (Amestar 25 %).

B. Benzanilide group;

- Flutolanil (Moncut, 25 % W.P)

3. SDS-PAGE Method;

The protein content of each sample was determined by the **Bradford method (1979).** While the prepairing for method of each sample was based on that described by **Laemi (1970)** with a stacking gel buffer of 0.125 mol Tris-HCl, pH 6.8, a separating gel buffer of 0.375 mol Tris-HCl, pH 8.8, and tank buffer of 0.025 mol Tris-HCl, 0.192 mol glycine pH 8.3.

Numbers of strains which used in this study were ordered in Figs. as;

I. Molecular weight of standard (marker).

II. The wild type inoculated on fungicide free medium (control).

III. The wild type inoculated on medium containing 1400 pm Trifloxystrobin fungicide.

IV. The wild type inoculated on medium containing 1400 ppm Azoxystrobin fungicide,

V. The wild type inoculated on medium containing 1400 ppm Flutolanil fungicide,

VI. F3 or F22 strain inoculated on medium containing 1400 ppm Trifloxystrobin fungicide,

VII. F3 or F22 strain inoculated on medium containing 1400 ppm Flutolanil fungicide,

VIII F11 or F33 strain inoculated on medium containing 1400 ppm Azoxystrobin fungicide,

IX. F11or F33 strain inoculated on medium containing 1400 ppm Flutolanil fungicide.

RESULTS AND DISCUSSION

a)- The protein profiles of ***F. equesti*** by (SDS – PAGE).

SDS-PAGE for water soluble protein of eight strain of ***F.equesti*** is illustrated in Table (1) and Fig. (1). In this study SDS-PAGE of ***F. equesti*** protein subunits and investigate genetic diversity among the wild type of ***F. equesti*** , F3 and F11 strains which grown on fungicide free medium or media containing different concentrations on each of Trifloxystrobin, Azoxystrobin or Flutolanil fungicides, respectively.

About 25 clearly detectable mycelial protein bands over a wide range of molecular weight 20.77 to 145.42 KDa were recognized. The best results were obtained with freshly prepared extracts. Protein of : 20.77, 25.51, 27.11, 31.46, 33.47, 36.60, 40.75, 45.47, 47.49, 50.02, 53.18, 62.23, 68.01, 72.52, 75.44, 80.02, 88.63, 91.56, 94.56, 97.17, 101.16, 113.64, 125.36, 130.14 and 145.42 KDa were present but their frequencies varied among these strains. Protein mobility in the gel allowed to the separation of 25 different proteins, and this separation was enough to propose 24 protein patterns based on the absence or presence of specific bands.

The protein profiles were as follows:

pattern II (control), presence of the bands; 2(130.14), 4(113.64), 5(101.16), 9(88.63), 12(72.52), 16(50.02), 18(45.47), 20(36.60), 22(31.46), and 25(20.77)KDa; pattern III absence of the bands; 2(130.14), 5(101.16), 9(88.63), 12(72.52), 16(50.02), and 25(20.77)KDa ;and presence of new bands as; 8(91.56), 14(62.23), 17(47.49), 21(33.47) and 24(25.51) KDa; pattern IV absence of the bands 2(130.14), 5(101.16), 9(88.63), 12(72.52), 18(45.47), and 22(31.46); and presence of new bands as; 7(94.56), and 14(62.23) KDa ; pattern V absence of bands ; 2(130.14), 5(101.16), 9(88.63), 12(72.52), 16(50.02),18(45.47), 22(31.46) and 25(20.77)KDa ; and presence of new bands as 8(91.56), 14(62.23), 17(47.49) and 21(33.47) KDa ; pattern VI absence of band 2(130.14), 12(72.52), 16(50.02), and 25(20.77)KDa ; and presence of new bands ; 1(145.42), 14(62.23) and 21(33.47)KDa; pattern VII absence of bands 2(130.14), 5(101.16), 9(88.63) and 12(72.52)KDa and presence of new bands ;3(125.36), 8(91.56), 11(75.44), 13(68.01), 14(62.23), 17(47.49), 21(33.47) and 23(27.61) KDa; pattern VIII absence of bands; 5(101.16), 9(88.63) and 18(45.47)KDa and presence of new

Table (1): The banding pattern presence (+), presence new (*+) and absence (-) of ***F. equesti*** (WT), F3 and F11strains grown on different concentrations of Trifloxystrobin, Azoxystrobin or Flutolanil fungicides.

