Pak. J. Bot., 45(2): 635-642, 2013.

PREVALENCE OF *PYTHIUM APHANIDERMATUM* IN AGRO-ECOSYSTEM OF SINDH PROVINCE OF PAKISTAN

A. MUBEEN LODHI[1*], M. ALI KHANZADA[1], S. SHAHZAD[2] AND A. GHAFFAR[2]

[1]*Sindh Agriculture University Tandojam, Pakistan,*
[2]*Department of Agriculture, University of Karachi, Pakistan*
[*]*Corresponding author, e-mail: mubeenlodhi@gmail.com*

Abstract

P. aphanidermatum is cosmopolitan in distribution and one of the most common plant parasitic pathogen of a number of different crop plants in warmer parts of the world. In the present investigation, *P. aphanidermatum* was isolated in very high frequency from different locations of Sindh province and from various sources. The complete ITS region DNA sequence (ITS-1, 5.8S, ITS-2) and partial LSU (D1-D3) of *P. aphanidermatum* consists of 2171 base pairs (bp). ITS-1 consisted of 174 (6-179), 5.8S of 159 (180-338), ITS-2 of 444 (339-782) and partial LSU of 1389 (783-2171) bp. Sequences of all our *P. aphanidermatum* isolates were 99-100% identical except ML1634 which showed 98.2% similarity with ML700. Phylogenetically, *P. aphanidermatum* belong to basal clade A of Lévesque & de Cock (2004). In ITS (ITS-1 & 2 and 5.8S) sequence analysis our isolates of *P. aphanidermatum* were found to be 99-100% identical to sequences of *P. aphanidermatum* deposited in GenBank. In present studies, *P. aphanidermatum* was isolated from millet, banana, cucurbits, coriander, wheat, sugarcane, tomato, papaya, squash, bottle gourd, chilies, guava, betel vine, mung bean, cotton, muskmelon, brinjal, berseem, okra and radish, as well as from water canal and ponds.

Introduction

The *Pythium* species (kingdom Straminopila; phylum Oomycota; class Oomycetes; subclass Peronosporomycetidae; order Pythiales and family Pythiaceae) are worldwide in distribution and associated with a wide variety of habitats ranging from terrestrial or aquatic environments, in cultivated or fallow soils, in plants or animals, in saline or fresh water. The genus *Pythium* is one of the largest oomycete genus and consists of more than 130 recognized species which are isolated from different regions of the world (Plaats-Niterink, 1981; Dick, 1990, de Cock & Lévesque, 2004; Paul *et al*., 2006; Bala *et al*., 2010; Robideau *et al.,* 2011). Some species of *Pythium* are beneficial while most species are known to parasitize and cause infections in the roots of crop plants and ultimately damage them (Plaats-Niterink, 1981; Kucharek and Mitchell, 2000; Hendrix and Campbell, 1973; Krober, 1985). Pythium diseases of vegetables and field crops are considered an important limiting factor in successful cultivation of crop plants throughout the world. It is estimated that diseases caused by *Pythium* species in different crops are responsible for losses of multibillion dollar worldwide (van West *et al*., 2003).

Among the *Pythium* species, *P. aphanidermatum* is cosmopolitan in distribution and one of the most common plant parasitic pathogen of a number of different crop plants in warmer parts of the world. *P. aphanidermatum* is known to cause infection on a wide range of plant species, belonging to different families *viz.,* Amaranthaceae, Amaryllidaceae, Araceae, Basellaceae, Bromeliaceae, Cactaceae, Chenopodiaceae, Compositae, Coniferae, Convolvulaceae, Cruciferae, Cucurbitaceae, Euphorbiaceae, Gramineae, Leguminosae, Linaceae, Malvaceae, Moraceae, Passifloraceae, Rosaceae, Solanaceae, Umbelliferae, Violaceae, Vitaceae, Zingiberaceae (Waterhouse & Waterston ,1964). The diseases caused by the *P. aphanidermatum* varies with the host plant and it may be the causal agent of pre and post emergence damping-off of various seedlings, seedling rots, root rot, cottony-leak, cottony blight, stalk rot etc. Moreover, new host and diseases caused by this destructive pathogen are continuously reported. A significant number of studies on *Pythium aphanidermatum* have been carried out in different parts of the world (Plaats-Niterink, 1981), whereas little work has been done on the host range, pathogenicity and control of diseases caused by *Pythium aphanidermatum* in Pakistan especially in Sindh Province (Shahzad & Ghaffar, 1993; Abdul Haq & Shahzad, 1998; Mushtaq & Hashmi, 1997, Haq *et al.,* 2012). Because *P. aphanidermatum* is an aggressive pathogen at high temperature (Gold & Stanghellini, 1985), it could be widely distributed in the Sindh province agro eco-system, causing severe crop losses without being diagnosed properly. Keeping in view the scarcity of information about the subject, the present studies therefore plan to determine the occurrence and distribution of *Pythium aphanidermatum* in the Sindh agro-ecosystem. The resulting knowledge is not only important for local agriculture but would be of great interest to researchers and taxonomists in other parts of the world.

Materials and Methods

Collection of samples: Soil and water samples were collected at regular intervals from different districts of the province of Sindh, Pakistan. Samples were taken from the rhizospheres of different cultivated and wild plant species, riverside, water canals, water channels, lakes, natural and man-made ponds, fish ponds, road side stagnant water and tube wells. During the survey, algae, root portions, plant leaves and residues, and insects floating on the surface of the water were also collected. The soil core samples were collected at random up to a depth of 12 cm. Samples from a given field collection site were pooled to obtain representative sample. The collected samples were immediately brought to the laboratory for the isolation of *P. aphanidermatum*.

