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# Occurrence and distribution of indigenous isolates of Pythium species in northern India

## Abstract

Twelve different species of Pythium were isolated from different soil types, water types, vegetables and ornamentals of Delhi. Pythiumultimum and P. vexans were common in soil than in water. P. middletonii was more common in water than soil followed by P. deliense. The rhizosphere samples collected from the nursery bed of chilli showed the maximum diversity of Pythium species, out of the 12 species studied. The ornamentals like marigold, canna and chrysanthemum were more proned to Pythium. The occurrence of Pythium species in soil, water, vegetable and ornamentals fluctuated with seasonal variations. Results obtained from the taxonomic investigations showed that out of 12 species of Pythium studied except in P. afertile, P. catenulatum and P. graminicola where the characters of sporangia were tallied with the original description and while rest of the species did not tallied. Description of the other species of Pythium were amended accordingly. In the case of P. aphanidermatum, P. aquatile, P. debaryanum, P. dissotocum, P. diclinus mostly occurred in several localities of Delhi. The size of the oogonia found to be larger than reported in literature. The filamentous sporangia of P. afertile which was similar to vegetative hyphae differed from the original description by its increase in length. Intercalary catenulate oogonia with papilla of P. dissotocum was recorded for the first time. P. graminicola was very well predominent in the samples from irrigation channels mostly near by the paddy fields, conforming the previous reports. The isolates of P. middletonii obtained during summer season produced bigger sporangia than those of winter and rainy season isolates. P. ultimum and P. vexans was mostly similar in their globose nature of sporangia, which was recorded for the first time from the area of Delhi, but they differed in their nature of oogonia and antheridial attachment.

**Keywords:** *pythium*, oomycetes, fungal characterization, *pythium* morphology

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## Introduction

The genus *Pythium* was erected by Pringsheim in 1858 and placed in the family Saprolegniaceae. By the end of 19th century taxonomic details of the genus had been clarified and many new species had been described. The relationship with other Oomycetes was established and the genus was included in a new family, Pythiaceae, by Schroter in 1897. The fungus *Pythium* belongs to the subdivision Mastigomycotina, class Oomycetes, order Peronosporales and family Pythiaceae. The genus, its taxonomical position, and relationship to other fungi have remained unchanged since that time. There are almost 307 described *Pythium* species (www.mycobank.org) which are classified in the Kingdom Straminopila.[1] The genus perception of *Pythium* is still a continuing debate and there is no harmony on the alternative genera.[2–4] Several other researchers made an attempt to work on *Pythium* spp. into other genera based on the sporangial shapes.[5]

*Pythium* are soil inhabiting organisms that are cosmopolitan in nature. *Pythium* is generally considered a primitive parasite because it attacks juvenile or succulent tissues of young seedlings. The fungus quickly attacks seeds and emerging radicles causing seed decay and pre-emergence damping-off respectively. It also attacks young seedlings above the soil level causing post-emergence damping-off. Such symptoms can be prominently seen in nursery beds, greenhouse and row crops because the mortality is sudden and fast.[6,7] *Pythium* spp. are generally considered to be root pathogens, but occasionally, some species of this fungus also causes severe foliage bight, root necrosis of higher plants. Middleton JT[8] reported that about 60 species of this organism have the potential to initiate infection in higher plants both in cultivated and forest soils. However, they are most commonly encountered in cultivated soils where they pose serious problems for various agronomically important crops, causing pre[9] and post emergence damping-off and other root rot diseases. The species of *Pythium* are potent producers of pectic and cellolytic enzymes which was proved effective for its pathogenesis.[10]

*Pythium* affects most of the economically important crops which ranges from wheat, mustard, forest pine trees to grasses.[11,12] It causes notable economic losses to tomato, chilli, cotton, leguminaceous crops and trees like citrus, peach, pear and apple. Soft fleshy plants and plant parts such as squash, cabbage, beans and potatoes also may be rotted by certain species of *Pythium*. They often cause great havoc in green houses, seed beds and nurseries. Another interesting feature of genus *Pythium* is apart from its nature of pathogenicity,[13] a certain species of *Pythium* have an ability to grow as a mycoparasite,[14,15] which may probably act as natural regulators of the populations of other fungi. The ability of the *Pythium* to grow as a mycoparasite was tested on 0.1% CMA against a range of filamentous fungi selected to represent most of the major taxa.[16] Some species of *Pythium* are even found to cause disease in mammals. *Pythiuminsidiosum* causes a disease called Pythiosis, a cosmopolitan granulomatous disease common in horses, cattle and dogs and are even occasionally reported in humans.[17]

