Mycobiology 32(1): 35-41 (2004)

Kinds, Abundance and Pathogenicity of *Pythium* Species Isolated from Maize Rhizosphere of Various Habitats in El-Minia Governorate, Egypt

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(Received July 30, 2003)

A total of 374 *Pythium* isolates were isolated and identified from the rhizosphere soils of maize plants grown in 100 different agricultural fields in nine provinces at El-Minia Governorate, Egypt. Five *Pythium* spp. of *P. deliense*, *P. graminicola*, *P. irregulare*, *P. oligandrum* and *P. splendens* were obtained. *P. deliense* and *P. oligandrum* were predominant in all of the locations with 48.1% and 41.4% of total counts, respectively. *P. graminicola*, *P. irregulare* and *P. splendens* were not isolated in 4, 2 and 4 provinces out of 9 provinces with 5.3%, 3.5%, and 1.6% of isolation percentage from total counts, respectively. Number of *Pythium* isolates in each were 34, 31, 34, 33, 34, 96, 37, 37 and 38 out of 374, for locations of El-Edwa, Maghagha, Beni-Mazar, Matai, Samalout, El-Minia city, Abou-Querquas, Mallawi and Der Mawas, respectively. Pre- and post-emergence pathogenicity tests indicated that only *P. deliense* was highly pathogenic to germinating grains and seedlings of maize whereas *P. oligandrum* was non-pathogenic.

KEYWORDS: Egypt, Maize, *Pythium deliense*, *Pythium oligandrum*, Rhizospere soil

Many species of *Pythium* can attack a variety of hosts, and other species appear to be restricted to specific host species (Plaats-Niterink, 1981). *Pythium* was recognized as a major pathogen of Graminae in several countries, and to be reducing yields by almost 30% (Abdelzaher *et al.*, 1997). In Egypt, little information is available concerning losses in maize yield due to infection by *Pythium* despite of its frequent isolation reports from Egyptian soils (Abdelzaher *et al.*, 2000; Elnaghy *et al.*, 2002).

P. deliense was reported to be the main causal agent of maize damping-off in the U.S.A. (Plaats-Niterink, 1981), Germany (Jochems, 1927), and Egypt (Abdelzaher *et al.*, 2000).

In many parts of the world the importance of *Pythium* in the root-disease complexes of the crops has been overlooked. Techniques for isolating fungi from diseased roots often discriminated against *Pythium* and it was frequently missed. However, studies on productivity losses of important commercial crops e.g. maize, wheat, rice and cotton, where feeder root necrosis seems now seen to be the major limiting factor for the production, have focussed their attention back onto *Pythium*. It is now believed that *Pythium* is a major pathogen in these crops and occurrence of these fungi in the rhizosphere of such plants can cause significant yield losses especially when climatic and soil conditions favour its activity (Bouhot, 1988; Abdelzaher *et al.*, 1997).

The aim of this work is to determine the kinds and abundance of various species of *Pythium* in rhizosphere soil of maize plants cultivated in nine provinces in El-Minia Governorate, Egypt and assesses the variability in virulence of the isolates obtained.

Materials and methods

Soil samples. A total of 100 rhizosphere soil samples were used for the isolation of *Pythium* spp., obtained from 100 maize cultivating in nine different provinces belong to El-Minia Governorate, Egypt, namely El-Edwa (10 samples), Maghagha (10 samples), Beni-Mazar (10 samples), Matai (10 samples), Der-Mawas (10 samples), Abou-Qurquas (10 samples), Mallawi (10 samples) and El-Minia city with 10 and 20 samples obtained per province, respectively (Fig. 1).

Fungal isolation.

