An- Najah National University
Faculty of Graduate Studies

The Use of Multivariate Analysis of Variance to Aid Species separation in the genus *Pythium*

By
Hadeel Rasheed Thaher Al-Thaher

Supervisor
Prof. Dr. Mohammed S. Ali-Shtayeh

Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Environmental Science, Faculty of Graduate Studies, at An-Najah National University, Nablus; Palestine.
2008

The Use of Multivariate Analysis of Variance to Aid Species separation in the genus *Pythium*

By

Hadeel Rasheed Thaher shawahneh

This thesis was successfully defended on 26/ 5/ 2008 and approved by:

Committee members	**Signature**
1. Prof. Dr. Mohammed S. Ali-Shtayeh (Supervisor)	....
2. Dr. Munqez J. Shtaya (Internal Examiner)	....
3. Dr. Hazem Sawalha (External Examiner) **Arab American University of Jenin**	....

DEDICATION

To

My great and lovely family father, mother and brother (ehab) for their support and encouragement, my dear husband (Khaled) for his patience and endurance during difficult times with beauty and nice flower to describe my respect and love.

Acknowledgments

I would like to express my sincere special thanks and gratitude to my supervisor, Professor Dr. Mohammed S. Ali-Shtayeh for his supervision, encouragement, guidance and help throughout this study.

I am also indebted for the generous help and facilities supplied by the Biodiversity and Environmental Research Center, BERC, Til, Nablus, throughout this work.

Special thanks are due to my father, mother, brother and husband for their help, patience encouragement and endless support.

Also special thanks for colleagues at An-Najah University and my school mates in various schools, and my best and great friend Raeda Yassin, for her help and encouragement. Thanks are also due to the engineer Mahdy AL-Khather for his assistance in the laboratory.

And I never forget my neighbours (Zahra Khodruj and Wejdan Taha) for their helping in language.

إقـــــرار

أنا الموقع/ة أدناه, مقدم الرسالة التي تحمل العنوان:

The Use of Multivariate Analysis of Variance to Aid Species separation in the genus *Pythium*

أقر بأن ما اشتملت عليه هذه الرسالة إنما هي نتاج جهدي الخاص، باســتثناء مـــا تمــت الإشارة إلية حيثما ورد، وان هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أي درجة أو لقب علمي أو بحثي لدى أية مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher’s own work, and has not been submitted elsewhere for any other degree or qualification.

Student’s Name: اسم الطالب:

Signature: التوقيع:

Date: التاريخ:

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

The Use of Multivariate Analysis of Variance to Aid Species Separation in the Genus *Pythium*

By

Hadeel Rasheed Thaher Al-Thaher

Supervisor

Prof. Dr. Mohammed S. Ali-Shtayeh

ABSTRACT

One hundred and forty seven isolates belonging to forty one species of *Pythium* were used in this study. *Pythium* isolates were recovered from soil from the Palestinian West Bank and Gaza using the VP3 selective medium and the surface-soil-dilution plating technique.

The isolates were used to test the robustness of the biometric approach and to examine the taxonomic value of biometric characters.

Morphological characteristics including oogonium and oospore diameters, oospore wall thickness and derived indices (oospore wall index and aplerotic index) were used.

The biometric parameter set which consists of oogonium and oospore diameters and oospore wall thickness were all of taxonomic value for the separation of species. This was clearly shown from canonical variate plots and cluster analysis.

By using multivariate analysis of variance in this study inter and intra specific variations among *Pythium* species were demonstrated. However, according to dendrogram and canonical variate plots some of *Pythium* species were separated. Despite intra-specific variation, good species separation was obtained.

Multivariate analysis of variance is shown in this study to be a useful technique of taxonomic value for the separation of *Pythium* species.

Chapter One

Introduction

1.1 Taxonomy of *Pythium*

The genus *Pythium* include many species that are pathogens of plants and some are parasitic on other fungi (Plaats-Niterink. 1981). *Pythium*, like others in the family Pythiaceae, are usually characterized by their production of coenocytic hyphae, hyphae without septations. Oogonia generally contain a single oospore and antheridia contain an elongated and club-shaped antheridium. Originally, the genus *Pythium* was placed in the Family Saprolegniaceae by Pringsheim in 1858 (Hendrix and Campbell, 1973). Currently, *Pythium* species are placed in the Family of Pythiaceae, Class of Oomycota. The first *Pythium* sp. reported in the United States was *P. anandrum,* which emerged in 1930s. The genus *Pythium* belong to the family *Pythiaceae* (Waterhouse, 1973; Plaats-Niterink, 1981). The scientific classification of this genus as follows (Dick, 1990).

Domain: Eukaryota
Class: Oomycetes
Order: Peronosporales
Family: Pythiaceae
Genus: *Pythium*

1.2 *Pythium* life cycle

Pythium root rot and wilt is caused by various fungus-like pathogens in the genus *Pythium*. The diseases are favored by high soil moisture and moderate to high temperature. Varietal susceptibility will also influence disease development. Pathogen survival and inoculum buildup are favored by soils with high organic matter and poor drainage. Susceptibility can be increased if roots are damaged during cultivation or by other soil-related problems, such as nematode feeding.

The fungus can be transported within and between fields by contaminated irrigation water. Occurrence is also favored by improper crop rotation. The pathogen survives pathogenically on weeds and in the soil as dormant oospores (Schwartz and Gent, 2005).

Pythium survives in the soil as oospores, hyphae and sporangia. The fungus can survive unfavorable soil moisture and temperatures for several years as oospores. Oospores may form a germ tube directly to infect the plant or they may form sporangia. The sporangia produce zoospores, which are the motile form of the fungus. The zoospores swim around briefly before encysting and forming a germ tube, which can cause infection. Sporangia that have developed on plant tissue can germinate in a similar manner as the oospores, by either germinating directly or by forming zoospores (Hendrix and Campbell, 1973).

The pathogen is dispersed when infected debris is transported to uninfected areas and when the soil moisture is enough to allow the zoospores to swim freely (Bruehl, 1987).

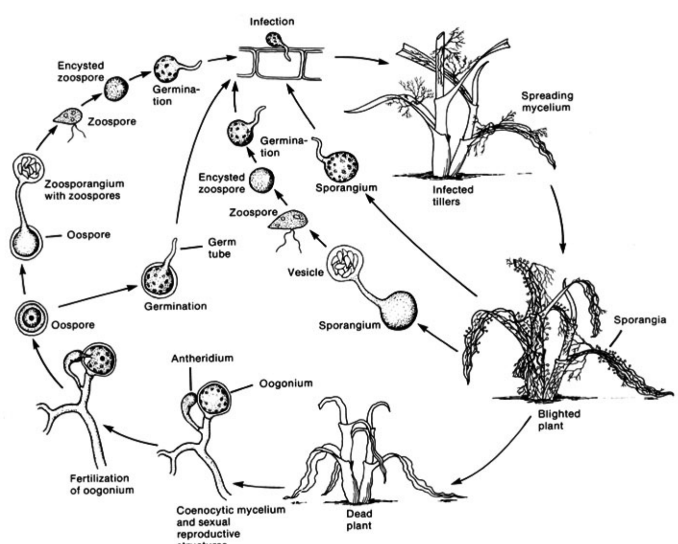

Figure (1.1): General depiction of oomycete life cycle (Adopted from plant clinic - Cornell Education).

1.3 Epidemiology of *Pythium*

There are many *Pythium* species with varying degrees of host specificity and pathogenicity. Diseases can be expressed as seed decay, pre- or post-emergence damping off and infection of the roots or stems of young plants. The pathogen is inclined to moist soils typical of those subjected to excess irrigation, poor drainage and very high humidity

Hosts susceptible to Pythium root rot include cucurbits (melon, squash, cucumber, watermelon and pumpkin), onion, lettuce, beans, mungbeans, soybeans, maize, potatoes, coffee, pineapple and sugarcane. The seedlings of many plant species are susceptible to damping off.

The composition of the pathogen complex can affect *Pythium* species because the biological characteristics such as pathogencity of each species are different, because the factor usually affecting epidemicity may modify the behavior of each species. The pathogenic capacity is largely determined by the available enzymes. Many species can attack a variety of hosts, others are restricted to one host species. Infection depends on several factors: inoculums density, soil water content, temperature, pH, light intensity, cation content and presence of micro- organisms. Sufficient amount or excess of soil water often favor infection and the severity of attack. The influence of temperature depends on the species of *Pythium* involved and the optimum for infection is not always the same as that for growth in pure culture (Bagnall, 2007).

Pythium species may infect planted seeds prior to germination, germinating seedlings, young plants, or older plants during blossoming and fruit formation (*Pythium* spp. also produces root diseases in adult plants

(Hendrix and Campbell, 1973). It is one of the pathogens capable of causing seed decay and seedling death. Initial root rot symptoms appear as elongated water-soaked areas on the hypocotyl and roots, within one to three weeks after planting. The pathogen will extensively prune roots, reduce overall plant growth, and destroy much of the hypocotyl and main root system. The water-soaked region may extend several inches above the soil line, with little, if any, visible evidence of the fungus. These regions eventually dries out, becomes somewhat sunken, and tan to brown in color. Plants then wilt and die. Disease development later in the season results when fruit pods in contact with the soil become infected. These pods exhibit a watery soft rot, or a mass of white fungal growth, but black sclerotic do not form as with white mold (Bagnall, 2007).