*Pythium* also produces sexual spores in the roots or in the soil that are resistant to adverse environmental conditions such as

drying or cold temperatures and can survive in a dormant state for months or years. These spores germinate in the presence of a susceptible host when environmental conditions are favorable. The hyphae then penetrate the host root, to continue the disease cycle. The fungus *Pythium* is successful in its infectivity even in coexistence with other microorganisms. Interaction between *Pythium aphanidermatum* and *Meloidogyne incognita* indicated severe pre and post emergence damping off in chilli and brinjal.[18] The coexistence of *P. aphanidermatum* with *Pratylenchuscoffae* showed a significantly high inoculum level.[19]

*Pythium* are true fungi having coenocytic branched mycelium, and phylogenetic comparisons place them close to algae and higher plants.[20] Unlike most of the eumycetes, the members of this genus remain diploid throughout their life cycles with meiosis occurring in the gametangia before fertilization.[21] Both asexual and sexual reproductive phases are very distinct in this genus.

Species separation in the genus *Pythium* has been mainly dependent upon morphological structures, since they are relatively easy to observe. The morphological criteria most often used are the mature structure and size of the oogonia, oospores, sporangia and hyphal swellings. The female gametangia (oogonia) is usually smooth walled, but species having ornamental oogonia are not rare for the genus, the most commonly occurring species with such oogonia is *P. echinulatum*. The wall ornamentations are usually spines and are merely extensions of the oogonial wall.[22] The spines may be short or long, conical or curved with sharp or blunt apices or may be digitate, mammiform or papillate.[8] However, the emphasis placed on these different structures as valid taxonomical criteria has varied considerably. In recent days, the morphological descriptions of a species are increasingly supplemented by its molecular characteristics.[23] Physical and nutritional factors play a paramount importance in governing reproductive phases. An accurate knowledge of factors triggering production of reproductive bodies by *Pythium* can be helpful in formulating a successful control strategy. A large number of workers have conducted detailed studies on the genus *Pythium*[16,24] but so far not much work has been done for a detailed study on its characterization. Although there are number of species recorded from India which is mostly based on its host specificity and pathogenicity viz., *P. irregular,*[25] *P. butleri*and, *P. myriotylum,*[26] *P. aconthophoron,*[16] Very few works has been done in its taxonomical aspects.

## Materials and methods

To study the taxonomy of genus *Pythium* the methods adopted were similar to Butler EJ[27] and of Harvey JV.[28] The species of *Pythium* are found in water, soil and are associated with roots at seedling stage, as well as along with deep primary and secondary roots of annual or perennial plants. Random sampling was adopted for collection of samples. Method of collection, isolation and propagation of the fungus *Pythium* were presented in a few significant publications of Butler EJ,[27] Harvey JV,[28] Raper JR[29] and Sparrow FK.[30] The procedure used for baiting the fungus *Pythium* from water and soil were essentially similar to the techniques used by Johnson TW,[31] Scott WW,[32] Klemmer HW et al.[33] and Banihashemi Z.[34] The study was carried out during the period 2000 to 2005.

### Microscopic examination

A piece of agar with mycelium was floated in a sterile water which was treated as baits and observation of different stages required for identification are made by making micro slide preparation.

### Preparation of slides

**Whole mount preparation:** Slides were prepared by scrapping the diseased material with the help of a blade on to the slide in a drop of lactophenol-cotton blue solution. The slides were slightly heated over a bunsen flame and covered by slip. Excess mounting fluid was drained off with clean blotting stripes. In case of the baited samples the fresh hyphal tips was used for slide preparation.

**Hand sections:** Free hand thin sections of the infected materials were cut with the help of blade for getting the sporangia stages, which are the most important features for identification of the fungus *Pythium*.