Fungal isolation was done using two different methods: I-Portions of the soil adjacent to the root system of different maize plants were collected and placed in Petri-dishes containing "vancomycin, penicillin, pentachloronitrobenzene, pimarcin" selective medium (VP^3) (Ali-Shtayeh *et al.*, 1986) for the selective isolation of *Pythium* species, which was composed of (g/l): sucrose, 20; corn meal agar, 17; agar, 23; $CaCl_2$, 0.01; $MgSO_4 \cdot 7H_2O$, 0.01; $ZnCl_2$, 0.001; micro-elements (mg/l): $CuSO_4 \cdot 5H_2O$, 0.02; MoO_3, 0.02; $MnCl_2$, 0.02; $FeSO_4 \cdot 7H_2O$, 0.02; antibiotics (mg/l): pimarcin, 5; vancomycin, 75; penicillin, 50; PCNB, 100, and thiamine-HCl was added at a rate of 100 $\mu g/l$. The emerging hyphal tips from VP^3 medium were transferred to water agar (WA). A small block of

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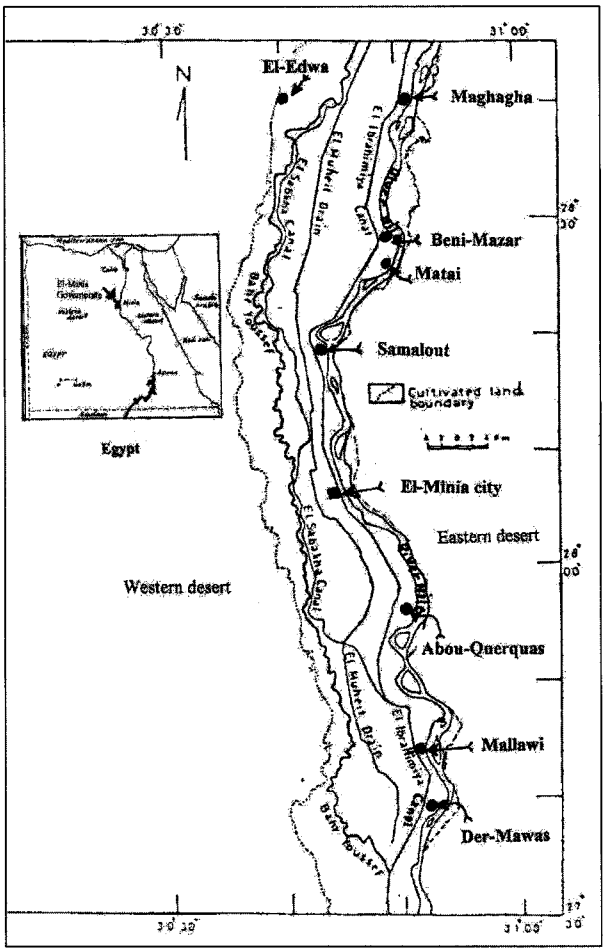

Fig. 1. Location map of samples collected from nine provinces in El-Minia Governorate, Egypt.

agar medium from the distal end of a colony growing in the VP^3 medium was cut and re-inoculated on 2.5~3% WA medium in a Petri-dish to obtain a colony of about 1 cm diameter. The whole agar medium in the Petri-dish was then turned upside-down with a flamed forceps in the same Petri-dish and incubated until the colony reached before the dish wall. During this procedure the non-contaminated mycelia penetrated the agar medium and reached the top of the agar medium. Thin pieces of the agar containing a single hyphal tip of the fungus were taken from the margin of the colony and transferred to corn meal agar (CMA) slants for maintaining the fungus and to CMA plates supplemented with 500 μg/ml wheat germ oil to check the formation of sexual structure. A fungal block taken from the slant culture (after growth for 7 days at 25°C) was placed in Nutrient broth (NB) for confirmation of further bacterial contamination check (Abdelzaher *et al.*, 1994).

II-Rhizosphere soil (5 g) was placed in sterilized 9 cm diam Petri-dishes. Ten ml of sterile water was added to enable the baits to float on the surface (Abdelzaher *et al.*, 1997). Autoclaved cucumber seeds and *Zea mays* leaf discs were used as baits. After 5 days of incubation at 25°C, the baits were removed, washed thoroughly with sterile distilled water and blotted dry with sterile filter paper. Four baits were then placed on the edge of a Petri-dish containing VP^3 (Ali-Shtayeh *et al.*, 1986) for isolation of *Pythium* spp. The plated baits were incubated at 20°C for 3 days or until the appearance of the colonies. Bacterial contamination was removed as described previously.