Isolation: *P. aphanidermatum* was isolated from soil and water using different isolation techniques described below. Isolation from plant parts was made by plating the plant pieces on agar medium.

a. Baiting techniques

Petri plate method: The Petri plates method as described by Harvey (1925) was used. Soil samples in polyethylene bags were moistened by the addition of autoclaved water and thoroughly mixed to obtain a paste-like consistency. A sterilized teaspoon was used to take the moistened soil and place it at one side in each of the three sterilized Petri plates, which were then flooded with 10-15 ml of autoclaved water. Two grass blades (3 cm long) were placed in each Petri plate, one near the soil and the other about 6 cm away from the soil. Dicot leaves, hemp seeds, sesame seeds, lemon leaf and young cucumber stems were also used as baits. The Petri plates were incubated at room temperature (about 22-25°C). Faint halo of fungal threads were observed on the baits after 5-8 days of incubation. The baits were rinsed in sterilized water to remove excess soil particles and transferred into fresh sterilized Petri plates half-filled with sterile water. New fresh baits were then added and monitored daily for colonization by *P. aphanidermatum*. After 2 days of incubation, the baits colonized by *P. aphanidermatum* were transferred on corn-meal agar (CMA) medium for purification.

b. Serial dilution method

The serial dilution method as described by Wakesman & Fred (1922) was used. Soil samples (2 g) were weighed and added to 18 ml of 0.1% autoclaved water agar (WA) amended with Streptomycin (0.2 gL^{-1}) and Penicillin (100,000 U L^{-1}) to obtain 1/10 dilution. A series of 1/100, 1/1000, 1/10,00 and 1/100,00 dilutions were prepared by adding 2 ml of a dilution to 18 ml of sterile 0.1% WA using a fresh sterile pipette. One ml aliquot sample from each dilution was plated and spread with sterile glass rod on CMA, potato sucrose agar (PSA) or selective media. The plates were incubated at room temperature (22-25°C) for 3-5 days and growing mycelial colonies were transferred to Petri plates containing WA or PSA.

Purification of cultures

Bacteria-free cultures: Bacteria-free cultures were obtained using the glass-ring method reported by Raper (1937). A glass ring of about 15 mm diameter was placed in the center of 90mm Petri plate and autoclaved. CMA medium was then poured into the plate so as to cover about half of the height of ring. After solidification of the medium, a small piece of agar culture plug was placed into the ring, incubated at room temperature (22-25ºC) and monitored daily. After 24-48 hours of incubation, mycelial mat growing outside the glass ring, on the agar surface, was aseptically transferred onto the surface of a second Petri plate containing CMA medium. Repeating of this procedure produced bacteria-free cultures.

Pure cultures: To obtain a pure culture, 6-8 ml of water agar was poured into Petri plates. After solidification, a small disc of agar culture was placed at the center of the plates containing CMA and incubated at room temperature for 15-24 hours. Tips of growing hyphae were then cut with the aid of microscope in the laminar flow hood and transferred onto the surface of a fresh plate containing CMA medium.

Biometric values: Biometric values for *P. aphanidermatum* were calculated. Aplerotic index, ooplast index and wall index were determined for 20 oogonia with the method described by Shahzad *et al*. (1992).

Identification of isolated fungi: Water cultures of fungi were prepared by adding an inoculum disc and a grass blade to sterile water in a Petri plate and incubating at 25ºC. Oomyceteous species were identified based on structure of sporangia, zoospore and sexual structures as described by Sparrow (1960), Plaats-Niterink (1981), Dick (1990), Johnson (1956), Tucker (1931), Seymour (1970), Erwin & Ribeiro (1996) and Khulbe (2001).

Molecular studies

Extraction of DNA: To obtain DNA of high purity and which could be stored for extended periods of time, FastDNA extraction kit (BIO 101 Systems; QBiogene, Carlsbad, CA, USA) was used. All isolates were cultured in tubes containing 6-ml of potato dextrose broth (Que lab, Canada) and incubated at room temperature (23-26ºC) for 5-10 days to allow for sufficient mycelial mat development. The mycelial mats were removed from the tubes using sterilized forceps and scrappers, blotted on autoclaved filter paper and placed in tubes containing FastDNA extraction matrix. DNA was extracted from the mycelium of 250 isolates following the manufacturer's instructions along with a FastPrep FP120 cell disrupter (QBiogene, USA) as described by Paulitz & Adams (2003). The amount of DNA extracted was determined using NanoDrop Spectophotometer ND-1000 (NanoDrop Technologies, USA). The extracted total DNA was stored at -20ºC for future use.

DNA amplification by Polymerase chain reaction (PCR): The internal transcribed spacers were amplified by PCR in a total volume of 10 µl. A universal forward primer Un-Up 18S42 (5'-CGTAACAAGGTTTCC GTAGGTGAAC-3') and a universal reverse primer Un-LO28S1220 (5'- GTTGTTACACACTCCTTAGCGGAT -3') at final concentration of 0.04 µM were used in a reaction mixture containing a final concentrations of Titanium Taq DNA polymerase (BD Bioscience Clonetech, Palo Alto, USA) 1mM, dNTPs, and 1X Titanium Taq PCR buffer (Allain-Boulé *et al*., 2004). DNA amplification was performed using a MasterCycler ep gradient thermocycler (Eeppendorf, Germany). The PCR profile used to amplify the ITS region included: 1 cycle of 3 min., at 95ºC followed by 40 cycles of 30 sec., at 95ºC, 45 sec., at 68ºC and 2 min., at 72ºC; and a final extension of 8 min., at 72ºC.

In order to evaluate the quality of amplified ITS fragment visual quantification was made by comparison to a DNA low mass ladder (Invitrogen, Carlsbad, CA, USA) after electrophoresis on a 1.5% agarose gel (Seakem LE Agarose, Cambrex Bio Science Rockland, USA) stained with Ethidium bromide (Sigma-Aldrich, Canada). For gel electrophoresis, 1 µl of amplified DNA was loaded on the agarose gel and run in a BRL Horizon 58 (Life Technologies, USA) gel box for 42 minutes at 60 volts. The resulting gel image was captured using the Alphamager 2200 gel documentation unit. (Alpha Innotech, USA). Finally, the amount of DNA amplicons generated was quantified using the Spectophotometer ND-1000 (NanoDrop Technologies, USA).

