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Use of Serological Interactions to Differentiate between *Trichoderma harzianum* and *Trichoderma longbrachiatum* Isolated from Cotton Roots

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ABSTRACT

Double diffusion (DD) and immunoelectrophoresis (IE) techniques were used to differentiate among *Trichoderma* isolates. Each of *T. longbrachiatum* and *T. harzianum* isolates tended to group together based on their antigenic composition, which indicated a remarkable overall serological similarity among isolates of each species. However, the serological differences among the isolates did not always reflect the taxonomic differences because some isolates of *T. longbrachiatum* and *T. harzianum* showed considerable serological similarity. Thus, DD and IE were not useful techniques in identification of *Trichoderma* isolates.

INTRODUCTION

Trichoderma Pers. is a genus of hyphomycetes. Its species are among the most commonly encountered soil fungi (Roiger *et al*., 1991). *Trichoderma* has been shown to act as a mycoparasite against a range of economically important aerial and soilborne plant pathogens. Different factors involved in the antagonistic properties of *Trichoderma* have been identified, including antibiotics (Dennis and Webster, 1971a, b) and hydrolytic enzymes, such as β-(1, 3) glucanases, proteases and chitinases (Elad *et al* ., 1984; Geremia *et al*., 1993). The intial interaction between *Trichoderma* and its host is characterized by the chemotrophic growth of hyphae of the mycoparasite towards the host (Chet and Elad, 1983). When the mycoparasite reaches the host, its hyphae often coil around it or are attached by hook-like structures (Elad *et al*., 1983 a). Following these interactions, the mycoparasite penetrates the host mycelium, apparently by partially degrading its cell wall. Susceptible host mycelia show rapid vacuolation, collapse and disintegration (Elad *et al*., 1983b; Benhamou and Chet, 1993).

Trichoderma spp. exert their beneficial effect on plant growth by producing a growth-promoting factor that increases the rate of seed germination and dry weight of shoots and roots (Baker *et al*., 1984; Windham *et al*., 1986; Chang *et al*., 1986; Menzies, 1993; Hanson, 2000). *Trichoderma* spp. play an important role in biological control thanks to their advantageous ecological and physiological properties: a good environmental fitness, which includes the ability to exploit competitively many different nutritional sources and high antagonistic ability against soil microorganisms (Howell, 2005; Sariah *et al*., 2005, Vergara *et al*., 2006; Abd-Elsalam *et al*., 2010; Saba *et al*., 2012.).

The usefulness of serological interactions in fungal taxonomy is well documented in the literature. For instance, Polyclonal antibodies were raised from whole cells, wall components, soluble proteins, and ribosomes of different fungal species. It has been demonstrated that species-specific polyclonal antibodies are present in antisera raised against fungi and can be useful for detection and quantification purpose (Srivastava and Arora, 1997).
Hornok (1980) used immunoelectrophoresis 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 view of the genus.

Iannelli *et al.* (1982) showed that *F. oxysporum, F. moniliforme,* and *F. xylarioides* possessed distinct antigenic characteristics. In addition, they describe how four different formae speciales of *F. oxysporum* (*dianthi, melonis, pisi, lycopersici*) and the physiological races of *F. oxysoprum* 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 tandum-crossed immunoelectrophoresis. They found that the two races had apparently almost identical antigenic patterns differing only in one antigen specific to race 3.

Barak *et al*. (1985) raised antisera against conidia of several *Trichoderma* isolates in rabbits and tested them by agglutination and immunofluorescence. Six serotypes were characterized and the differences in their surface properties studied. The serological differences among the isolates did not always reflect their taxonomic differences. Serological similarities were found in several instances between conidia and hyphae of the same isolate.

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, they were not sufficient for an unequivocal statement that fungi belong to the same species.

Hussein *et al.* (1996) compared *F. oxysporum*, *F. moniliforme,* and *F. solani*, isolates from cotton seedlings infected with damping-off, by double diffusion (DD) and immunoelectrophoresis (IE) techniques to determine their serological relationships. On the basis of serological relationships, isolates were grouped by cluster analysis and the results were expressed as phenograms. The taxonomic relationships established based on DD matched those based on modern system of morphological classification. DD technique in comparison with 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.

Kratka *et al*. (1997) studied specificity and sensitivity of polyclonal antibodies after immunization of rabbits with antigens of 18 monospore isolates of *F. culmorum* (FCU). Antigens of FCU isolates showed similar reactions. Anti-FCU IgG reacted with antigens of other *Fusarium* spp. (*F. oxysporum, F. solani, F. equiseti, F. nivale, F. sambucinum, F. poae, F. avenaceum*). Differences were quantitative. Reactions of antisera and

IgG with antigens were evaluated by agar double diffusion and ELISA.

The present investigation was initiated to determine whether *T. harzianum* and *T. longibrachiatum* isolated from cotton roots (Table 1) can be distinguished by their serological protein patterns separated by double diffusion (DD) and immunoelectrophoresis (IE) techniques

Table 1. Geographic origins of *Trichoderma* spp. isolated from cotton seedlings and used in the present study.