Band	Marker (I) KDa	II	III	IV	V	VI	VII	VIII	IX
1	145.42					*+			
2	130.14	+	-	-	-	-	-	+	-
3	125.36						*+		
4	113.64	+	+	+	+	+	+	+	+
5	101.16	+	-	-	-	+	-	-	-
6	97.17							*+	
7	94.56			*+					
8	91.56		*+		*+		*+	*+	
9	88.63	+	-	-	-	+	-	-	--
10	80.02							*+	
11	75.44						*+		
12	72.52	+	-	-	-	-	-	+	-
13	68.01						*+		
14	62.23		*+	*+	*+	*+	*+	*+	*+
15	53.18							*+	*+
16	50.02	+		+	-	-	+	+	+
17	47.49		*+		*+		*+		*+
18	45.47	+	+	-	-	+	+	-	-
19	40.75							*+	*+
20	36.60	+	+	+	+	+	+	+	+
21	33.47		*+		*+	*+	*+		*+
22	31.46	+	+	-	-	+	+	+	-
23	27.11						*+	*+	*+
24	25.51		*+					*+	
25	20.77	+	-	+	-	-	+	+	+
Total		10	9	6	6	9	14	15	10

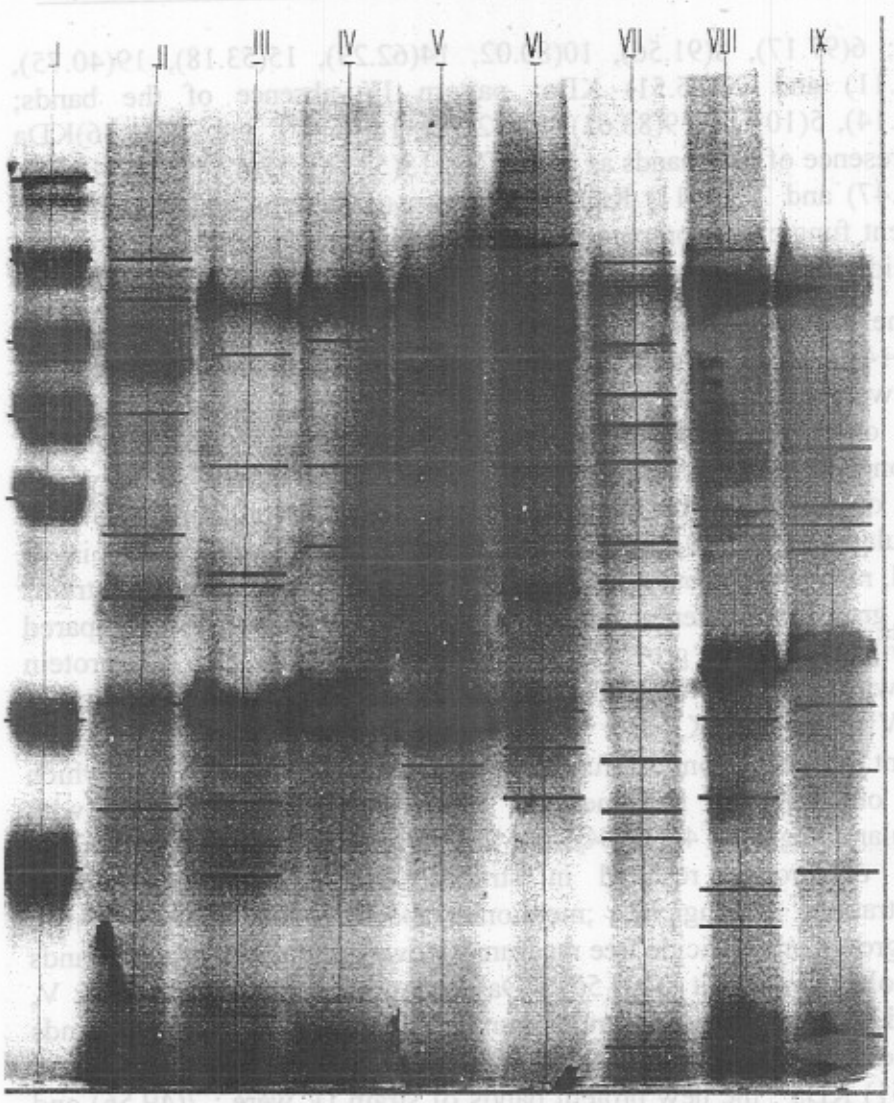

Fig (1); SDS-PAGE shows banding pattern of soluble protein of the wild type ***F.equesti***, F3 and F11 strains which grown either fungicide free medium or media containing different concentrations of Trifloxystrobin, Azoxystrobin and Flutolanil fungicides.

bands; 6(97.17), 8(91.56), 10(80.02, 14(62.23), 15(53.18), 19(40.75), 23(27.11) and 24(25.51) KDa; pattern IX absence of the bands; 2(130.14), 5(101.16), 9(88.63), 12(72.52), 18(45.47), and 22(31.46)KDa and presence of new bands as 14(62.23), 15(53.18), 17(47.49), 19(40.75), 21(33.47) and 23(27.11) KDa, in all case of patterns which grown on different fungicides compared with the wild type strain which grown on fungicide free medium.

The polymorphic protein band (non sole) with molecular weight of 2(130.14) and 3(125.36) KDa were present in VIII and VII, respectively, but it was absent in all other protein of ***F. equesti*** (F3 and F11) which grown on media containing different concentrations of the fungicides mentioned above when it was available of the wild type strain which grown on fungicide free medium. Additionally, the protein bands with molecular weight 5(101.16) and 9(88.63) or 12(72.52) were present in VI or VIII, respectively, but it was absent in all other protein bands of strains which grown on different concentrations of