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USE OF SEROLOGICAL INTERACTIONS TO IDENTIFY THE TELEOMORPH OF *OIDIUM LINI* INVOLVED IN POWDERY MILDEW OF FLAX

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ABSTRACT

Two methods were used to identify the teleomorph of *Oidium lini*. In the first method, antiserum of flax infected with *O. lini* interacted against antigens of healthy plants (flax, okra, sunflower, tobacco, peas, alfalfa, and cocklebur). In the second method, antiserum of flax infected with *O. lini* interacted against antigens of the same hosts infected with powdery mildew. Cluster analysis of the antigenic patterns, obtained in each method, indicates that *Erysiphe polygoni* is the teleomorph of *O. lini*.

INTRODUCTION

Powdery mildew of flax (*Linum usitatissimum* L) is caused by the obligate parasite *Oidium lini* Škoric. The fungus is found on flax in Egypt only in its imperfect (conidial) stage. The pathogen infects all aboveground flax organs including stems, leaves, flowers, and capsules. One obvious gap in our current knowledge of *O. lini* is the lack of consensus regarding the identification of its teleomorph which has been referred to as *Erysiphe polygoni* (McKay, 1947; Dickson, 1956, and Nyval, 1981) or *E. cichoracearum* (McKay, 1947; Dickson, 1956, and Agrios, 1988).

Serology has been widely used to study the relationship between plant pathogens and their hosts and to determine the phylogenetic relationship among pathogens. For example, Charudattan and DeVay (1972) demonstrated a common antigen relationship among *Fusarium* species and both wilt-susceptible and wilt-tolerant varieties of cotton. When the common antigenic substance was isolated and purified, it was found to be a polysaccharide-protein complex. Azad and Shaad (1988) found that preparations of membrane protein complex of *Xanthomonas compestris* pv. *translucens* appeared to contain a highly specific immunogen at the pathovar level. Furthermore, Ouchterlony double diffusion was an effective method for accurate identification of *X. compestris* pv. *translucens*. Hussein *et al.* (1996) compared *Fusarium oxysporum*, *F. moniliforme*, and *F. solani*, isolated from cotton seedlings infected with damping-off, by double diffusion (DD) and immunoelectrophoresis (IE) technique, species were grouped 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. Hussein *et al.* (1997) compared proteins of *Rhizoctonia solani* with those of host and nonhost plants by DD test. Cotton, flax, and kenaf were used as host plants. In the reactions of antiserum of *R. solani* with homologous antigens and plant antigens, among the four bands formed in the homologous reaction, two were common with the antigens of host plant. No common antigens were shared between *R. solani* and any of the nonhost plants. These results tend support to the concept that the greater the antigenic (protein) parity between the host and the pathogen, the greater will be the susceptibility of the host to the pathogen.

The objective of the present study was to identify the teleomorph of *O. lini* by comparing its serological protein patterns with those of *E. polygoni* an *E. cichoracearum*.

MATERIALS AND METHODS

Extraction of proteins from flax seeds

Protein extract was prepared according to Hussein (1992) in the following way: Seeds of healthy plants of flax cultivar Giza 7 were slightly ground and defatted by diethyl either or chloroform 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 19000 rpm for 30 minutes at 0°C. The protein content in supernatant was adjusted to a concentration of 3 to 4 mg/ml according to Bradford spectrophotometric method (1976) by using bovine serum albumin as a standard protein. If protein concentration was low, protein would be precipitated from the clarified supernatant by adding ammonium sulfate at 70% of saturation (60 g/100 ml), then kept in the refrigerator for 30 hours. Pellets, collected by centrifugation at 11000 rpm for 30 minutes were resuspended in phosphate buffer pH 8.3 and subjected to dialysis for 24 hours against the buffer and centrifugation at 11000 rpm for 30 minutes. Native protein was estimated in the obtained supernatant (from 3 to 4 mg/ml).

Extraction of proteins from healthy and powdery mildew infected-hosts

Healthy and infected fresh whole plants of flax, okra, sunflower, tobacco, peas, alfalfa and cocklebur were ground in liquid nitrogen to a fine powder. The grounded plants were suspended in a solution (1-3 ml/g plants) consisting of 12.5 g glucose and 1 g ascorbic acid dissolved in 100 ml phosphate buffer pH 8.3 and centrifuged at 19000 rpm for 30 minutes at 0°C. The protein content in supernatant was adjusted according to Bradford (1976) as previously mentioned.