Isolate code	Geographic origin	Identification
T3	Daqahliya, Simbellawain	*T. harzianum*
T4	Assiut, Assiut	*T. longibrachiatum*
T5	Assiut, Assiut	*T. longibrachiatum*
T6	Daqahliya, simbellawain	*T. harzianum*
T9	Unknown	*T. longibrachiatum*
T10	Daqahliya, Simbellawain	*T. harzianum*
T14	Giza, Giza	*T. longibrachiatum*
T18	Minufiya, Minouf	*T. longibrachiatum*
T23	Gharbiya, El Mahalla El Kobra	*T. harzianum*
T27	Gharbiya, El Mahalla El Kobra	*T. harzianum*
T29	Daqahliya, Simbellawain	*T. harzianum*
T31	Daqahliya, Simbellawain	*T. harzianum*
T38	Minufiya, Shibeen El Kom	*T. longibrachiatum*
T39	Minufiya, Shibeen El Kom	*T. longibrachiatum*
T42	Giza, Giza	*T. longibrachiatum*

MATERIALS AND METHODS

1. Extraction of antigens (proteins) from *Trichoderma* isolates

Antigens were prepared according to Guseva and Gromova (1982), Rataj-Guranowska *et al.* (1984), and Hussein (1992), while the protein content in supernatant was estimated according to Bradford (1976) by using bovine serum albumin as a standard protein.

2. Immunization and preparation of antisera

New Zealand, rabbits 3-4 kg weight were immunized by antigens of isolates no_s. 9 (*T. longibrachiatum*) and 31 (*T. harzianum*) to produce antisera. The first injection was given intraacutaneously 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. After one week, each animal was received 4 ml protein administered intramuscularly every third day in the thigh in a series of twelve injections. One week after the last injection, the animals were bled and antibodies in serum were assayed by double diffusion technique (Hussein, 1992).

3. Double diffusion (DD) technique

The techniques was carried out according to Outcheterlony and Nilsson (1978). One percent ionagar, melted in saline and supplemented with merthiolate (1:10,000), was poured into 9-cm-diameter Petri dishes to obtain a layer of agar 1-2 mm thick. The diameter of the central and of the 4 peripheral wells were 5 and 3 mm, respectively. The distance between the central well and the peripheral ones was 15 mm. The central well was filled with antiserum and the peripheral wells with antigens. Dishes were kept in humid conditions at room temperature (18-24 ^{o}C) in the dark for one week. The developing precipitin lines were examined and recorded by hand drawing and photography.

4. Immunoelectrophoresis (IE) technique

The technique was carried out according to Grabar and Williams (1953). In this technique, proteins in antigen were first separated by electrophoresis in agar. Antiserum was then allowed to diffuse from a trough cut in the gel parallel to the direction of electrophoresis. Immunoprecipitin arches were observed where antigen-antibody interactions occurred. Glass slides 3.5 cm x 7.5 cm were covered with a thin layer (2 mm) of buffered agar gel. The gel was prepared by incorporating 1% ionagar in sodium barbital buffer pH 8.6 to which merthiolate (1:10,000) was added to give final concentration of 0.1%. Sample wells 4 mm in diameter were cut about 3.5 cm from the cathode. After filling the wells with antigen solution (10 µl), the slide was placed in an electrophoresis apparatus, which received 150 ml of the run buffer solution (sodium barbital buffer) into each of the two troughs of the electrophoresis tank, the necessary electrical connections were made with filter paper wicks. The gel was covered with a glass plate to prevent surface evaporation. The electrophoresis was performed at 3 mA for each sample for about 2 hrs. The slides were removed from the apparatus, after termination of the electrophoresis. A channel (5cm x 2mm) was cut between the two wells to act as trough for homologous or heterologous antiserum. The trough was filled with about 100µl of antiserum solution. The slides were kept under humid conditions of room temperature for 4 days in the dark. The developing precipitin arches were recorded by hand drawing and photography, and identified according to Ghobrial (1981) and Johnstone and Thorpe (1982).

5. Cluster analysis

Pearson's correlation coefficient (r) was calculated for each pair of isolates. Based on these data, a correlation matrix was constructed and from this matrix isolates were clustered by the unweighted pair-group method based on arithmetic mean (UPGMA). Cluster analysis was performed using SPSS 6.0 software package.

RESULTS

1. DD technique

Double diffusion technique was used to differentiate among *Trichoderma* isolates. The reaction of the antiserum of isolate T9 of *T. longibrachiatum* against antigens of 15 isolates of *Trichoderma* are

shown in Fig. 1 and Table 2. Linear correlation coefficient (r) was calculated for assessing similarity among isolates in their serological protein patterns (Table 3). In this method, r values were calculated by using all the resulting common antigens (specific and nonspecific). Fig. 2 showed the phenogram constructed based on taxonomic distances (TDs) generated from cluster analysis of r values. In this phenogram, the smaller the TD, the more closely the isolates were related in their antigenic composition. The phenogram was divided into 3 unrelated clusters. The first one included isolates T29, T31, T10, T23, T27, T5, T42, T9, and T18. The second cluster included only isolate T6. Isolates T3, T14, T38, T39, and T4 were members of the third cluster.