fungicides used compared with wild type which grown on fungicide free medium, also, the protein bands with molecular weight of (50.02) and 25(20.77) KDa were present in IV, VII, VIII and IX, but it were absent in III, V and VI when grown on different concentrations of fungicides compared with wild type which grown on fungicide free medium, however, the protein bands with molecular weight as 4(113.64) and 20(36.60) KDa were present in all pattern of protein resulted in strains which grown on different concentrations of fungicides ;mentioned above; compared with control which grown on fungicide free medium . However, the new protein bands with molecular weight 8(91, 56) KDa were present in strains no. III, V, VII and VIII, additionally, strains number III gives a new protein bands with molecular weight as; 8(91.56).14(62.23), 17(47.49), 21(33.47) and 24(25.51) KDa ; the new protein bands of strain IV were ; 7(49.56) and 14(62.23) KDa; strains no. V was gave new bands as; 8(91.56), 14(62.23), 17(47.49) and 21(33.47) KDa; bands no. 1(145.42), 14(62.23), and 21(33.47) KDa were appared in strain no. VI, meanwhile, strain no. VII was gave 3(125.36), 8(91.56), 11(75.44), 13(68.01), 17(47.49), 21(33.47) and 23(27.11) KDa as a new protein bands. The VIII strain gave new bands 6(97.17), 8(91.56), 10(80.02), 15(53.18), 19(40.75), 23(27.11) and 24(25.51) KDa, while strain no. IX gave; 14(62.23), 15(53.18), 17(47.49), 19(40.75), 21(33.47) and 23(27.11) KDa as a new bands. All strains were grown on different concentrations of fungicides

mentioned above compared with control of the wild type which grown on fungicide free medium.

The combined SDS-PAGE of total protein of genotypes ***F.equesti***, F3 and F11 strains which grown on fungicide free medium or media containing different concentrations of fungicides mentioned above were applied to the computer using PSS statistical system programe to get the similarity matrix of genotype of all strains mentioned above (Table 2 and Fig. 2).