Immunization and preparation of antisera

New Zealand rabbits, 3-4 kg weight, were immunized by flax antigens (seeds of healthy plants or infected whole plants) to produce antiserum. 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 incomplete 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 animals were bleed 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, subdivided into small samples, placed in serum vials, and stored frozen until the time of use. Antibodies in the obtained antiserum were assayed by double diffusion technique (Hussein, 1992).

Double diffusion technique

The technique was carried out according to Ouchterlony and Nilsson (1978). 1% ionagar (Sigma), melted in saline (8.5 g NaCl to 1.000 ml) and supplemented with merthiolate (1:10.000), was poured into 9-cm diameter petri dishes to obtained a layer of agar 1-2 mm thick. The diameter of the central and of 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 flax antiserum healthy seeds or infected hole plants and the peripheral wells with antigens of healthy or infected plants of flax, okra, sunflower, tobacco, cocklebur, peas, alfalfa. Plates were kept in humid conditions at room temperature (18-24°C) in the dark for one week. Agar was stained with Commassie Brilliant Blue R-250 (Weeke, 1973), the developing precipitin lines were examined and recorded by hand drawing and photography.

Statistical analysis of the data

Simple matching coefficient (SSM) was determined for each pair of hosts (healthy and infected) as described by Sokal and Michener (1958) by the formula: SSM = m/(m+u), where: m = the number of pairs of precipitin lines found in common between the two hosts, and u = the total number of precipitin lines unique to each host. The resulting similarity matrix was subjected to cluster analysis (Joseph *et al.*, 1992) by the average linked technique (unweighted pair-group method).

RESULTS

Figs. 1 and 2 clearly showed the lack of any antigenic relationships between flax and any of the other hosts. Double-diffusion data (Tables 1 and 3) were established based on common antigens shown in Figs. 3-14. These data were used for calculating simple matching coefficients (SSM) shown in Tables 2 and 4. A phenogram (Fig. 15) was constructed based on taxonomic

distances (TD) generated from SSM established among seven healthy hosts (Table 2). The smaller the TD, the more closely related the hosts were. In this phenogram, all hosts susceptible to genus *Erysiphe* belonged to a single cluster (TD = 17). This cluster was subdivided into two subclusters, the first one (TD = 4.8) included all hosts susceptible to *E. polygoni* (alfalfa, peas and flax), while the second one (TD = 0.6) included all hosts susceptible to *E. cichoracearum* (okra, sunflower and tobacco).

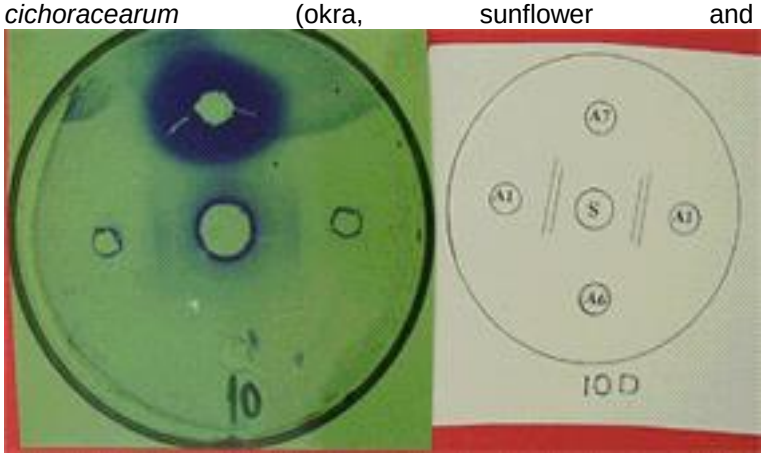

Fig. (1): Photograph (10 P) and diagram (10 D) showing the double-diffusion reactions of the antiserum (S) of flax seeds (in central well) against antigens of healthy host plants (in peripheral wells). Antigens are flax (A1), cocklebur (A6), and Peas (A7).