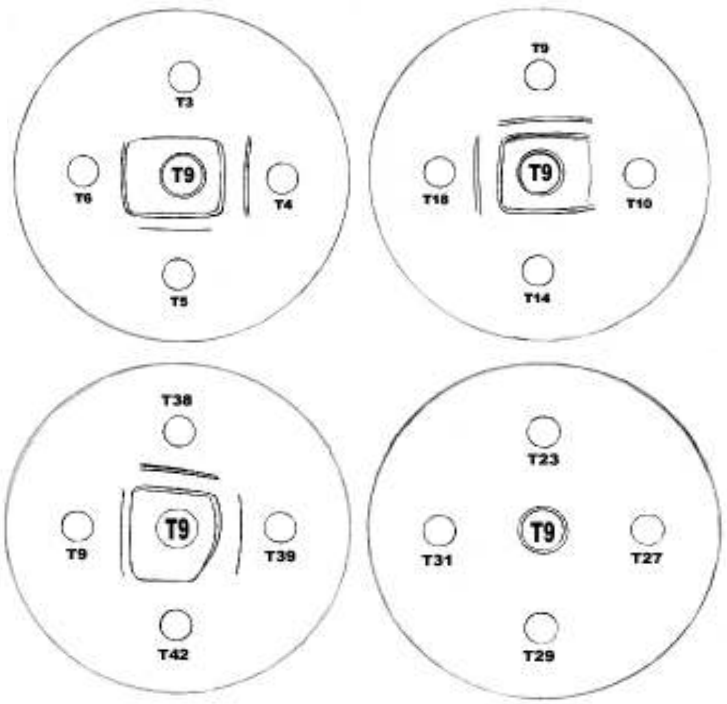

Fig. 1. Diagram showing the double diffusion reactions of the antiserum of isolate no. T9 of *Trichoderma longibrachiatum* from cotton (in central well) against antigens of isolates from cotton (in peripheral wells). Identification of the isolates is shown in Table 1

Table 2. Number and distribution of protein fractions obtained by double-diffusion reaction of antiserum of *Trichoderma* isolate no. T9 against antigens of *Trichoderma* isolates from cotton.

Protein fraction		Antiserum of isolate no. T9 x antigens of isolate[b] no.														
No.	Distance [a] (mm)	T3	T4	T5	T6	T9*	T10	T14	T18	T23	T27	T29	T31	T38	T39	T42
1	1	+ [c]	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	4	+	+	+	-	-	-	+	+	-	-	-	-	+	+	-
3	5	-	+	+	-	+	-	+	+	-	-	-	-	+	+	-
4	6	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
5	8	-	-	+	-	+	-	-	+	-	-	-	-	-	-	+
6	9	-	-	-	+	+	-	-	+	-	-	-	-	+	-	-
7	10	-	+	-	+	-	-	-	-	-	-	-	-	+	+	-
8	11	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-

[a] **Migration distance of the protein fraction from the central well, which contained the antiserum.**
[b] **Isolates nos. T3, T6, T10, T23, T27, T29, and T31 were *T. harzianum,* while isolates nos. T4, T5, T9, T14, T18, T38, T39, and T42 were *T. longibrachiatum*.**
[c] **Protein fraction was present (+) or absent (-).**
(*) Homologous reaction.

The present study included seven isolates of *T. harzianum*, of these isolates, five (71.43%) were found in the first cluster showing the highest level of similarity in their antigenic composition (TD= 0.0). Isolates of *T. longibrachiatum* were divided into 2 unrelated groups, one group (4 isolates) was found in the first cluster, while the other group (4 isolates) was found in the third cluster.

Table 3. Correlation among serological protein patterns when antiserum of *Trichoderma* isolate no. T9 interacted against antigens of 15 isolates of *Trichoderma* spp.

Trichoderma isolate	*Trichoderma* isolate														
	T3	**T4**	**T5**	**T6**	**T9**	**T10**	**T14**	**T18**	**T23**	**T27**	**T29**	**T31**	**T38**	**T39**	**T42**
T3	---	0.447	0.197	0.149	-0.149	0.655	0.745	0.447	0.655	0.655	0.655	0.655	0.447	0.577	0.333
T4	0.447[a]	---	0.088	0.067	-0.600	0.293	0.600	-0.067	0.293	0.293	0.293	0.293	0.467	0.775	-0.149
T5	0.197	0.088	---	-0.088	0.577	0.487	0.509	0.631	0.487	0.487	0.487	0.487	0.088	0.289	0.744
T6	0.149	0.067	-0.088	---	0.067	0.488	-0.067	0.067	0.488	0.488	0.488	0.488	0.600	0.258	0.149
T9	-0.149	-0.600	0.577	0.067	---	0.293	0.067	0.467	0.293	0.293	0.293	0.293	-0.067	-0.258	0.447
T10	0.655	0.293	0.487	0.488	0.293	---	0.488	0.293	1.000	1.000	1.000	1.000	0.293	0.378	0.655
T14	0.745	0.600	0.509	-0.067	0.067	0.488	---	0.600	0.488	0.488	0.488	0.488	0.600	0.775	0.149
T18	0.447	-0.067	0.631	0.067	0.467	0.293	0.600	---	0.293	0.293	0.293	0.293	0.467	0.258	0.447
T23	0.655	0.293	0.487	0.488	0.293	1.000	0.488	0.293	---	1.000	1.000	1.000	0.293	0.378	0.655
T27	0.655	0.293	0.487	0.488	0.293	1.000	0.488	0.293	1.000	---	1.000	1.000	0.293	0.378	0.655
T29	0.655	0.293	0.487	0.488	0.293	1.000	0.488	0.293	1.000	1.000	---	1.000	0.293	0.378	0.655
T31	0.655	0.293	0.487	0.488	0.293	1.000	0.488	0.293	1.000	1.000	1.000	---	0.293	0.775	0.655
T38	0.447	0.467	0.088	0.600	-0.067	0.293	0.600	0.467	0.293	0.293	0.293	0.293	---	0.775	-0.149
T39	0.577	0.775	0.289	0.258	-0.258	0.378	0.775	0.258	0.378	0.378	0.378	0.378	0.775	---	0.000
T42	0.333	-0.149	0.744	0.149	0.447	0.655	0.149	0.447	0.655	0.655	0.655	0.655	-0.149	0.000	---

