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SEROLOGICAL STUDIES ON THE RELATIONSHIP BETWEEN *FUSARIUM* SPECIES AND COTTON PLANTS

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ABSTRACT

Nine *Fusarium* species, isolated from cotton seedlings infected with damping-off, were compared by double diffusion (DD) technique to determine their serological relationships. On the basis of serological relationships, species were grouped by cluser analysis and the results were expressed as phenograms. *Fusarium* species were divided into two distinct groups. The first group included *F. subglutinans, F. moniliforme, F. semitectum,* and *F. solani,* while the second group included *F. sambucinum, F. oxysporum, F. sporotrichoides,* and *F. poae*. The use of common antigens or common specific antigens gave the same taxonomic relationships. Regression analysis was used to quantify the relationship between the no. of common antigens (x) shared by *Fusarium* species and cotton cultivars and pathogenicity (Y) of these species on these cultivars. Coefficient of determination (R^2) of the obtained models ranged from 0.81 to 0.90. These results indicate that DD test constituted a positive test to quantify pathogenicity of *Fusarium* species on cotton cultivars.

INTRODUCTION

Immunological techniques for the detection and identification of particular micro-organisms are of great value because of the specificity of the reaction between the antigens of the organisms and the corresponding antibodies, which are produced in the serum of animals inoculated with the organism (Ouchterlony, 1949). Hornok (1980) used immuno-electrophoresis in a study of 13 *Fusarium* species belonging to Sections Discolor and Gibbosum, with two or three strains representing each species. Four groups were evident, corresponding with section Gibbosum, Section Discolor and with *F. buharicum* and *F. heterosporum* coming out as different from all the others. The results, therefore, corresponded with morphological views of the genus Marziano *et al.* (1981) reported that immunological techniques complement morphological criteria in defining the taxonomy of the genus *Fusarium*. They used double diffusion test for the serological analysis of *Fusarium* and reported that it was possible to confirm the taxonomic affinity of *F. oxysporum* and *F. moniliforme*. Iannelli *et al.* (1982) showed that *F. oxysporum, F. moniliforme* and *F. xylarioides* possessed distinct antigenic characteristics. In addition, they described how four different formae speciales of *F. oxysporum* (*dianthi, melonis, pisi,* and *lycopersici*) and the physiological races of *F. oxysporum* f. sp. *melonis* (races 1, 2, 3) can be differentiated by serological techniques. Rataj-Guranowska *et al.* (1984) compared between race 2 and race 3 of *F. oxysporum* f. sp. *lupini* by tandem-crossed immuno-electrophoresis. They found that the two races had

apparently almost identical antigenic patterns differing only in one antigen specific to races 3.

Rataj-Guranowska and Wolko (1991) compared *F. oxysporum* and *F. oxysporum* var. *redolens* serologically. Although their results indicated a strong similarity between the two fungi, it was not sufficient for an unequivocal statement that the fungi belong to the same species. When Centurion and Kimati (1992) used Ouchterlony gel agar double-diffusion method for serological characterization of *F. moniliforme* and *F. moniliforme* var. *subglutinans* isolates, they found that this method appeared to be ineffective for serological characterization of these isolates. When the indirect immunofluorescence (1F) test was used in reaction between antiserum of *F. avenaceum* with spores of some other species. The highest serological affinity with *F. avenaceum* was found for *F. poae* and the lowest for *F. tricinctum* (Mierzwa *et al.*, 1996). Hussein *et al.* (1996) compared *F. oxysporum, F. moniliforme* and *F. solani* isolated from damped-off cotton seedlings by double diffusion (DD) and immuno-electrophoresis (IE) technique. Species were groupped by cluster analysis and the results were expressed as phenograms. The taxonomic relationships established based on DD matched those based on modern systems of morphological classification. Double diffusion technique, in comparison to IE technique, proved to be more sensitive as a serotaxonomic tool provided that the use of specific antigens for comparisons, in combination with cluster analysis of the resulting similarity indexes.

Serology has been widely used to study the relationship between plant pathogens and their hosts. Charudattan and DeVay (1972) demonstrated a common antigen relationship among *Fusarium* spp. and wilt-susceptible or wilt-tolerant varieties of cotton, when the common antigenic substance was isolated and purified, it was found to be a polysaccharide-protein complex. It was postulated that the common antigen may be involved in the establishment and survival of *Fusarium* isolates in host tissue. When the seed globulins of cotton cultivars susceptible to *Fusarium* wilt reacted with antiserum of *F. oxysporum* f. sp. *vasinfectum*, more precipitation lines were formed than with the resistant cultivars. On the other hand, no obvious reaction was detected in case of antiserum of *F. moniliforme*, which was nonpathogenic on cotton (Abd-El-Rehim *et al.*, 1988). Shady *et al.* (2000) compared proteins of *F. oxysporum* f. sp. *vasinfectum* with those of wilt-resistant (Dandara, Giza 70, Giza 75, Giza 77 and Giza 85) and wilt susceptible (Giza 71 and Giza 74) cotton cultivars by double diffusion test. They found that common antigens were detected only between *F. oxysporum* f. sp. *vasinfectum* and the two susceptible cultivars, while no common antigens were detected between this pathogen and the resistant ones.

Proteins of cotton seeds cultivar Giza 75 were compared with those of pathogenic and non-pathogenic isolates of *F. oxysporum, F. moniliforme* and *F. solani*, involved in damping-off of cotton seedlings, by double diffusion (DD) test. Linear correlation coefficient (r) was calculated to measure the degree of association between common antigens shared by *Fusarium* isolates and cotton cultivar Giza 75 in DD test, and pathogenicity of the isolates on this cultivar. There was a positive significant correlation ($r = 0.84$, p

< 0.09) between antigens and pathogenicity of isolates (susceptibility of cotton). Regression analysis indicated that the common antigens accounted for 70% of the explained (model) variation in pathogenicity of isolates (susceptibility of cotton). These results indicate that DD test constituted a positive test for quantifying pathogenicity of *Fusarium* isolates on Giza 75 or susceptibility of Giza 75 to these isolates (Hussein *et al.*, 2001).

MATERIALS AND METHODS

1. Differentiation among *Fusarium* species using double diffusion technique:

1.1. Extraction of fungal protein:

Protein extract was prepared according to Rataj-Guranowsk *et al.* (1984) in the following way: fungal isolates were grown for 22 days at 25-30°C on liquid Czapeck medium. The mycelium was harvested by filtration through cheese cloth, washed with distilled water several times, and freeze-dried. The frozen mycelium was suspended in phosphate buffer pH 8.3 (1-3 ml/g mycelium), ground in liquid nitrogen to a fine powder. The ground mycelium was centrifuged at 14.000 rpm for 30 min. at 0°C. The protein content in supernatant was estimated according to Bradford (1976) by using bovine serum albumin (BSA) as a standard protein. If protein concentration was low, it would be precipitated from the clarified supernatant by adding ammonium sulfate at 70% of saturation (60 g/100 ml water) then kept in the refrigerator for 30 hrs. Pellets, collected by centrifugation at 11.000 rpm for 30 min., resuspended in phosphate buffer pH 8.3 and subjected to dialysis for 24 hrs. against the buffer then centrifuged at 11.000 rpm for 30 min. and protein was assayed in the obtained supernatant.

1.2. Immunization and preparation of antisera:

New Zealand rabbits, 3-4 kg weight, were immunized by antigens of 9 isolates for 9 species of *Fusarium* (one isolate for each species) to produce antiserum. The first injection was given intracutaneously in the back between ears. This injection consisted of 0.5 mg protein suspended in 1 ml phosphate buffer and mixed in 1 ml Freund's complete adjuvant (Difco). After one week, each animal was received 4 mg protein administered intramuscularly every third day in the thigh in a series of twelve injections. One week after the last injection, the animal was bled in the marginal ear vein. Collected blood was kept at room temperature for 1 to 2 hr. clots were then gently loosened and stored over night at 4°C. Antisera were then decanted and clarified by centrifugation at 10000 rpm for 30 minutes. Boric acid was added to supernatant serum and subdivided into small portions.

1.3. Double diffusion technique:

The technique was carried out according to Ouchterlony and Nilsson (1978). Melted 2% ionagar (sigma), in saline (8.5 g NaCl to 1 liter D.W) and supplemented with merthiolate (1:10.000, 37°C), was poured into 7 cm diameter Petri dishes to obtained a layer of agar 1-2 mm thick. The diameter of the central and the peripheral wells was 10 and 5 mm, respectively. The distance between the central well and the peripheral ones was 15 mm. The central well was filled with antiserum of *Fusarium* spp. (9 species) and the peripheral wells were filled with antigens of *Fusarium* spp. Plates were kept in

humid conditions at room temperature (18-24°C) in the dark for 48-72 hours. The developing precipitin lines were examined and recorded by hand drawing (Hussein, 1992).