Pythium damping off is a very common problem in fields and greenhouses, where the organism kills newly emerged seedlings (Jarvis, 1992). Many *Pythium* species, along with their close relatives, Phytophthora species are plant pathogens of economic importance in agriculture. *Pythium* spp. tend to be very generalistic and unspecific in their host range. They infect a large range of hosts, while Phytophthora spp. are generally more host-specific. For this reason, *Pythium* spp. are more devastating in the root rot they cause in crops, because crop rotation alone will often not eradicate the pathogen (nor will fallowing the field, as *Pythium* spp. are also good saprotrophs, and will survive for a long time on decaying plant matter).

The damage caused by *Pythium* spp. in the field crops is limited to the area affected. This is because the motile zoospores need ample surface water to travel long distances and the capillaries formed by soil particles act as a natural filter. In hydroponic systems inside greenhouses, where extensive monocultures of plants are maintained in plant nutrient solution, that is continuously re-circulated to the crop, *Pythium* spp. cause extensive and devastating root rot and is often difficult to prevent or control. The root rot affects entire operations within two to four days due to the inherent nature of hydroponic systems where roots are nakedly exposed to the water medium, in which the zoospores can move freely (Jarvis, 1992; Owen-Going *et al.,* 2003; Bagnall, 2007).

1.3.1 Management of *Pythium* plant diseases

In recent years there has been considerable interest in exploiting soil microorganisms for the control of fungal and bacterial plant diseases. A substantial body of research has clearly shown that microorganisms introduced onto subterranean plant parts will colonize seeds and roots, where they provide promising levels of biological disease control of seed- and root-infecting fungal plant pathogens (Handelsman and Stabb, 1996). Current models for microbial control of soil borne plant diseases have focused on antibiotic biosynthesis, parasitism, induced systemic resistance, and microbial competition (Whipps, 1997). At present there is evidence to support those models involving antibiotic biosynthesis (Cook *et al.,* 1995), parasitism (Jeffries, 1995), and induced systemic resistance (Van Loon *et al.*, 1998).

For competitive interactions to occur, both the introduced microbial strain and the plant pathogen must compete in time and/or space for some common resource. Recent study by Van Dijk and Nelson, (2000), based on study system in which a common resource is known to be utilized by *Pythium ultimum*, and the introduced bacterium, *Enterobacter cloacae*, showed that *E. cloacae* can suppresses *Pythium* seed rot. The study focused on *P. ultimum* because it is one of the most destructive plant pathogens, causing seed and root diseases of a wide variety of economically important plants (Martin and Loper, 1999). Despite their virulence, *Pythium* spp. are generally considered to be poor microbial competitors in the presence of other plant-associated bacteria (Elad and Chet, 1987; Tedla and Stanghellini, 1992). To compensate for this, *Pythium* spp. rely on the production of survival propagules such as oospores and sporangia (Stanghellini and Hancock, 1971; Stanghellini, 1974), which germinate rapidly in response to fatty acids present in the exudates of germinating seeds (Ruttledge and Nelson, 1997). This allows *Pythium* species to infect seeds at a time when microbial activities, and thus competitive interactions, are low around the surface of the seed.

An approach for reducing this rapid response to exudates and the subsequent infection of plants by *Pythium* species has been to introduce bacteria on the surface of the seed at the time of planting. One of the more effective bacterial species studied for its *Pythium* suppressiveness is *E. cloacae* (Martin and Loper, 1999). A unique feature of some *E. cloacae* strains is that they can rapidly reduce the germination of survival propagules and subsequent seed colonization and infection by *P. ultimum* (Van Dijk and Nelson, 1998). The researchers observations suggest a biological control process mediated by competition between *E. cloacae* and *P. ultimum* for specific molecules such as fatty acids released by germinating seeds, which results in the suppression of *Pythium* propagule germination and subsequent plant infection.

Several *Pythium* species, including *P. oligandrum*, *P. nunn*, *P. periplocum*, and *P. acanthicum* are mycoparasites of plant pathogenic fungi and oomycetes, and have received interest as potential biocontrol agents.

1.4 Identification of *Pythium* species

1.4.1 Morphological criteria

Colony morphology is rarely used as a criterion for identifying *Pythium* species, however the feature shown by some species is distinctive colonies on CMA and reveals various patterns depending on species and temperature from an indefinite pattern through radiation to chrysanthemum and rosette (Yong-nian and Guo-zhong, 1989). The hyphae of *Pythium* are cylindrical usually irregularly branched, often hyaline, sometimes lustrous occasionally slightly colored. In young hyphae protoplasmic streaming is often clearly visible. The most important criteria for *Pythium* species delimitation is morphological characteristics which are: hyphal swellings, sporangia, zoospores, antheridia, oogonia and oospores (Yong-nian, and Guo-zhong, 1989).

1.4.1.1 Hyphal swellings

In many *Pythium* species hyphal swellings are formed and may be globose, subglobose, limoniform, elliptical or irregularly shaped. In some species hyphal swellings are formed in chains. In most cases they are difficult to distinguish from young sporangia or oogonia and can germinate and form a new thallus.

Appressoria are essentially hyphal swellings which are frequently found in agar cultures, especially in species with filamentous sporangia.

They may be simple or complex, straight, curved, inflated; knob-like which become flattened when applied to surface (Yong-nian and Guo-zhong, 1989).

1.4.1.2 Sporangia and zoospores

There are three well know types of sporangia among *Pythium* species. These include:

1- Filamentous sporangia: consist of fertile variable length separated from adjacent mycelium by a cross-wall. They may be simple or branched with similar dimension that can hardly be distinguished from vegetative hyphae, unless the vesicle and zoospores indicate the location of the sporangial part.

2- Lobulate sporangia: strongly inflated, forming lobulate, dactyloyed or toruloid elements, which extends outward in all directions.

3- Spheroid sporangia: vary in shape size and position on the supporting hyphae. The sporangia may be spherical to subspherical, ovate to obovate, ellipsoid, pyriform and truncate, acrogenous, intercalary. Spherical elements may join together in short chains to form catenulate sporangia (Yong-nian, and Guo-zhong, 1989).

Figure (1.2): Spherical sporangia of *P. ultimum*. (Allen *et al.* 2004, http://www.apsnet.org)

1.4.1.3 Antheridia and oogonia

Morphology of antheridia is used as an important tool for identification of *Pythium* species. In general, there are three recognized types of antheridia among *Pythium* species. These include: monoclinous, diclinous and hypogenous. Antheridia vary in shape, size, number and place. They may be calvate or allantoid, globose or oblong, sub-orbicular or trumpet-shaped and may borne terminally or intercalary. For each oogonium there are one to twenty five or more antheridia (Yong-nian, and Guo-zhong, 1989).

The oogonia are usually spherical to sub-spherical when acrogenous and ellipsoid to limoniform when intercalary. The oogonial wall may be smooth or ornamented with projections which may be short or long, straight or curved, digitate or conical, mammiform or papillate.

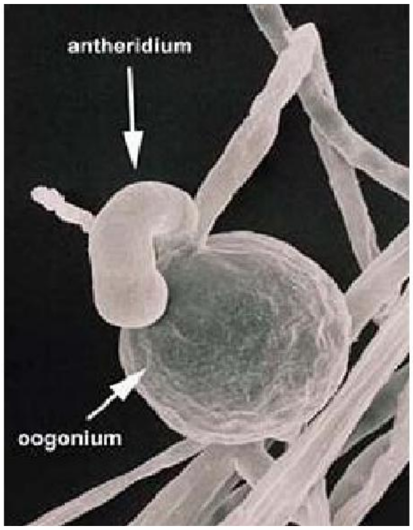

Figure (1.3): Oogonium and antheridium of *P. ultimum* (http://www.apsnet.org/education/IllustratedGlossary/default.htm)

1.4.1.4 Oospores

Oospores produced after fertilization which takes place inside the oogonia. The oospores were usually globose and rarely spherical.

1.4.2 Morphological characteristics

Species identification in the genus *Pythium* depends mainly upon morphological observation of various structures. The morphological criteria most often used are the mature structure and size of the oogonia, oospores, sporangia and hyphal swellings. Morphological variations among the different structures were used as taxonomic tool for differentiation of the different species (Sideris, 1932; Middleton, 1943). For species differentiation Middleton (1943) depended on morphological characters such as the origin and morphology of the antheridium; the sporangial form; the plerotic or aplerotic condition of the oospore; the thickness of the oospore wall; the smooth or echinulate character of the oogonial wall, and cardinal temperatures. Oogonial size and dimensions were of no taxonomic value. Matthews (1931) and Waterhouse (1967) relied heavily on reproductive cell size for species differentiation, however, Hendrix & Campbell (1974) showed that the size of reproductive structures changed with time in culture, and therefore doubted the validity of spore sizes as a valid taxonomic character. Ho (1975) reported that oospore size is stable character under different cultural conditions and a number of *Pythium* isolates showed considerable intra-specific variations.

As taxonomic disjunctions can only be deduced from studying different isolates taken from various places at different times, under unified standard culture condition, most previous studies were unable to asses intra-specific variations and a major weakness in these studies that they depended on single isolates in some cases.

Plaats-Niterink, in 1981, utilized more detailed morphological characters such as reproductive cell size and some physiological characters such as maximum temperature and daily growth rate of eighty five *Pythium* species including heterothallic species.

1.4.2.1 Multivariate analysis to aid identification of *Pythium* species

Statistical methods are available for analyzing data consist of multiple variables (James and McCulloch, 1990; Stevens, J. 1992; Hair, *et al.,* 1998; and Kenkel, *et al.,* 2002). Such methods use three major tools: (i) ordination, which aims at describing data by identifying a reduced data dimension of a few variables that account for the greatest amount of variability in the data; (ii) discrimination, which aims at delineating experimental groups or classifying observations into experimental groups based on a set of variables; and (iii) canonical, which aims at describing and predicting the relationship between two sets of variables.