**Petri-dish culture:** The soil and water samples, also the diseased specimen were placed in a petridish with 75ml of sterile distilled water. After 5-7days incubation following baiting at room temperature, the bait is observed microscopically for hyphal and zoo sporangial growth.

**Stains and mounting media:** Temporary slides were prepared by using following stains and mounting media for microscopic examinations in order to observe the sporangial and reproductive structures.

**Lacto phenol-cotton blue preparation:** For identifying the hyaline nature of sporangia 0.05-0.1% cotton blue solution was added.[12,35]

**D.P.X. mountant:** For preparing permanent slides, D.P.X. mountant was used. It has the advantage of rapid setting quality and does not require sealing with the wax or nail polish. D.P.X. is a colourless oily liquid which can be used directly for materials stained in lactophenol cotton blue. For mounting stained material, the D.P.X. mountant is placed over the material. Due to its viscous nature, excess cotton blue diffuses out from the material thus giving a coloureless black ground to the hand cut sections. The stain used in my experiment was Nigrofuschin.

Media used for Pythiaceous fungi: Before an attempt in identification of *Pythium* by means of the key was made, it was found essential to determine whether a culture will produce zoosporangia and sex organs. Therefore, to induce production of zoosporangia the fungus was grown on corn meal agar or/on Schmitthennr's medium; small cubes (3mm) in water (distilled 2; filtered pond 1, autoclaved in parts) was placed in contact with 2.5cm pieces of grass blades (boiled for ten minutes) and incubated at room temperature. When grass blades become infected they were transferred to fresh dishes. Emerson R[36] For winter season isolates if no zoospores were produced at room temperature (18-20°C) the same when placed for an hour or two in a refrigerator at 8-10°C resulted in zoospores formation. But for those isolates obtained in summer and rainy season, incubation at 25-30°C was found most suitable.

## Results

The genus *Pythium* was established in 1858 by Pringsheim and became the type genus of family Pythiaceae. The genus *Pythium* is antedated by *Pythium* authority Nees 1823. *Pythium* was erected with *Pythiummonospermum* (Pringsheimia) Butler as the type species. The genus due to vesicle formation was included in a new family Pythiaceae from Saprolegniaceae by Schroter in 1885. The sporangia

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of the organism remain attached to the hyphae and germinate *in situ*. Germination is either by zoospores or by germ tube. Production of zoospores is preceded by formation of a bubble like vesicle at the tip of a long tube which issues from sporangium. The sporangial protoplast flows into the vesicle through the tube and differentiation of the zoospores takes place in the vesicle. Zoospores are liberated on bursting of vesicle in surrounding medium, then they encyst and germinate by producing germ tube into somatic hyphae.

The Genus *Pythium* has been mono graphed by Butler EJ,[27] Matthews VD,[37] Sideris CP[38,39] and Middleton JT[8] Since 1943, thirty-one new specific or sub specific taxa have been described and these species have been scattered in literature and some of them are difficult or almost impossible to locate. However a collection of the *Pythium* species has been made from Indian Agricultural Research Institute field as well as some of locations of New Delhi. These species have been identified and described on the following pages in alphabetical order. The results of the isolation and taxonomic studies of *Pythium* species were made by collecting the samples from the field of IARI and other locations of Delhi, which is presented as under:

## Isolation of Pythium species

***Pythium* species isolated from different water types in and around Delhi:** The taxonomical studies were done by collecting different water samples from pond water, irrigation water, drain water and field water (cultivated soil and uncultivated soil). From these water samples collected twelve different species of *Pythium* were isolated. After the isolation it was found that *P. afertile, P. quatile, P. catenulatum, P. deliense, P. middletonii* and *P. ultimum* were common in pond water, while in the case of irrigation channel water *P. aphanidermatum, P. catenulatum, P. dissotocum, P. diclinus, P. graminicolum, P. middletonii* and *P. vexans* were common. In the case of drain water only four species of *Pythium* was found, they are *P. afertile, P. deliense, P. middletonii* and *P. vexans*. The isolation of the genus *Pythium* from the field water-cultivated and uncultivated soil showed three species of *Pythium* were common in both the samples, the species are *P. dissotocum, P. diclinus, P. ultimum*. While *P. aphanidermatum, P. aquatile, P. catenulatum, P. debaryanum, P. graminicolum, P. middletonii, P. ultimum* were found only in field water of the cultivated soil. Similarly, *P. afertile* was found only in the field water of uncultivated soil (Table 1).