1.4. Cluster analysis:

Similarity indexes between isolates were calculated according to the following formula:

$$\text{Similarityindex} = \frac{\text{Number of common antigens}}{\text{Number of antigens in homologous reaction}} \times 100$$

From these data, similarity matrixes were constructed and, from the matrixes, isolates were clustered (Joseph *et al.*, 1992) by the average linked technique (unweitghed pair-group method). And the results were expressed as phenograms. Cluster analysis was performed with a computerized program. In this analysis, clustering began with the fusion of the two most similar isolates and proceeded until all isolates were fused into clusters and/or all clusters fused. The clustering process was represented in the form of a phenogram (tree) in which the top branch indicated the highest fusion level, and so on. For reference purposes, the fusion levels were designated 1, 2, ... from top to bottom, respectively.

2. Serological relationship between *Fusarium* spp. and cotton cultivars

2.1. Extraction of proteins from cotton seeds:

Protein extract was prepared according to Hussein (1992) in the following way: seeds of cotton cultivars Giza 45, Giza 70, Giza 80, Giza 83, Giza 85, Giza 86, Giza 87, Giza 88, Giza 89 and Giza 90 were slightly ground and defatted by diethyl either for 4 to 5 days. After drying at room temperature. Ground seeds were suspended in a solution (1-3 ml/g seeds) consisting of 12.5 g glucose and 1 g ascorbic acid dissolved in 100 ml phosphate buffer (pH 8.3) and ground in liquid nitrogen to a fine powder. After thawing, the powder suspended in buffer was centrifuged at 14.000 rpm for 30 minutes at 0°C. The protein content in the supernatant was adjusted to a concentration of 3 to 4 mg/ml according to Bradford (1976) using BSA as standard protein.

The serological interactions between *Fusarium* spp. and cotton cultivars were carried out as previously mentioned.

RESULTS AND DISCUSSION

1. Differentiation among *Fusarium* species using double diffusion technique:

The bands shown in Figures from 1 to 9 were used to construct the similarity indexes. Two methods of calculation were used for assessing the similarity among species. In one method, similarity indexes (Table 1) were calculated by using all the resulting common antigens (specific and non specific). In the other method, similarity indexes (Table 2) were calculated by using only the resulting specific antigens. The phenogram constructed based on taxonomic distances generated from similarity indexes calculated by the

first method showed in Fig. 10 in this phenogram highest homology percentage (up to 95%) was expressed between *F. moniliforme* and *F. subglutinans* followed by 85% homology between *F. fusarioides* and *F. sambucinum*.

Sixty percentage of homology was obtained between *F. fusarioides* and *F. oxysporum*. Less than 50% homology was provided among other fusaria. Four species of *Fusarium*, i.e., *subglutinans*, *moniliforme*, *semitectum* and *solani* showed homology among each other and could be placed in one group differed from other species e.g. *fusarioides*, *sambucinum*, *oxysporum*, *sporotrichioides* and *poae*, which showed no homology with the aforementioned group.

The phenogram of Fig. 9 was constructed based on taxonomic distances generated from similarity and indexes calculated by the second method (the use of specific antigens). In this phenogram, homology of group included *F. subglutinans*, *F. moniliforme*, *F. semitectum* and *F. solani* was 20% and homology (up to 35%) was expressed between *F. semitectum* and *F. solani*. Regarding serological studies, *Fusarium* species showed various homology percentages among each other. On the base of general antigens, up to 95% homology percentage was expressed between *F. moniliforme* and *F. moniliforme var. subglutinans*. *F. fusarioides* provided 85% homology with *F. sambucinum* while it gave 60% homology with *F. oxysporum*. Data strongly suggest that four species of *Fusarium* i.e., *moniliforme var. subglutinans*, *moniliforme*, *semitectum* and *solani* could be placed in one group differed from other species. Data are agreed with those reported previously by Centurion and Kimati (1992) and Bouznad *et al.* (1998) who demonstrated that serological methods are useful for detecting some plant pathogens. In contrast, on the base of nonspecific antigens less homology percentages were expressed between *Fusarium* species. Thus, identification and differentiation *Fusarium* species could be applied easily using the specific antigens. Hussein *et al.* (1996) concluded that the use of specific antigens for calculating similarity indexes is more reliable method for grouping isolates of each species of *Fusarium* than based on the use of all antigens in the calculation.

2. Serological relationship between *Fusarium* species and cotton cultivars:

Data summarized in Table (3) which derived from the double diffusion reactions (Figs. 10-12) showed various number of common bands (antigens) formed by *Fusarium* species depending on cotton cultivar tested. Generally, the least number of band was accompanied with the least susceptibility of cultivar to infection. In parallel increasing number of bands enhanced susceptibility of cultivars to infection with *Fusarium* species. In case of *F. fusarioides*, cotton cv. Giza 90 exhibited the highest number of band (12) and highest susceptibility (65%) followed by cv. Giza 85 that 10 bands with 62.5% disease severity. By *F. semitectum*, cvs. Giza 70 and Giza 88 appeared the highest number of bands (11) with 60 and 65 disease severity, respectively. *F. poae* gave the highest number of bands (10) with cotton cvs. Giza 45, Giza 80 and Giza 83 and elicited the highest pathogenicity

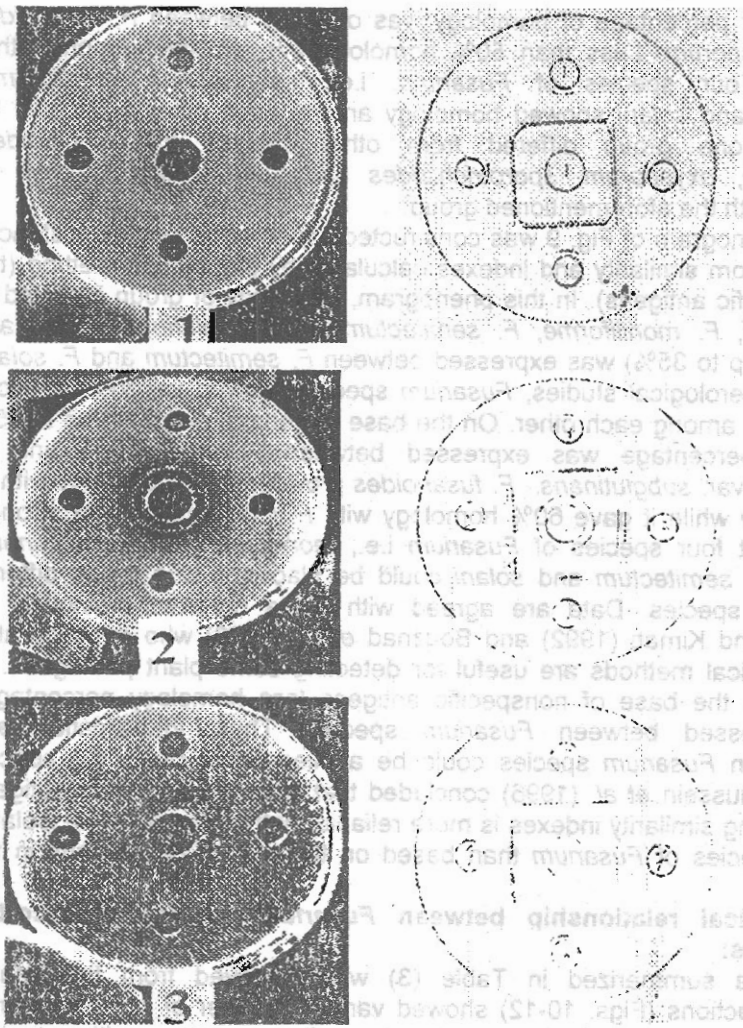

Figure 1: Double diffusion reaction of the antiserum of *F. fusarioides* (in the center well) against antigens of *Fusarium* spp. (in peripheral wells) *Fusarium* spp. were *F. fusarioides* (1) (homologous), *F. semitectum* (2), *F. poae* (3), *F. sambucinum* (4), *F. oxyporum* (5), *F. moniliforme var.subglutinans* (6), *F. moniliforme* (7), *F. solani* (8) and *F. sporotrichiodes* (9) (Heterologus).