[a] **Linear correlation coefficient (r). Tabulated value of r = 0.707 (p = 0.05) or 0.834 (p = 0.01).**

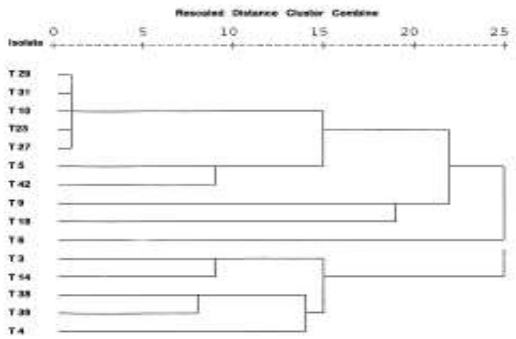

Fig. 2. Phenogram based on average linkage cluster analysis of serological protein patterns obtained by double diffusion technique from 15 isolates of *Trichoderma* spp. when their antigens interacted against antiserum of isolate no. T9 from cotton. Identification of the isolates is shown in Table 1.

Double diffusion reactions of the antiserum of isolate T31 of *T. harzianum* against antigens of 15 *Trichoderma* isolates are shown in Fig. 3 and Table 4. Calculated values of r are shown in Table 5. Fig. 4 showed the phenogram constructed based on TDs generated from cluster analysis of r values. The phenogram composed of three distinct clusters. The first one included isolates T18, T38, T42, T10, and T39. Isolates T9, T23, T27, T29, T5, and T31 were members of the second cluster. The third cluster included isolates T4, T6, and T14. Although each of these clusters was composed of a mixture of *T. longibrachiatum* and *T. harzianum* isolates, it was predominated by isolates of one species. Thus, 80% of the isolates in the first cluster were belonging to *T. longibrachiatum*, and 66.7% of the isolates in the second cluster was belonging to *T. harzianum*, and 66.7% of the isolates in the third cluster were belonging to *T. longibrachiatum*. A noteworthy peculiarity in the phenogram is the individuality of T3, which was unrelated the other isolates of *Trichoderma*.

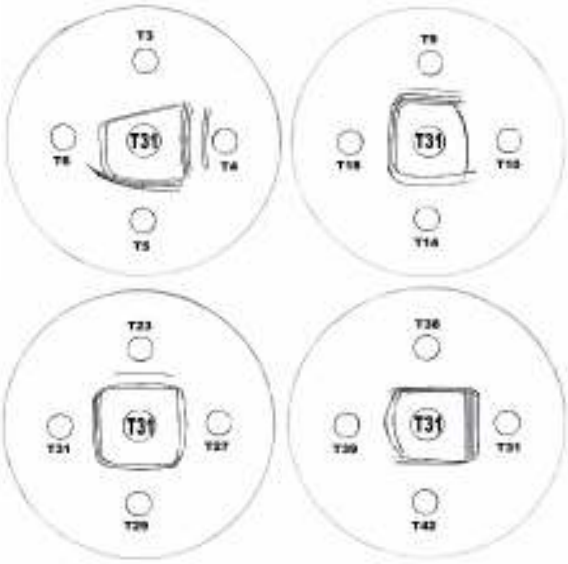

Fig. 3. Diagram showing the double diffusion reactions of the antiserum of isolate no. T31 of *Trichoderma harzianum* from cotton (in central well) against antigens of isolates from cotton (in peripheral wells). Identification of the isolates is shown in Table 1.

Table 4. Number and distribution of protein fractions obtained by double-diffusion reaction of antiserum of *Trichoderma* isolate no. T31 against antigens of *Trichoderma* isolates from cotton.

Protein fraction		Antiserum of isolate no. T31 x antigens of isolate[b] no.														
No.	Distance [a] (mm)	T3	T4	T5	T6	T9	T10	T14	T18	T23	T27	T29	T31*	T38	T39	T42
1	3	+ [c]	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	4	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	5	-	-	-	-	-	-	-	+	-	-	-	-	+	+	-
4	6	-	+	-	+	-	-	+	+	-	-	-	+	+	-	+
5	7	-	+	+	-	+	+	-	+	+	+	+	+	+	+	+
6	8	-	+	+	+	+	-	-	-	+	-	-	+	-	-	-
7	9	-	-	+	-	+	-	+	-	-	+	+	+	-	-	-
8	10	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-
9	11	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-
10	12	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
11	13	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-

[a] Migration distance of the protein fraction from the central well, which contained the antiserum.
[b] Isolate nos. T3, T6, T10, T23, T27, T29, and T31 are *T. harzianum,* while isolates nos. T4, T5, T9, T14, T18, T38, T39, and T42 are *T. longibrachiatum*.
[c] Protein fraction was present (+) or absent (-).
(*) Homologous reaction.

Table 5. Correlation among serological protein patterns when antiserum of *Trichoderma* isolate no. T31 interacted against antigens of 15 isolates of *Trichoderma* spp.