DNA sequencing: Sequencing reactions were prepared following Big Dye Terminator (BDT) chemistry version 3.1 protocol (Applied Biosystems, Foster City, CA, USA) with the forward and reverse primers to obtain complete ITS sequences. The reaction volume of 10 µl comprised a final concentration of 0.875X BDT Sequencing buffer, 0.125X BDT Sequencing mix 3.1, and 0.1 µM of the primer. About 20 ng of DNA template obtained by PCR were added to the mix. The following profile was used to amplify the DNA: 1 cycle of 3 min., at 95ºC; followed by 40 cycles of 30 sec at 95ºC, 15 sec at 58 ºC and 4 min., at 60 ºC using a MasterCycler ep gradient thermocycler (Eppendrof, Germany). The sequencing reactions were purified by ethanol precipitation re-suspended in formamide then run on an ABI 3130XL genetic analyzer (Applied Biosystems/Hitachi, Foster City, CA, USA). At least two complete sequences were obtained for each ITS region. Sequences were edited using SeqMan II, and MegAlign was used to perform final multiple alignments for phylogenetic analyses, both program being part of the Lasergene DNA and protein analysis software v6.1 (DNASTAR, Madison, WI, USA). A BLAST search was performed on GenBank to find closely related species for analysis. The ITS sequence alignments done with MegAlign were edited manually before the parsimony phylogenetic analysis was performed with PAUP version 4.0b10 for Macintosh (Swofford, 2001).

Results and Discussions

Fungal isolates: During the present studies, *P. aphanidermatum* was isolated in very high frequency from different locations of Sindh province and from various sources that are listed in Table 1.

Table 1. Details about isolates of *Pythium aphanidermatum* isolated during the study.

Strains	Source	Locality of origin
ML311 (68º27′ E, 25º30′ N)	Millet	TandoFazal, District Hyderabad
ML136 (68º30′ E, 25º25′ N)	Banana	Tandojam, District Hyderabad
ML700 (24º54′ E, 67º12′ N)	Cucurbits	Murad Memon, District Malir, Karachi
ML1618 (69º21′ E, 25º30′ N)	Coriander	Digri, District MirpurKhas
ML1713 (68º30′ E, 25º39′ N)	Wheat	Seakhat, District Mityari
ML2038 (68º31′ E, 25º10′ N)	Sugarcane	TandoMohammadKhan, District Hyderabad
ML2044 (68º29′ E, 25º25′ N)	Wheat	TandoMohammadKhan, District Hyderabad
ML76 (24º58′ E, 67º10′ N)	Tomato	Gadap, Karachi
ML104 (24º55′ E, 67º12′ N)	Papaya	Murad Memon Malir, Karachi
ML117 (24º56′ E, 67º14′ N)	Squash	Murad Memon Malir, Karachi
ML119 (24º55′ E, 67º14′ N)	Bottle gourd	Murad Memon Malir, Karachi
ML192 (68º31′ E, 25º26′ N)	Water canal	Tandojam, District Hyderabad
ML294 (68º26′ E, 25º24′ N)	Chillies	TandoFazal, District Hyderabad
ML342 (68º26′ E, 25º24′ N)	Guava	TandoFazal, District Hyderabad
ML395 (68º38′ E, 25º45′ N)	Betelvine	TandoAdam, District Sangher
ML437 (67º06′ E, 24º55′ N)	Guava	Safari Park, Karachi
ML516 (68º07′ E, 24º56′ N)	Mung bean	Karachi University, Karachi
ML647 (68º32′ E, 24º 41′ N)	Chillies	Aderolal, District TandoAllahaYar
ML671 (68º32′ E, 24º41′ N)	Cotton	Aderolal, District TandoAllahaYar
ML699 (24º56′ E, 67º14′ N)	Pond	Malir, Karachi
ML705 (24º54′ E, 67º13′ N)	Cucurbits	Malir, Karachi
ML955 (68º28′ E, 25º47′ N)	Water channel	BhitShah, District NawabShah
ML959 (68º30′ E, 25º41′ N)	Wheat	Kheber, District NawabShah
ML985 (68º29′ E, 26º51′ N)	Musk melon	TahruShah, District Naushero Feroz
ML1007 (68º29′ E, 26º51′ N)	Brinjal	TahruShah, District Naushero Feroz
ML1026 (67º59′ E, 26º38′ N)	Chillies	Moro, District Naushero Feroz
ML1032 (68º58′ E, 26º30′ N)	Wheat	Doulatpur Safan, District Naushero Feroz
ML1047 (68º05′ E, 26º18′ N)	Coriander	Qazi Ahmed, District NawabShah
ML1054 (68º05′ E, 25º35′ N)	Wheat	Sajawal, District Thatta
ML1088 (68º10′ E, 26º19′ N)	Berseem	Qazi Ahmed, District NawabShah
ML1107 (68º15′ E, 26º08′ N)	Chillies	Sakrand, District NawabShah
ML1110 (68º26′ E, 25º49′ N)	Okra	Hala, District NawabShah
ML1115 (68º30′ E, 25º41′ N)	Bottle gourd	Kheber, District Hyderabad
ML1121 (68º06′ E, 26º18′ N)	Radish	Qazi Ahmed, District NawabShah
ML1127 (68º02′ E, 26º24′ N)	Tomato	Daulatpur Safan, District NausheroFeroz

Morphological characters: Mycelium non-septate, hyaline, branched, up to 8 µm wide. Sporangia filamentous inflated making toruloid structures of varying width and length. Abundant zoospores formed at relatively high temperatures i.e., 25-35ºC. Encysted zoospores 8-12 µm in diameter. Oogonia smooth, globose, mostly terminal, (-21.5)23-25(-26.5) (av. 24.85) µm in diameter. Oospores highly aplerotic, 20-23 (av. 21.29) µm in diameter. Ooplast 8.5-12 (av. 10.0) µm in diameter. Oospore wall 1-2 (av. 1.65) µm thick. Antheridia 1-2 per oogonium, monoclinous and diclinous, typically intercalary occasionally terminal, bell shaped, making broad apical contact with the oogonium (Figs. 1 & 2).