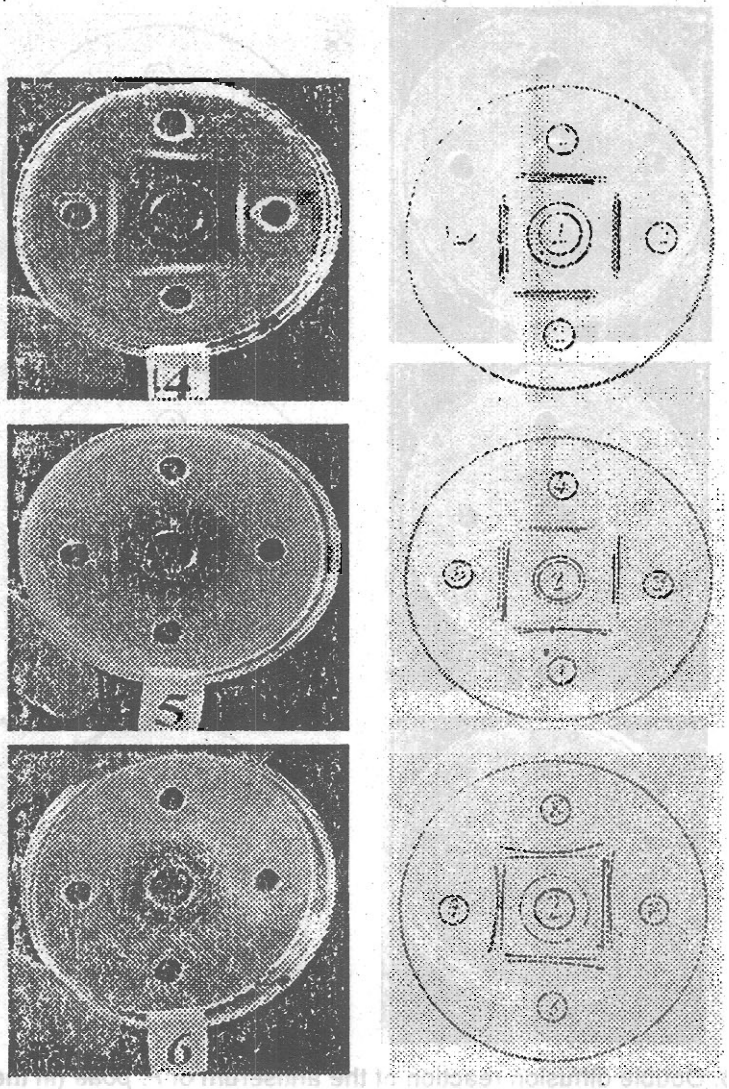

Figure 2: Double diffusion reaction of the antiserum of *F. semitctum* (in the center well) against antigens of *Fusarium* spp. (in peripheral wells) *Fusarium* spp. were *F. semitectum* (2) (homologous), *F. fusarioides* (1) *F. poae* (3), *F. sambucinum* (4), *F. oxyporum* (5), *F. moniliforme var. subglutinans* (6), *F. moniliforme* (7), *F. solani* (8) and *F. sporotrichiodes* (9) (Heterologus).

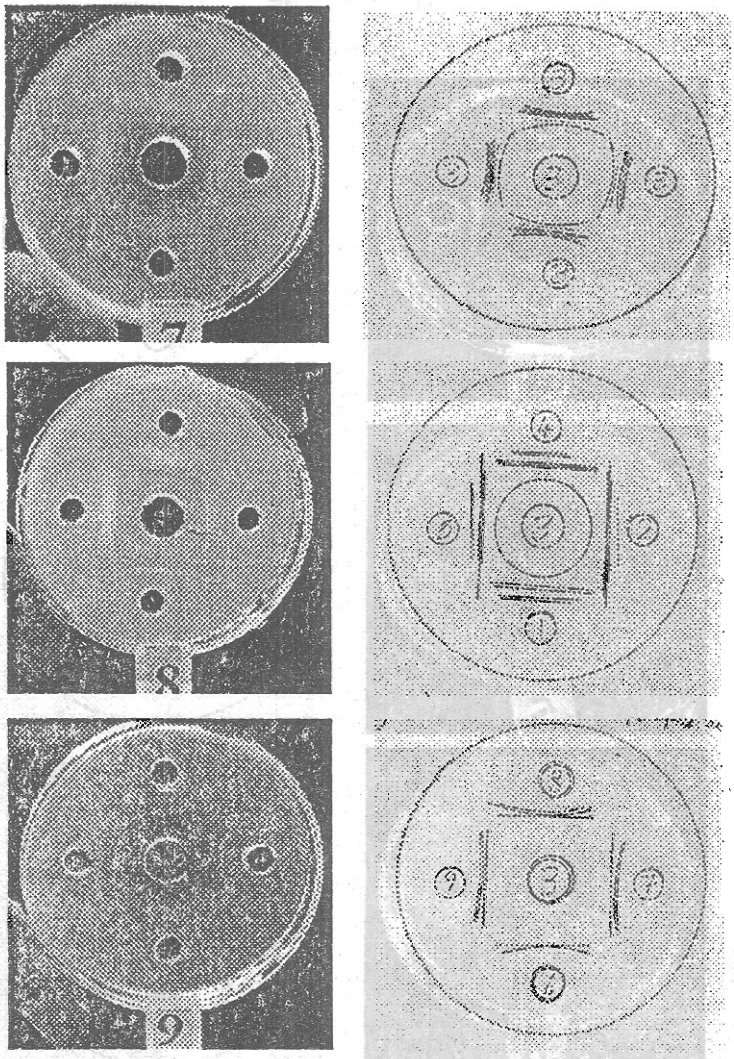

Figure 3: Double diffusion reaction of the antiserum of *F. poae* (in the center well) against antigens of *Fusarium* spp. (in peripheral wells) *Fusarium* spp. were *F. poae* (3) (homologous), *F. fusarioides* (1), *F. semitectum* (2) *F. sambucinum* (4), *F. oxyporum* (5), *F. moniliforme var. subglutinans* (6), *F. moniliforme* (7), *F. solani* (8) and *F. sporotrichiodes* (9) (Heterologus).

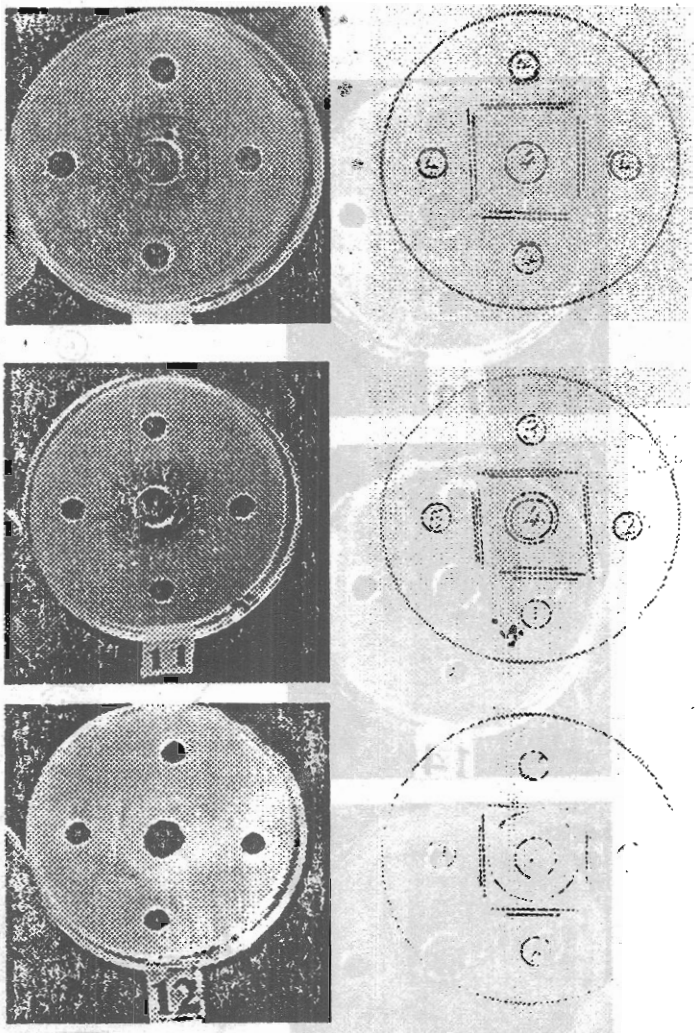

Figure 4:Double diffusion reaction of the antiserum of *F. sambucinum* (in the center well) against antigens of *Fusarium* spp. (in peripheral wells) *Fusarium* spp. were *F. sambucinum* (4) (homologous), *F. fusarioides* (1) *F. semitectum* (2), *F. poae* (3), *F. oxyporum* (5), *F. moniliforme var. subglutinans* (6), *F. moniliforme* (7), *F. solani* (8) and *F. sporotrichiodes* (9) (Heterologous).