Trichoderma isolate	*Trichoderma* isolate														
	T3	T4	T5	T6	T9	T10	T14	T18	T23	T27	T29	T31	T38	T39	T42
T3	---	0.430	-0.356	-0.222	-0.356	-0.149	-0.222	-0.289	-0.289	-0.222	-0.222	-0.430	-0.289	-0.222	-0.222
T4	-0.430[a]	---	0.069	0.516	0.069	0.346	0.043	0.261	0.261	0.043	0.043	0.267	0.261	0.043	0.516
T5	-0.356	0.069	---	0.134	0.607	0.418	0.134	-0.039	0.386	0.624	0.624	0.828	-0.039	0.134	0.134
T6	-0.222	0.516	0.134	---	0.134	-0.149	0.389	0.241	0.241	-0.222	-0.222	0.516	0.241	-0.222	0.389
T9	-0.356	0.069	0.607	0.134	---	0.418	0.134	-0.039	0.810	0.624	0.624	0.449	-0.039	0.134	0.134
T10	-0.149	0.346	0.418	-0.149	0.418	---	-0.149	0.516	0.516	0.671	0.671	0.346	0.516	0.671	0.671
T14	-0.222	0.043	0.134	0.389	0.134	-0.149	---	0.241	-0.289	0.389	0.389	0.516	0.241	-0.222	0.389
T18	-0.289	0.261	-0.039	0.241	-0.039	0.516	0.241	---	0.083	0.241	0.241	0.261	1.000	0.770	0.770
T23	-0.289	0.261	0.386	0.241	0.810	0.516	-0.289	0.083	---	0.241	0.241	0.261	0.083	0.241	0.241
T27	-0.222	0.043	0.624	-0.222	0.624	0.671	0.389	0.241	0.241	---	1.000	0.516	0.241	0.389	0.389
T29	-0.222	0.043	0.624	-0.222	0.624	0.671	0.389	0.241	0.241	1.000	---	0.516	0.241	0.389	0.389
T31	-0.430	0.267	0.828	0.516	0.449	0.346	0.516	0.261	0.261	0.516	0.516	---	0.261	0.043	0.516
T38	-0.289	0.261	-0.039	0.241	-0.039	0.516	0.241	1.000	0.083	0.241	0.241	0.261	---	0.770	0.770
T39	-0.222	0.043	0.134	-0.222	0.134	0.671	-0.222	0.770	0.241	0.389	0.389	0.043	0.770	---	0.389
T42	-0.222	0.516	0.134	0.389	0.134	0.671	0.389	0.770	0.241	0.389	0.389	0.516	0.770	0.389	---

[a] Linear correlation coefficient (r). Tabulated value of r = 0.602 (p = 0.05) or 0.735 (p = 0.01).

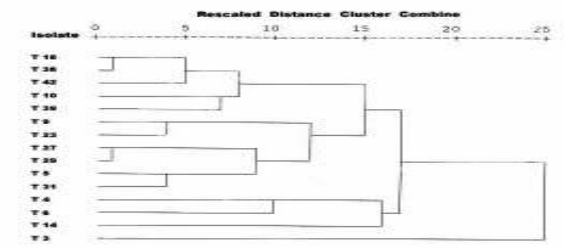

Fig. 4. Phenogram based on average linkage cluster analysis of serological protein patterns obtained by double diffusion technique from 15 isolates of *Trichoderma* spp. when their antigens interacted against antiserum of isolate no. T31 from cotton. Identification of the isolates is shown in Table 1.

2. IE technique

Immunoelectrophoretic data (Table 6) were established based on Immunoelectrophoretically fractioned proteins diagramed in Fig. 5. These data were used for calculating r values shown in Table 7. A phenogram (Fig. 6) was constructed based on TDs generated from cluster analysis of r values. In this phenogram, the isolates under investigation formed two unrelated main groups. The first group (TD = 15.9) comprised mainly isolates of *T. longibrachiatum*. The second group included isolates nos. T29, T31, T23, and T27, which were belonging to *T. harzianum*.

Table 6. Number and distribution of immunoglobulins obtained by immunoelectrophoresis of antiserum of *Trichoderma* isolate no. T9 against antigens of *Trichoderma* isolates from cotton.

Immunoglobulin		Antiserum of isolate no. T9 x antigens of isolate[a] no.														
No.	**Identification**	**T3**	**T4**	**T5**	**T6**	**T9***	**T10**	**T14**	**T18**	**T23**	**T27**	**T29**	**T31**	**T38**	**T39**	**T42**
1	Haptoglopulin	-[b]	+	+	+	+	-	+	+	-	-	-	-	+	+	+
2	Transferrin	+	+	+	-	+	+	+	+	-	-	-	-	+	+	+
3	α-Lipoprotein	-	-	+	-	+	-	+	-	-	-	-	-	+	-	-
5	Igm Globulin	-	-	+	-	-	-	-	+	+	+	+	+	+	+	+

[a] Isolates nos. T3, T6, T10, T23, T27, T29, and T31 were *T. harzianum,* while isolates nos. T4, T5, T9, T14, T18, T38, T39, and T42 were *T. longibrachiatum*.

[b] Immunoglobulin was present (+) or absent (-).

(*) Homologous reaction.