Fig.(2) determined that all strains were found in two main families; the 1st were strains no. 2 and 6 with 51% relationship, the rest strains were related to the 2nd family, which divided to three main groups; a) strains no. 4 and 5 (83%) and strain no 3 with relationship (69%), b) the two strains no.8 and 9 (75%) were related with the first group with (64%) and with strain no. 7 (group no. c) with (54%). The two main groups were related to each other with (39%).

Results for the relation between strains were agreed with **Sparkes *et al* (1994).**

b. The protein profiles of ***F. moniliforme*** by (SDS – PAGE).

SDS-PAGE for water soluble protein of eight strain of ***F.moniliforme*** is illustrated in Table (3) and Fig. (3). In this study SDS-PAGE of ***F. moniliforme*** protein subunits and investigate genetic diversity among the wild type of ***F.moniliforme*** , F22 and F33 strains which grown on fungicide free medium or media containing different concentrations from each of Trifloxystrobin, Azoxystrobin or Flutolanil fungicides, respectively.

About 33 clearly detectable mycelial protein bands over a wide range of molecular weight 17.46 to 423.48 KDa were recognized. The best results were obtained with freshly prepared extracts. Protein of : 17.46, 18.18, 19.77, 20.48, 22.62, 23.37, 26.54, 33.23, 34.39, 36.00, 38.55, 40,39, 41.34, 49.64, 51.18, 53.22, 58.59, 76.66, 80.20, 92.47,137.78, 304.95, 312.00, 368.20, 412.35 & 423.48 KDa were present but their frequencies varied among these strains . Protein mobility in the gel allowed to the separation of about 16 different proteins, and this separation was enough to propose 16 protein patterns based on the absence or presence of specific bands.

Table (2); Similarity matrix of genotype of ***F.equesti*** strains; W.T, F3 & F11 as mentioned by protein banding on SDS – PAGE

	VIII	IX	VII	IV	V	II	III	VI
VIII	100	75.5	64.4	61.2	59.6	57.6	56.5	40.7
IX	75.5	100	58.3	69.5	65.5	46.5	70.2	27.1
VII	64.4	58.3	100	47.8	47.8	52.9	51.4	28.9
IV	61.2	69.5	47.8	100	82.8	47.3	71.9	23.1
V	59.6	65.5	47.8	82.8	100	38.7	66.2	31.4
II	57.6	46.5	52.9	47.3	38.7	100	41.2	51.3
III	56.5	70.2	51.4	71.9	66.2	41.2	100	27.9
VI	40.7	27.1	28.9	23.1	31.4	51.3	27.9	100

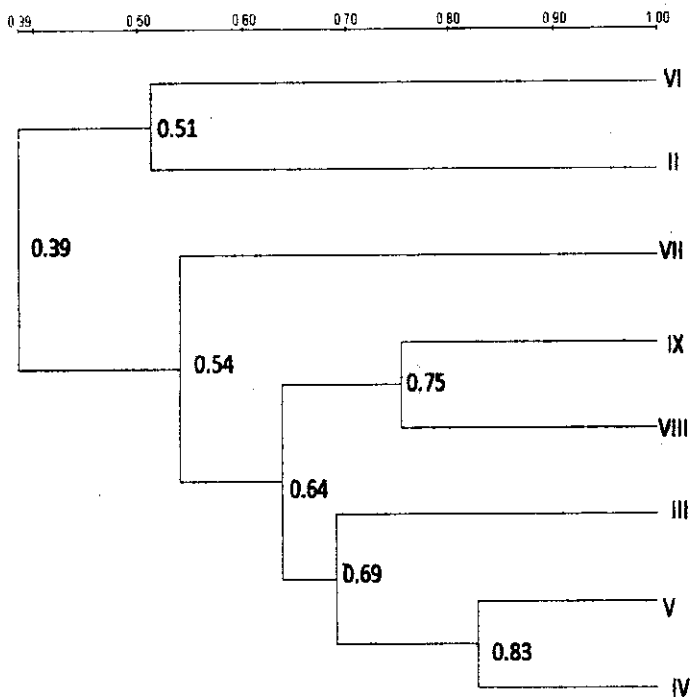

Fig.(2); Dendrograme of ***F.equesti*** strains; W.T, F3 & F11as mentioned by protein banding on SDS - PAGE.