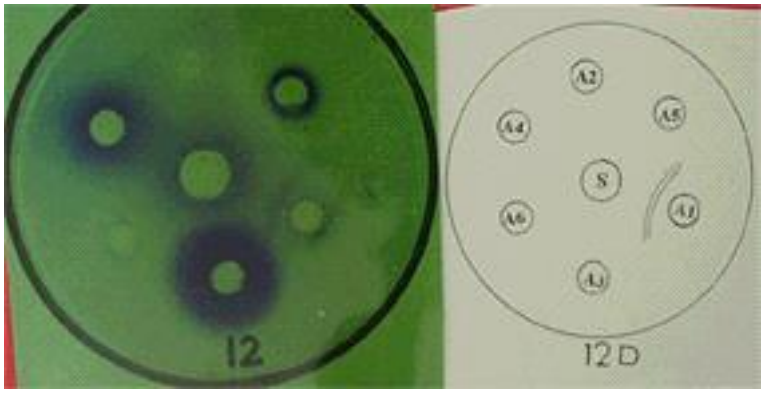

Fig. (2): Photograph (12 P) and diagram (12 D) showing the double-diffusion reactions of the antiserum (S) of flax seeds (in central well) against antigens of healthy host plants (in peripheral wells). Antigens are flax (A1), Sunflower (A2), Alfalfa (A3), okra (A4), Tobacco (A5), and cocklebur (A6).

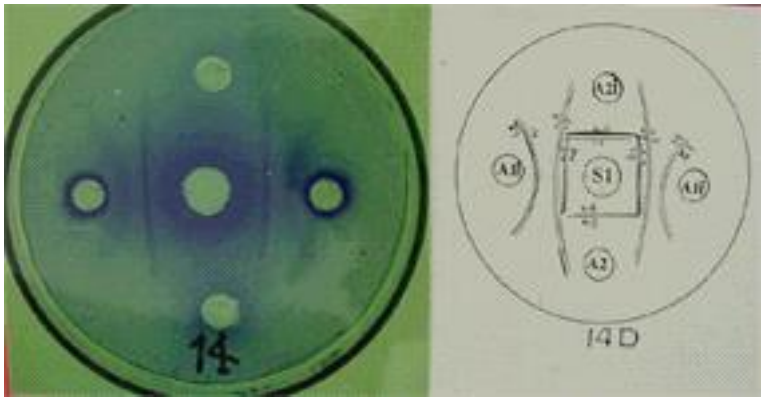

Fig. (3): Photograph (14 P) and diagram (14 D) showing the double-diffusion reactions of the antiserum (S1) of flax infected with powdery mildew (in central well) against antigens of healthy and infected (i) host plants (in peripheral wells). Antigens are flax (A1) and Sunflower (A2).

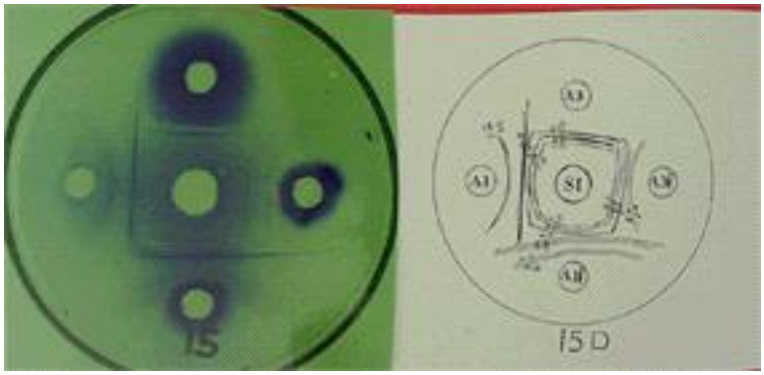

Fig. (4): Photograph (15 P) and diagram (15 D) showing the double-diffusion reactions of the antiserum (S1) of flax infected with powdery mildew (in central well) against antigens of healthy and infected host plants (i) (in peripheral wells). Antigens are flax healthy (A1) and infected (A1i), alfalfa healthy (A3) and infected (A3i).

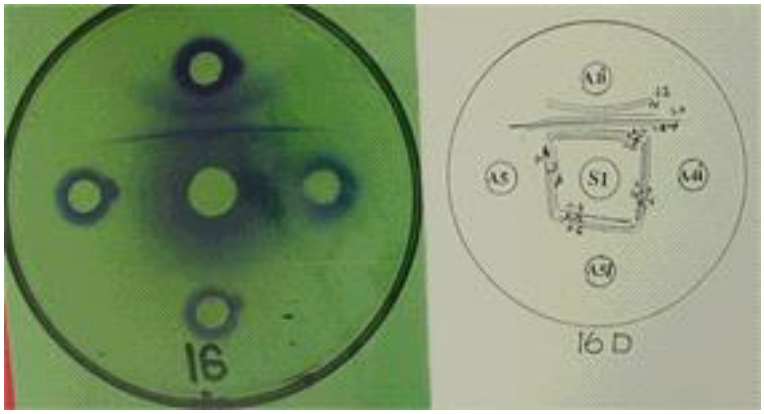

Fig. (5): Photograph (16 P) and diagram (16 D) showing the double-diffusion reactions of the antiserum (S1) of flax infected with powdery mildew (in central well) against antigens of healthy and infected (i) host plants (in peripheral wells). Antigens are flax infected (A1i), okra infected (A4i), tobacco healthy (A5) and infected (A5i).