Multivariate data analysis is based on two features: (1) linear combinations of variables or variates, and (2) distances or measures of association. In addition to these features (Hair , *et al.,* 1998) defined two notions that need to be considered in multivariate analysis. First, is the nature of variables, which may be either metric (quantitative or numerical) or nonmetric (qualitative or categorical). Second, is the notion of dependency or interdependency. In the dependency context, one or several variables designated as dependent variables are influenced in magnitude by another set of variables designated as independent variables. In the interdependency context, variables are not designated as either dependent or independent. On the basis of these features, several multivariate statistical tools have been developed (Hair *et al.,* 1998; James and McCulloch, 1990).

Multivariate tools have been employed to varying degrees in the phytopathological literature. A bibliometric search of the Biological Abstract database (Silver Platter Information, Norwood, MA), from 1997 to 2003, indicates that ordination and discrimination tools have, by far, been used frequently compared with canonical analyses. Among the ordination methods, correspondence analysis was the least used, and multivariate analysis of variance (MANOVA) was the least used among discrimination methods.

1.4.2.1.1 Multi analysis of variants (MANOVA)

MANOVA is a procedure for assessing differences among several nonmetric independent variables based on the linear combination of several metric dependent variables. This procedure enables the simultaneous examination of several dependent variables. Golinski *et al.,* (2002) used MANOVA to assess the effect of two pathogens (*Fusarium avenaceum* and *F. culmorum*) on three yield components (1,000-kernel weight and weight and number of kernels per winter wheat head) of 14 winter wheat cultivars in a 2-year study. The dependent variables were the three yield components and the independent variables were pathogens, years, and cultivars, including interaction terms among these three factors. Similarly, Evans *et al.*, (1997) used MANOVA to examine differences in host penetration and colonization by *Alternaria linicola* in three genotypes (susceptible, moderately resistant, and resistant) of *Linum usitatissimum*.

1.4.2.1.2 Canonical correlation

The goal of canonical correlation is to describe the association between two sets of variables. For example, it may be desired to relate differences in pathogenic microflora at a given geographical location to differences in botanical composition or environmental/edaphic conditions at the same location. Schlosser *et al.,* (2000) working on rice blast (caused by *Pyricularia grisea*) in six upland rice cultivars, used canonical correlation to characterize the relationship between plant morphological variables (plant height, leaf length, leaf area, and plant growth rate) and disease variables.

1.4.1.3 Molecular differentiation of *Pythium* species

Identification of *Pythium* species, especially those with similar morphological features is often difficult. In 1991, Brasier showed that *Phythophthora* species, a close relative of *Pythium,* could evolve relatively rapidly and changes in morphology might lag behind pathological capability. These observations emphasizes the need for more advanced techniques rather than morphologic features for differentiation purposes.

Recently, advances in molecular biology contributed several important tools for confirmation of the validity of species. These approaches aid to help answer question as to whether certain morphological features are valid for differentiating species and whether certain species that are similar morphologically actually are similar genetically. Many tools were used to differentiate *Pythium* species. Among these; protein electrophoresis, isoenzyme patterns, and restriction fragment length polymorphism (RFLPs).

Using synthetic oligo-nucleotide probes complementary to specific diagnostic regions, the phylogeny of 116 species and varieties of *Pythium* was studied using parsimony and phenetic analysis of the ITS region of the nuclear ribosomal DNA (Lévesque, and Cock, 2004), more than 100 species were differentiated. Hybridization patterns included cluster-specific oligonucleotides that facilitated the recognition of these species (Erwin and Ribeiro, 1996). The use of polymerase chain reaction technique (PCR) was also used as a taxonomic tool for differentiation of various *Pythium* species (Kageyama, *et al.,* 1997).

1.5 Use of multivariate analysis for differentiation of *Pythium* species

Multivariate analysis of variance techniques have only been used by few workers (Ali-Shtayeh, 1982; Shahzad, 1992 and MØller and Hochenhull, 2001) in assessing inter- and intra-specific variation in *Pythium* species or in solving the practical problems of interpreting biometric data. This might be due to lack of enough adequately estimated characters for different isolates from each of the different species.

A multivariate analysis of variance has been employed by these researchers to examine inter- and intra-specific variation for those taxa available. It cannot be a complete analysis because not all species hd been isolated and not all species possess the characters which these studies had used. The number of characters was, in any case, well below that normally regarded as reasonable for numerical taxonomy in other groups. It has, never-the less, claimed to be a useful tool for separating groups of isolates. The characters used were oogonia, oospore dimensions and oospore wall thickness.

More speacies and isolates were used in this work, the characteristics which are able to measuring were select ; for example ooplast diameter wasn’t used because it difficult to measure and in one oospore may be found more than one ooplast. Samples were collecting from different environmental society . Adding to biometric characteristics morphological characteristics were used and species were divided in groups according to morphological characteristics which are (oogonia type, sporangia type and aplerotic index).

1.6 Objectives

The present study aimed at:

1- Assessing inter- and intra-specific variations in *Pythium* species.

2- Differentiation of various *Pythium* species using of maltivariate analysis of variance.

3- Examine the taxonomic value of biometric characters from a practical perspective.

4- Investigate whether the biometric data alone would still delimit single species well. And possibly solving practical problems of interpreting biometric data by using multivariate analysis of variance techniques.

Chapter Two

Materials and Methods

2.1 Isolates of *Pythium* species examined

One hundred and forty seven isolates belonging to species of *Pythium* were used in this study (Table A.1). The majority (144) of the isolates were obtained from Professor’s Ali-Shtayeh’s fungus culture collection at An-Najah National University. These isolates were recovered by Prof. M. S. Ali-Shtayeh during the course of ecological work he carried out where a large number of *Pythium* isolates were recovered from soil from the Palestinian West Bank and Gaza. Many of these have been kept as axenic isolates. The remaining isolates (3) were recovered by the author from soil, using the VP3 selective medium and the surface-soil-dilution plating technique (Ali-Shtayeh et al., 1986).

2.2 Preparation of isolates for examination

Uni-fungal isolates were prepared and grown on wet-sterilized, natural substrates in water (Dick, 1990). For microscopic examination, mounts of living material in water mounts ringed with a temporary sealant were prepared. Hyphae were cut from baits using rocking movement of a large round-edged scalpel blade. Excised material was floated over a partly submerged square cover glass. The cover glass was gently raised out of water keeping it almost horizontal, so that excess water drains from the edges and spreads out the hyphae. Underside excess of water was then drained and the cover glass was inverted over a drop of water on a slide. Excess water was blotted away from the edges of the mount using folded tissue held against the edge of the cover glass. A well-made temporary preparation remains in healthy living conditions for at least 24h, especially if refrigerated (Dick, 1990).

2.3 Collection of biometric and descriptive data

2.3.1 Direct observations

For each isolate, a total of 25 individual growth from each slide was examined separately and the mean of three measurements was used for each character. The following measurements and criteria were used in data collection:

1. Diameter of the oogonium, ignoring spines or papillae was recorded. Measurements of the diameter of up to 25 oogonia (og) were recorded and the mean value was used.

2. Measurements of the diameter of up to 25 oospores was carried out. The mean value for oospores (os) and the standard deviation was determined.

3. Oospore wall thickness (ot) was determined and measurements of up to 25 oospores were used.

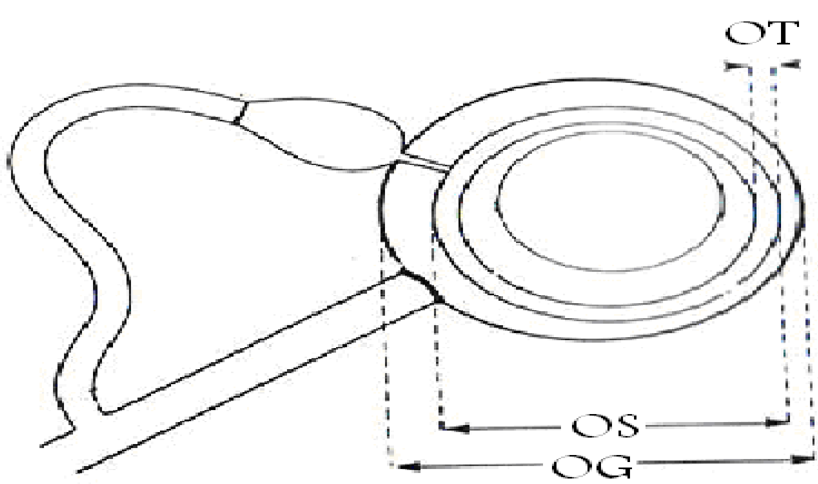

Figure (2.1): Diagram of measurable oogonial criteria: oogonial diameter, OG; oospore diameter, OS; oospore wall thickness, OT.

2.3.2 Derived data

1- Aplerotic index

For the purpose of the current research, plerotic was used for an oospore which occupies more than 75% of the oogonial volume; nearly plerotic was used for an oospore which occupies from 66% to 75% of the oogonial volume; and aplerotic was used for an oospore which occupies less than 65% of the oogonial volume.

The aplerotic index was calculated using the following formula (Dick, 1990).

$$100 \text{ x } \left[\frac{\sum \dfrac{\left(\dfrac{os1}{2}\right)^3}{\left(\dfrac{og1}{2}\right)^3}, \ldots\ldots\ldots, \dfrac{\left(\dfrac{os25}{2}\right)^3}{\left(\dfrac{og25}{2}\right)^3}}{25} \right] \%$$

2- Wall index

The relative volume of the oospore occupied by wall material was called the wall index.