Table (3): The banding pattern presence (+), presence new (*+) and absence (-) of ***F. moniliforme***,F22 and F33 strains grown on different concentrations of Trifloxystrobin, Azoxystrobin and Flutolanil fungicides

Band	Marker (I) KDa	II	III	IV	V	VI	VII	VIII	IX
1	423.48	+	+	-	-	-	-	-	-
2	412.35	+	-	-	-	-	-	-	-
3	368.20	+	+	-	-	-	-	-	-
4	312.00	+	-	-	-	-	-	-	-
5	304.95		*+						
6	137.78	+	+	+	+	+	+	+	+
7	92.47	+	-	-	-	-	-	-	-
8	80.20						*+	*+	*+
9	76.66	+	+	-	+	+	-	-	-
10	58.59	+	-	-	-	-	*+	*+	*+
11	53.22		*+	*+	*+	*+	*+	*+	*+
12	51.81	+	-	-	-	-	-	+	+
13	49.64	+	+	+	-	+	+	+	-
14	41.43	+	+	+	+	+	+	+	+
15	40.39					*+	*+	*+	
16	38.55	+	+	-	+	+	+	+	+
17	36.00					*+	*+		
18	34.39						*+	*+	*+
19	33.23	+	+	-	-	-	+	+	+
20	26.54	+	+	+	-	-	-	-	-
21	23.37	+	-	+	+	-	-	+	-
22	22.62					*+	*+	*+	*+
23	20.48	+	-	-	-	+	+	+	+
24	19.77							*+	
25	18.18		*+			*+	*+	*+	*+
26	17.46						*+		*+
Total		16	12	5	6	11	15	16	13