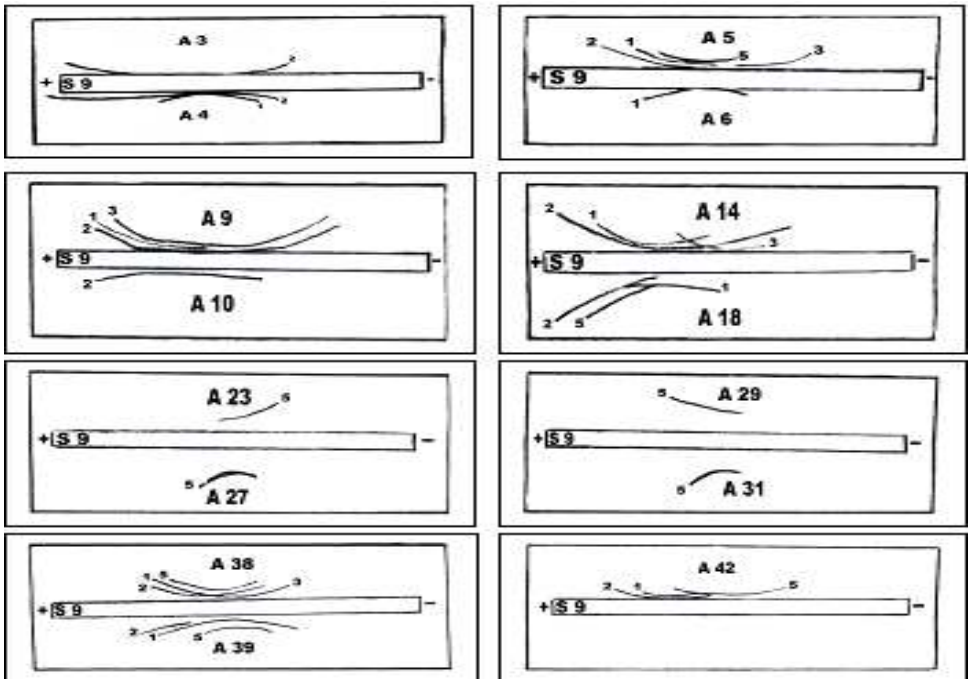

Fig. 5. Diagram of the immunoelectrograms of the fractionated proteins resulting from the reactions of the antiserum (S) of *Trichoderma* isolate no. T9 against antigens (A) of 15 *Trichoderma* isolates from cotton. Identification of *Trichoderma* isolates and the fractionated proteins (immunoglobulins) are shown in Table 6.

Table 7. Correlation among immunoglobulin patterns when antiserum of *Trichoderma* isolate no. T9 interacted against antigens of 15 isolates of *Trichoderma* spp.

Trichoderma	*Trichoderma* isolate														
isolate	**T3**	**T4**	**T5**	**T6**	**T9**	**T10**	**T14**	**T18**	**T23**	**T27**	**T29**	**T31**	**T38**	**T39**	**T42**
T3	---	0.612	0.250	-0.250	0.408	1.000	0.408	0.408	-0.250	-0.250	-0.250	-0.250	0.250	0.408	0.408
T4	0.612[a]	---	0.408	0.612	0.667	0.612	0.667	0.667	-0.408	-0.408	-0.408	-0.408	0.408	0.667	0.667
T5	0.250	0.408	---	0.250	0.612	0.250	0.612	0.612	0.250	0.250	0.250	0.250	1.000	0.612	0.612
T6	-0.250	0.612	0.250	---	0.408	-0.250	0.408	0.408	-0.250	-0.250	-0.250	-0.250	0.250	0.408	0.408
T9	0.408	0.667	0.612	0.408	---	0.408	1.000	0.167	-0.612	-0.612	-0.612	-0.612	0.612	0.167	0.167
T10	1.000	0.612	0.250	-0.250	0.408	---	0.408	0.408	-0.250	-0.250	-0.250	-0.250	0.250	0.408	0.408
T14	0.408	0.667	0.612	0.408	1.000	0.408	---	0.167	-0.612	-0.612	-0.612	-0.612	0.612	0.167	0.167
T18	0.408	0.667	0.612	0.408	0.167	0.408	0.167	---	0.408	0.408	0.408	0.408	0.612	1.000	1.000
T23	-0.250	-0.408	0.250	-0.250	-0.612	-0.250	-0.612	0.408	---	1.000	1.000	1.000	0.250	0.408	0.408
T27	-0.250	-0.408	0.250	-0.250	-0.612	-0.250	-0.612	0.408	1.000	---	1.000	1.000	0.250	0.408	0.408
T29	-0.250	-0.408	0.250	-0.250	-0.612	-0.250	-0.612	0.408	1.000	1.000	---	1.000	0.250	0.408	0.408
T31	-0.250	-0.408	0.250	-0.250	-0.612	-0.250	-0.612	0.408	1.000	1.000	1.000	---	0.250	0.408	0.408
T38	0.250	0.408	1.000	0.250	0.612	0.250	0.612	0.612	0.250	0.250	0.250	0.250	---	0.612	0.612
T39	0.250	0.667	0.612	0.408	0.167	0.408	0.167	1.000	0.408	0.408	0.408	0.408	0.612	---	1.000
T42	0.408	0.667	0.612	0.408	0.167	0.408	0.167	1.000	0.408	0.408	0.408	0.408	0.612	1.000	---

[a] Linear correlation coefficient (r). Tabulated value of r = 0.950 (p = 0.05) or 0.990 (p = 0.01).

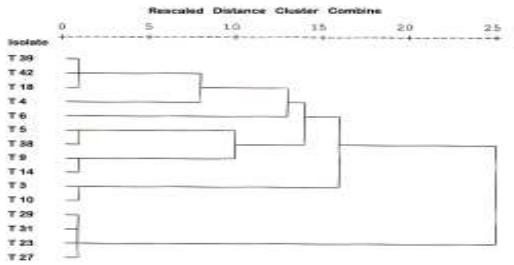

Fig. 6. Phenogram based on average linkage cluster analysis of serological protein patterns obtained by immunoelectrophoresis technique from 15 isolates of *Trichoderma* spp. when their antigens interacted against antiserum of isolate no. T9 from cotton. Identification of the isolates is shown in Table 1.