***Pythium* species isolated from different soil types in and around Delhi:** The isolation of *Pythium* species from different soil types in and around Delhi was also undertaken. In this study soil samples collected were of six different types. From which twelve different species of *Pythium* were isolated from different soils. The six different soil types from which the *Pythium* species isolated are marshy soil, dry soil, cultivated soil, grassland soil, uncultivated soil and sandy soil. In the isolation study it was found that *P. afertile* was found in dry soil and uncultivated soil, *P. aphanidermatum* and *P. aquatile*, both were found in marshy soil, cultivated soil and grassland soil. *P. catenulatum* found in dry soil, cultivated soil and grassland soil. *P. debaryanum* found in marshy soil, cultivated soil and grassland soil. *P. deliense* found in dry soil, uncultivated soil and sandy soil. *P. dissotocum* was common in dry soil, cultivated soil and uncultivated soil, while *P. diclinus* was found only in grassland soil. *P. graminicolum* was present in cultivated soil and grassland soil. *P. middletonii* found in marshy soil, dry soil and uncultivated soil, while *P. ultimum* and *P. vexans* were found in marshy soil, cultivated soil, and grassland soil (Table 2).

***Pythium* species isolated from seedling/nursery beds in and around Delhi region:** Isolation of *Pythium* was also studied from the planting materials from seedlings on nursery beds. The rhizosphere portions of the seedlings or the roots were taken for isolation. The samples were collected from different nurseries of vegetables. After the isolation it was found the samples collected from tomato nursery consist of *P. debaryanum, P. middletonii* and *P. vexans*. The samples collected from the nursery of chilli and onion contained *P. aphanidermatum, P. catenulatum, P. deliense, P. dissotocum, P. graminicolum* and *P. middletonii*, while *P. afertile* and *P. aquatile* was found only in the nursery of chilli. *P. vexans* was found only in the nursery of onion. The samples collected from the nursery of cauliflower contain *P. afertile, P. aquatile, P. catenulatum, P. deliense, P. middletonii* and *P. ultimum*. Samples from the nursery of spinach consists of *P. aphanidermatum, P. catenulatum, P. ultimum* and *P. vexans*, while *P. afertile, P. aquatile, P. catenulatum, P. dissotocum, P. graminicolum, P. ultimum* and *P. vexans* were found in the samples collected from the nurseries of carrot (Table 3).

***Pythium* species isolated from rhizosphere/earthern pots of ornamentals of Delhi:** The isolation was also carried out for the ornamentals by collecting the samples from the rhizosphere. The samples in the case of ornamentals were collected from the earthern pots also. In the isolation, it was found *P. afertile* was found in the samples of roses, marigold and tuberose. *P. aphanidermatum*, found in the samples of gladiolus, marigold and chrysanthemum while *P. aquatile* was found in the samples of roses, canna and jasmine. *P. catenulatum* and *P. debaryanum* were found in the samples of canna and tuberose, while *P. catenulatum* was found in marigold. *P. deliense* found in gladiolus, jasmine and chrysanthemum. *P. dissotocum* was found in the samples of gladiolus and Jasmine. *P. diclinus* and *P. graminicolum* both were observed in chrysanthemum, while *P. diclinus* were isolated from the samples of roses. *P. middletonii* and *P. vexans* were found in the samples of gladiolus and marigold, while *P. vexans* in roses, canna and tuberose, *P. middletonii* in Jasmine were observed. *P. ultimum* was isolated from the samples of canna and chrysanthemum (Table 4).

*Datasoutside the bracket indicates the number of samples collected and the datas within the bracket indicates the number of samples shown *Pythium* yield.

+Indicates the presence of *Pythium* in the samples.

-Indicates the absence of *Pythium* in the samples.