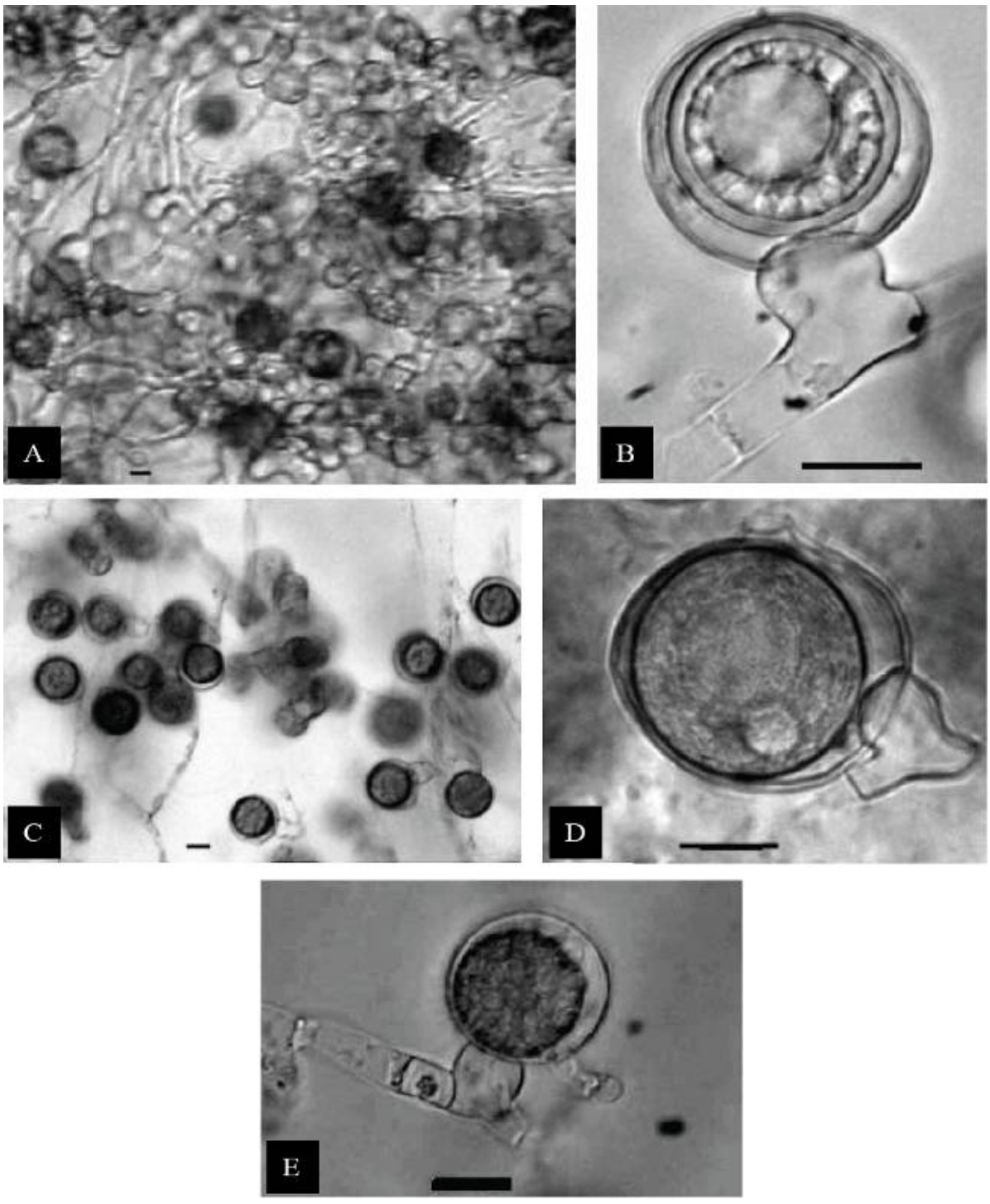

Fig. 1. *Pythium aphanidermatum*: (A) toruloid sporangia; (B & D) oogonia with intercalary antheridia and aplerotic oospore; (C) oogonia in water culture; (E) immature oogonium with intercalary antheridium. Scale bar = 10 µm