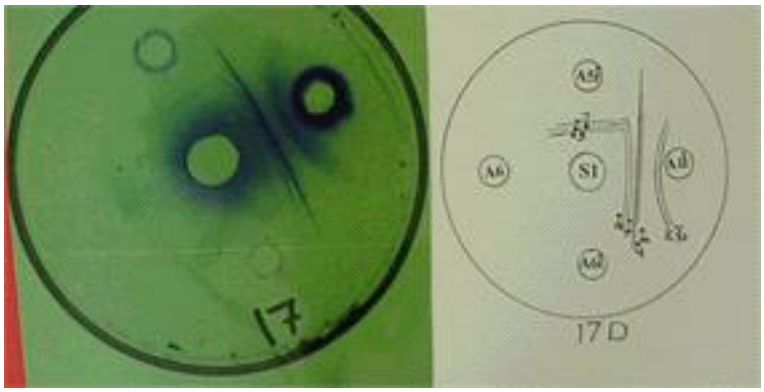

Fig. (6): Photograph (17 P) and diagram (17 D) showing the double-diffusion reactions of the antiserum (S1) of flax infected with powdery mildew (in central well) against antigens of healthy and infected (i) host plants (in peripheral wells). Antigens are flax infected (A1i) cocklebur infected (A6i) and healthy (A6), tobacco infected (A5i).

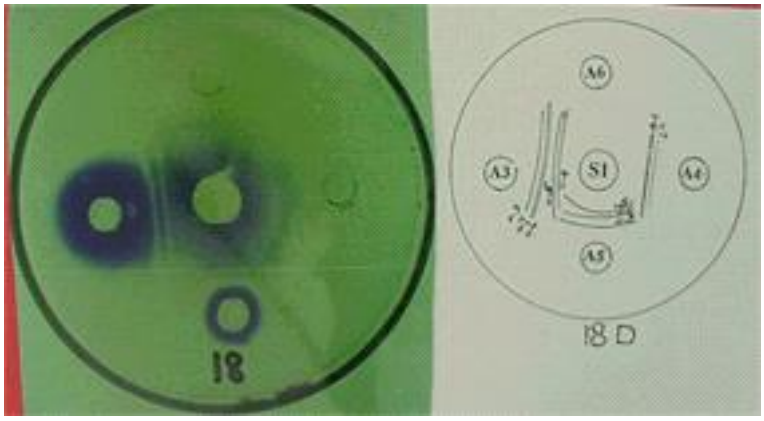

Fig. (7): Photograph (18 P) and diagram (18 D) showing the double-diffusion reactions of the antiserum (S1) of flax infected with powdery mildew (in central well) against antigens of healthy host plants (in peripheral wells). Antigens are okra (A4), tobacco (A5) alfalfa (A3) and cocklebur (A6).

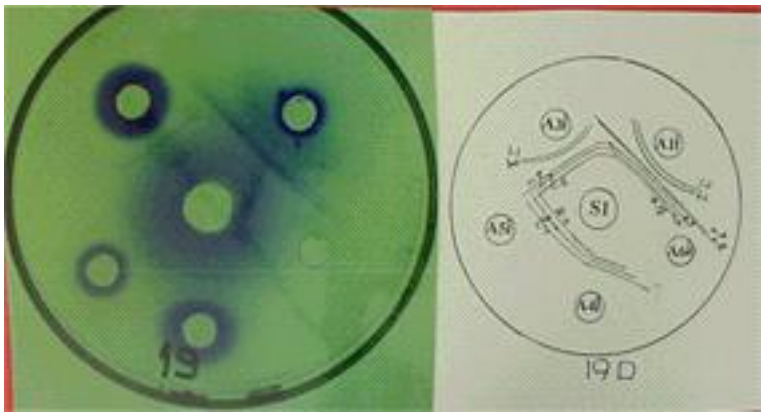

Fig. (8): Photograph (19 P) and diagram (19 D) showing the double-diffusion reactions of the antiserum (S1) of flax infected with powdery mildew (in central well) against antigens of infected (i) host plants (in peripheral wells). Antigens are flax (A1i), cocklebur (A6i), okra (A4i) tobacco (A5i) and alfalfa (A3i)