Immunoelectrophoretic data (Table 8) were established based on immunoelectrophoretically fractionated proteins diagramed in Fig. 7. These data were used for calculating r values shown in Table 9. A phenogram (Fig. 8) was constructed based on TDs generated from cluster analysis of r values. This phenogram was divided into two unrelated clusters. The first one (TD = 23.2) included 10 isolates, while the second one (TD = 11.8) included only five isolates. Although each cluster was composed of a maximum of *T. longibrachiatum* and *T. harzianum* isolates, it was predominated by isolates of one species. Thus, 70% of the isolates in the first cluster were belonging to *T. longibrachiatum*, while 80% of the isolates in the second cluster were belonging to *T. harzianum*

Table 8. Number and distribution of immunoglobulins obtained by immunoelectrophoresis of antiserum of *Trichoderma* isolate no. T31 against antigens of *Trichoderma* isolates from cotton.

Immunoglobulin		Antiserum of isolate no. T31 x antigens of isolate[a] nos.														
No.	**Identification**	**T3**	**T4**	**T5**	**T6**	**T9**	**T10**	**T14**	**T18**	**T23**	**T27**	**T29**	**T31***	**T38**	**T39**	**T42**
1	Haptoglopulin	+[b]	+	-	-	+	-	+	+	+	-	-	-	+	-	-
2	Transferrin	+	+	-	-	-	+	-	-	-	+	-	-	+	+	+
3	α-Lipoprotein	+	+	-	-	-	-	-	+	+	+	+	+	+	+	+
4	α-Macroglubin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
5	Igm Globulin	-	-	+	+	-	+	+	+	-	+	-	+	-	-	-

[a] **Isolate nos. T3, T6, T10, T23, T27, T29, and T31 were *T. harzianum,* while isolates nos. T4, T5, T9, T14, T18, T38, T39, and T42 were *T. longibrachiatum*.**

[b] **Immunoglobulin was present (+) or absent (-).**

(*) Homologous reaction.

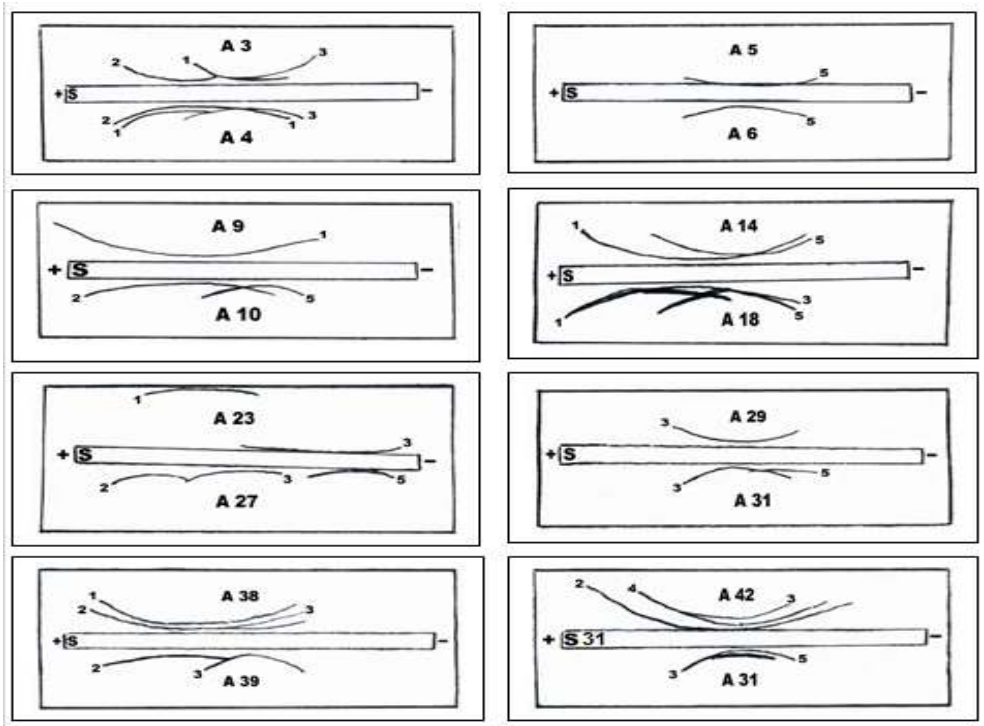

Fig. 7. Diagram of the immunoelectrograms of the fractionated proteins resulting from the reactions of the antiserum (S) of *Trichoderma* isolate no. T31 against antigens (A) of 15 *Trichoderma* isolates from cotton. Identification of *Trichoderma* isolates and the fractionated proteins (immunoglobulins) are shown in Table 8.

Table 9. Correlation among immunoglobulin patterns when antiserum of *Trichoderma* isolate no. T31 interacted against antigens of 15 isolates of *Trichoderma* spp.