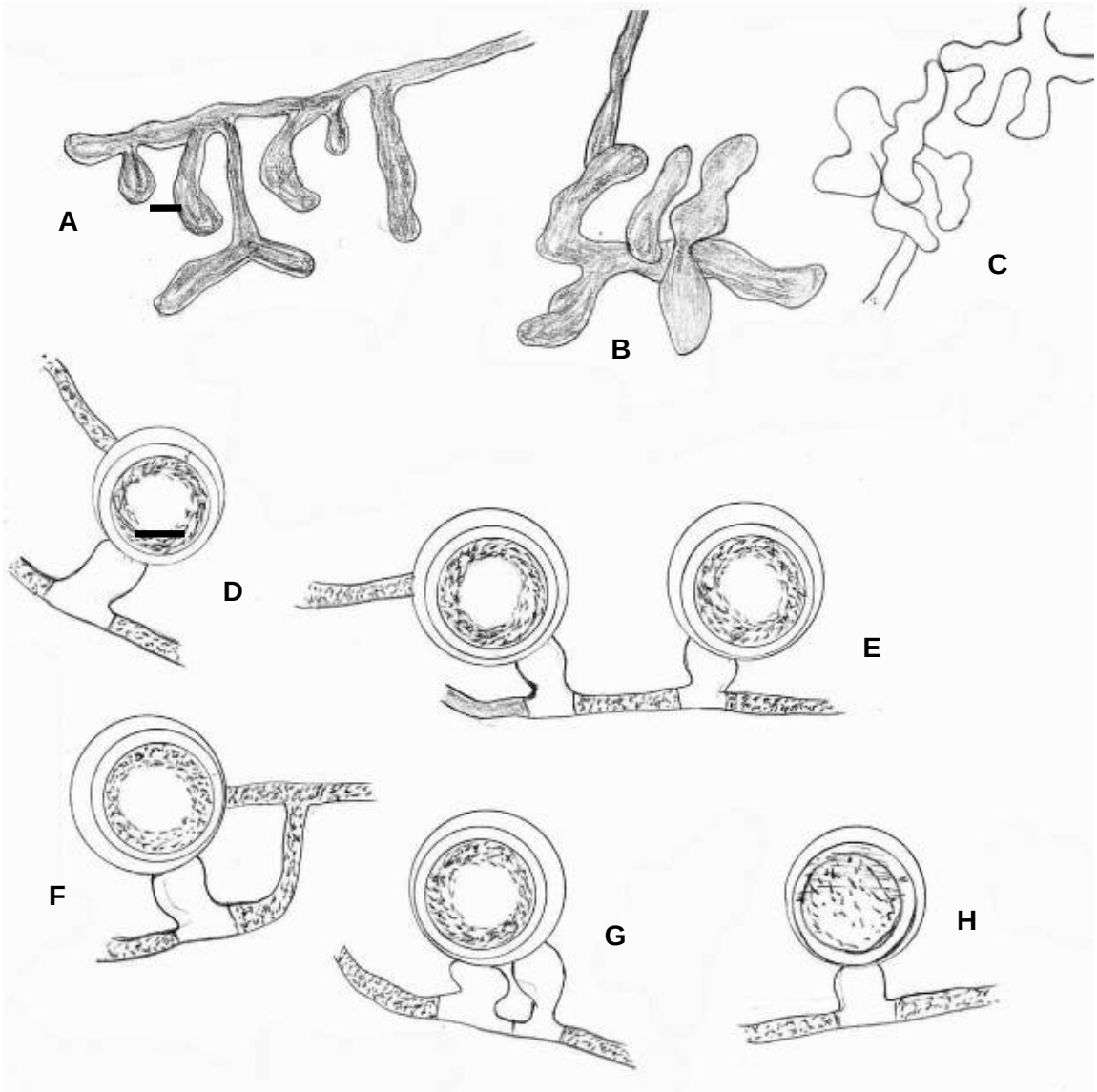

Fig. 2. *Pythium aphanidermatum*: (A-C) filled and empty sporangia; (D-H) oogonia with intercalary antheridia and aplerotic oospore. Scale bar = 10 µm

Biometric values: Aplerotic index 63%, ooplast index 17.5% and wall index 39.5%.

Colony characteristics: *P. aphanidermatum* produces light aerial growth on CMA without any special pattern, on PDA and CMDA thick white cottony growth and on PCA submerged mycelium. Daily growth rate at 25ºC: 51.5mm on CMA, 52 mm on PCA, 62 mm on PDA and 72 mm on CMDA. On CMA, the optimum temperature for growth 25-40ºC, minimum <15ºC and maximum >40ºC.

Sequence analysis (ML700): The complete ITS region DNA sequence (ITS-1, 5.8S, ITS-2) and partial LSU (D1-D3) of *P. aphanidermatum* consists of 2171 base pairs (bp). ITS-1 consisted of 174 (6-179), 5.8S of 159 (180-338), ITS-2 of 444 (339-782) and partial LSU of 1389 (783-2171) bp. Sequences of all our *P. aphanidermatum* isolates were 99-100% identical except ML1634 which showed 98.2% similarity with ML700. Phylogenetically, *P. aphanidermatum* belong to basal clade A of Lévesque & de Cock (2004). In ITS (ITS-1 & 2 and 5.8S) sequence analysis our isolates of *P. aphanidermatum* were found to be 99-100% identical to sequences of *P. aphanidermatum* deposited in GenBank (Fig. 3A). One of our strains ML1634 and one from GenBank GI 28883555 were slightly different from the representative strains of *P. aphanidermatum* as well as *P. deliense*. The ITS sequence of *P. butleri* (GI 2863080) perfectly matched with ITS sequences of our isolates of *P. aphanidermatum.* Lévesque & de Cock (2004) and Wang & White (1997) also designated *P. butleri* as the synonym of *P. aphanidermatum*. In LSU rDNA sequence analysis all of our isolates were 99.8-100% identical except ML1634, which had 99.4% homology to the representative strains. There were about 100% homologies between our isolates of *P. aphanidermatum* and GenBank representative strain GI 51235476 (Fig. 3B). There was only 1 bp difference in between LSU sequences of ML700 and GI 51235476.