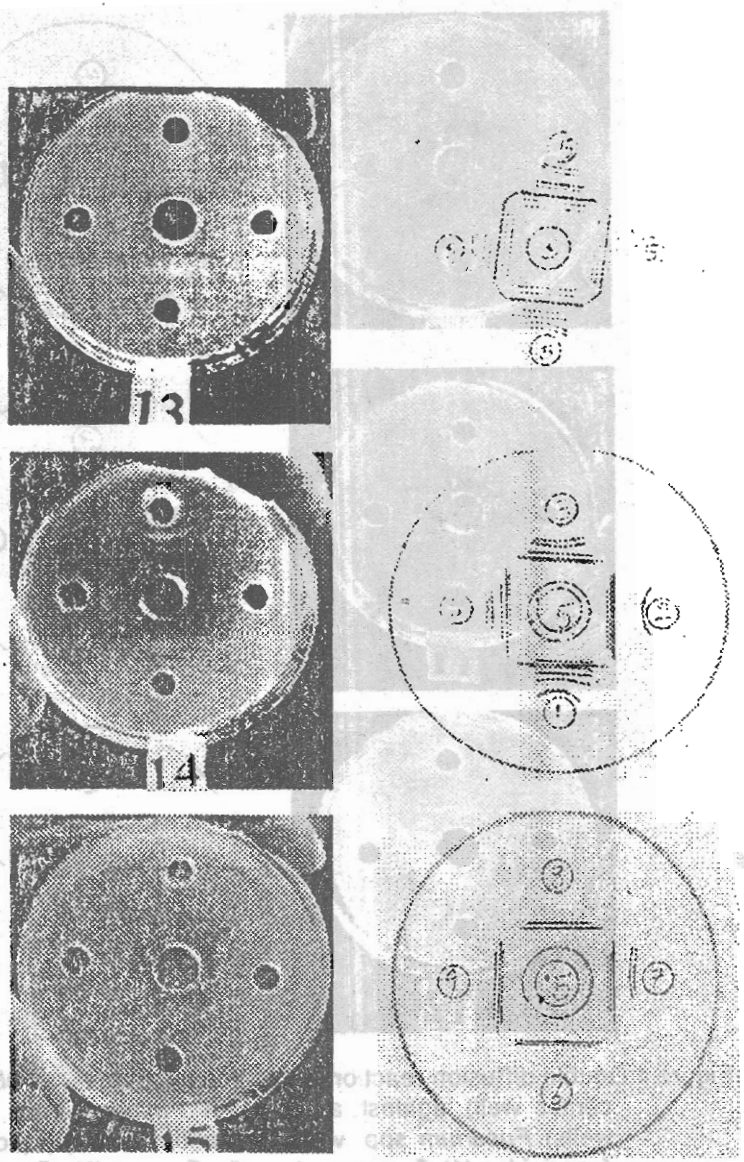

Figure 5:Double diffusion reaction of the antiserum of *F. oxysporum* (in the center well) against antigens of *Fusarium* spp. (in peripheral wells) *Fusarium* spp. were *F. oxyporum* (5) (homologous), *F. fusarioides* (1), *F. semitectum* (2), *F. poae* (3), *F. sambucinum* (4), *F. moniliforme var. subglutinans* (6), *F. moniliforme* (7), *F. solani* (8) and *F. sporotrichiodes* (9) (Heterologus).

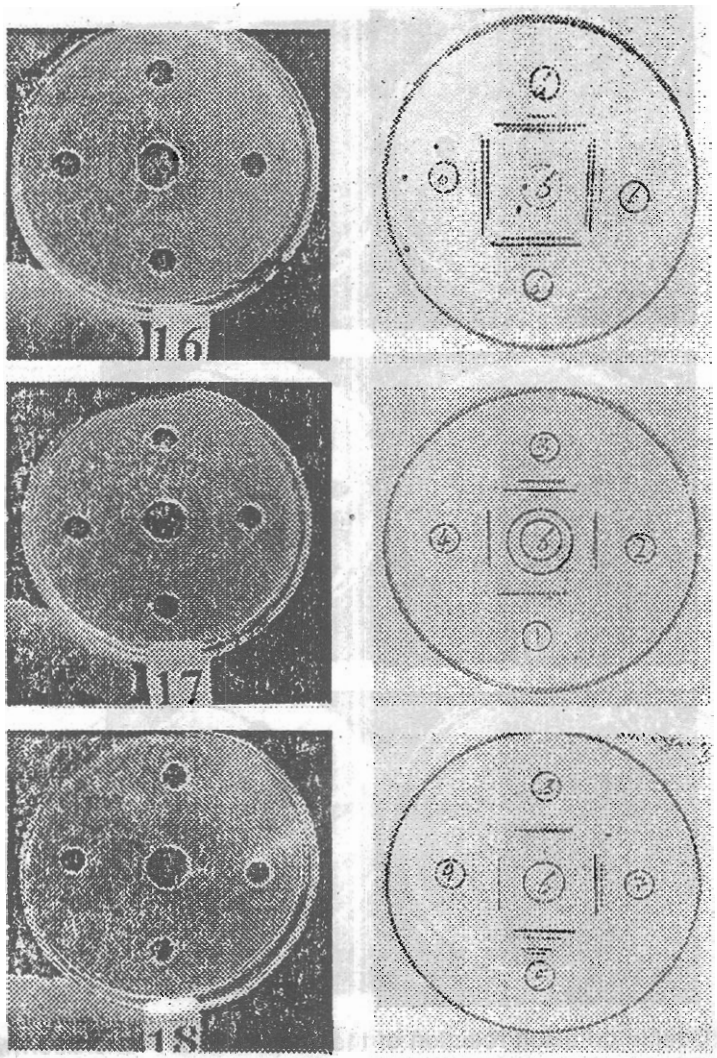

Figure 6: Double diffusion reaction of the antiserum of *F. moniliforme var. subglutinana* (in the center well) against antigens of *Fusarium* spp. (in peripheral wells) *Fusarium* spp. were *F. moniliforme var. subglutinans* (6) (homologous); *F. fusarioides* (1), *F. semitectum* (2), *F. poae* (3), *F. sambucinum* (4), *F. oxyporum* (5) , *F. moniliforme* (7), *F. solani* (8) and *F. sporotrichioides* (9) (Heterologus).

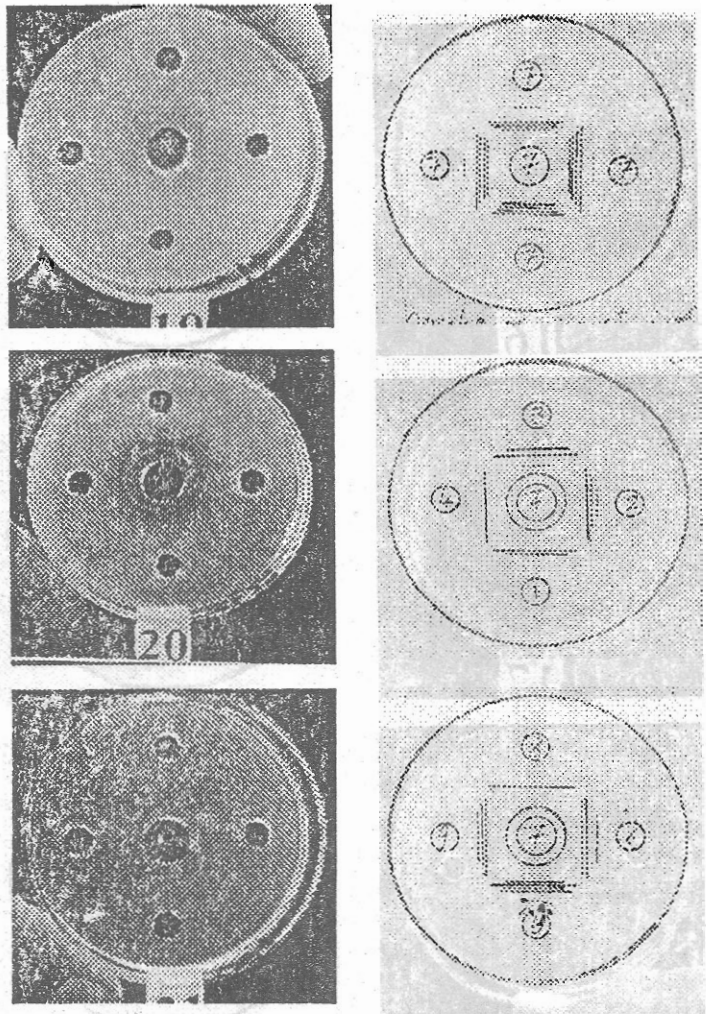

Figure 7:Double diffusion reaction of the antiserum of *F. moniliforme* (in the center well) against antigens of *Fusarium* spp. (in peripheral wells) *Fusarium* spp. were *F. moniliforme* (7) (homologous), *F. fusarioides* (1) *F. semitectum* (2), *F. poae* (3), *F. sambucinum* (4), *F. oxysporum* (5), *F. moniliforme var. subglutinans* (6), *F. solani* (8) and *F. sporotrichioides* (9) (Heterologus).