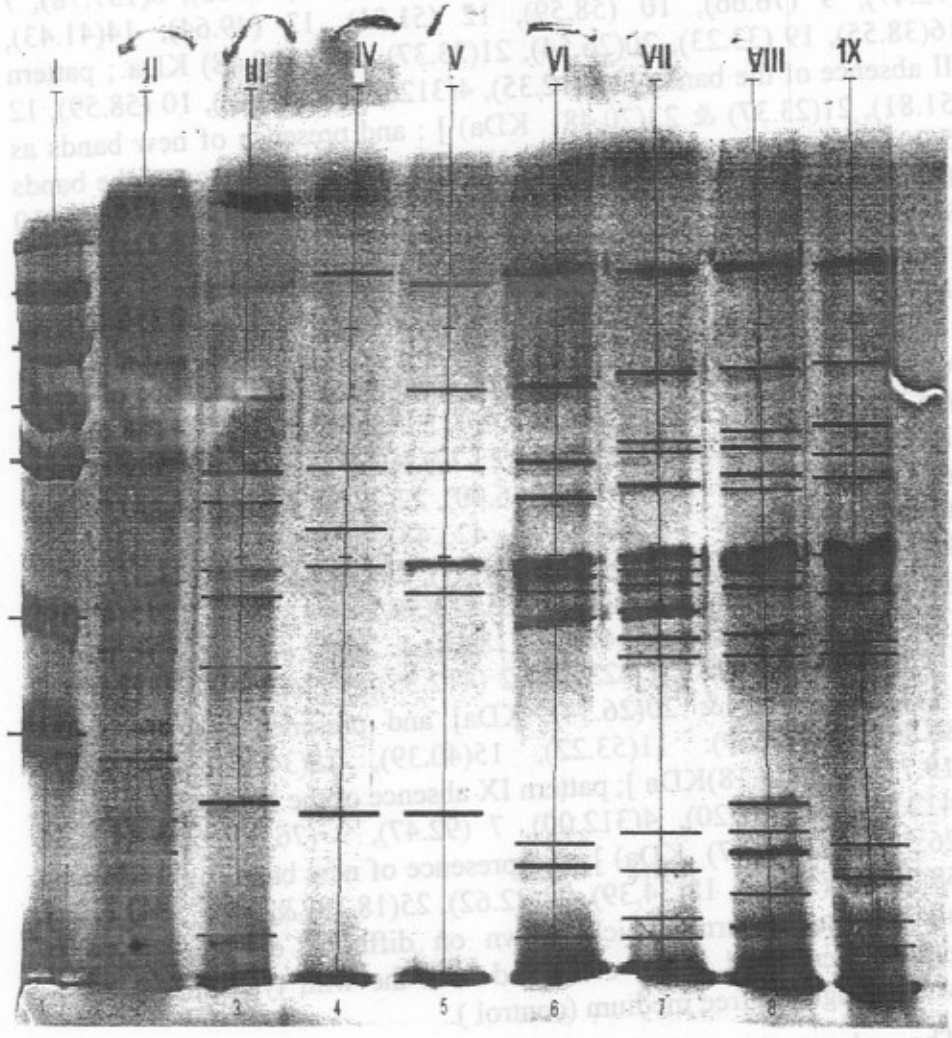

Fig (3); SDS-PAGE shows banding pattern of soluble protein of the wild type ***F.moniliforme***, F22 and F33 strains which grown either fungicide free medium or media containing different concentrations of Trifloxystrobin, Azoxystrobin and Flutolanil fungicides.

The protein profiles were as follows: pattern II (control) , presence of the bands [1(423.48), 2 (412.35), 3 (368.20), 4(312.00), 6(137.78), 7 (92.47), 9 (76.66), 10 (58.59), 12 (51.81), 13 (49.64), 14(41.43), 16(38.55), 19 (33.23), 20(26.54), 21(23.37) & 23 (20.48) KDa ; pattern III absence of the bands [2 (412.35), 4(312.00), 7 (92.47), 10 (58.59), 12 (51.81), 21(23.37) & 23(20.48) KDa)] ; and presence of new bands as [5(304.95), 11(53.22) & 25(18.18) KDa]; pattern IV absence of the bands [1(423.48), 2 (412.35), 3 (368.20), 4(312.00), 7 (92.47), 9 (76.66), 10 (58.59), 12(51.81), 16(38.55), 19 (33.23) & 23 (20.48)KDa)] and presence of new bands as [11(53.22) KDa)] ; pattern V absence of bands [1(423.48), 2 (412.35), 3 (368.20), 4(312.00), 7 (92.47), 10 (58.59), 12 (51.81), 13 (49.64), 19 (33.23), 20(26.54) & 23 (20.48) KDa]; and presence of new bands as; [11(53.22) KDa]; pattern VI absence of bands [1(423.48), 2 (412.35), 3 (368.20), 4(312.00), 7 (92.47), 10 (58.59), 12 (51.81), 19 (33.23), 20(26.54) & 21(23.37) KDa)]; and presence of new bands as [11(53.22), 15(40.39), 17(36.00), 22(22.62), & 25(18.18) KDa)]; pattern VII absence of bands [1(423.48), 2 (412.35), 3 (368.20), 4(312.00), 7 (92.47), 9 (76.66), 12 (51.81), 20(26.54) & 21(23.37) KDa] and presence of new bands [8(80.20, 10(58.59), 11(53.22), 15(40.39), 17(36.00), 18(34.39), 22(22.62), 25(18.18) & 26(17.46) KDa)]; pattern VIII absence of bands [1(423.48), 2 (412.35), 3 (368.20), 4(312.00), 7 (92.47), 9 (76.66) & 20(26.54), KDa] and presence of new bands [8(80.20), 10(58.59). 11(53.22), 15(40.39), 18(34.39), 22(22.62), 24(19.77) & 25(18.18)KDa]; pattern IX absence of the bands [1(423.48), 2 (412.35), 3 (368.20), 4(312.00), 7 (92.47), 9 (76.66), 13 (49.64), 20(26.54) & 21(23.37) KDa)]; and presence of new bands as [8(80.20), 10(58.59), 11(53.22), 18(34.39), 22(22.62), 25(18.18) & 26(17.46) KDa], in all case of patterns which grown on different concentrations of fungicides mentioned above compared with the wild type strain which grown on fungicide free medium (control).