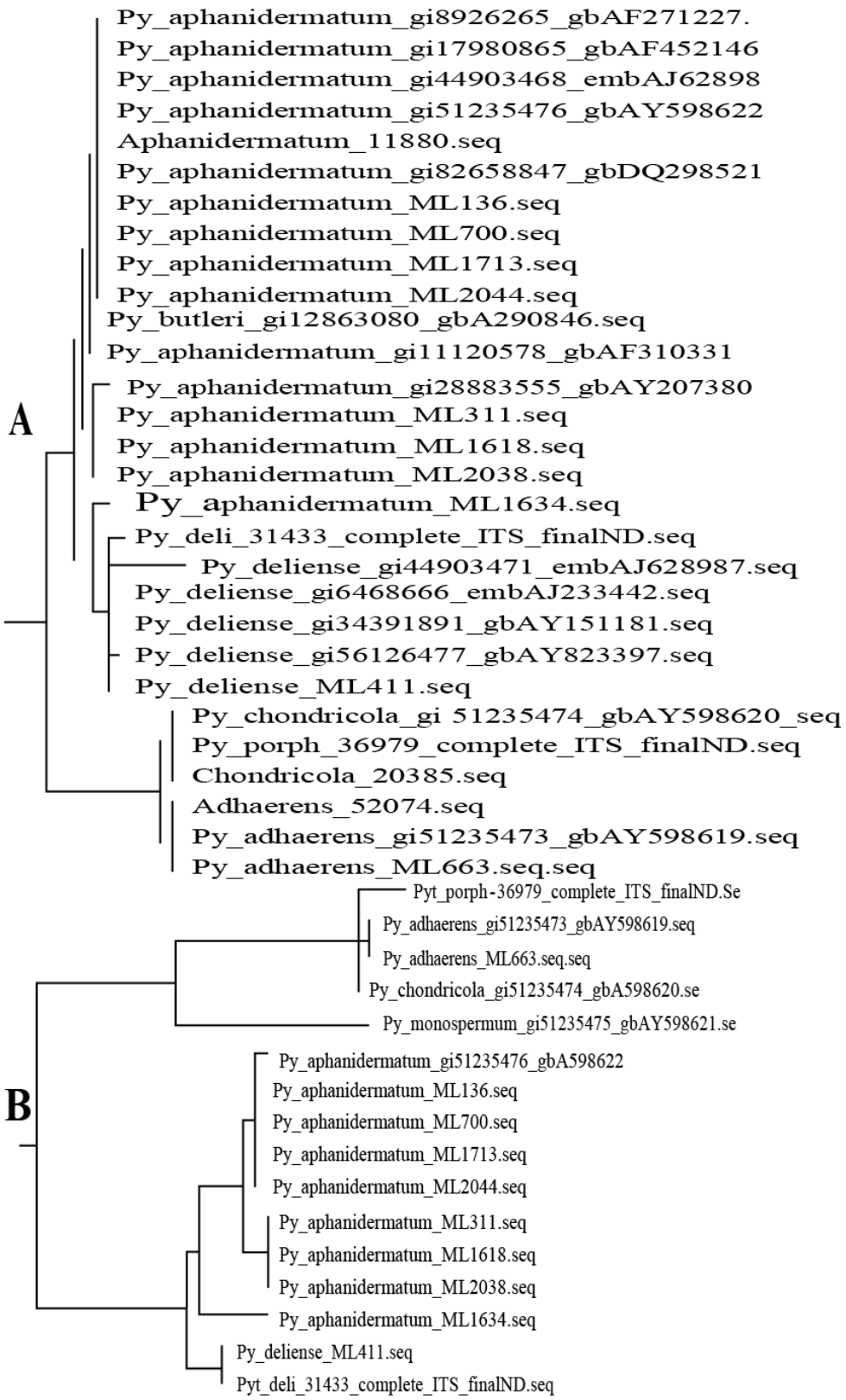

Fig. 3. Trees of *Pythium* species from clade A using some *Phytophthora* species as outgroups. The part of overall tree constituting clade A is presented. Our isolates are given in bold letters. (A) One out of 10,000 (maximum set) most parsimonious trees is shown after a heuristic search from ITS (1 and 2) and the 5.8S gene of nuclear rDNA (Length= 4511, CI = 0.476, RCI = 0.439, and RI =

0.923). (B). One out of 10,000 (maximum set) most parsimonious trees is shown after a heuristic search from LSU rDNA (Length= 1355, CI = 0.431, RCI = 0.391, and RI = 0.909).

The three *Pythium* species *viz., P. aphanidermatum*, *P. deliense* and *P. indigoferae* were characterized by possessing the toruloid sporangia, highly aplerotic oospores and intercalary antheridia (Plaats-Niterink, 1981). Our isolate differ from *P. deliense* and *P. indigoferae* that in both species oogonial stalks curve towards the antheridia. Also, the sporangium of *P. aphanidermatum* is more complex than those of *P. deliense.* The size of oogonia (24.85 µm) and oospores (21.29 µm) of our isolate were also greater to those of *P. deliense* (oogonia 21.9 µm and oospore 17.0 µm) and *P. indigoferae* (oogonia 10-20 µm and oospore 18.0 µm). Morphologically, our isolate closely resemble to the *P. aphanidermatum* and therefore identified as such for now (Plaats-Niterink, 1981). If more strains are found, a multigene phylogeney would be required to determine if this is a different species.

P. aphanidermatum has been reported to cause diseases in a large number of crop plants in warmer parts of the world. It has also been isolated from different areas of the world such as root rot of vegetable pea in Australia (Lin *et al*., 2002), foot rot of ulluco in Japan (Tomioka *et al*., 2002), leaf rot of *A. fasciata* in China (Rongyi *et al*., 2003), rot on *Brassica campestris* chinensis group in Japan (Tanina *et al*., 2004), damping-off, vascular wilt and root rot of groundnuts in Australia (Bellgard & Ham, 2004), damping-off of cauliflower in India (Sharma & Sain, 2005), damping-off in fenugreek in India (Mehra, 2005), rot of leaves, stems and roots of *B. rubra* in Japan (Tojo *et al*., 2006), root and stem rot of poinsettia in Argentina (Palmucci & Grijalba, 2007), stem canker of *Amaranthus caudatus* in Argentina (Noelting & Sandoval, 2007), cottony leak of scarlet runner bean in Japan (Aoki *et al*., 2007), soft rot ginger in India (Kavitha & Thomas, 2007), damping-off of cucumber in Oman (Deadman *et al*., 2007), root and crown rot of melon plants in Honduras (Cara *et al*., 2008), root and crown necrosis of adult bean plants in Spain (Serrano *et al*., 2008), *Pythium* rot of figmarigold in Japan (Kawarazaki *et al*., 2008), rhizome rot of ginger in India (Sagar *et al*., 2008), damping-off and root rot of soybean in USA (Rosso *et al*., 2008), damping-off of chilli in India (Saha *et al*., 2008), damping-off of *Cucumis melo* in China (Juan *et al*., 2009), damping-off disease in tobacco seedbeds in India (Subhashini & Padmaja, 2009), watermelon "sudden death" of greenhouses in Spain (Guirado *et al*., 2009), post-emergence damping-off in chili in India (Muthukumar *et al*., 2009), rhizome rot disease to turmeric crops in India (Radhakrishnan & Balasubramanian, 2009), damping-off disease of *Aquilaria agallocha* seedlings in India (Tabin *et al*., 2009), root rot of turf grass in China (Juan *et al*., 2009) and damping-off of cabbage seedlings in Japan (Kubota, 2010).

The diseases caused by *P. aphanidermatum* are not commonly recognized in Pakistan. It appears that sufficient work has not yet been taken up in Pakistan especially in Sindh. The present study revealed that *P. aphanidermatum* is widely distributed in the cultivated soils of Pakistan and may be causing severe crop losses to various crops that should be looked into.

Acknowledgements

We are grateful for the technical support and scientific advice provided by Nicole Désauniers and C. André Lévesque from Agriculture and Agri-Food Canada, Ottawa.

References

Abdul Haq, M. and S. Shahzad. 1998. Oomycetes from soil of Bajour Agency, FATA, Pakistan. *Pak. J. Bot.*, 30: 305-306.

Allain-Boulé, N., R. Tweddel, M. Mazzola, R. Bélanger and C.A. Lévesque. 2004. *Pythium attrantheridium* sp. nov. taxonomy and comparison with related species. *Mycological Research,* 108: 795-805.