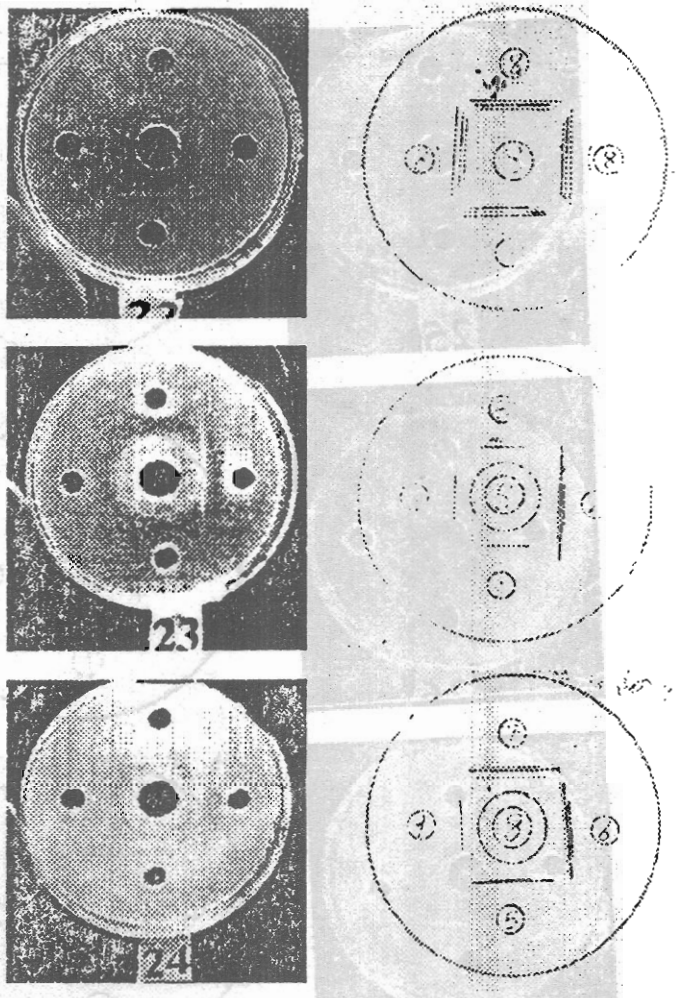

Figure 8: Double diffusion reaction of the antiserum of *F. solani* (in the center well) against antigens of *Fusarium* spp. (in peripheral wells) *Fusarium* spp. were *F. solani* (8) (homologous), *F. fusarioides* (1) *F. semitectum* (2), *F. poae* (3), *F. sambucinum* (4), *F. oxyporum* (5), *F. moniliiforme var. subglutinans* (6), *F. moniliforme* (7), and *F. sporotrichiodes* (9) (Heterologus).

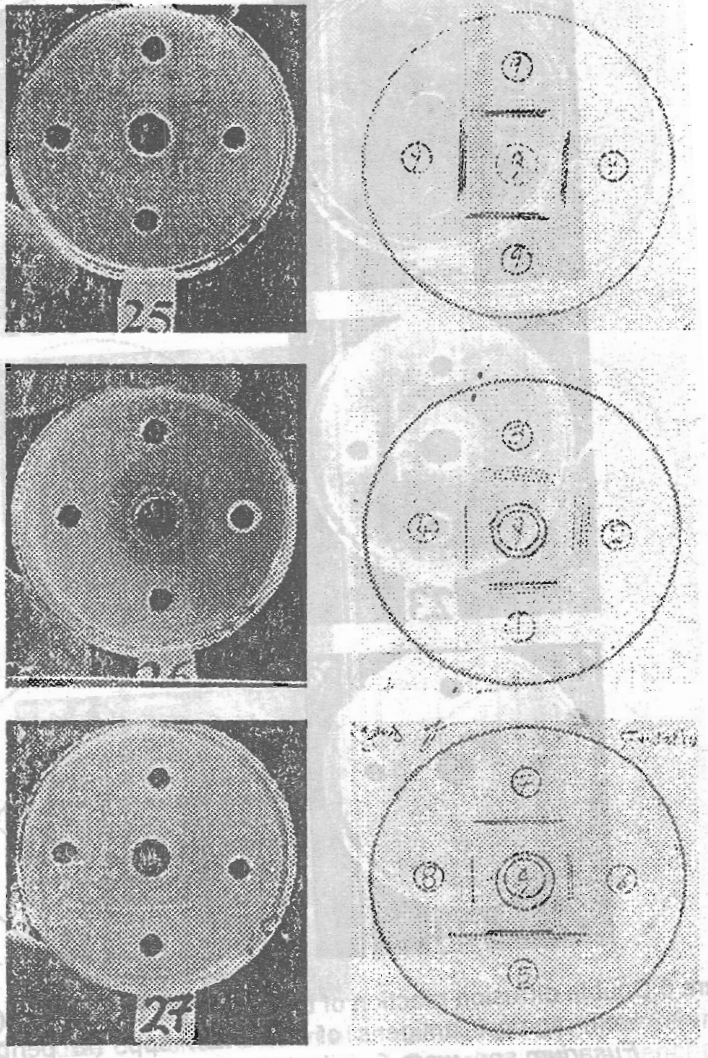

Figure 9 : Double diffusion reaction of the antiserum of *F. sporotrichioidas* (in the center well) against antigens of *Fusarium* spp. (in peripheral wells) *Fusarium* spp. were *F. sporotrichiodes* (9) (homologous), *F. fusarioides* (1) *F. semitectum* (2), *F. poae* (3), *F. sambucinum* (4), *F. oxyporum* (5), *F. moniliforme* var. *subglutinans* (6), *F. moniliforme* (7), and *F. solani* (8) (Heteroiogus).

Table 1:Matrix containing similarity indexes (Ssm)[a] established among *Fusarium* species based on data obtained from double diffusion test technique.

Antisera	Antigens								
	F. fusarioides	*F. semitectum*	*F. poae*	*F. sambacinum*	*F. oxysporum*	*F. subglutinans*	*F. moniliforme*	*F. solani*	*F. sporotrichioides*
F. fusarioides	100	40	60	66.66	71.43	20	20	25	66.66
F. semitectum	25	100	60	33.33	57.14	40	20	50	33.33
F. poae	25	40	100	33.33	71.43	40	20	50	66.66
F. sambacinum	50	40	80	100	57.14	20	20	25	33.33
F. oxysporum	75	60	60	66.66	100	60	60	50	66.66
F. subglutinans	25	40	40	33.33	42.86	100	60	50	33.33
F. moniliforme	50	60	40	33.33	71.43	60	100	50	66.66
F. solani	25	60	60	33.33	28.57	20	20	100	33.33
F. sporotrichioides	50	80	60	66.66	28.57	20	20	25	100

[a] Similarity index (Ssm) was calculated according to the formula

$$Ssm = \frac{\text{Number of common antigens}}{\text{Number of antigens in homologous reaction}} \times 100$$

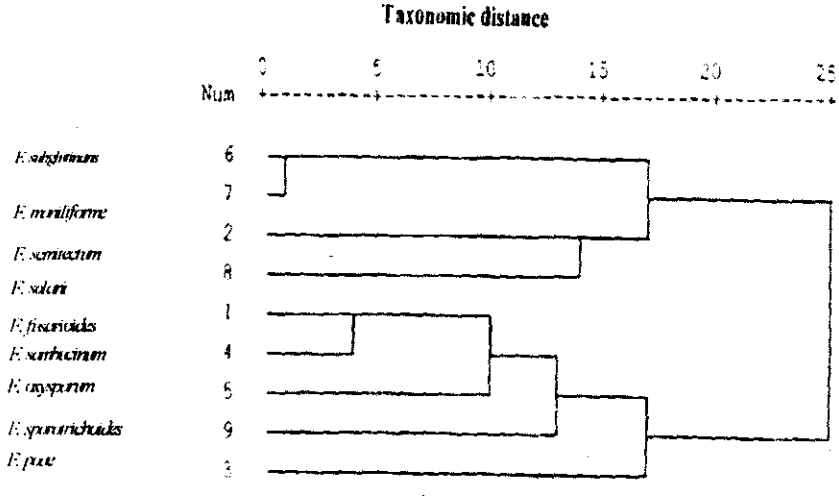

Figure 10: Phenogram based on average linkage cluster analysis of serological protein patterns obtained by double diffusion from isolates of nine *Fusarium* spp. based on common antigens.

Table 2:Matrix containing similarity indexes (Ssm)[a] established among *Fusarium* species based on data obtained from double diffusion test technique.