The wall index was calculated using the following formula (Dick, 1990).

$$100 \text{ x } \left[1 - \left[\frac{\sum \dfrac{\left[\left(\dfrac{os1}{2}\right) - ot1\right]^3}{\left(\dfrac{ot1}{2}\right)^3}, \ldots\ldots\ldots, \dfrac{\left[\left(\dfrac{os25}{2}\right) - ot25\right]^3}{\overset{\%}{\left(\dfrac{ot25}{2}\right)^3}}}{25} \right] \right] \%$$

2.4 Statistical analysis

Correlations between pairs of variables within upper triangle and between lower triangle for means of measured diameters (oogonia and oospores), oospore wall thickness and distribution of oospore diameters and oospore wall thickness for all isolates were determined.

According to the morphological characteristics of *Pythium* isolates, the species were divided into three main groups according to their aplerotic index.

Based on morphological characteristics of both sporangia and oogonia, *Pythium* species were classified to four major groups including:

1- Smooth oogonia and globose or subglobose sporangia

2- Spiny oogonia and globose sporangia and/or hyphal swellings

3- Filamentous (non-swollen) sporangia

4- Lobulate sporangia

Collected data concerning the various groups mentioned above was then analyzed using multivariate analysis of variance.

Data concerning the studied four variables were collected from the 147 isolates. Twenty five individual measurements were used for each variable in each isolate. Data concerning the various groups resulted from morphological findings was analyzed using SPSS. Clustering analysis and canonical variate analysis were used in order to differentiate between *Pythium* species.

2.4.1 Clustering analysis

In the present study, average linkage clustering techniques was used in order to avoid extremes that might be introduced by single and complete linkage clustering techniques (Kerbs, 1998). Cluster analysis made use of the computer program statistical program for social sciences (SPSS – Version 13.0, 2005).

2.4.2 Canonical variate analysis

The first canonical variate defines the combination of measures used and gives the best greatest separation of isolates. The second canonical variate defines the next best dimension for separating isolates. The number of possible canonical variate corresponds to the number of variables. The value of a specific parameter for the group discrimination may be assessed by its correlation to the different canonical variate and these correlations were calculated. Since groups (species or isolates) are discriminated by their differences in canonical variate, and the variates are non-correlated, the extent to which the parameters separate groups may be well visualized by plotting, for example, the first varieties against the second varieties, since the varieties are collective expressions of several parameters. Such plots were made where appropriate. When species are used as group centers in the analysis, the fractions of single observations correctly allocated to their species of origin and fractions misclassified to other species are calculated on the basis of distance evaluations, thus giving a more detailed evaluation of how will the biometric parameters separate species compared with what could be obtained from plots of canonical variates (MØller and Hockenthull, 2001).

Chapter Three

Results

3.1 Results

According to analysis, the approach has been to start by considering each variable on its own, then proceeding to all of them together. The results showed that there are real differences between isolates in the means of each of the three variables. However, this is of little value when there is a continuum of isolates between the two extremes, e.g., a small oospore diameter and large oospore diameter.

Figure 3.1 and Figure 3.2 show the distribution of means of oospore diameter, and oospore wall index. The distribution of each variable is remarkably uniform, with a few outlying species at the lower and upper extremes of each variable. From plots it can be seen that discontinuities between isolate means are few and extremely narrow (Moller & Hockenthull, 2001).

The use of any of these variables, on its own, will not allow species or isolates to be separated without introducing arbitrary cut-off points, with some isolates falling on either side of the cut-off. I therefore considered all variables together.

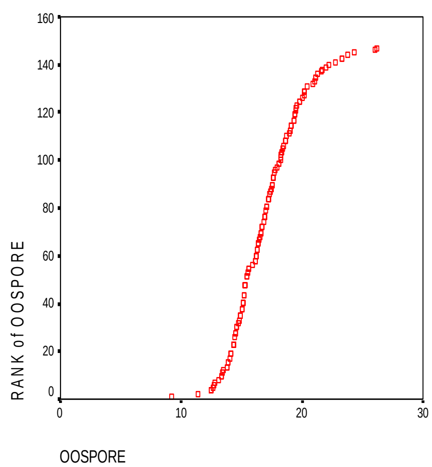

Figure (3.1): Distribution of oospore diameters for all isolates on scale 0-160

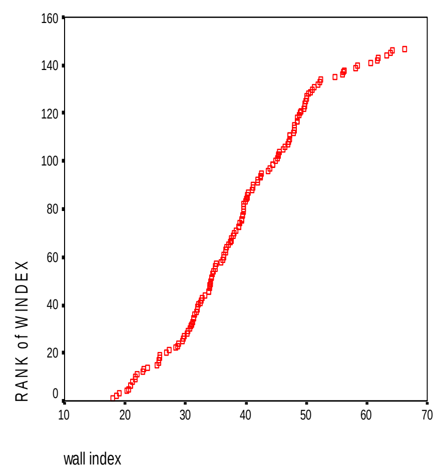

Figure (3.2): Distribution of oospore wall index for all isolates on scale 0-160.

Table 3.1 shows high correlation between the two diameters and moderate correlation between the diameters and wall thickness. Thus large oogonium diameter tends to go with a large oospore diameter. This is in agreement with the findings of Shahzad *et al*. (1992).

The correlation between the original variables and derived index are much smaller. Correlations between measured parameters and their derived indices are low within isolates and genarly low between isolates, although higher than those found by Shahzad *et al* (1992),

Table (3.1): Correlations between pairs of variables within (upper triangle and between lower triangle) for means of species isolates

Character	Oogonium diameter	Oospore diameter	Oospore wall thickness	Wall index
Oogonium diameter	1	.914	.506	.047
Oospore diameter	.914	1	.589	.167
Oospore wall thickness	.506	.589	1	.771
Wall index	.047	.167	.771	1

We now consider all three variables and show the value of considering them jointly when describing the isolates canonical variate analysis is used.

The technique is used for finding variables or combinations of variables that best discriminate between groups of individuals. Here oogonia are the individuals, and isolates are the groups. I am looking for measures that allow isolates, or at least isolates of different species, to be separated. The first canonical variate defines the combination of measures used which gives the greatest separation power of isolates. The second canonical variate defines the next best dimension for separating isolates (Korzeniowski, 1987).

A canonical variate analysis was carried out using all three variables (oogonium diameter, oospore diameter, and oospore wall index). A dendrogram using average linkage (between groups) was constructed for each analysis.

Of the forty one species (Table A.3) used in this work, twenty three possessed smooth oogonia and globose or subglobose sporangia and/or hyphal swellings, six possessed spiny oogonia, seven species possessed filamentous (non-swollen) sporangia, five species possessed lobulate sporangia, eighteen species possessed plerotic oospores, four possessed nearly plerotic oospore, and nineteen possessed aplerotic oospores.

3.2 Canonical variate analysis using all three variables for all *Pythium* species used in this study.

3.2.1 Canonical variate analysis using means of isolates

Twelve groups of isolates (Table A.1.4) can be seen from the plots of means of isolates of all *Pythium* species on canonical variates 1 (horizontal) which explained 63.5 % and 2 (vertical) (Figure 3.3) which explained 27.5 % of the variance (three characters, oogonium dimension, oospore dimension, and oospore wall thickness were employed).

And from the constructed dendrogram (Figure 3.4) six groups of isolates (Table 3.2) can be seen. *Pythium graminicola* was the only species that could be easily segregated on the basis of their oogonial and oospore dimensions, having significantly larger oogonia and oospores.

However, two isolates of *Pythium* species although formed a group on it characters one isolate of *Pythium dissimile* and the other isolate of *P.*

tenue. Groups 1-4 consist of species which are obviously difficult to separate on the basis of their oospore and oogonial dimensions alone.

Based on studied criteria only one species separated from the other. Inter and intra specific variations in the species studied were large enough to mask the differences between species and therefore, one species could be separated on these basis (*P. graminicola)* which has the largest oogonia and oospore.

