

## **PYTHIUM ULTIMUM VAR. ULTIMUM, A NEW RECORD FROM PAKISTAN**

**A. MUBEEN LODHI, A. QAYOOM, S. SHAHZAD AND A. GHAFAR**

*Pest & Disease Research Lab., Department of Botany,  
University of Karachi, Karachi-75270, Pakistan.*

### **Abstract**

A *Pythium* species without sporangia and zoospores, having terminal as well as intercalary oogonia with a few papilla, monoclinal but occasionally declinal antheridia and aplerotic oospores was isolated from the rhizosphere of date palm. This species is identified as *P. ultimum* var. *ultimum* Trow and appears to be a new record from Pakistan.

### **Introduction**

*Pythium* is a complex genus containing over 120 species that grow in a variety of terrestrial and aquatic habitats (Plaats-Niterink, 1981; Dick, 1990). *Pythium* species such as *P. aphanidermatum*, *P. debaryanum*, *P. myriotylum* and *P. ultimum* cause damping-off, root rot, seedling blight and stem rot of many plants (Kucharek & Mitchell, 2000). *Pythium ultimum* var. *ultimum* was first isolated and described by Trow (1901), who considered it non-parasitic. Lehman & Wolf (1926) identified it as a soybean parasite. *P. ultimum* var. *ultimum* is one of the most common soil borne species that has a worldwide distribution and causes diseases on a number of plants (Plaats-Niterink, 1981; Hancock, 1977; Johnson, 1971). During the present studies on Oomycetous fungi of Sindh, a *Pythium* species isolated from the rhizosphere of date palm growing in Khairpur, Sindh closely resembled *P. ultimum* var. *ultimum* with few exceptions. The culture has been deposited at the Karachi University Culture Collection (KUCC) as KUCC-OOP-03019. It appears to be the first report of *P. ultimum* var. *ultimum* from Pakistan (Sultan *et al.*, 1997; Abdul-Haq & Shahzad, 1998). The species is described and illustrated herein.

### **Materials and Methods**

Soil samples were collected at random from a depth of 0-5 inches of soil from the date palm field and mixed to obtain a composite sample. Isolation was done by baiting technique (Harvey, 1925). Soil sample kept in a polyethylene bag was moistened by the addition of sterile water and mixed thoroughly to get a paste like consistency. With the help of a sterilized teaspoon, the soil was placed at one side in each of the three sterilized Petri plates and 10-15 ml sterilized water was added. Two grass blades (3 cm long) were placed in each Petri plate, one near the soil and the other away from the soil. The Petri plates were incubated at room temperature. After 5-8 days faint halo of fungal threads were observed on the baits. The baits were rinsed in sterilized water to remove soil particles, and placed into fresh sterilized Petri plates half-filled with sterile water and fresh baits were added. After 2 days of incubation, the baits colonized by Oomycetous fungi were transferred on the corn-meal agar (CMA) medium amended with 100 ppm PCNB for purification.

Radial growth and colony characteristics were observed on potato dextrose agar (PDA), corn-meal agar (CMA), potato-carrot agar (PCA) (Plaats-Niterink, 1981) and corn-meal dextrose agar (CMDA) (Lodhi *et al.*, 2004) at 25°C. Water culture of fungi was prepared by adding an inoculum disc and grass blades to sterile water in a Petri plate and incubating at 25°C. Cold shocks for zoospore releases were given by incubating the culture in a refrigerator for a few hours and then incubating at room temperature. The fungus was identified after reference to Sparrow (1960), Plaats-Niterink (1981) and Dick (1990). Biometric values viz., aplerotic index, ooplast index and wall index were determined after Shahzad *et al.*, (1990).

### Morphological characters (Fig. 1)

Main hyphae up to 7µm wide. Sporangia and zoospores not observed. Hyphal swellings of varying size. Oogonia smooth with occasionally a few papilla, globose intercalary as well as terminal (17-)20-24(-26) (av. 22) µm in diameter. Antheridia 1-2 per oogonium, crook necked, closely monoclinal, occasionally declinal, mostly emerging very close to the oogonial stalk. Oospore single, globose aplerotic, (15-)19-22(-24.5) (av. 19.9) µm in diameter. Ooplast 6-10(av. 8.9) µm in diam. Oospore wall thin, 1µm in thickness.

Biometric values: Aplerotic index 72.9%, Ooplast index 13% and Wall index 27.4%.

**Colony characteristics:** The *P. ultimum* var. *ultimum* produces cottony aerial mycelial growth on CMA and PDA, radiate pattern on PCA and thick cottony and superficial growth on CMDA. Daily growth rate at 25 °C on PDA 3.3 cm, PCA 3.7 cm, CMA 4 cm and CMDA 4.7 cm.

### Discussion

In the group of homothallic species of *Pythium* producing smooth walled oogonia and no sporangia and zoospores, there are two species viz., *P. ultimum* var. *ultimum* and *P. violae*. *P. violae* differs from our isolate by having larger oogonia and oospore, more number of antheridia per oogonium and branched antheridial stalk.