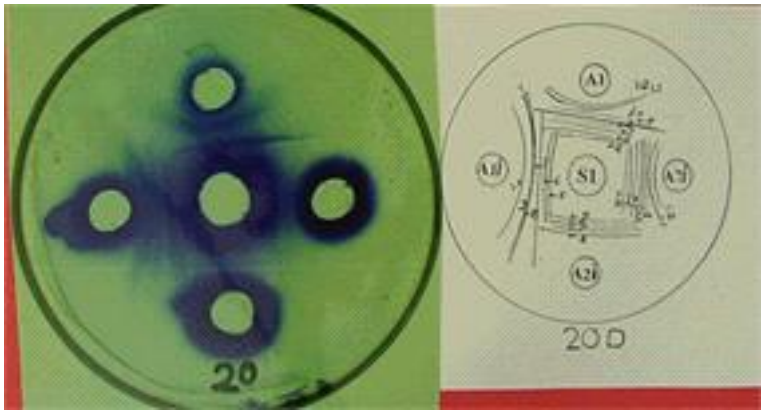

Fig. (9): Photograph (20 P) and diagram (20 D) showing the double-diffusion reactions of the antiserum (S1) of flax infected with powdery mildew (in central well) against antigens of healthy and infected (i) host plants (in peripheral wells). Antigens are flax healthy (A1) and infected (A1i), peas infected (A7i) and infected sunflower (A2i).

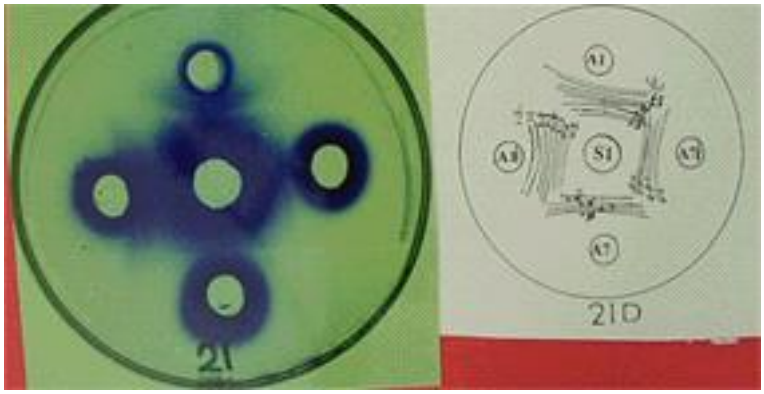

Fig. (10): Photograph (21 P) and diagram (21 D) showing the double-diffusion reactions of the antiserum (S1) of flax infected with powdery mildew (in central well) against antigens of healthy and infected (i) host plants (in peripheral wells). Antigens are flax healthy (A1) and infected (A1i), peas infected (A7i) and healthy (A7).

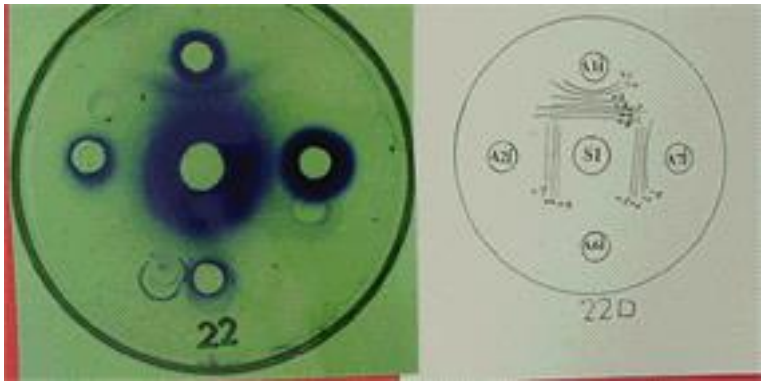

Fig. (11): Photograph (22 P) and diagram (22 D) showing the double-diffusion reactions of the antiserum (S1) of flax infected with powdery mildew (in central well) against antigens of infected host plants (in peripheral wells). Antigens are flax (A1i), peas (A7i), cocklebur (A6i) and sunflower (A2i).