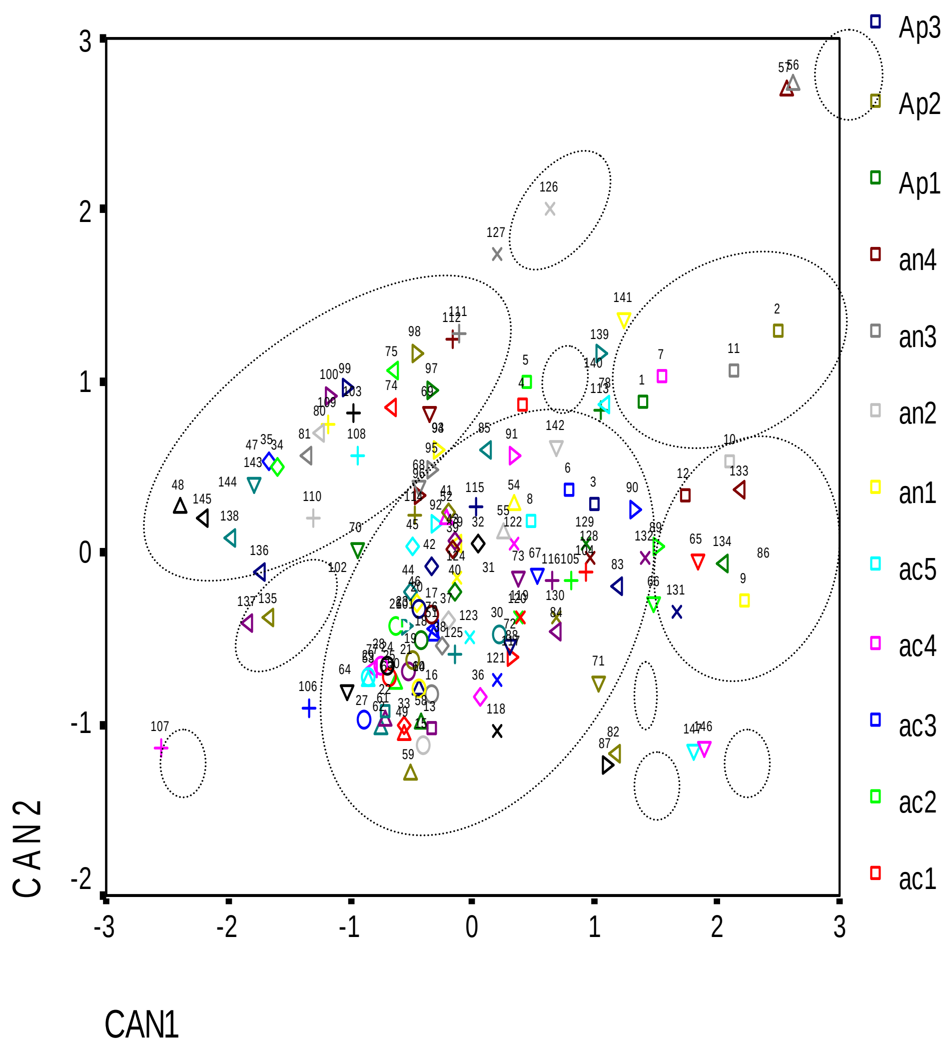

Figure (3.3): Plots of 147 isolate of Pythium species, on two canonical variates 1 (horizontal), 2 (vertical); characters used are: oogonia diameter, oospore diameter, oospore wall thickness.

Rescaled Distance Cluster Combine

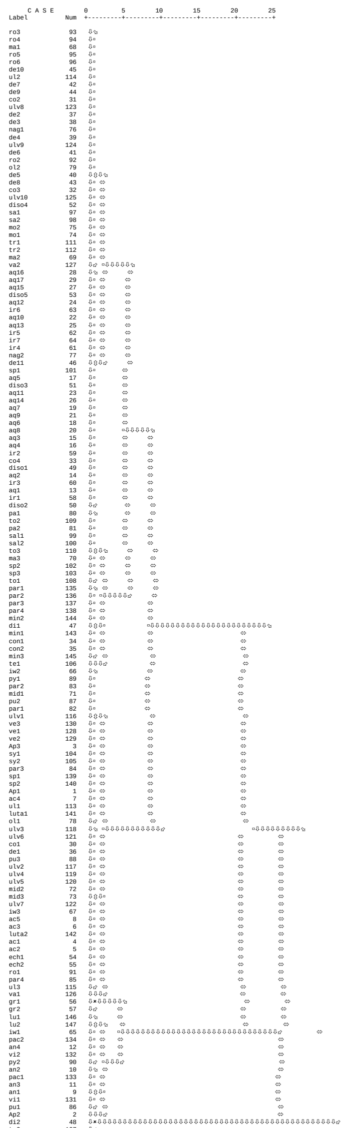

Figure (3.4): Dendrogram for all isolates of Pythium species, characters used are: oogonia diameter, oospore diameter, and oospore wall thickness.

Table (3.2): Cluster groups for all isolates of Pytium species

	cluster 1	cluster 2	cluster 3	cluster 4	cluster 5	cluster 6
	P.acanthicum(1-5)	*P.anandrum* (1-4)	*P.aquatile*(1-17)	*P.conidiophorum*(1,2)	*P.dissimile (2)*	*P.graminicola (1,2)*
	P.aphanidermatum(1,3)	*P.aphanidermatum*(2)	*P.coloratum*(2-4)	*P.dissimile*(1)	*P.tenue(2)*	
	P.coloratum (1)	*P.iwayami* (1)	*P.debaryanum*(2-11)	*P.mamillatum* (3)		
	P.debaryanum (1)	*P.lucens* (1,2)	*P.dissotocum*(1-5)	*P.papillatum* (1,2)		
	P.echinulatum (1,2)	*P.pachycaule* (1,2)	*P.irregulare*(1-7)	*P.parvum* (1-4)		
	P.iwayam (2,3)	*P.pulchrum* (1)	*P.mamillatum* (1,2)	*P.salpingophorum*(1,2)		
	P.middletonii (1-3)	*P.pyrilobum* (2)	*P.monospermum* (1,2)	*P.spinosum* (2,3)		
	P.oligandrum (1)	*P.violae* (1,2)	*P.nagaii*(1,2)	*P.tenue*(1)		
	P.paroecandrum(1-4)		*P.oligandrum* (2)	*P.torulosum* (1-3)		
	P.pulchrum (2,3)		*P.rostratum* (2-6)	*P.minus* (1-3)		
	P.pyrilobum (1)		*P.salinum* (1,2)			
	P.rostratum (1)		*P.spinosum*(1)			
	P.sylvaticum (1,2)		*P.tracheiphilum* (1,2)			
	P.ultimum.var.sporangi iferum (1-7)		*P.ultimum.var.sporan giiferum* (8-10)			
	P.ultimum var ultimum(1,3)		*P.ultimum var ultimum*(2)			
	P.vexans(1-3)		*P.vantrepoolii*(2)			
	P.vantrepoolii (1)					
	P.sp (ma2153)(1,2)					
	P.lutarium (1,2)					
Range of oogonia	19.6-24.8	24.3-28.5	16.8-20	12.6-16.8	11.4-11.8	26-26.2
Range of oospore	15.6-20.44	19.8-24.3	13.8-18.6	12.5-16.2	9.2-11.4	26-26.2
Range of oospore wall thickness	0.86-2.8	0.8-3.23	0.5-2.5	0.5-1.9	0.9-1	2.5

3.2.2 Canonical variate analysis using means of species

From the constructed dendrogram (Figure 3.4) five groups of isolates (Table 3.3) can be seen. *Pythium graminicola* was the only species that could be easily segregated on the basis of their oogonial and oospore dimensions, having significantly largest oogonia and oospores. Groups 1-4 consist of species which are obviously difficult to separate on the basis of their oospore and oogonial dimensions alone.

When species were clustered by using the mean of isolates the result wasn't change. Inter and intra specific variations in the species studied were large enough to mask the differences between species and there for one species could be separated on these basis (*P. graminicola)* which has the largest oogonia and oospore.

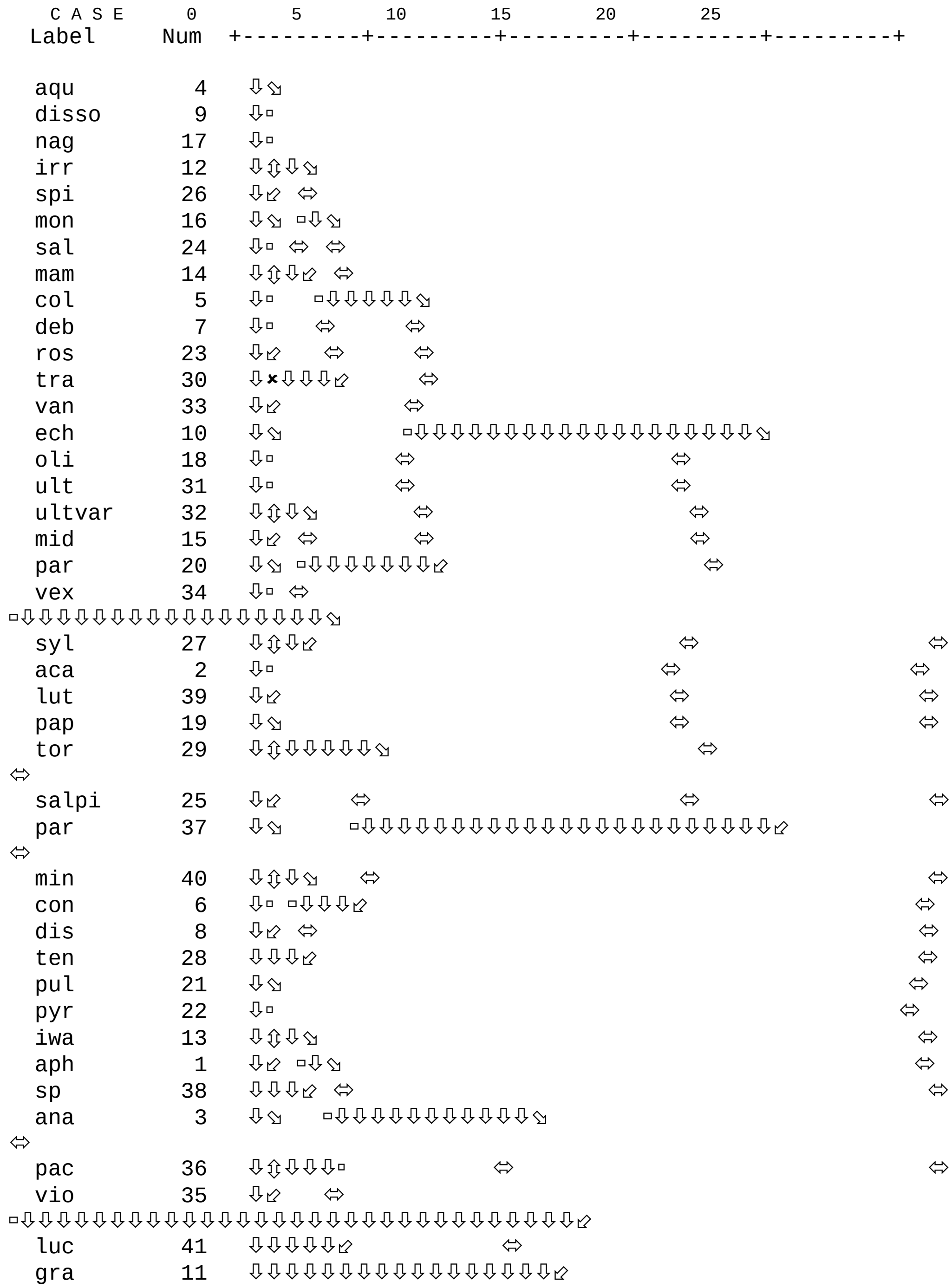

Figure (3.5): Dendrogram for isolates of Pythium species means, characters used are: oogonia diameter, oospore diameter, and oospore wall thickness.