Aoki, K., M. Tojo, K. Watanabe, S. Uzuhashi and M. Kakishima. 2007. Cottony leak of scarlet runner bean caused by *Pythium aphanidermatum*. *J. General Plant Pathology*, 73(6): 408-410.

Bala, K., G. Robideau, A.W.A.M. de Cock, Z.G. Abad, A.M. Lodhi, S. Shahzad, A. Ghaffar, M.D. Coffey and C.A. Lévesque. 2010. *Phytopythium* Abad, de Cock, Bala, Robideau, Lodhi & Lévesque, *gen. nov*. *Persoonia*, 24: 136-137.

Bellgard, S. and C. Ham. 2004. *Common Diseases of Peanuts in the Top end of the NT (Arachis hypogaea* L). Department of Primary Industries and Fisheries, Northern Territory of Australia.

Cara, M.de., V. López, M. Santos and J.C. Tello Marquina. 2008. Association of *Pythium aphanidermatum* with root and crown rot of melons in Honduras. *J. Plant Disease*, 92(3): 483.

de Cock, A.W.A.M. and C.A. Lévesque. 2004. New species of *Pythium* and *Phytophthora*. *Studies in Mycology*, 50: 481-487.

Deadman, M., J. Perret, S. Al-Jabri, Y. Al-Maqbali, A. Al-Sa'di, K. Al-Kiyoomi and H. Al-Hasani. 2007. Epidemiology of damping-off disease in greenhouse cucumber crops in the Sultanate of Oman. *Acta Horticulturae*, 731: 319-326.

Dick, M.W. 1990. *Keys to Pythium.* Department of Botany, School of Plant Sciences, University of Reading, Reading, U.K.

Erwin, D.C. and O.K. Ribeiro. 1996. *Phytophthora Diseases Worldwide*. American Phytopath. Soc., St. Paul, Minn.

Guirado, M.L., Y. Sáez, E. Serrano and J. Gómez. 2009. Aetiology watermelon "sudden death" of greenhouses in the southeast of Spain. *Boletín de Sanidad Vegetal, Plagas*, 35(4): 617-628.

Inam-ul-Haq, M., S. Mehmood, H.M. Rehman, Z. Ali and M.I. Tahir. 2012. Incidence of root rot diseases of soybean in Multan Pakistan and its management by the use of plant Growth promoting rhizobacteria. *Pak. J. Bot*., 44(6): 2077-2080.

Gold, S.E. and M.E. Stanghellini. 1985. Effects of temperature on pythium root rot of spinach *Spinacia oleracea* grown under hydroponic conditions. Phytopathology, 75: 333-7.

Harvey, J.V. 1925. A study of the water molds and *Pythiums* occurring in the soil of Chapel Hill. *J. of Elisha Mitchell Scientific Society*, 41: 151-164.

Hendrix, F.F. and W.A. Campbell. 1973. *Pythiums* as plant pathogens. *Ann. Rev. of Phytopathology*, 11: 77-98.

Johnson, T.W.Jr. 1956. *The Genus Achlya*: *Morphology and Taxonomy*. Univ. Michigan Press: *Ann Arbor*. 180 pp.

Juan, Z., Q.H. Xue and M. Tang. 2009. Screening of antagonistic actinomycetes against 'Jiashi' *Cucumis melo* L. damping-off. *J. Northwest A & F University - Natural Science Edition,* 37(5): 144-148.

Kavitha, P.G. and G. Thomas. 2007. Evaluation of Zingiberaceae for resistance to ginger soft rot caused by *Pythium aphanidermatum* (Edson) Fitzp. *J. Plant Genetic Resources Newsletter,* 152: 54-57.

Kawarazaki, H., Y. Nara, T. Kijima and M. Goto. 2008. Pythium rot of figmarigold (*Lampranthus spectabile*) caused by *P. aphanidermatum*. *J. Gen. Pl. Pathol.,* 74(1): 94-95.

Khulbe, R.D. 2001. *A Manual of Aquatic Fungi (Chytridiomycetes & Oomycetes)*. Delhi, India: Daya Publishing House. 255 p.

Krober, H. 1985. *Erfahrungen mit Phytophthora de Bary und Pythium Pringsheim*. Biologische Bundesanstalt fur Land- und Forstwietschaft, Institut fur Mikrobiologie, Berlin-Dahlem, 225 pp.

Kubota, M. 2010. Diseases of cabbage plug seedlings in Japan and control of the diseases. *Bulletin of the National Institute of Vegetable and Tea Science,* 9: 57-112.

Kucharek, T. and D. Mitchell. 2000. *Diseases of agronomic and vegetable crops caused by Pythium.* Plant Pathology Fact Sheet, University of Florida; p. 53, http://128.227.207.24/takextpub/FactSheet/pp53.pdf.

Lévesque, C.A. and A.W.A.M. de Cock. 2004. Molecular phylogeny and taxonomy of the genus *Pythium*. *Mycologial Research*, 108: 1363-1383.

Lin, Y.S., Y.H. Gung and J.H. Huang. 2002. Control of *Pythium* root rot of vegetable pea seedlings in soilless cultural system. *ISHS*, 578: 221-229.

Mehra, R. 2005. Seed spices: diseases and their management. *Indian J. of Areca nut, Spices & Medicinal Plants,* 7(4): 134-143.

Mushtaq, M. and M.H. Hashmi. 1997. Fungi associated with wilt disease of *Capsicum* in Sindh. *Pak. J. Bot*., 29(2): 217-222.

Muthukumar, A., A. Eswaran and K. Sanjeevkumar. 2009. Effect of different soil types on the incidence of chilli damping-off incited by *Pythium aphanidermatum*. *Agricultural Science Digest*, 29(3): 215-217.

Noelting, M.C.I. and M.C. Sandoval. 2007. First report of stem canker affecting *Amaranthus caudatus* in Argentina. *J. Australasian Plant Disease Notes*, 2(1): 5.

Palmucci, H.E. and P.E. Grijalba. 2007. Root and stem rot caused by *Pythium aphanidermatum* on poinsettia in a soil-less culture system in Buenos Aires Province, Argentina. *J. Australasian Plant Disease Notes*, 2(1): 139-140.