Identification of *Pythium* spp. To induce sporangial formation, the colonized leaf blade portions of *Zea mays* were placed in Petri-dishes (7 cm diam) containing 10 *ml* of sterile distilled water and incubated at different temperatures (5, 10, 15, 20, 25 and 30°C). Sexual reproduction is commonly abundant in water cultures. Descriptions were made based on comparison of both water cultures and solid agar media such as corn meal agar (CMA) and potato carrot agar (PCA), each supplemented with 500 μg/ml wheat germ oil.

Thirty measurements of each structure were made for each isolate whenever possible. Since structures such as antheridia and sporangia may be formed rapidly and then degenerate, cultures were observed about 12 h after inoculation and then periodically observed for all possible characters.

Identification is principally based on the keys proposed by Plaats-Niterink (1981) and Dick (1990). Keys and descriptions by Waterhouse (1967, 1968) and Middleton (1943) were also consulted for comparison and confirmation of identifications.

Description of *P. deliense* and *P. oligandrum* were based on El-U720 and El-U820, repectively, and they were deposited in the Botany Department, Faculty of Science, El-Minia University, Egypt. Isolation was done on VP^3 medium at 20°C by Hani M. A. Abdelzaher.

Pathogenicity tests.

I-Pre-emergence damping-off: Preparation of inocula, was followed by the modified method of Tojo *et al.* (1993) in which an inoculum concentration of 2.5% was employed. Bent grass seeds (1 g) were moistened by adding 10 *ml* distilled water in 250-*ml* Erlenmeyer flask. After autoclaving at 121°C for 20 min, each flask was inoculated with three discs (7 mm, diam) of water agar containing growing margins of the test *Pythium*. The inoculated bent grass seeds were held at 25°C for 10 days. One gram of colonized bent grass seeds was mixes thoroughly in the Erlenmeyer flask with 50 g of oven-dried (70~80°C for 2 days) clay loam soil using a sterilized mortar and pestle and this mixture (MX) was designated as 100% inoculum density. To obtain 2.5% inoculum concentration 2.5 g of this mixture (MX) were added to 97.5 g of clay sterile loam (CL) soil which was previously ster-

ilized by autoclaving at 121°C for 60 min (pH 7 after autoclaving) and kept in a plastic bag for 2~3 weeks at room temperature with 25% water content prior to use.

Plastic pots (200 *ml* capacity and 7 cm diam) were filled with the soil (300 g) inoculated with each of the fungal isolates and 5 maize grains were sown in each pot and five pots were used for each test.

II-Post-emergence damping-off: Post-emergence damping-off was tested using 5% inoculum concentration in which the colonized bent grass seeds were mixed thoroughly (1 g seeds or grains with 50 g oven dried (70~80°C for 2 days) clay loamy soil) in a sterilized mortar with pestle. Five grams of the mixture (MX) were spread on 95 g CL soil around the seedlings in a plastic pot.

Soil (300 g) with inocula of each fungal isolate was distributed into 5 replicates pots (200 *ml* capacity and 7 cm diam). The inocula were added around the seedlings.

Post-emergence damping-off was determined from the number of diseased plants as a percentage of those emerged. The experiments were repeated twice.

Pre- and post-emergence damping-off experiments were carried out in a growth illuminated cabinet (Precision, United States) at 30°C with 12 h photoperiod (91 μmol m^{-2} s^{-1}) under humid conditions. Pre-emergence damping-off was determined as the difference in emergence between non-inoculated control soil and inoculated soil.