Table (3.3): Cluster groups for means of Pytium species

	cluster1	cluster 2	cluster 3	cluster 4	cluster 5
	P.anandrum	*P.acanthicum*	*P.aquatile*	*P.conidiophorum*	*P.graminicola*
	P.aphanidermatum	*P.echinulatum*	*P.coloratum*	*P.dissimile*	
	P.iwayami	*P.lutarium*	*P.debaryanum*	*P.minus*	
	P.lucens	*P.middletonii*	*P.dissotocum*	*P.papillatum*	
	P.pachycaule	*P.oligandrum*	*P.irregulare*	*P.parvum*	
	P.pulchrum	*P.paroecandrum*	*P.mamillatum*	*P.salpingophorum*	
	P.pyrilobum	*P.sylvaticum*	*P.monospermum*	*P.tenue*	
	P.sp (ma2153)	*P. ultimum var. sporangiiferum*	*P.nagaii*	*P.torulosum*	
	P.violae	*P.ultimum var ultimum*	*P.rostratum*		
		P.vexans	*P.salinum*		
			P.spinosum		
			P.tracheiphilum		
			P.vantrepoolii		
Range of oogonia	22.55-26.6	20.03-23	17.07	12.4-15.67	26.1
Range of oospore	19.53-22.9	17.21-19.55	14.6-18.75	10.95-15.76	26.1
Range of oospore wall thickness	0.85-2.523	1.15-2.3	0.85-2.65	0.5-1.85	2.5

3.2.3 Canonical variate analysis using means of isolates for Smooth oogonia and globose or subglobose sporangia

Eight groups of isolates (Table A.4.4) can be seen from the plots of means of isolates of all *Pythium* species on canonical variates 1 (horizontal) which explained 52.8 % and 2 (vertical) (Figure 3.6) which explained 28.9 % of the variance.

And from the constructed dendrogram (Figure 3.7) five groups of isolates (Table 3.4) can be seen. *Pythium conidiophorum* was the only species that could be easily segregated on the basis of their oogonial and oospore dimensions, having significantly smallest oogonia and oospores.

Based on studied criteria only one species separated from the other. Inter and intra specific variations in the species studied were large enough to mask the differences between species and therefore, one species could be separated on these basis (*P. conidiophorum)* which has the largest oogonia and oospore.

However, one isolate of *Pythium ultimum var ultimum* although formed a group on it characters, because it has significantly smaller oogonia and oospores between the other isolates of the same species. Intra specific variations in this species studied were large enough to mask the differences between isolates of *Pythium ultimum var ultimum* and other species. Groups 2-4 consist of species which are obviously difficult to separate on the basis of their oospore and oogonial dimensions alone.

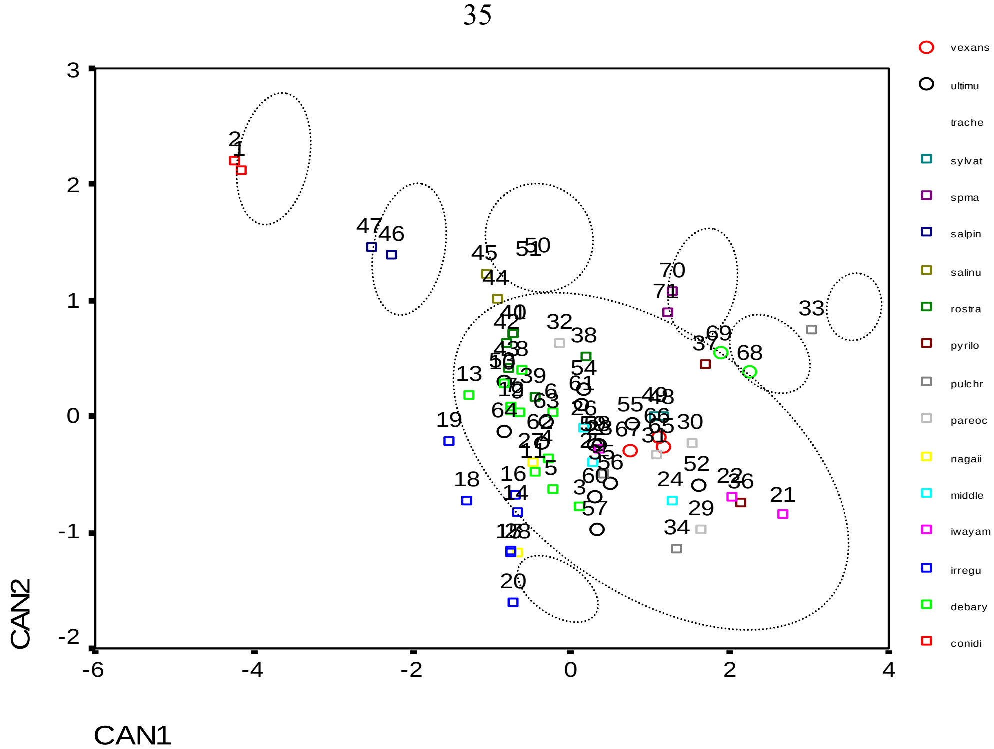

Figure (3.6): Plots of 76 isolates with smooth oogonia and globose or subglobose species of Pythium species, on two canonical variates 1 (horizontal), 2 (vertical); character used are: oogonia diameter, oospore diameter, oospore wall thickness.

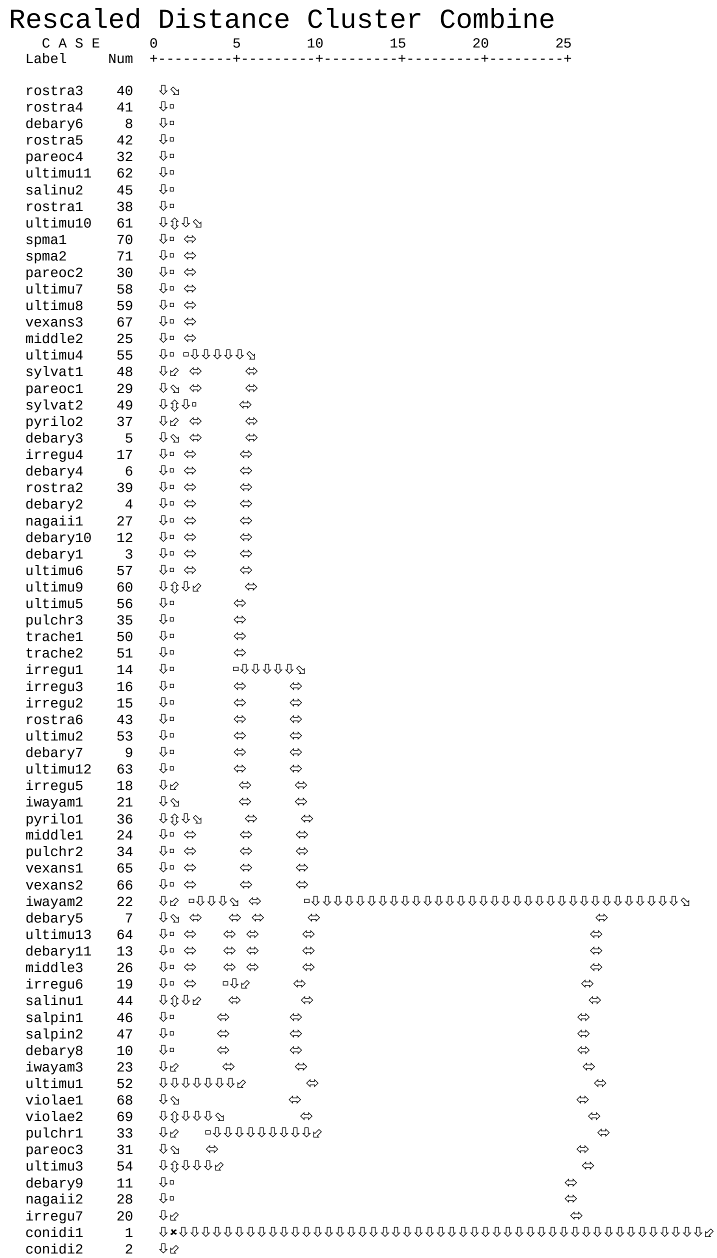

Figure (3.7): Dendrogram for smooth oogonia and globose or subglobose isolates of Pythium species, characters used are: oogonia diameter, oospore diameter and oospore wall thickness.