Antisera	Antigens								
	F. fusarioides	*F. semitectum*	*F. poae*	*F. sambacinum*	*F. oxysporum*	*F. subglutinans*	*F. moniliforme*	*F. solani*	*F. sporotrichioides*
F. fusarioides	100	25	33.33	50	66.66	0.00	0.00	0.00	50
F. semitectum	0.00	100	33.33	0.00	50	25	0.00	33.33	0.00
F. poae	0.00	25	100	0.00	66.66	25	0.00	33.33	50
F. sambacinum	33.33	25	66.66	100	50	0.00	0.00	0.00	0.00
F. oxysporum	66.66	50	33.33	50	100	50	50	33.33	50
F. subglutinans	0	25	0.00	0.00	3.33	100	50	33.33	0.00
F. moniliforme	33.33	50	0.00	0.00	66.66	50	10	33.33	50
F. solani	0.00	50	33.33	0.00	16.66	0.00	0.00	100	0.00
F. sporotrichioides	33.33	75	33.33	50	16.66	0.00	0.00	0.00	100

[a] Similarity index (Ssm) was calculated according to the formula

$$Ssm = \frac{\text{Number of common specific antigens}}{\text{Number of specific antigens in homologous reaction}} \times 100$$

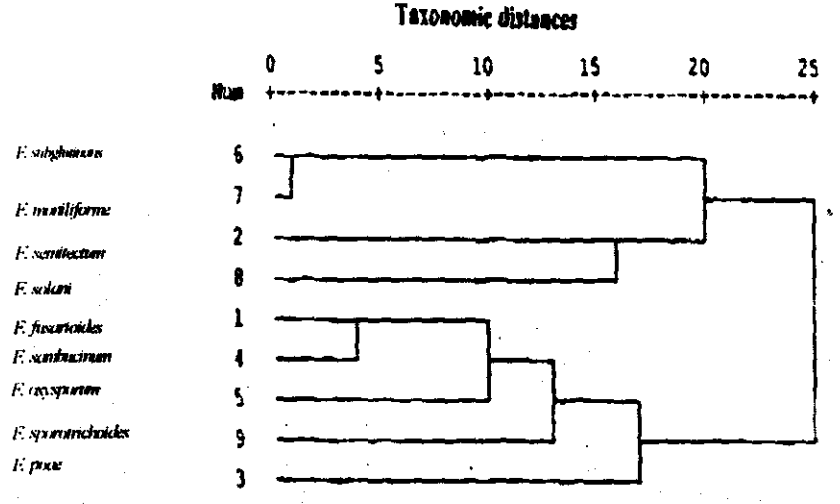

Figure 11:. Phenogram based on average linkage cluster analysis of serological protein patterns obtained by double diffusion from isolates of nine *Fusarium* spp. based on common specific antigens.

Table 3: The relationship between number of common antigens (bands) formed between *Fusarium* spp. and cotton cultivars and pathogenicity of *Fusarium* spp. on these cultivars.

Fusarium spp.	*F. fusarioides*		*F. semitectum*		*F. poae*		*F. sambucinum*		*F. oxysporum*		*F. moniliforme* var. *subglutinans*		*F. moniliforme*		*F. solani*		*F. sporotrich-oides*	
Cultivars	No. of bands	Pathogenicity (%)	No. of bands	Pathogenicity (%)	No. of bands	Pathogenicity (%)	No. of bands	Pathogenicity (%)	No. of bands	Pathogenicity (%)	No. of bands	Pathogenicity (%)	No. of bands	Pathogenicity (%)	No. of bands	Pathogenicity (%)	No. of bands	Pathogenicity (%)
Giza 45	8	50	9	52.5	10	52.5	10	55	11	60	10	52.5	9	52.5	12	62.5	10	52.5
Giza 70	7	35	11	60	9	50	9	47.5	9	50	9	47.5	10	57.5	11	60	9	45
Giza 80	8	50	7	42.5	10	60	12	65	14	67.5	10	57.5	8	45	11	62.5	9	55
Giza 83	7	47.5	6	45	10	55	8	45	13	60	8	40	9	42.5	10	50	12	57.5
Giza 85	10	62.5	7	40	9	52.5	9	50	10	57.5	6	35	10	60	10	55	12	65
Giza 86	8	47.5	9	50	6	40	11	60	9	55	9	52.5	6	37.5	10	57.5	11	60
Giza 87	7	37.5	8	45	6	35	9	47.5	9	50	9	45	7	40	9	52.5	9	50
Giza 88	7	40.0	11	65	8	45	8	42.5	10	55	10	52.5	11	60	10	57.5	8	45
Giza 89	7	42.5	8	45	9	50	11	57.5	10	55	8	50	10	57.5	11	62.5	11	60
Giza 90	12	65	9	57.5	9	52.5	11	60	10	57.5	11	62.5	10	62.5	13	70	12	65
X	8		8.5		8.6		9.8		10.5		9		9		10.7		10.3	

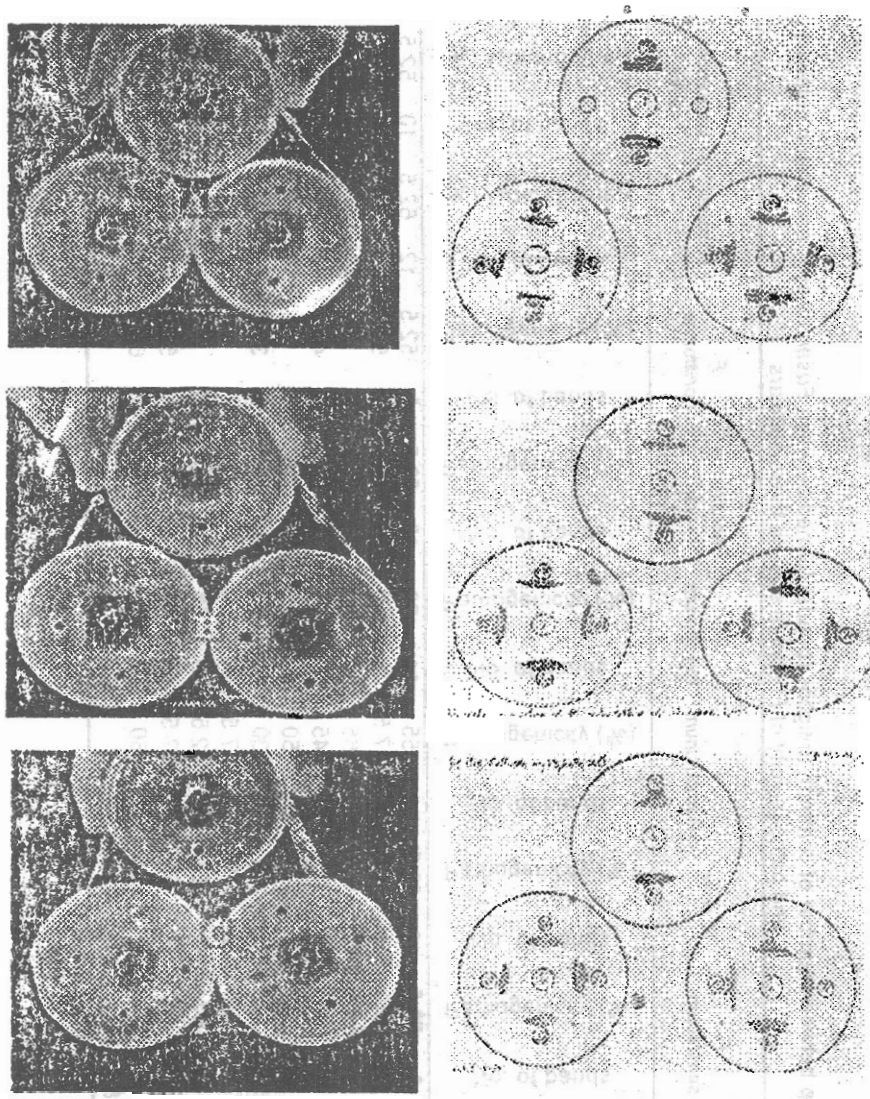

Figure 12: Double diffusion reaction of the antiserum of *Fusarium* spp.(in the center well) against antigens of cotton cultivars (in peripheral wells) *Fusarium* spp. were *F. fusarioides* (1) *F. poae* (2) and *F. sambucinum* (3), cotton cultivars were Giza 45, Giza 70, Giza 80, Giza 83, Giza 85, Giza86, Giza87, Giza88, Giza89 and Giza90.

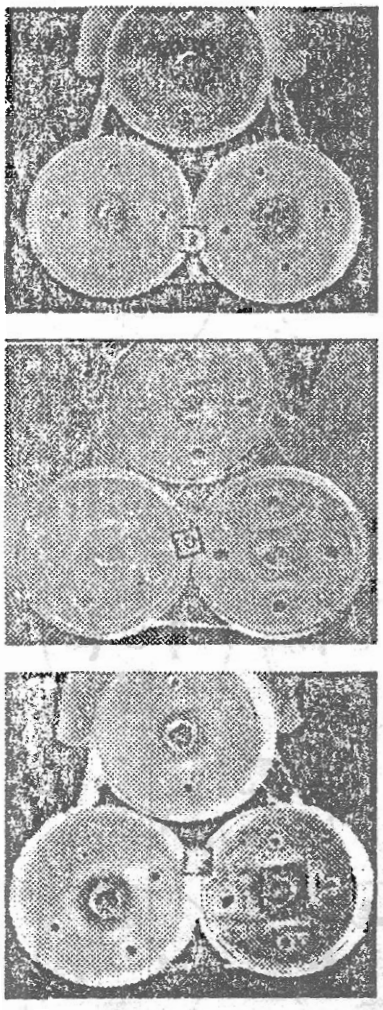

Figure 13: Double diffusion reaction of the antiserum of *Fusarium* spp., (in the center well) against antigens of cotton cultivar (in peripheral wells) *Fusarium* spp. were *F. oxyporum* (4), *F. moniliforme* var. *subglutinans* (5), *F. moniliforme* (6), cotton cultivars Giza 45, Giza 70, Giza 80, Giza 83, Giza 86, Giza 87, Giza 88, Giza 89 and Giza 90.