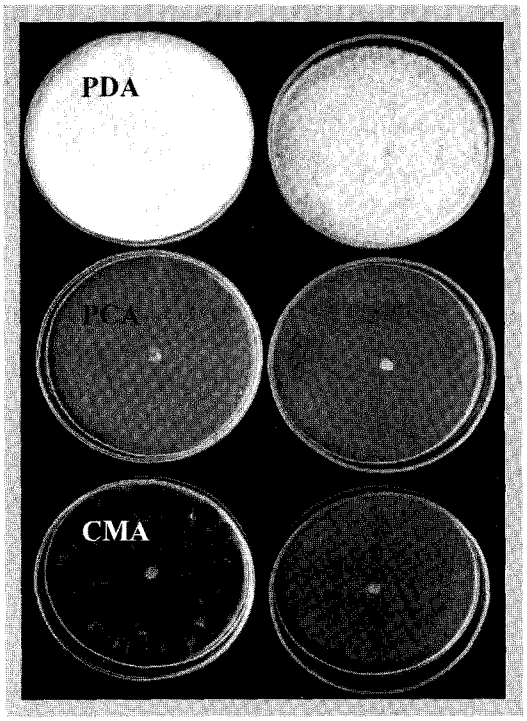

Fig. 2. Colony morphology of *Pythium deliense* El-U720 (left) and *P. oligandrum* El-U820 (right) after 7 days growth at 30°C on PDA, Potato carrot agar and Difco-CMA media.

Results

Occurrence of different *Pythium* spp. in rhizosphere soil of maize plants. A total of 374 *Pythium* isolates were obtained and identified into 5 taxa. Some *Pythium* were not detected from 10 samples. These soils may be free from that *Pythium* or contain very low populations to be detected. Other locations yielded three to five taxa per sample.

Five *Pythium* spp. are listed in Table 1 with numbers of samples yielding the respective fungi in the nine locations assayed. For example, *P. deliense* and *P. oligandrum* were predominant in all of the locations with 48.1% and 41.4% of total counts, respectively. *P. graminicola*, *P. irregulare* and *P. splendens* were not isolated in 4, 2 and 4 out of 9 provinces with 5.3%, 3.5%, and 1.6% of total counts, respectively. Number of isolates of *Pythium* species were 34, 31, 34, 33, 34, 96, 37, 37 and 38 out of 374, for each location in El-Edwa, Maghagha, Beni-Mazar, Matai, Samalout, El-Minia city, Abou-Querquas, Mallawi and Der Mawas, respectively.

Identification of *Pythium* spp. Results of *Pythium* isolation from rhizosphere soil of maize plants indicated that, *P. deliense*, *P. graminicola*, *P. irregulare*, *P. oligandrum*, and *P. splendens* were present in soil adhering to the maize roots grown in nine locations studied. *P. deliense* and *P. oligandrum* were the most abundant pythia and were isolated from all of the samples.

Table 1. *Pythium* species identified and numbers of soil samples yielding the respective fungi in the nine provinces assayed of El-Minia Governorate

Location	El-Edwa	Maghagha	Beni-Mazar	Matai	Samalout	El-Minia	Abou-Qurquas	Mallawi	Der-Mawas	TC%[b]
P. deliense	17	16	18	18	16	47	15	16	17	48.1
P. graminicola	–[a]	–	–	–	1	7	4	5	3	5.3
P. irregulare	1	–	–	1	1	4	1	1	4	3.5
P. oligandrum	15	14	15	14	16	36	17	15	13	41.4
P. splendens	1	1	1	–	–	2	–	–	1	1.6
Number of isolates	34	31	34	33	34	96	37	37	38	374

[a]No *Pythium* detected.

[b]Percentage of total counts.