Table (3.4): Cluster groups for Smooth oogonia and globose or subglobose Pythium isolates

	group 1	group 2	group 3	group 4	group 5
	P.Conidiophorum (1,2)	*P.debaryanum* (1-4,6,7,10)	*P.debaryanum* (5,8,11)	*P.debaryanum* (9)	*P.ultimum var ultimum* (1)
		P.irregulare (1-5)	*P.irregulare* (6)	*P.irregulare* (7)	
		P.middletonii (2)	*P.iwayamai* (1-3)	*P.nagaii* (2)	
		P.nagaii (1)	*P.middletonii* (1,3)	*P.pareocandrum* (3)	
		P.pareocandrum (1,2,4)	*P.pulchrum* (2)	*P.pulchrum* (1)	
		P.pulchrum (3)	*P.pyrilobum* (1)	*P.ultimum var ultimum* (3)	
		P.pyrilobum (2)	*P.salinum* (1)	*P.violae* (1,2)	
		P.rostratum (1-6)	*P.salpingophorum* (1,2)		
		P.salinum (2)	*P. ultimum var. sporangiiferum* (10)		
		P.sp.(ma2153) (1,2)	*P.vexans* (1,2)		
		P.sylvaticum (1,2)			
		P.tracheiphilum (1,2)			
		P.ultimum var. sporangiiferum (1-9)			
		P.ultimum var ultimum (2)			
		P.vexans (3)			
Range of oogonia	13-13.2	17.3-24.8	14.9-25.6	17.3-28.5	21.93
Range of oospore	12.5	14.1-21.3	14.3-21	14.3-23.8	19.52
Range of oospore wall thickness	1.8-1.9	0.8-1.5	1.2-2	0.6-1.4	2.34

3.2.4 Canonical variate analysis using means of isolates for Spiny oogonia and globose sporangia and/or hyphal swellings

Six groups of isolates (Table A.5.4) can be seen from the plots of means of isolates of all *Pythium* species on canonical variates 1 (horizontal) which explained 89.6 % and 2 (vertical) (Figure 3.8) which explained 9.8 % of the variance.

And from the constructed dendrogram (Figure 3.9) three groups of isolates (Table 3.5) can be seen. *Pythium anandrum* was the only species that could be easily segregated on the basis of their oogonial and oospore dimensions, having significantly larger oogonia and oospores.

Based on studied criteria only one species separated from the other. Inter and intra specific variations in the species studied were large enough to mask the differences between species and therefore, one species could be separated on these basis (*P. anandrum)* which has the largest oogonia and oospore.

Groups 1,3 consist of species which are obviously difficult to separate on the basis of their oospore and oogonial dimensions alone.

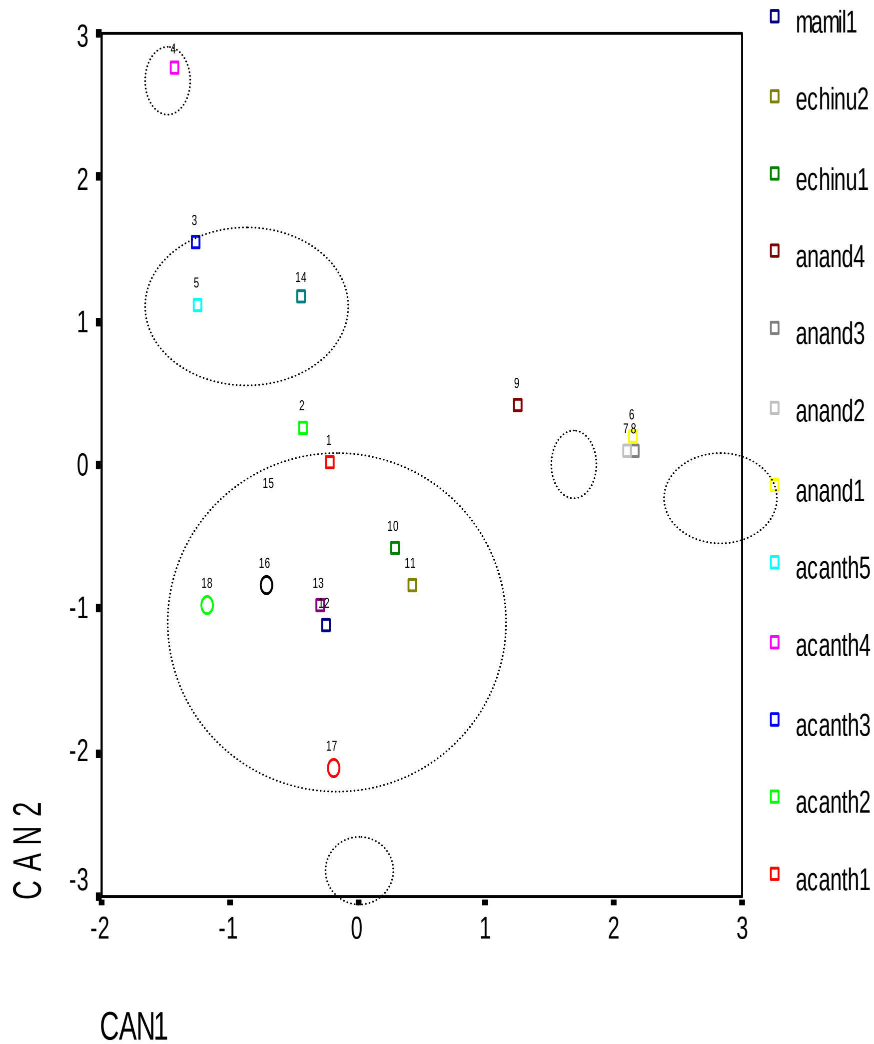

Figure (3.8): Plots of 18 isolates with spiny oogonia and globose sporangia and/or hyphal swellings of Pythium species, on two canonical variates 1 (horizontal), 2 (vertical); character used are: oogonia diameter, oospore diameter, oospore wall thickness.

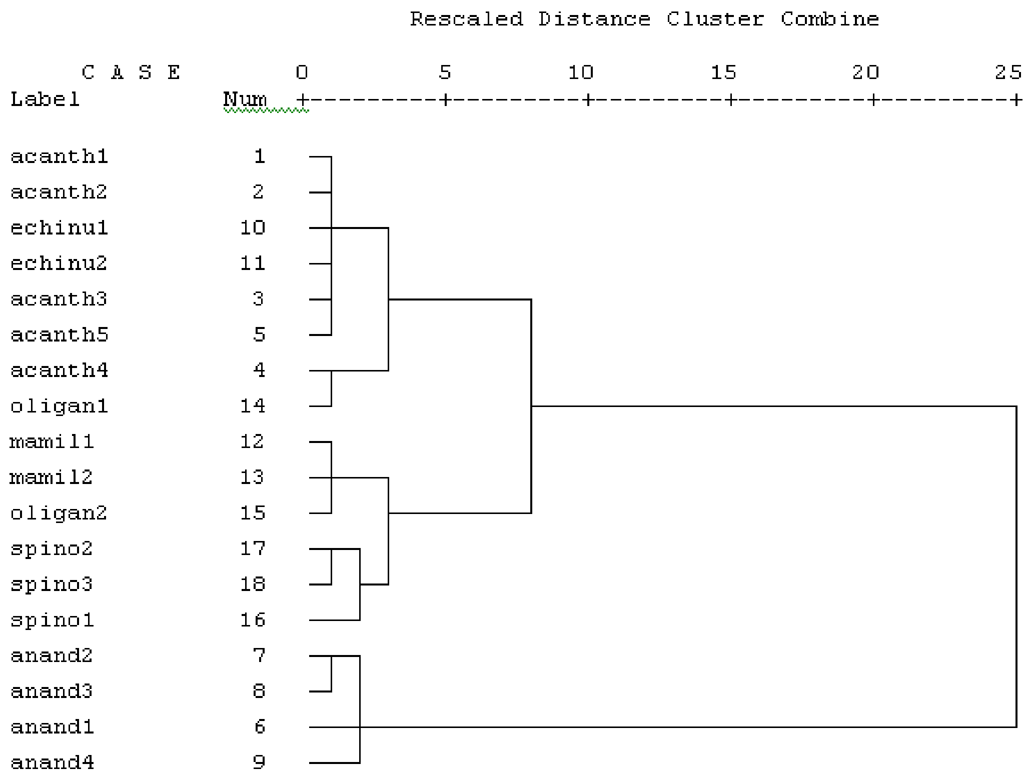

Figure (3.9): Dendrogram for spiny oogonia and globose sporangia and/or hyphal swellings isolates of Pythium species, characters used are: oogonia diameter, oospore diameter, and oospore wall thickness.

Table (3.5): Cluster groups for Spiny oogonia and globose sporangia and/or hyphal swellings Pythium isolates

	cluster 1	cluster 2	cluster 3
	P.acanthicum (1-5)	*P.anandrum* (1-4)	*P.mamillatum* (1,2)
	P.echinulatum (1,2)		*P.oligandrum* (2)
	P.oligandrum (1)		*P.spinosum* (1-3)
Range of oogonia	20.5-23.13	25.3-27.4	16.2-19.2
Range of oospore	17.45-20.44	21.7-24.3	15.1-17.9
Range of oospore wall thickness	1.1-2.8	1.4-1.7	0.5-1.4

3.2.5 Canonical variate analysis using means of isolates for Filamentous (non-swollen) sporangia

Nine groups of isolates (Table A.6.4) can be seen from the plots of means of isolates of all *Pythium* species on canonical variates 1 (horizontal) which explained 81.4% and 2 (vertical) which explained 18.3 % of the variance (three characters, oogonium dimension, oospore dimension, and oospore wall thickness were employed) (Figure 3.10).

Nine groups of isolates can also been seen from the constructed dendrogram (Figure 3.11) (Table 3.6). No *Pythium* species could be easily separated because they have comparable oogonia and oospores diameters.

Based on studied criteria no species could be separated from the other. Inter specific variations in the species studied weren't large enough to mask the differences between species.

However, three isolates of *Pythium* species one isolate of *Pythium tenue,* one isolate of *Pythium coloratum*, and one isolate of *Pythium papillatum* although formed a group on it characters, because *(Pythium tenue* and *Pythium papillatum* have significantly smaller oogonia and oospores between the other isolates of the other species and *Pythium coloratum* has significantly larger oogonia and oospores between the other isolates of the other species). Intra specific variations in this species studied were large enough to mask the differences between isolates of *Pythium tenue*, *Pythium coloratum*, *Pythium papillatum* and other species.Groups 1-4 consist of species which are obviously difficult to separate on the basis of their oospore and oogonial dimensions alone.