The polymorphic no. of protein bands (non sole) with molecular weight as; 2(412.35), 4(312.00), 7 (92.47) KDa were absent in all proteins of ***F.moniliforme***, F22 and F33 strains which grown on media containing different concentrations of the fungicides mentioned above but it was available of the wild type strain which grown on fungicide free medium. Also, protein bands (non sole) with molecular weight of [5 (304.95 KDa] were absent in protein of strains No. II (control), IV, V, VI, VII, VIII and IX but it was available in protein of strain No. III. The

results demonstrated that protein band No. 6 with molecular weight (137.78 KDa) was appeared in all strains grown on fungicide free medium or media containing different concentrations of the fungicides mentioned above, also, the protein band No. 9 with molecular weight (76.66 KDa) was absent in strains No. IV, VII, VIII &IX but it was presented in strain No. III, V &VI, however, the protein band No. 10 with molecular weight (58.59 KDa) was absent in strains No. III, IV, V & VI but it was available in strains no. II, VII, VIII & IX, additionally, the protein band No. 13 with molecular weight (49.64 KDa) was absent in strains No. V & IX but it was available in all other strains, additionally, the protein band No. 16 with molecular weight (38.55 KDa) was absent in strains No. IV but it was available in all other strains, additionally, the protein band No. 19 with molecular weight (33.23 KDa) was absent in strains No. IV, V & VI but it was available in all other strains, additionally, the protein band No. 20 with molecular weight (26.54 KDa) was available in strains No. II, III &IV but it was absent in all other strains, however, the protein band No. 21 with molecular weight (23.37 KDa) was absent in strains No. III, VI, VII & IX but it was available in strains No. II, IV, V & VIII, however, the protein band No. 23 with molecular weight (20.48 KDa) was absent in strains No. III, IV & V but it was available in strains No. VI, VII, VIII & IX, moreover, the protein band No. 25 with molecular weight (18.18 KDa) was absent in strains No. II, IV& V but it was available in all other strains, additionally, the protein band No. 26 with molecular weight (17.46 KDa) was absent in strains No. III, IV, V, VI &VIII but it was available in strains No. VII & IX.

The combined SDS-PAGE of total protein of genotypes ***F.moniliforme*** , F22 and F33 strains which grown on fungicide free medium or media containing different concentrations of fungicides mentioned above were applied to the computer using PSS statistical system programe to get the similarity matrix of genotype of all strains mentioned above (Table 4 and Fig.4).

Fig.(4) determined that all strains were found in two families ; the 1st were strains no. 2 and 3 with 61 % relationship, the rest strains were related to the 2nd. Family which divided to three groups; a) strain no. 4 and 5 with (69%), b) strain no. 6 and 7 with (67%) and c) strain no. 8 and 9 with (69%), meanwhile the group (b) and group (c) with (59%) relationship, the group (a) was related with the two groups (b and c) by (49%). The mean to family were related with (42%).This results were

Table (4); Similarity matrix of genotype of ***F.moniliforme*** strains; W.T, F22 & F33 as mentioned by protein banding on SDS - PAGE .

	II	III	VIII	VII	V	VI	IX	IV
II	**100**	61.2	45.3	45.3	43.7	43	36	29.6
III	61.2	**100**	43.1	40.7	44	48.7	43.5	35.4
VIII	45.3	43.1	**100**	66.7	61.5	63.1	68.7	40.3
VII	45.3	40.7	66.7	**100**	50.8	67.3	58.4	32.7
V	43.7	44	61.5	50.8	**100**	64.9	53.3	68.8
VI	43	48.7	63.1	67.3	64.9	**100**	48.2	53.3
IX	36	43.5	68.7	58.4	53.3	48.2	**100**	33.4
IV	29.6	35.4	40.3	32.7	68.8	53.3	33.4	**100**