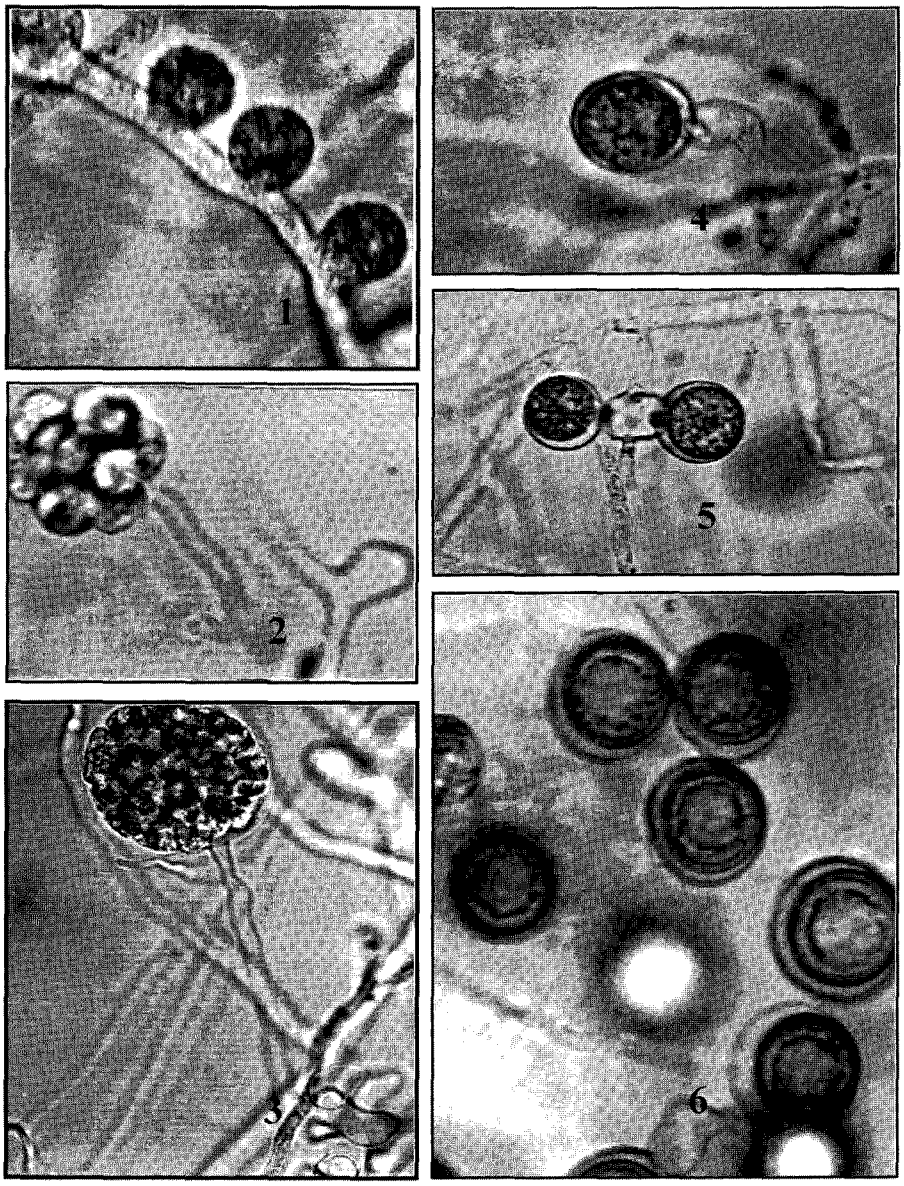

Fig. 3. Morphology of *Pythium deliense* El-U720. 1. Mycelia. 2, 3. Zoosporangia and vesicles. 4, 5. Antheridia and oogonia. 6. Oospores. Bar (20 μm) on photo 2 is applicable to the rest photos.

***Pythium deliense* meurs (Fig. 2 & 3).** Colonies on corn meal agar were formed a radiate pattern with a very little loose aerial mycelium, while on potato-dextrose agar they formed a thick cottony aerial mycelium (Fig. 2). Main hyphae were up to 10 μm wide (Fig. 3, 1). Zoosporangia were characterized by being extended, inflated filamentous structures, often with swollen side branches (Fig. 3, 1-3). Zoospores were formed at 20~25°C of which discharge tubes are of variable length (45~120 μm), mostly terminal (Fig. 3, 2, 3). Encysted zoospores were 8~12 μm, av. 10 μm in diam, and germinated by one germ tube. Oogonia were smooth, mostly terminal, globose 18~25 μm (av. 22 μm) diam, oogonial stalks obviously bended towards the antheridia (Fig. 3, 4). Antheridia were single, rarely two, with a straight stalk, terminal and intercalary, monoclinous, occasionally diclinous and antheridial cells are about 8×8 μm (Fig. 3, 4, 5). Oospores were aplerotic, 16~19 μm (av. 17 μm) in diam, with walls up to 2 μm thick (Fig. 3, 6).