Paul, B., K. Bala, L. Belbahri, G. Calmin, E. Sanchez-Hernandez and F. Lefort. 2006. A new species of *Pythium* with ornamented oogonia: morphology, taxonomy, ITS region of its rDNA, and its comparison with related species. *FEMS Microbiology Letters*, 254: 317-323.

Paulitz, T.C. and K. Adams. 2003. Composition and distribution of *Pythium* communities in wheat fields in eastern Washington State. *Phytopathology*, 93: 867-873.

Plaats-Niterink, A.J. Van Der. 1981. *Monograph of the Genus Pythium*. Studies in Mycology No. 21. Baarn, Netherlands: Centraal Bureau Voor Schimmelcultures.

Radhakrishnan, N. and R. Balasubramanian. 2009. Salicylic acid induced defence responses in *Curcuma longa* (L.) against *P. aphanidermatum* infection. *J. Crop Prot.*, 28(11): 974-979.

Raper, J.R. 1937. A method of freeing fungi from bacterial contamination. *Science*, 85: 342.

Robideau, G.P., A.W. De Cock, M.D. Coffey, H. Voglmayr, H. Brouwer, K. Bala, D.W. Chitty, N. Désaulniers, Q.A. Eggertson, C.M. Gachon, C.H. Hu, F.C. Küpper, T.L. Rintoul, E. Sarhan, E.C. Verstappen, Y. Zhang, P.J. Bonants, J.B. Ristaino and C.A. Lévesque. 2011. DNA barcoding of oomycetes with cytochrome c oxidase subunit I and internal transcribed spacer. Mol. Ecol. Resour., 11(6):1002-11.

Rongyi, Z., T. Zhiqiong and C. Shanying. 2003. First report of leaf rot caused by *Fusarium oxysporum* and *Pythium aphanidermatum* on *Aechmea fasciata* in Hainan Province, China. *Plant Disease*, 87(5): 599.

Rosso, M.L., J.C. Rupe, P.Y. Chen and L.A. Mozzoni. 2008. Inheritance and genetic mapping of resistance to Pythium damping-off caused by *Pythium aphanidermatum* in 'Archer' soybean. *J. of Crop Science,* 48(6): 2215-2222.

Sagar, S.D., S, Kulkarni and Y.R. Hegde. 2008. Survey, surveillance and etiology of rhizome rot of ginger in Karnataka. *J. Plant Disease Sciences*, 3(1): 37-39.

Saha, S., I, Naskar, D.K. Nayak and M.K. Sarkar. 2008. Efficacy of different seed dressing agents in the control of damping off disease of chilli caused by *Pythium aphanidermatum*. *J. of Mycopathological Research*, 46(1): 121-123.

Serrano, Y., M.L. Guirado, M.P. Carmona, J. Gómez and J.M. Melero-Vara. 2008. First report of root and crown necrosis of bean caused by *Pythium aphanidermatum* in Spain. *J. of Plant Disease*, 92(1): 174.

Seymour, R.L. 1970. The genus *Saprolegnia*. *Nova Hedwigia (Beiheft)*, 19: 1-124.

Shahzad, S. and A. Ghaffar. 1993. New record of *Pythium* species in Pakistan. *Pak. J. Bot*., 25: 237-238.

Shahzad, S., R. Coe and M.W. Dick. 1992. Biometry of oospores and oogonia of *Pythium* (Oomycetes): the independent taxonomic value of calculated ratios. *Botanical Journal of the Linnean Society*, 108: 143-165.

Sharma, P. and S.K. Sain. 2005. Use of biotic agents and abiotic compounds against damping off of cauliflower caused by *P. aphanidermatum*. *J. Indian Phytopath.,* 58(4): 395-401.

Sparrow, F.K. 1960. *Aquatic Phycomycetes*. Second Revised Edition. University of Michigan Press, Ann Arbor, Michigan, U.S.A.

Subhashini, D.V. and K. Padmaja. 2009. Exploitation of *Pseudomonas fluorescens* for the management of damping-off disease of tobacco in seedbeds. *Indian Journal of Plant Protection*, 37:147-150.

Swofford, D.L. 2001. *PAUP*: Phylogenetic analysis using parsimony (*and other methods).* 4 edn. Sinauer Associates, Sunderland, MA.

Tabin, T., A. Arunachalam, K. Shrivastava and K. Arunachalam. 2009. Effect of arbuscular mycorrhizal fungi on damping-off disease in *Aquilaria agallocha* Roxb. seedlings. *J. of Tropical Ecology*, 50(2): 243-248.

Tanina, K., M. Tojo, H. Date, H. Nasu and S. Kasuyama. 2004. Pythium rot of chingensai (*Brassica campestris* L. chinensis group) caused by *Pythium ultimum* var. *ultimum* and *Pythium aphanidermatum*. *J. of General Plant Pathology*, 70(3): 188-191.

Tojo, M., K. Yonemoto and A. Kawamura. 2006. First report of *Pythium aphanidermatum* on Basella rubra in Japan. *J. of Plant Disease*, 90(6): 830.

Tomioka, K., T. Sato and T. Nakanishi. 2002. Foot rot of ulluco caused by *Pythium aphanidermatum*. *J. of General Plant Pathology*, 68(2): 189-190.

Tucker, C.M. 1931. *Taxonomy of the Genus Phytophthora*. Univ. Mo. Agric. Exp. Stn. Res. Bull., 153.

van West P., A.A. Appiah and N.A.R. Gow. 2003. Advances in research on Oomycete root pathogens. *Physiological & Molecular Plant Pathology*, 62: 99-113.

Wakesman, S.A. and E.B. Fred. 1922. A tentative outline of the plate method for determining the number of microorganisms in the soil. *Soil Science*, 14: 27-28.

Wang, P.H. and J.G. White. 1997. Molecular characterization of *Pythium* species based on RFLP analysis of the internal transcribed spacer region of ribosomal DNA. *Physiological & Molecular Plant Pathology*, 51: 129-143.

Waterhouse, G.M. and G.M. Waterston. 1964. *Pythium aphanidermatum*. [*Descriptions of Fungi and Bacteria*]. CAB International. 4: Sheet 36.

(Received for publication 8 December 2011)