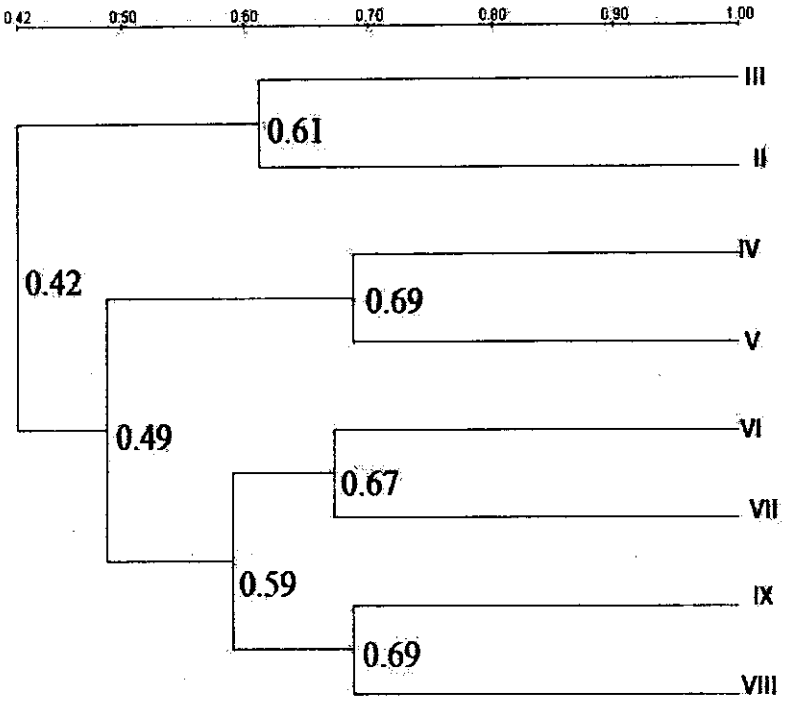

Fig.(4); Dendrograme of ***F.moniliforme*** strains ; W.T, F22 &F33 as mentioned by protein banding on SDS-PAGE.

obtained by **Sparkes *et al.*, (1994)** and **Lisette *et al.*,(2000).**

The results also demonstrated that the pronounced difference among fungus proteins of different strains of ***F. equesti*** and ***F. moniliforme*** was found. The results showed clearly that there are difference of proteins either ***F. equesti***, F3 and F11 strains or ***F. moniliforme***, F22 and F33 which grown on fungicide free medium or media containing different concentrations of fungicides mentioned above .In general, for protein fraction by SDS-PAGE electrophoresis, the data demonstrated that relying on the presence or absence of specific protein markers and relative concentration of common proteins are valuable tool for varietals characterization **(Mahmoud (1986), Lisette *et al.*, (2000)** and **Mahmoudii Rad *et al.*, (2001).**

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الملخص العربى

التغيرات الجزيئية فى مكونات البروتين كعلامات غيرمباشرة لتغيرات ال DNA الميكروبى بإستخدام ال SDS-PAGE

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- اجريت الدراسة على 16 سلالة من الفطرين *F. equesti* , *F. monilioforme* والتى نميت على بيئات تحتوى على تركيزات من المبيدات الفطرية الأتيه Trifloxystrobin، Azoxystrobin، Flotolanil وبيئات خالية من المبيدات تماما ، لاستخدامها فى الكشف عن التغيرات الجزيئية الحادثه فى شكل وعدد الحزم الخاصه ببروتين كل سلالة وذلك باستخدام تقنية SDS-PAGE .

- وقد اظهرت النتائج وجود 25 حزمة ذات وزن جزيئى يتراوح بين 20.77 الى 145 KDa بالنسبة لفطر *F. equesti* ووجود 26 حزمة ذات وزن جزيئى يتراوح بين 17.46 الى 423.48 KDa بالنسبة للفطر *F. moniliforme* مع تواجد عدد من الحزم ذات أوزان جزيئية متماثلة بين السلالات المختلفة كما لوحظ ان تواجد أو غياب عدد من الحزم بكل سلالة يعتبر صفة مميزة لهذة السلالة و ذلك مقارنة بالكنترول حيث يمكن اعتبار التغيرات الجزيئية فى مكونات البروتين كعلامات غير مباشرة لتغيرات ال DNA الميكروبى بإستخدام ال SDS-PAGE .