***P. oligandrum* drechsler (Fig. 2 & 4).** Colonies on Bacto-CMA were submerged while on PCA are with no special pattern appeared some aerial mycelium and on Bacto-PDA they showed a chrysanthemum pattern with aerial mycelium at all stages (Fig. 2). Main hyphae were up to 7.5 μm wide and septate when old (Fig. 4, 1). Zoosporangia were spherical or broadly ovoid, 12~28 μm × 3~-67 μm, 20×46 μm on average, contiguous, formed irregular aggregates of one or more subglobose elements with connected filamentous parts, mostly intercalary and

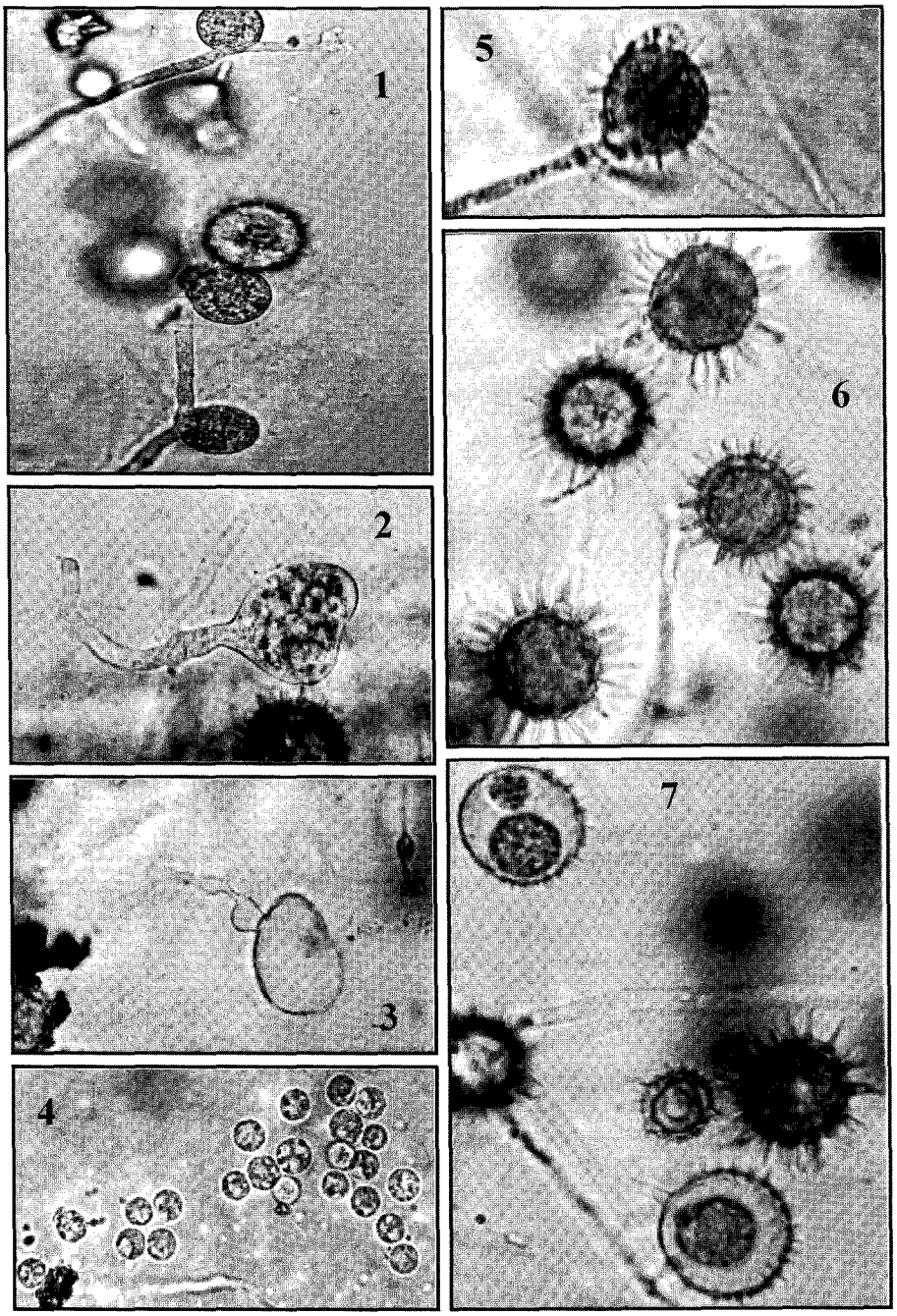

Fig. 4. Morphology of *Pythium oligandrum* El-U820. 1, 2. Young zoosporangia. 3. An empty zoosporangium. 4. Encysted zoospores. 5. An antheridium and an oogonium. 6, 7. Oospores. Bar (20 μm) on photo 7 is applicable to the rest photos.

occasionally were terminal (Fig. 4, 1-3). Germination occured directly by germ tubes or indirectly by zoospores. Zoospores were readily formed after 30 h at 20°C. Encysted zoospores were 8~11 μm in diam (Fig. 4, 4). Discharge tubes had a width of 4~6.5 μm and a length of 12~100 μm (Fig. 4, 2, 3). Oogonia were spherical, terminal or intercalary, 20~30 μm, 25 μm on average, walls with tapering sharply pointed