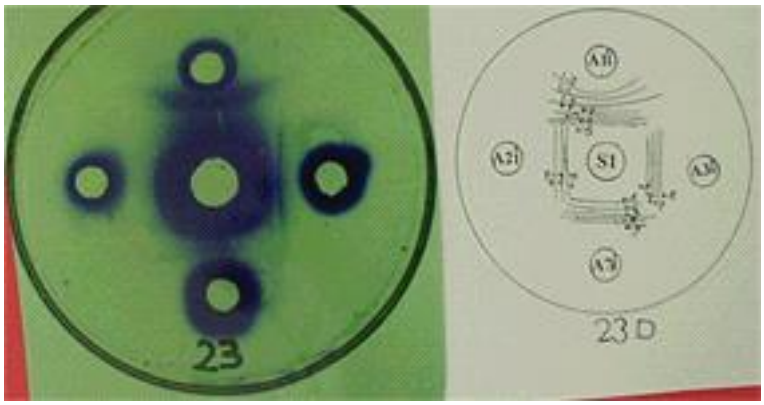

Fig. (12): Photograph (23 P) and diagram (23 D) showing the double-diffusion reactions of the antiserum (S1) of flax infected with powdery mildew (in central well) against antigens of infected host plants (in peripheral wells). Antigens are flax (A1i), alfalfa (A3i), peas (A7i) and sunflower (A2i).

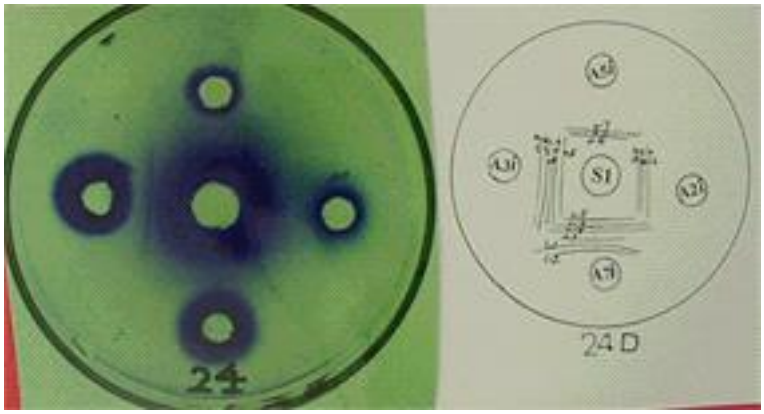

Fig. (13): Photograph (24 P) and diagram (24 D) showing the double-diffusion reactions of the antiserum (S1) of flax infected with powdery mildew (in central well) against antigens of infected host plants (in peripheral wells). Antigens are tobacco (A5i), tobacco (A2i), peas (A7i) and alfalfa (A3i).

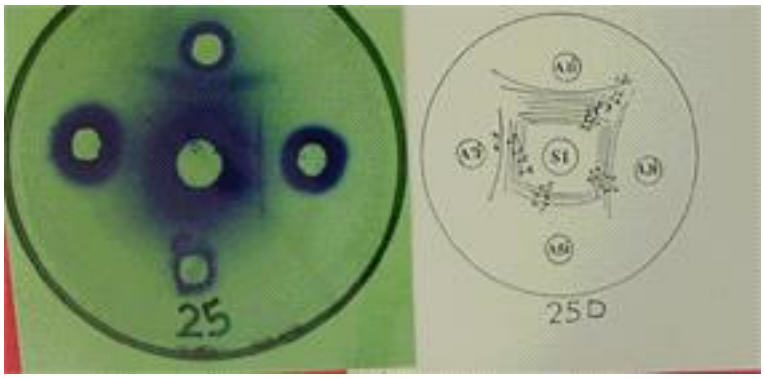

Fig. (14): Photograph (25 P) and diagram (25 D) showing the double-diffusion reactions of the antiserum (S1) of flax infected with powdery mildew (in central well) against antigens of infected host plants (in peripheral wells). Antigens are flax (A1i), alfalfa (A3i), tobacco (A5i) and peas (A7i).

Table (1): Number and distribution of protein fractions obtained during double-diffusion reaction of antiserum of flax infected with powdery mildew against antigens of healthy host plants [a].

Protein fraction No.	Antiserum of infected flax x antigens of healthy						
	Flax*	Peas	Okra	Alfalfa	cocklebur	Sunflower	Tobacco
1	+	+	+	+	-	+	+
2	+	+	+	+	-	+	+
3	+	+	+	+	-	+	+
4	+	+	-	+	-	-	-
5	+	+	-	+	-	-	-
6	+	-	-	-	-	-	-
7	+	-	-	-	-	-	-

[a] **All host plants were susceptible to powdery mildew.**

* **Homologous antiserum - antigen reaction.**

(-) Protein fraction was absent.