Trichoderma isolate	*Trichoderma* isolate														
	T3	**T4**	**T5**	**T6**	**T9**	**T10**	**T14**	**T18**	**T23**	**T27**	**T29**	**T31**	**T38**	**T39**	**T42**
T3	---	1.000	-0.612	-0.612	0.408	-0.167	-0.167	0.167	0.667	0.167	0.408	-0.167	1.000	0.667	0.167
T4	1.000[a]	---	-0.612	-0.612	0.408	-0.167	-0.167	0.167	0.667	0.167	0.408	-0.167	1.000	0.667	0.167
T5	-0.612	-0.612	---	1.000	-0.250	0.612	0.612	0.408	-0.408	0.408	-0.250	0.612	-0.612	-0.408	-0.612
T6	-0.612	-0.612	1.000	---	-0.250	0.612	0.612	0.408	-0.408	0.408	-0.250	0.612	-0.612	-0.408	-0.612
T9	0.408	0.408	-0.250	-0.250	---	-0.408	0.612	0.408	0.612	-0.612	-0.250	-0.408	0.408	-0.408	-0.612
T10	-0.167	-0.167	0.612	0.612	-0.408	---	0.167	-0.167	-0.667	0.667	-0.408	0.167	-0.167	0.167	-0.167
T14	-0.167	-0.167	0.612	0.612	0.612	0.167	---	0.667	0.167	-0.167	-0.408	0.167	-0.167	-0.667	1.000
T18	0.167	0.167	0.408	0.408	0.408	-0.167	0.667	---	0.667	0.167	0.408	0.667	0.167	-0.167	0.167
T23	0.667	0.667	-0.408	-0.408	0.612	-0.667	0.167	0.667	---	-0.167	0.612	0.167	0.667	-0.167	-0.167
T27	0.167	0.167	0.408	0.408	-0.612	0.667	-0.167	0.167	-0.167	---	0.408	0.667	0.167	0.667	0.167
T29	0.408	0.408	-0.250	-0.250	-0.250	-0.408	-0.408	0.408	0.612	0.408	---	0.612	0.408	0.612	0.408
T31	-0.167	-0.167	0.612	0.612	-0.408	0.167	0.167	0.667	0.167	0.667	0.612	---	-0.167	0.167	-0.167
T38	1.000	1.000	-0.612	-0.612	0.408	-0.167	0.167	0.167	0.667	0.167	0.408	-0.167	---	0.667	0.167
T39	0.667	0.667	-0.408	-0.408	-0.408	0.167	-0.667	-0.167	-.167	0.667	0.612	0.167	0.667	---	0.667
T42	0.167	0.167	-0.612	-0.612	-0.612	-0.167	1.000	-0.667	-0.167	0.167	0.408	-0.167	0.167	0.667	---

[a] **Linear correlation coefficient (r). Tabulated value of r = 0.878 (p = 0.05) or 0.959 (p = 0.01).**

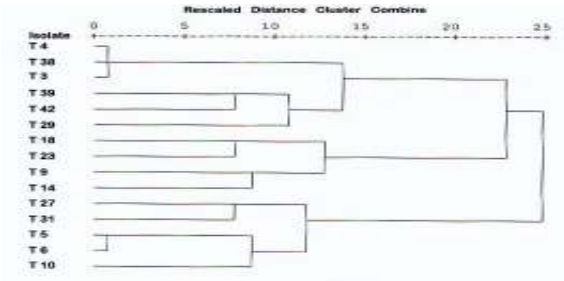

Fig. 8. Phenogram based on average linkage cluster analysis of serological protein patterns obtained by IE technique from 15 isolates of *Trichoderma* spp. when their antigens interacted against antiserum of isolate no.T31 from cotton. Identification of the isolates is shown in Table 1.

DISCUSSION

Each of *T. longibrachiatum* and *T. harzianum* isolates tended to group together based on their antigenic composition, which indicates a remarkable overall serological similarity among isolates of each species. However, the serological differences among the isolates did not always reflect the taxonomic differences because some isolates of *T. longibrachiatum* and *T. harzianum* showed considerable serological similarity. Thus, DD and IE were not useful techniques in identification of *Trichoderma* isolates.

A noteworthy pattern of reactivity could be observed when antigens of some isolates reacted against antisera of isolates T9 of *T. longibrachiatum* and isolates T31 of *T. harzianum*. In both cases the numbers of immunoglobulins obtained by the heterologous reactions against some isolates were greater than those obtained by homologous ones. Two points should be noted regarding these findings. (1) The antigenic nature of the fungus studied is complex and it carries on its surface a multitude of different membrane antigens, some of which may be common or similar in some of the isolates. (2) This study has been carried out using antisera raised *in vivo*, which contain many antibody populations directed against different antigenic epitopes. This polyspecificity of the antisera may explain their cross-reactivity, which in certain instances appears quantitatively to be synergistic (Barak *et al.,* 1985).

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استعمال التفاعلات السيرولوجية للتفرقة بين فطرى تريكوديرما هارزيانم وتريكودرما لونجيبراكياتم المعزولين من جذور القطن

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درست العلاقات السيرولوجية بين عزلات التريكودرما ، وذلك باستعمال طريقتى الإنتشار المزدوج والفصل الكهربى المناعى. استعمل أسلوب التحليل العنقودى لتصنيف هذه العزلات إلى أنواع بناءً على ما بينها من درجات قرابة سيرولوجية ، وتم التعبير عن النتائج فى فينوجرامات . أظهرت النتائج أن عزلات كل من تريكودرما لونجيبراكياتم وتريكودرما هارزيانم كانت تميل إلى التجمع معاً بناءً على محتواها الأنتيجينى مما يدل على أن هناك درجة عالية من التماثل الكلى السيرولوجى بين عزلات كل نوع. رغماً عن ذلك ، لوحظ أن الفروق السيرولوجية بين العزلات لم تكن دوماً متفقة مع علاقتها التقسيمية ، إذ أن بعض عزلات تريكودرما لونجيبراكياتم وتريكودرما هارزيانم أظهرت درجة عالية من التماثل السيرولوجى ، وعلى ذلك يمكن القول بأنه لا يمكن الإعتماد على الإنتشار المزدوج أو الفصل الكهربى المناعى – كأسلوبى تصنيف – للتفرقة بين نوعى التريكودرما.