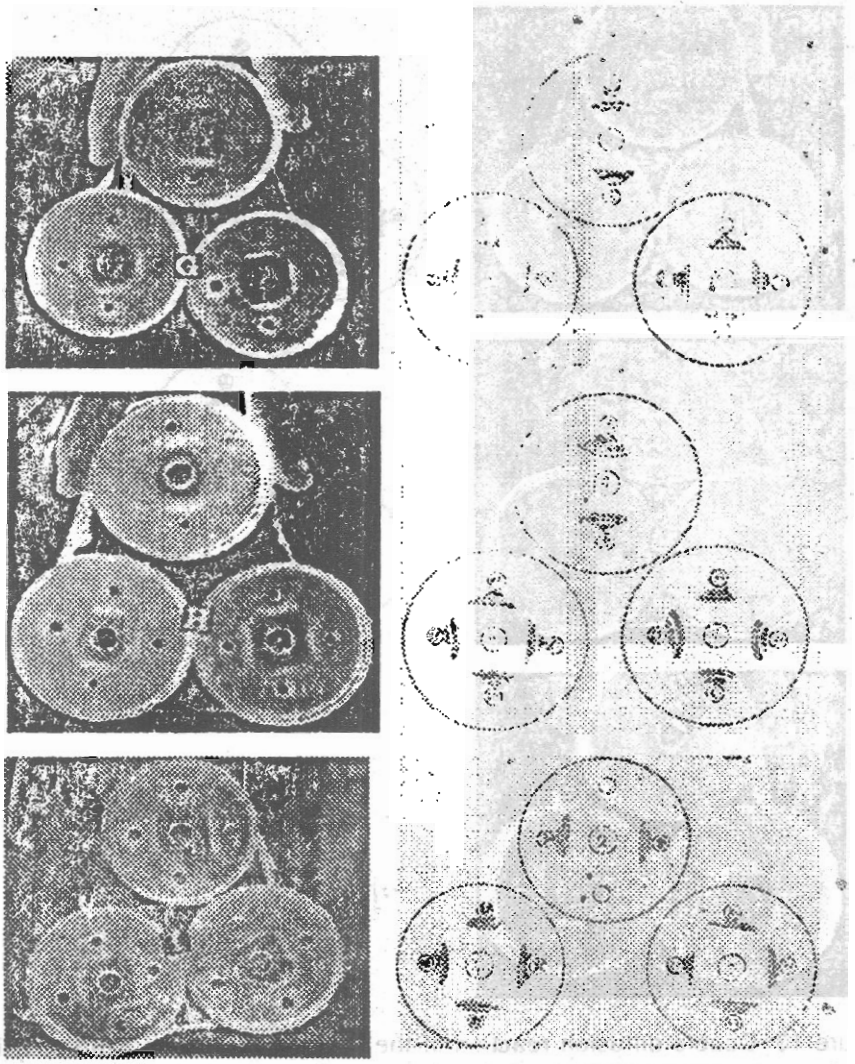

Figure 14:Double diffusion reaction of the antiserum of *Fusarium* spp. (in the center well) against antigens of cotton cultivars (in peripheral wells) *Fusarium* spp. were *F. solani* (7) (homologous), *F. sporotrichiodes* (8), *F. semitectum* (9), cotton cultivars Giza 45, Giza 70, Giza 80, Giza 83, Giza 86, Giza 87, Giza 88, Giza 89 and Giza 90.

to Giza 80 (60%) followed by Giza 83 (55%). *F. sambucinum* provided 12 bands with 65% disease severity with cotton cv. Giza 80 followed by 11 bands and 60% disease severity with cotton cvs. Giza 86 and Giza 90. *F. oxysporum* exhibited 14 bands and 67.5% disease severity with cv. Giza 80 followed by 13 bands and 60% disease severity with Giza 83 and 11 bands with 60% disease severity toward cv. Giza 45.

Fusarium subglutinans existed 11 bands and 62.5% disease severity with cotton cv. Giza 90 and 10 bands plus 57.5% disease severity with cotton cv. Giza 80. While, *F. moniliforme* showed 11 bands with 60% disease severity toward cv. Giza 88 it expressed 10 bands with 62.5% disease severity toward cv. Giza 90. *F. solani* formed.

Regression equations that describes the relationship between number of bands common antigens shared by *Fusarium* spp. and cotton cultivars and pathogenicity of *Fusarium* spp. on these cultivars are shown in Table (4) presented by Figs. 15, 16 and 17. R^2 of the equation ranged from 80-90%, which indicated that, the common antigens between *Fusarium* spp. and cotton cutlivars explained from 80 to 90% the total variation in pathogenicity of Fusaria on cotton cultivars.

A positive serological correlation between *Fusarium* species and cotton cultivars was established in the present study. Increasing number of common antigens of *Fusarium* species was paralleled with enhancing cultivar susceptibility to the same species and vise versa was correct in most cases. Data confirmed that serological methods are not only important for fungi identification (Osman-Eman, 1996; Aly *et al.*, 1997; Bouznad *et al.*, 1998; Burgess and Summerell, 2000 and Mishra *et al.*, 2003) but also important for determination plant host susceptibility to infection by the pathogens and for quantitative assessment of pathogens population in eithe soil or host tissues (Glazek, 2000; Chakraborty *et al.*, 2002 and Hussein *et al.*, 2003).

Table 4: Regression equations that describes the relationship between no. of common antigens shared by *Fusarium* spp. and cotton cultivars and pathogenicity (Y) of those species on those cultivars.

Fusarium spp	Regression equation	R^2	P>F
F. fusarioides	Y= 1.63 + 5.63 x	0.67	0.01
F. semitectum	Y= 11.65 + 4.54 x	0.82	0.01
F. poae	Y= 9.41 + 4.63 x	0.90	0.01
F. sambucinum	Y= 6.39 + 4.86 x	0.85	0.01
F. oxysporum	Y= 28.52 + 2.69 x	0.80	0.01
F. subglutinans	Y= 3.25 + 5.14 x	0.81	0.01
F. moniliforme	Y= 2.41 + 5.45 x	0.83	0.01
F. solani	Y= 10.81 + 4.50 x	0.81	0.01
F. sporotrichioides	Y= 10.15 + 4.40 x	0.80	0.01

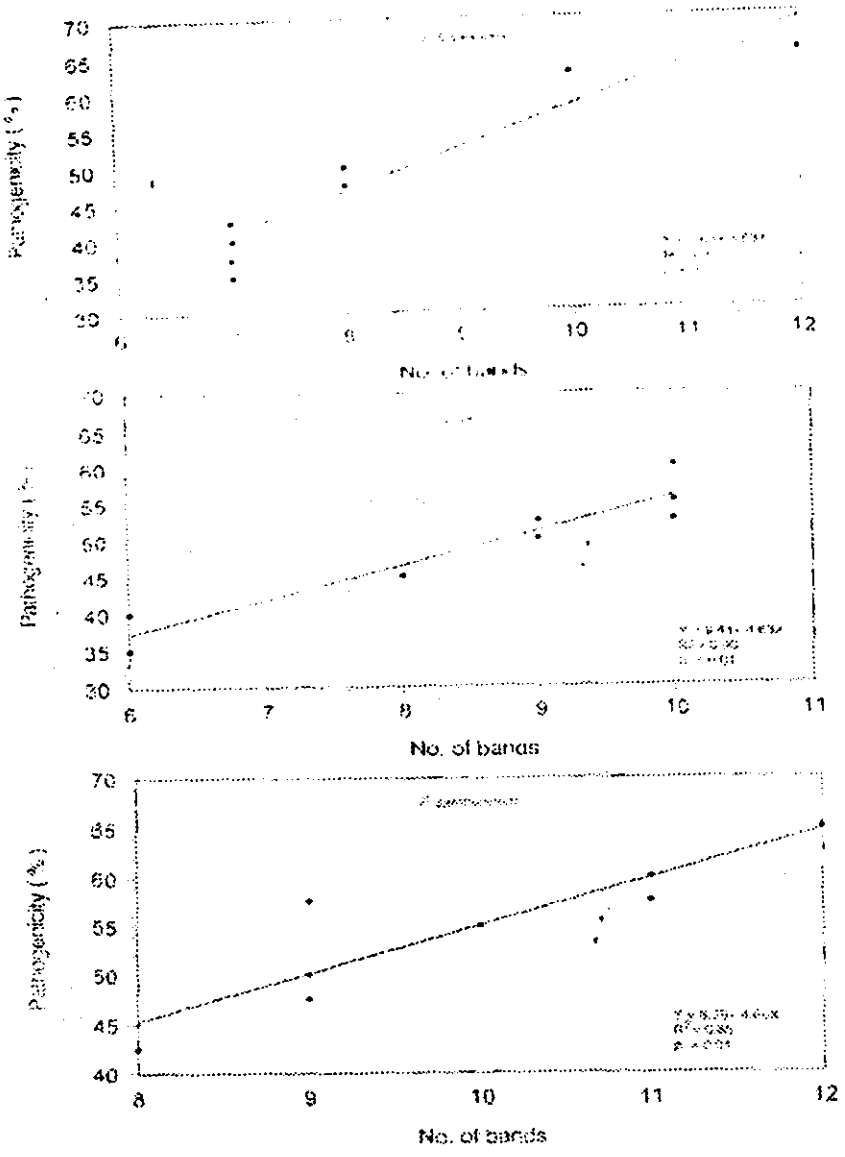

Figure 15: Regression equation that describes the relationship between no. of common antigens shared by *F. fusarioides*, *F. poae* and *F. sambucinum* and cotton cultivars and pathogenicity of those species on those cultivars.