**Seasonal variation and periodicity of the *Pythium* species during 2001 from Delhi region:** The seasonal variation and the periodicity of the *Pythium* species were also studied with reference to the year 2001 from the region of Delhi. The study was undertaken from January, 2001 to December, 2001. It was found that *P. aphanidermatum, P. aquatile, P. debaryanum, P. graminicolum, P. ultimum* and *P. vexans* were available during the month of January, February and March, while during the period from April to June *P. afertile, P. catenulatum, P. debaryanum, P. deliense, P. diclinus* and *P. ultimum* were common. *P. afertile, P. aquatile, P. catenulatum, P. deliense, P. dissotocum, P. graminicolum, P. middletonii* and *P. vexans* were common during the period of July to September. *P. aphanidermatum, P. catenulatum, P. debaryanum, P. diclinus, P. graminicolum, P. middletonii, P. ultimum* and *P. vexans* were present during the period from October to December (Table 5).

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**Table 1** *Pythium* species isolated from different water types in and around Delhi

| S.No. | Species | Pond water | Irrigation channel water | drain water | Field water cultivated soil | Uncultivated soil |
|---|---|---|---|---|---|---|
| 1 | *P. afertile* | + 11(6) | - 6(0) | + 4(2) | - 8(0) | + 7(4) |
| 2 | *P. aphanidermatum* | - 10(0) | + 10(4) | - 6(0) | + 8(6) | - 6(0) |
| 3 | *P. aquatile* | + 6(5) | - 2(0) | - 5(0) | + 10(6) | - 5(0) |
| 4 | *P. Catenulatum* | + 6(2) | + 2(1) | - 4(0) | + 9(5) | - 6(0) |
| 5 | *P. debaryanum* | - 3(0) | + 2(1) | - 6(0) | + 8(4) | - 8(0) |
| 6 | *P. deliense* | + 8(4) | - 2(0) | + 8(6) | - 9(0) | - 7(0) |
| 7 | *P. diclinus* | - 6(0) | + 4(3) | - 5(0) | + 8(4) | + 8(4) |
| 8 | *P. dissotocum* | - 7(0) | + 2(2) | - 7(0) | + 8(8) | + 7(4) |
| 9 | *P. graminicolum* | - 3(0) | + 9(7) | - 4(0) | + 7(3) | - 7(0) |
| 10 | *P. middletonii* | + 2(2) | + 10(8) | + 6(4) | + 6(4) | -6 (0) |
| 11 | *P. ultimum* | + 4(3) | - 6(0) | - 5(0) | + 8(3) | + 8(6) |
| 12 | *P. vexans* | - 6(0) | + 5(3) | + 6(2) | - 6(0) | - 5(0) |

**Datas outside the bracket indicates the number of samples collected and the datas within the bracket indicates the number of samples shown Pythium yield*
*+Indicates the presence of Pythium in the samples*
*-Indicates the absence of Pythium in the samples*

***Table 2*** *Pythium species isolated from different soil types of Delhi*

| S.No. | Species | Marshy soil | Dry soil | Cultivated soil | Grass land soil | Uncultivated soil | Sandy soil |
|---|---|---|---|---|---|---|---|
| 1 | *P. afertile* | - 8(0) | + 6(4) | - 6(0) | - 9(0) | + 6(5) | + 11(10) |
| 2 | *P. aphanidermatum* | + 9(7) | - 9(0) | + 10(8) | + 9(8) | - 5(0) | - 8(0) |
| 3 | *P. aquatile* | + 5(4) | - 3(0) | + 9(7) | + 7(7) | - 3(0) | - 6(0) |
| 4 | *P. catenulatum* | - 8(0) | + 6(6) | + 8(6) | + 5(3) | - 6(0) | - 9(0) |
| 5 | *P. debaryanum* | + 10(9) | - 7(0) | + 6(6) | + 11(10) | - 11(0) | - 5(0) |
| 6 | *P. deliense* | - 7(0) | + 11(8) | - 5(0) | - 10(0) | + 6(4) | + 8(6) |
| 7 | *P. diclinus* | - 10(0) | - 8(0) | - 10(0) | + 11(8) | - 6(0) | - 3(0) |
| 8 | *P. dissotocum* | - 5(0) | + 12(11) | + 11(10) | - 8(0) | + 7(6) | - 6(0) |
| 9 | *P. graminicolum* | - 6(0) | - 3(0) | + 7(6) | + 8(8) | - 7(0) | - 11(0) |
| 10 | *P. middletonii* | + 12(9) | + 6(5) | - 6(0) | - 9(0) | + 6(4) | - 8(0) |
| 11 | *P. ultimum* | + 11(10) | - 11(0) | + 3(3) | + 10(8) | - 4(0) | - 10(0) |
| 12 | *P. vexans* | + 6(4) | - 9(0) | + 5(4) | + 6(5) | - 3(0) | - 3(0) |