projections, 5~8 μm long, 3~4 μm diam at the base (Fig. 4, 5, 6). Antheridia were mostly lacking, but sometimes 1~2 (-3) per oogonium, diclinous, occasionally monoclinous, rarely hypogenous, often adhering lengthwise to the oogonium, often with one or two transverse constrictions and appeared lobate at the early growth stages of the culture (Fig. 4, 5). Oospores were plerotic, sometimes aplerotic, 19~29 μm, 24 μm on average with wall 1~2 μm in thickness (Fig. 4, 6, 7).

Pathogenicity. Pre-emergence damping-off pathogenicity experiments using maize germinating seeds and seedlings indicated that the three isolates of each of *P. deliense*

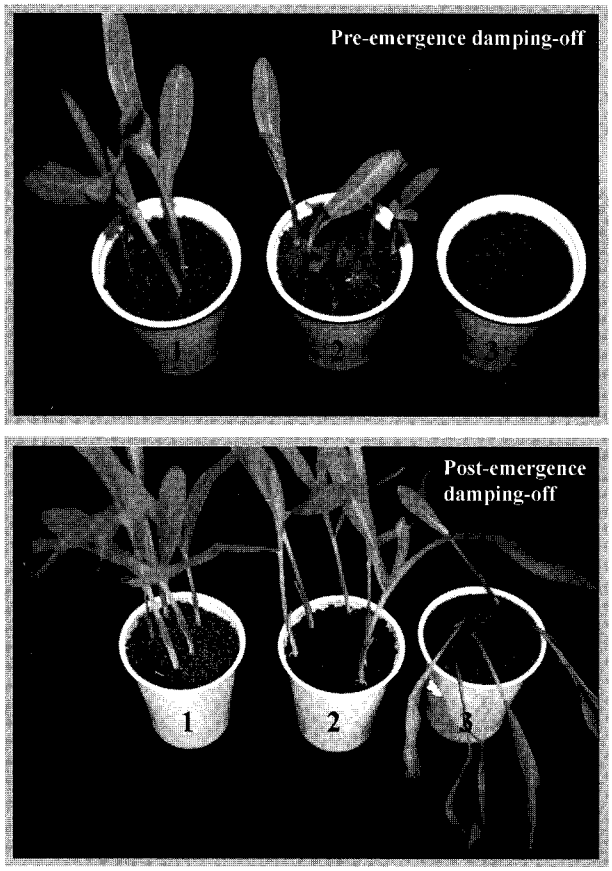

Fig. 5. Pre- and post-emergence of maize seedlings caused by *P. deliense* (3), and *P. oligandrum* (2) isolated from the rhizosphere soils of maize plants. The designation 1, 2, 3 means control (non-infested soil), avirulent *P. oligandrum*, stronghly virulent *P. deliense*, respectively.

and *P. oligandrum* can be divided according to their virulence against the test plant into two categories: *P. oligandrum* is belong to avirulent *Pythium* with 0% damping-off, and strong virulent *Pythium* with 100% damping-off is *P. deliense* (Fig. 5).