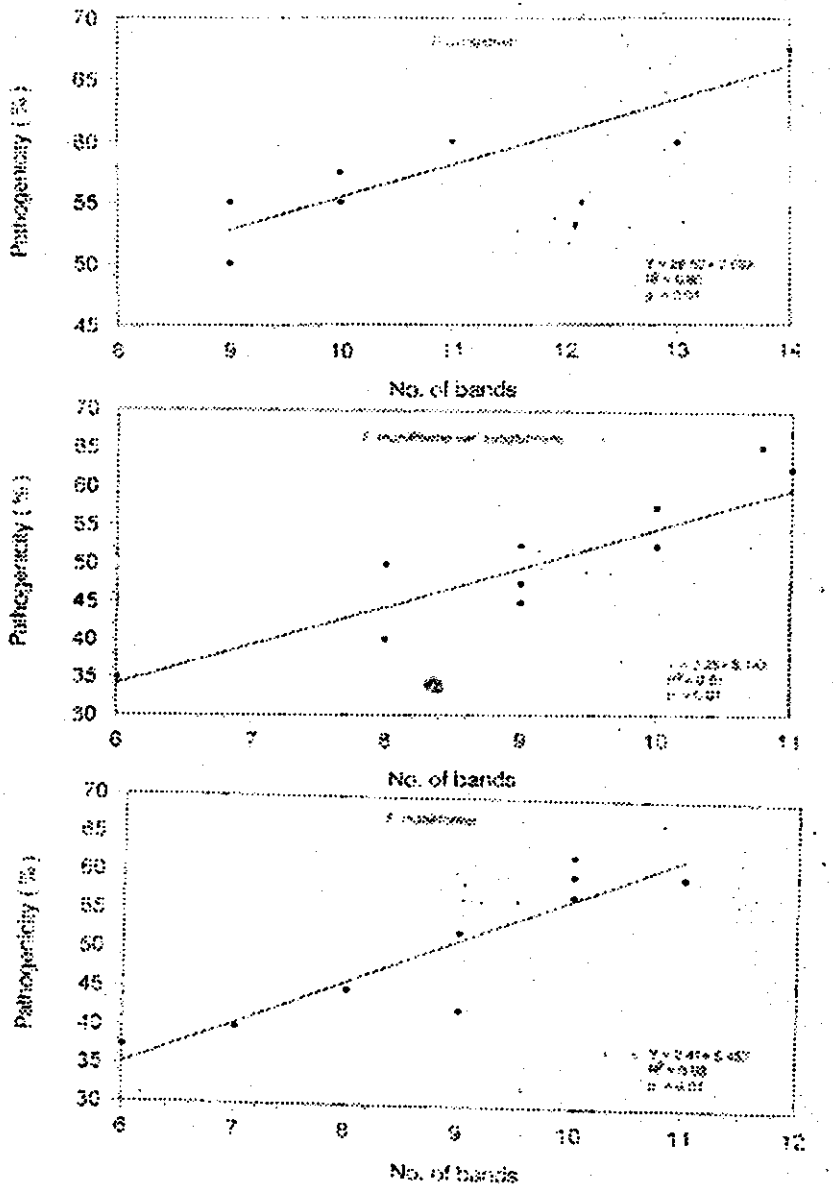

Figure 16: Regression equation that describes the relationship between no. of common antigens shared by *F. oxysporum*, *F. moniliforme* var. *subglutinans* and *F. moniliforme* and cotton cultivars and pathogenicity of those species on those cultivars.

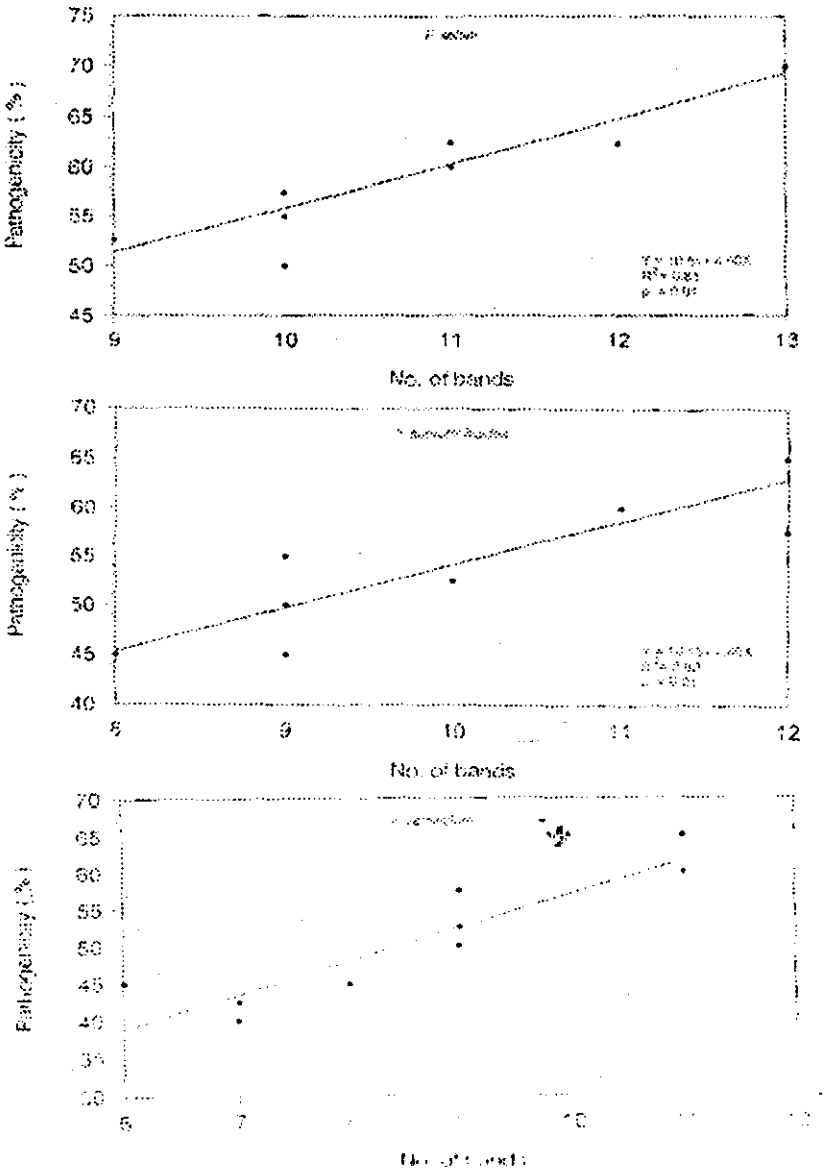

Figure 17: Regression equation that describes the relationship between no. of common antigens shared by *F. solani*, *F. sporotrichoides* and *F. semitectum* and cotton cultivars and pathogenicity of those species on those cultivars.

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دراسات سيرولوجية على العلاقة بين بعض انواع الفيوزاريوم ونباتات القطن

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**قسم أمراض النبات-كلية الزراعة-جامعة المنيا- المنيا- مصر.

درست العلاقات السيرولوجية بين تسعة أنواع تابعة لجنس الفيوزاريوم – معزولة من بادرات قطن مصابة بمرض موت البادرات – وذلك باستعمال أسلوب الإنتشار المزدوج. استعمل أسلوب التحليل العنقودى لتصنيف هذه الأنواع بناءاً على ما بينها من درجات قرابة سيرولوجية وتم التعبير نت النتائج فى فينوجرامات. أمكن تقسيم الأنواع لإلى مجموعتين محددتين إشتملت الأولى على الأنواع التالية: سبجلوتيناتز ومونيليفورمى وسميتكتم وسولانى فى حين إشتملت المجموعة الثانية على الأنواع: سميبوسينم وأوكسىسبورم وسبوروتريكويدز وبويا.

علماً بان استخدام الإنتيجينات الغير متخصصة أو المتخصصة لم يغير من طبيعة العلاقات التقسيمية ، أظهر تحليل الإنحدار أن مابين ٨١ إلى ٩٠% من التباين في القدرة المرضية للأنواع على أصناف القطن من الممكن أن تعزى إلى الإنزيمات المشتركة بين هذه الأنواع وأصناف القطن تدل هذه النتائج على إمكانية استخدام اختبار الإنتشار المزدوج للتعبير الكمى عن القدرة المرضية لأنواع الفيوزاريوم على أصناف القطن.