**Datas outside the bracket indicates the number of samples collected and the datas within the bracket indicates the number of samples shown Pythium yield.*
*+Indicates the presence of Pythium in the samples*

*-Indicates the absence of Pythium in the samples*

**Species of *Pythium* isolated from different substrate in and around Delhi region:** The *Pythium* species were isolated from different substrate like water, soil and planting material and the substrate or the samples collected were mainly in and around Delhi region. In an overall account twelve species of *Pythium* was isolated viz., *P. afertile, P. aquatile, P. aphanidermatum, P. catenulatum, P. debaryanum, P. deliense, P. diclinus, P. dissotocum, P. graminicolum, P. middletonii, P. ultimum* and *P. vexans* and the taxonomic study was undergone for the species. It was found from the data that some species were common in few areas, while some species were specific to a particular area. The area of the samples collected was mainly from IARI fields and some other places of Delhi region. There was several numbers of samples collected and during isolation, the presence of *Pythium* species were observed only in few numbers of samples, but not in all samples (Table 6).

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***Table 3*** *Pythium species also from seedlings/nursery beds around Delhi region.*

| S.No. | Species | Tomato | Chilies | Onion | Cauliflower | Spinach | Carrot |
|---|---|---|---|---|---|---|---|
| 1 | *P. afertile* | - 10(0) | + 10(6) | - 6(0) | + 6(5) | - 9(0) | + 10(6) |
| 2 | *P. aphanidermatum* | - 8(0) | + 11(4) | + 7(4) | - 6(0) | + 10(4) | - 7(0) |
| 3 | *P. aquatile* | - 8(0) | + 8(7) | - 8(0) | + 5(3) | - 10(0) | + 10(8) |
| 4 | *P. catenulatum* | - 7(0) | + 7(4) | + 9(5) | + 6(5) | + 11(4) | + 11(10) |
| 5 | *P. debaryanum* | + 5(3) | - 6(0) | - 10(0) | - 8(0) | - 9(0) | - 7(0) |
| 6 | *P. deliense* | - 8(0) | + 9(5) | + 11(8) | + 9(4) | - 8(0) | - 9(0) |
| 7 | *P. diclinus* | - 10(0) | - 11(0) | - 10(0) | - 11(0) | - 6(0) | - 9(0) |
| 8 | *P. dissotocum* | - 7(0) | + 10(6) | + 12(10) | - 10(0) | - 7(0) | + 10(8) |
| 9 | *P. graminicolum* | - 10(0) | + 9(6) | + 12(4) | + 12(12) | - 6(0) | + 8(4) |
| 10 | *P. middletonii* | + 8(4) | + 9(5) | + 10(5) | + 12(6) | - 8(0) | - 7(0) |
| 11 | *P. ultimum* | - 11(0) | - 4(0) | - 10(0) | - 10(0) | + 8(4) | + 8(6) |
| 12 | *P. vexans* | + 12(5) | - 8(0) | + 8(4) | - 10(0) | + 8(7) | + 8(8) |

**Datas outside the bracket indicates the number of samples collected and the datas within the bracket indicates the number of samples shown Pythium yield. +Indicates the presence of Pythium in the samples*