Plaats-Niterink (1981) also mentioned that *P. ultimum* var. *ultimum* rarely produces zoospores at 5 °C. Under this condition, within a group of *Pythium* species producing smooth, globose, non proliferating sporangia, our isolate is more close to *P. ultimum* var. *sporangiiferum*, *P. okanoganense*, *P. macrosporum* and *P. irregulare*. Our isolate of *P. ultimum* var. *ultimum* closely resembles *P. ultimum* var. *sporangiiferum* but differs in regular production of sporangia and zoospores, by these species at room temperature. *P. okanoganense* differs from our isolate by producing proliferating sporangia, monoclinal antheridia originating at a distance below the oogonia, swollen antheridial stalk and large, almost plerotic oospores. *P. macrosporum* differs from our isolate by having only crook necked declinal antheridium and larger Oospores. *P. irregulare* is characterized by its irregular, occasionally ornamented oogonia and sessile antheridia.

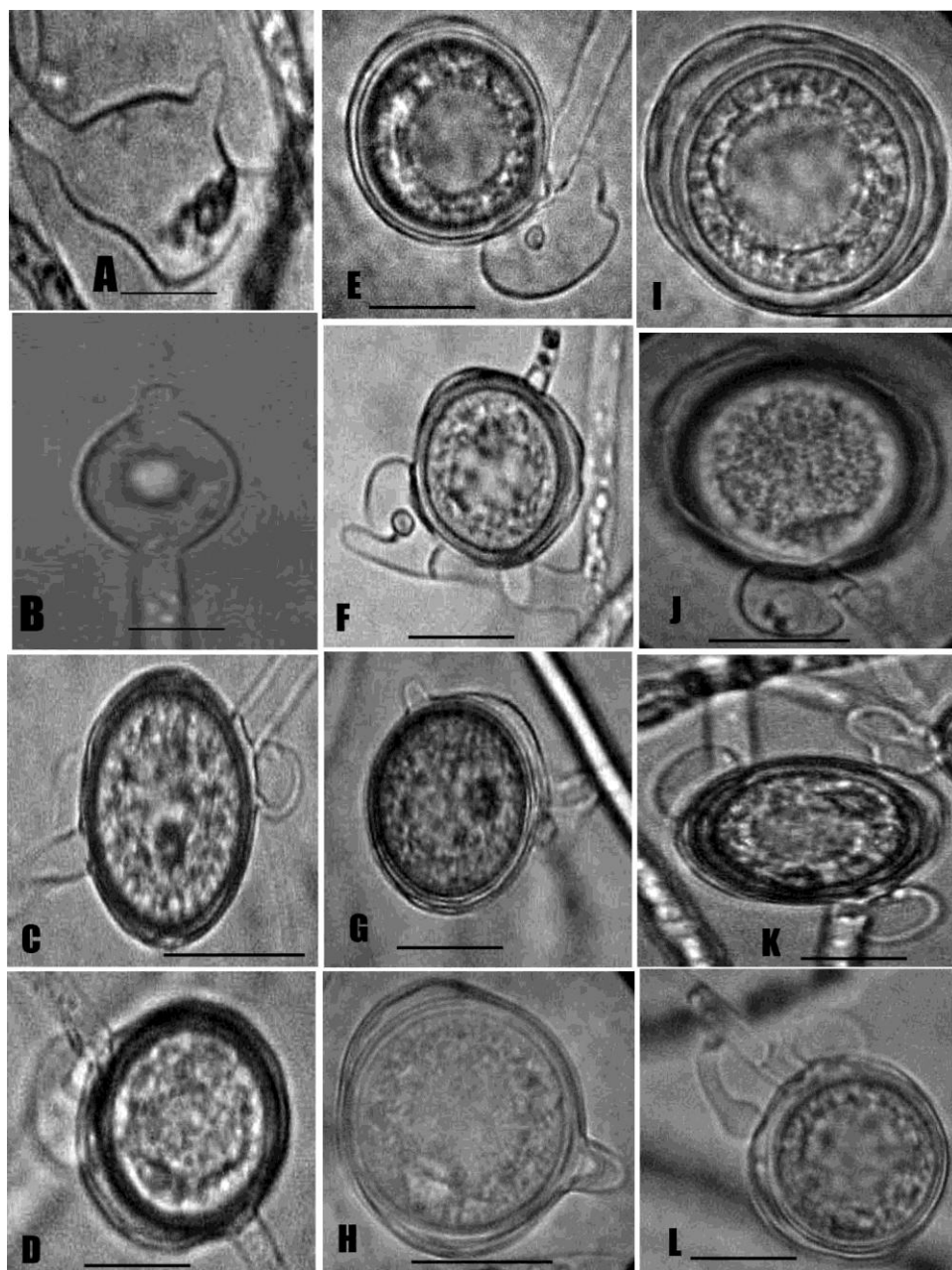


Fig.1. Sexual and asexual structures of *P. ultimum* var. *ultimum*. A & B, hyphal swellings; C-E, intercalary and terminal oogonia with closely monoclinal antheridia; F-H, Oogonia with papillae; I-J, aplerotic Oospore; K-L, closely monoclinal antheridia. Scale bar = 10  $\mu$ m

*P. ultimum* var. *ultimum* has also been viewed as a synonym of *P. debaryanum* (Drechsler 1927; Plaats-Niterink, 1981). Both species have androgynous antheridial branches, but in *P. debaryanum* these branches are long and originate at some distance from the oogonial cell. However, in our isolate mostly monoclinal antheridium originate from immediately below the oogonium. *P. debaryanum* is considered to be a valid taxon by Dick (1990).

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