Similarly, the post-emergence damping-off test indicated that the two *Pythium* spp. examined according to their virulence using maize plants into two categories as the same as the pre-emergence damping-off test (Fig. 5).

Discussion

In the year of 2000, Abdelzaher *et al.* reported the damping-off disease of maize caused by *Pythium deliense* in a village near El-Minia city, Egypt. They pointed out that *P. deliense* was behind a loss in maize yield during that season (Abdelzaher *et al.*, 2000). They further postulated that *P. deliense* was isolated for the first time in Egypt from the rhizosphere soil and rhizoplane of healthy and infected corn roots. For this reason, studying the occurrence, abundance and pathogenicity of *P. deliense* in the rhizosphere soil of maize is an importance in order to determine the occurrence of such dangerous taxa in the rhizosphere of this important crop.

This study showed that 374 fungal isolates belonging to 5 *Pythium* species were isolated from the rhizosphere soil of maize plants grown in different localities in El-Minia Governorate, Egypt, indicating the widespread occurrence of pythiaceous fungi in the rhizosphere soil of maize.

Our results revealed that *P. deliense* and *P. oligandrum* were predominant in rhizosphere of maize plants in all of the locations studied with 48.1% and 41.4% of total counts of pythia obtained. Other pythia of *P. graminicola*, *P. irregulare* and *P. splendens* were isolated accidentally with 5.3%, 3.5% and 1.6% of total counts of pythia obtained.

Previous studies indicated that *P. deliense* is a typical plant parasite of plants in warm regions. They have been isolated from rhizosphere soil of many healthy and diseased plants such as maize, vegetables and number of ornamentals (Jochems, 1927; Plaats-Niterink, 1981; Mazen *et al.*, 1988). Recently, they have been isolated from maize rhizosphere in Egypt (Abdelzaher *et al.*, 2000; Elnaghy *et al.*, 2002).

P. oligandrum is a soil borne fungus occurring in various climates including tropical countries, central africa, south Australia, Hawaii, USA, Germany, Japan, the Netherlands and Egypt (Plaats-Niterink, 1981; Abdelzaher, 1999). *P. oligandrum* is an aggressive hyperparasite against other fungi and is used as a biocontrol agent against many plant diseases (Abdelzaher *et al.*, 1997; Abdelzaher and Elnaghy, 1998).

In this work, experiments of testing the pathogenicity of three isolates of *P. deliense* and three isolates of *P. oligandrum* indicate that isolates of *P. deliense* were strongly virulent against maize, whereas isolates of *P. oligandrum* showed avirulent against maize plants. Previous studies revealed that *P. deliense* proved to be pathogenic to maize (Plaats-Niterink, 1981) while *P. oligandrum* was avirulent *Pythium* species. Abdelzaher *et al.* (1997) reported that *P. oligandrum* is a mycoparasitic and non pathogenic to maize and it was used as a biocontrol agent against many fungal diseases.

Pythium species can live saprophytically or parasitically. Their parasitic role often depends on the external factors. When the environmental conditions are favorable for growth of the fungus but less for the growth of the host, *Pythium* species become more pathogenic and cause severe damping-off to seeds and seedlings or even attack the feeder roots of the old plants which eventually lead to reduce plant growth (Plaats-Niterink, 1981; Bouhot, 1988). For these reasons, occurrence of *P. deliense* in the rhizosphere soils of maize plant creates a hostile environment to the plant especially when the conditions are more favorable for growth of the fungus than the plant growth.

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