*-Indicates the absence of Pythium in the samples*

**Table 4** Species of Pythium isolated from Rhizosphere/earthern pots of ornamentals in Delhi area.

| S.No. | Species | Gladiolus | Roses | Marigold | Canna | Jasmine | Tuberose | Chrysanthemum |
|---|---|---|---|---|---|---|---|---|
| 1 | *P. afertile* | - 5(0) | + 4(3) | + 5(3) | - 4(0) | - 10(0) | + 10(4) | - 10(0) |
| 2 | *P. aphanidermatum* | + 8(4) | - 8(0) | + 8(4) | - 5(0) | - 9(0) | - 12(0) | + 10(8) |
| 3 | *P. aquatile* | - 9(0) | + 7(6) | - 8(0) | + 7(4) | + 9(8) | - 11(0) | - 9(0) |
| 4 | *P. catenulatum* | - 8(0) | - 8(0) | + 7(4) | + 8(5) | - 8(0) | + 10(5) | - 9(0) |
| 5 | *P. debaryanum* | - 9(0) | - 7(0) | - 8(0) | + 8(7) | - 7(0) | + 10(7) | - 8(0) |
| 6 | *P. deliense* | - 10(0) | - 8(0) | + 8(4) | -9(0) | + 8(6) | - 4(0) | + 8(7) |
| 7 | *P. diclinus* | - 10(0) | + 8(4) | - 7(0) | + 6(5) | - 8(0) | - 7(0) | + 10(6) |
| 8 | *P. dissotocum* | + 10(5) | - 9(0) | - 8(0) | - 5(0) | + 8(7) | - 5(0) | - 9(0) |
| 9 | *P. graminicolum* | - 11(0) | - 7(0) | - 6(0) | - 7(0) | - 8(0) | - 8(0) | + 11(5) |
| 10 | *P. middletonii* | + 6(5) | - 8(0) | + 5(4) | - 8(0) | + 9(5) | - 9(0) | - 10(0) |
| 11 | *P. ultimum* | - 7(0) | - 5(0) | - 6(0) | + 8(6) | - 10(0) | - 9(0) | + 10(0) |
| 12 | *P. vexans* | + 8(4) | + 7(6) | + 6(5) | + 8(3) | - 9(0) | + 9(8) | - 8(0) |

**Table 5** Seasonal variation and periodicity of Pythium species during the year 2001 from Delhi

| S.No. | Species | Jan - March | April - June | July - Sept. | Oct. - Dec. |
|---|---|---|---|---|---|
| 1 | *P. afertile* | - | + | + | - |
| 2 | *P. aphanidermatum* | + | - | - | + |
| 3 | *P. aquatile* | + | - | + | - |
| 4 | *P. catenulatum* | - | + | + | + |
| 5 | *P. debaryanum* | + | + | - | + |
| 6 | *P. deliense* | - | + | + | - |
| 7 | *P. diclinus* | - | + | - | + |
| 8 | *P. dissotocum* | - | - | + | - |
| 9 | *P. graminicolum* | + | - | + | + |
| 10 | *P. middletonii* | - | - | + | + |
| 11 | *P. ultimum* | + | + | - | + |
| 12 | *P. vexans* | + | - | + | + |

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**Table 6** Species of Pythium isolated from different substrates around Delhi region

| S.No. | Name of the species | Isolated from | Locality | No. of samples collected | No. of Pythium species yielded |
|---|---|---|---|---|---|
| 1 | *P. afertile* | Dry soil, uncultivated soil, sandy soil, pond water, drain water, field, water-uncultivated soil, chilies, cauliflower, carrot, roses, marigold, tuberose | BudhaJayanti Park, Indian Agricultural Research Institute fields, Green Park, QutbMinar park, Panjabi Bagh area, JantarMantar Park, Nehru Stadium area, Kalkaji Temple area, India Gate area, Delhi University, South campus area, Lotus temple area | 181 | 58 |
| 2 | *P. aphanidermatum* | Marshy soil, cultivated soil, grassland soil, irrigated channel water, field water-cultivated soil, chilies, onion, spinach, gladiolus, marigold, chrysanthemum | - do - | 199 | 61 |
| 3 | *P. aquatile* | Marshy soil, cultivated soil, grassland soil, pond water, field water & cultivated soil, chilies, cauliflower, carrot, roses, canna, jasmine | - do - | 170 | 65 |
| 4 | *P. catenulatum* | Dry soil, cultivated soil, grassland soil, pond water, irrigation channel water, field water-cultivated soil, chilies, onion, cauliflower, spinach, marigold, canna, tuberose. | - do - | 178 | 65 |
| 5 | *P. debaryanum* | Marshy soil, cultivated soil, grassland soil, irrigation channel water, field water-cultivated soil, tomato, canna, tuberose | - do - | 179 | 47 |
| 6 | *P. deliense* | Dry soil, uncultivated soil, grassland soil, irrigation channel water, field water-cultivated soil, tomato, canna, tuberose | - do - | 190 | 62 |
| 7 | *P. diclinus* | Grassland soil, irrigation channel water, field water-cultivated soil, roses, canna chrysanthemum | - do - | 192 | 34 |