(+) Protein fraction was present.

Table (2): Matrix containing simple matching coefficients (SSM)[a] established among seven healthy plants [b] when their antigens interacted against antiserum of flax infected with *O. lini*.

	Host	Host 1	2	3	4	5	6	7
1	Flax	100.00	71.43	42.86	71.43	0.00	42.86	42.86
2	Peas	71.43	100.00	60.00	100.00	0.00	60.00	60.00
3	Okra	42.86	60.00	100.00	60.00	0.00	100.00	100.00
4	Alfalfa	71.43	100.00	60.00	100.00	0.00	60.00	60.00
5	cocklebur	0.00	00.00	0.00	0.00	0.00	0.00	0.00
6	Sunflower	42.86	60.00	100.00	60.00	0.00	100.00	100.00
7	Tobacco	42.86	60.00	100.00	60.00	0.00	100.00	100.00

[a] **Simple matching coefficient (SSM) was determined for each pair of hosts as described by Sokal and Michener (1958) by the following formula:**

$$SSM = \frac{m}{m + u} \times 100$$

where m = the number of pairs of precipitin lines found in common between the two hosts, and u = the total number of precipitin lines unique to each host.

[b] **All hosts were susceptible to powdery mildew.**

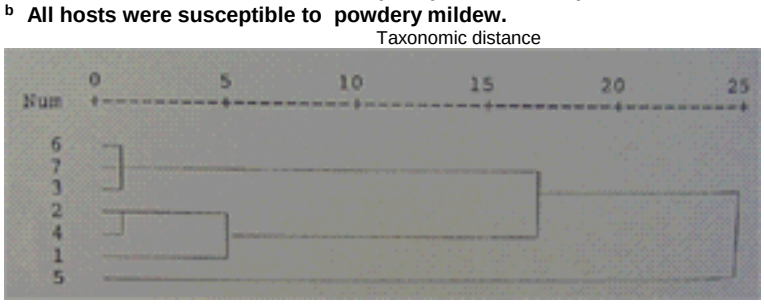

Fig. (15): Phenogram based on average linkage cluster analysis of serological protein patterns obtained by double-diffusion technique from seven healthy hosts when their antigens interacted against antiserum of flax infected with *O. lini*.
Hosts are flax (1), peas (2), okra (3), alfalfa (4), cocklebur (5), sunflower (6), and tobacco (7).

Table (3): Number and distribution of protein fractions obtained during double-diffusion reactions of antiserum of flax infected with powdery mildew against antigens of host plants infected with powdery mildew.

Protein fraction No.	Antiserum of infected flax x antigens of infected						
	Flax*	Peas	Okra	Alfalfa	Cocklebur	Sunflower	Tobacco
1	+	+	+	+	-	+	+
2	+	+	+	+	-	+	+
3	+	+	+	+	-	+	+
4	+	+	+	+	-	+	+
5	+	+	-	+	-	-	-
6	+	+	-	+	-	-	-
7	+	+	-	+	-	-	-
8	+	+	-	+	-	-	-
9	+	-	-	-	-	-	-

* **Homologous antiserum - antigen reaction.**
(-) Protein fraction was absent.
(+) Protein fraction was present.

Cocklebur, which is susceptible to *Sphaerotheca* sp. was found at a separate cluster unrelated to the other cluster, which included *Erysiphe*-susceptible hosts. Fig. 16 showed a phenogram constructed based on TD generated from SSM established among seven hosts infected with powdery mildew fungi (Table 4). In this phenogram, all hosts infected with genus *Erysiphe* belonged to a single cluster (TD = 23). This cluster was subdivided into two subclusters, both at TD = 0.6. One cluster included sunflower, tobacco, and okra, which were infected with *E. cichoracearum*. The other subcluster included peas and alfalfa, which were infected with *E. polygoni*. Flax infected with *O. lini* was found at this cluster. Cocklebur, which was infected with *Sphaerotheca* sp. was found at a separate cluster unrelated to the other clusters, which included *Erysiphe*-infected hosts.

Table (4): Matrix containing simple matching coefficients (SSM)[a] established among seven hosts infected with powdery mildew when their antigens interacted against antiserum of flax infected with *O. lini*.

	Host	Host						
		1	2	3	4	5	6	7
1	Flax	100.00	88.89	44.44	88.89	0.00	44.44	44.44
2	Peas	88.89	100.00	50.00	100.00	0.00	50.00	50.00
3	Okra	44.44	50.00	100.00	50.00	0.00	100.00	100.00
4	Alfalfa	88.89	100.00	50.00	100.00	0.00	50.00	50.00
5	cocklebur	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	Sunflower	44.44	50.00	100.00	50.00	0.00	100.00	100.00
7	Tobacco	44.44	50.00	100.00	50.00	0.00	100.00	100.00

[a] **Simple matching coefficient (SSM) was determined for each pair of hosts as described by Sokal and Michener (1958) by the following formula:**

$$SSM = \frac{m}{m + u} \times 100$$

where m = the number of pairs of precipitin lines found in common between the two hosts, and u = the total number of precipitin lines unique to each host.

Taxonomic distance

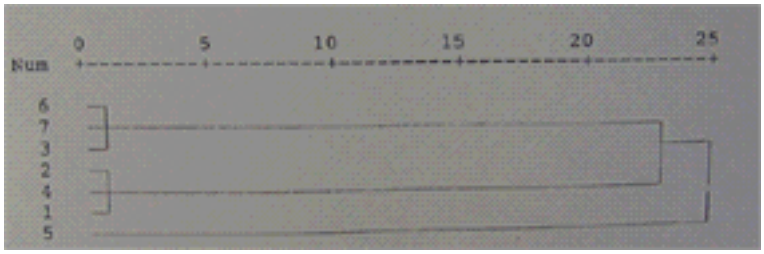

Fig. (16): Phenogram based on average linkage cluster analysis of serological protein patterns obtained by double-diffusion technique from seven hosts infected with powdery mildew when their antigens interacted against antiserum of flax infected with *O. lini*.

Hosts are flax (1), peas (2), okra (3), alfalfa (4), cocklebur (5), sunflower (6), and tobacco (7).

DISCUSSION

Two methods were used to study the serological relatedness between *O. lini* and the anamorphos of some powdery mildew fungi. In the first method, antiserum of flax infected with *O. lini* interacted against antigens of healthy plants. The lack of antigenic relationships between flax and any of the other hosts indicates that the observed bands were attributed only to the antigenic relationship between *O. lini* and the tested hosts - that is, these bands represent the common antigens between *O. lini* and each of the tested hosts. Antigenic patterns resulting from the interaction of *O. lini* against flax were closely related to those resulting from the interaction of *O. lini* against each of alfalfa and peas. It is well established that *E. polygoni* is the causal agent of powdery mildew on alfalfa (Graham *et al.*, 1979) and peas (Farahat, 1980). Therefore, it seems reasonable to conclude that the teleomorph of *O. lini* also belongs to *E. polygoni*.

In the second method, antiserum of flax infected with *O. lini* interacted against antigens of the same hosts infected with powdery mildew. The increase in the number of bands in this method could be ascribed to the interaction of the antiserum of *O. lini* on flax against antigens of the other powdery mildew fungi on the infected hosts. The phenogram constructed by this method is almost identical to that constructed by the first method. Therefore, the previously mentioned conclusions of the first method hold true.

ACKNOWLEDGEMENT

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إستعمال التفاعلات السيرولوجية لتعريف الطور الكامل لفطر أويديم لينـاى المسبب لمرض البياض الدقيقى فى الكتان

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إستعملت طريقتان لتعريف الطور الكامل لفطر أويديم لينـاى. اعتمدت الطريقة الاولى على تفاعل السيرم المحتوى على الأجسام المضادة لكل من الكتان وفطر أويديم لينـاى مع أنتيجينات نباتات خاليـة مـن الإصـابة بالبيـاض الـدقيقى (الكتـان والباميـة وعبـاد الشـمس والـدخان والبسـلة والبرسـيم الحجـازى والشبيط) ، أمـا الطريقـة الثانيـة فقد اعتمدت علـى تفاعـل السيرم المحتوى علـى الأجسـام المضادة لكل من الكتان وفطر أويديم لينـاى مع انتيجينات نفس العوائل بعد إصابتها بالبيـاض الـدقيقى. أظهر التحليل العنقودى للأنماط الأنتيجينية المتحصل عليها فى كل من الطريقتين أن فطر إيريزيف بوليجونـاى هو الطور الكامل لفطر أويديم لينـاى.