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Table Continued

| S.No. | Name of the species | Isolated from | Locality | No. of samples collected | No. of Pythium species yielded |
|---|---|---|---|---|---|
| 8 | *P. dissotocum* | Dry soil, cultivated, uncultivated soil, irrigation channel water, field water-cultivated and uncultivated soil, chilies onion, carrot, gladiolus, jasmine | - do - | 190 | 77 |
| 9 | *P. graminicolum* | Cultivated soil, grassland soil, irrigation channel water, field water - cultivated soil, chilies, onion, cauliflower, carrot, chrysanthemum | - do - | 187 | 55 |
| 10 | *P. middletonni (or) P. proliferum* | Marshy soil, dry soil, uncultivated soil, pond water, irrigation channel water, drain water, field water-cultivated soil, tomato, chilies, onion, cauliflower, gladiolus, marigold, jasmine | - do - | 186 | 70 |
| 11 | *P. ultimum* | Pond water, field water-cultivated soil, uncultivated soil, marshy soil, cultivated soil, grassland soil, spinach, carrot, canna, chrysanthemum | - do - | 186 | 55 |
| 12 | *P. vexans* | Marshy soil, cultivated soil, grassland soil, irrigation channel water, drain water, tomato, onion, spinach, carrot, gladiolus, roses, marigold, canna, tuberose | - do - | 169 | 68 |

## Discussion

Different species of *Pythium* were identified based on their nature of sporangia, attachment of sex organs and were compared with the species described in relevant literature, description were amended wherever it was found necessary. The collection of different water samples yielded six different *Pythium* species, mainly having filamentous, lobulate, irregularly swollen sporangia, not differing from vegetative hyphae in appearance (*P. afertile, P. aquatile, P. catenulatum, P. deliense, P. middletonii),* except in case of *P. ultimum* the flamenous sporangia were not seen. The samples collected from irrigation channel were also having similar type of filamenous sporangia except *P. vexans*, where the sporangia were globose. All the species from drain water had only filamentous sporangium. Twelve different species of *Pythium* were isolated from six different soil types (marshy, dry, cultivated, grass land, uncultivated, sandy). Samples from dry soil, sandy soil, uncultivated soil yielded less number of *Pythium* species, while cultivated soil yielded maximum number of species, followed by grass land soil and marshy soil.

The *Pythium* flora along the vegetable rhizosphere showed that out of twelve species maximum diversity in number of species were observed in chilli nursery bed followed by onion and carrot (seven species), cauliflower (six species), spinach (four species) and least number from tomato nursery bed. The *Pythium* species associated with ornamentals have indicated that gladiolus, tuberose, jasmine, roses have less association of *Pythium* species, while marigold, canna as well as chrysanthemum are more prone to *Pythium*. Seasonal

variation due to temperature, humidity, moisture, rainfall play an important role in development of sporangia and oogonium. From July to December have shown the presence of maximum number of species, while the decline in temperature from January to June have shown comparatively less in number of *Pythium* species in Delhi.

The taxonomic investigations in *Pythium* species have been observed with the variation in sporangia formation starting from filamentous non-inflated, filamentous dendroid, filamentous inflated and Proliferating. There was lot of variation in hyphal swelling chlamydospores and aspersoria formation. The species of *Pythium* recorded from this Delhi area were only with smooth oogonial wall. None of the species observed with ornamented with obtuse or blunt projections. The most significant challenges during this course of work is the identification. The only broad identification key for *Pythium* species available is of van der Plaats-Niternk, 1981, where only 120 was covered out of 307 reported species. Similarly, there are no graphic sheets, no online database and no DNA based barcode system for *Pythium* species. On the other hand, depending on molecular identification not much has been exploited, the existing species-specific primers are for only around 20 species for plant pathogens.[40] Moorman GW et al.,[41] made some efforts for creating web-based communicating keys using Lucid Builder platform, however, it is a alteration of van der Plaats-Niternk identification key.

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## Conflict of interest

The author declares no conflict of interest.

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