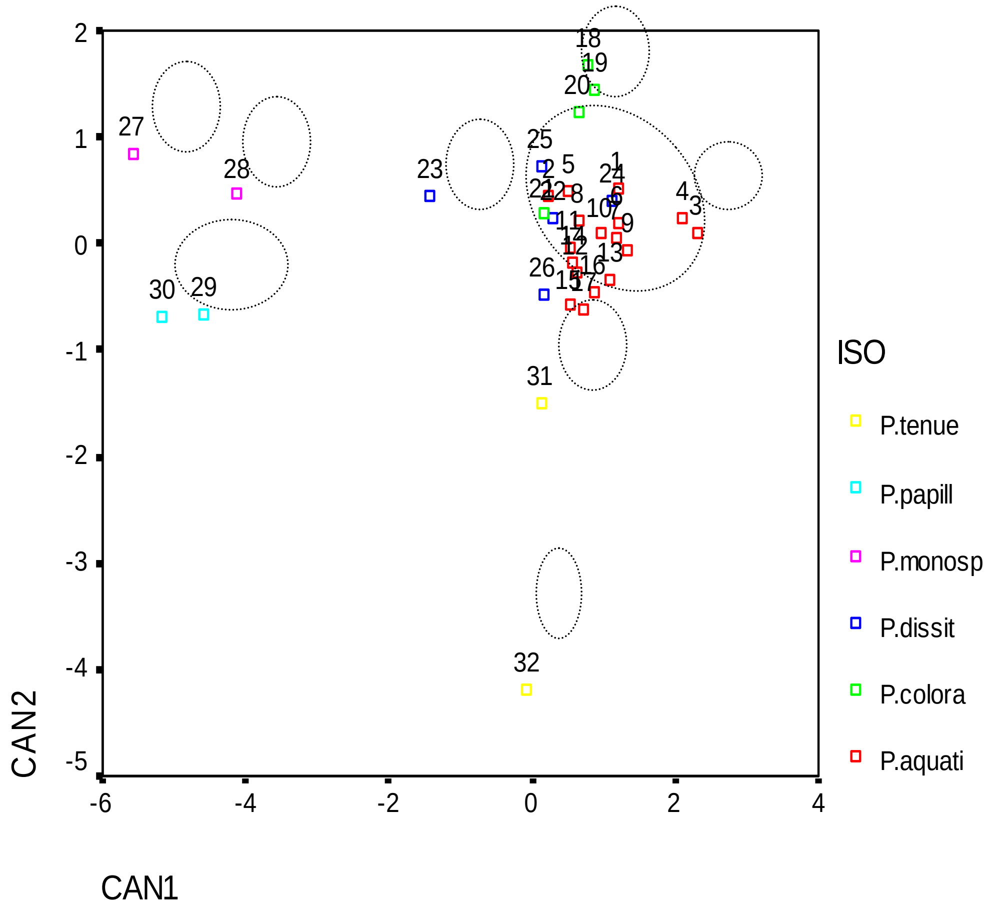

Figure (3.10): A plot of 30 isolates with filamentous (non-swollen) sporangia of Pythium species, on two canonical variates 1(horizontal), 2(vertical); character used are: oogonia diameter, oospore diameter, oospore wall thickness.

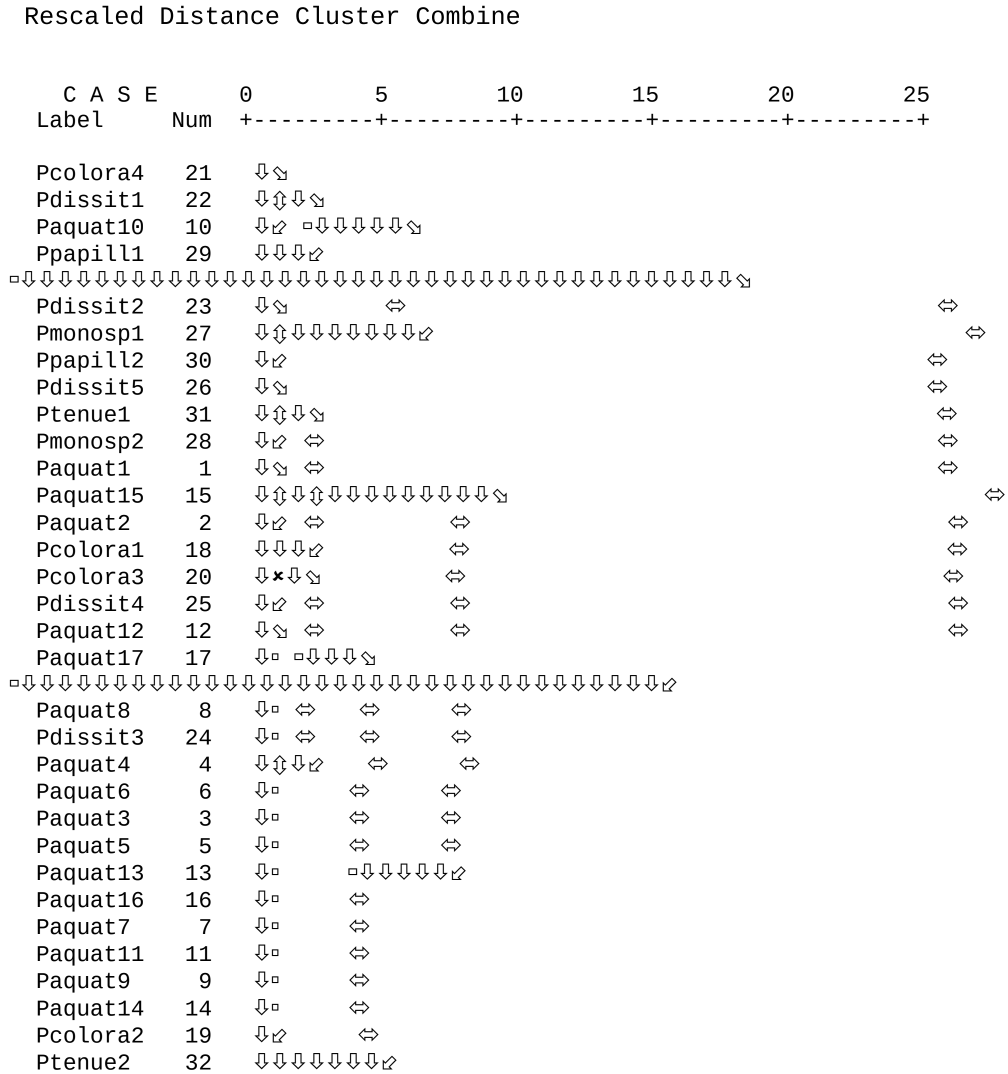

Figure (3.11): Dendrogram for filamentous (non-swollen) sporangia isolates of Pythium species, characters used are: oogonia diameter, oospore diameter, and oospore wall thickness.

Table (3.6): Cluster groups for Filamentous (non-swollen) sporangia Pythium isolates

	cluster 1	cluster 2	cluster 3	cluster 4	cluster 5	cluster 6	cluster 7	cluster 8	cluster 9
	P.aquatile (1,2,15)	*P. coloratum* (3)	*P.aquatile* (3-9,11-14,16, 17)	*P. papillatum* (1)	*P.tenue* (2)	*P.aquatile* (10)	*P. dissitocum* (2)	*P. monospermum* (2)	*P. coloratum* (1)
		P.dissitocum (4)	*P.coloratum* (2)			*P .coloratum* (4)	*P. monospermum* (1)	*P.dissitocum* (5)	
			P.dissitocum (3)			*P. dissitocum* (1)	*P. papillatum* (2)	*P.tenue (1)*	
Range of oogonia	17.5-19.4		17.2-20	15.3	11.8	17.8-18.9	15.3-18.8	*17.6-15.9*	20.7
Range of oospore	13.8-15.3		13.8-16.6	15.3	9.2	13.9-14.9	15.3-17.6	*12.7-17.6*	16.9
Range of oospore wall thickness	1-1.1		1-1.7	0.9	0.9	0.8-1.3	0.5-0.6	*0.6-1.1*	1.4

3.2.6 Canonical variate analysis using means of isolates for lobulate sporangia

On canonical variates 1 (horizontal) which explained 76.9 % and 2 (vertical) (Figure 3.12) which explained 18.6 % of the variance, five groups of isolates (Table A.7.4) can be seen from the plots of means of isolates of all *Pythium* species.

It can also been seen from the constructed dendrogram (Figure 3.13) six groups of isolates (Table 3.7). *Pythium graminicola* and *Pythium vanterpoolii* could be easily segregated on the basis of their oogonial and oospore dimensions, having significantly larger oogonia and oospores.

Based on studied criteria two species were separated from one another. Inter and intra specific variations in the species studied were large enough to mask the differences between species and therefor two species could be separated on these basis (*P. graminicola* and *P. vanterpoolii)* which each one of them have the same oogonia and oospore dimentions.

However two isolates of *Pythium* species although formed a group. One isolate of *Pythium dissimile* and the other isolate of *Pythium aphanidermatum* because *Pythium dissimile* has significantly smaller oogonia and oospores between the other isolates of the other species and because *Pythium aphanidermatum* has significantly larger oogonia and oospores between the other isolates of the same species. Intra specific variations in this species studied were large enough to mask the differences between isolates of *Pythium dissimile* and *Pythium aphanidermatum*. Groups 1, 3 consist of species which are obviously difficult to separate on the basis of their oospore and oogonial dimensions alone.

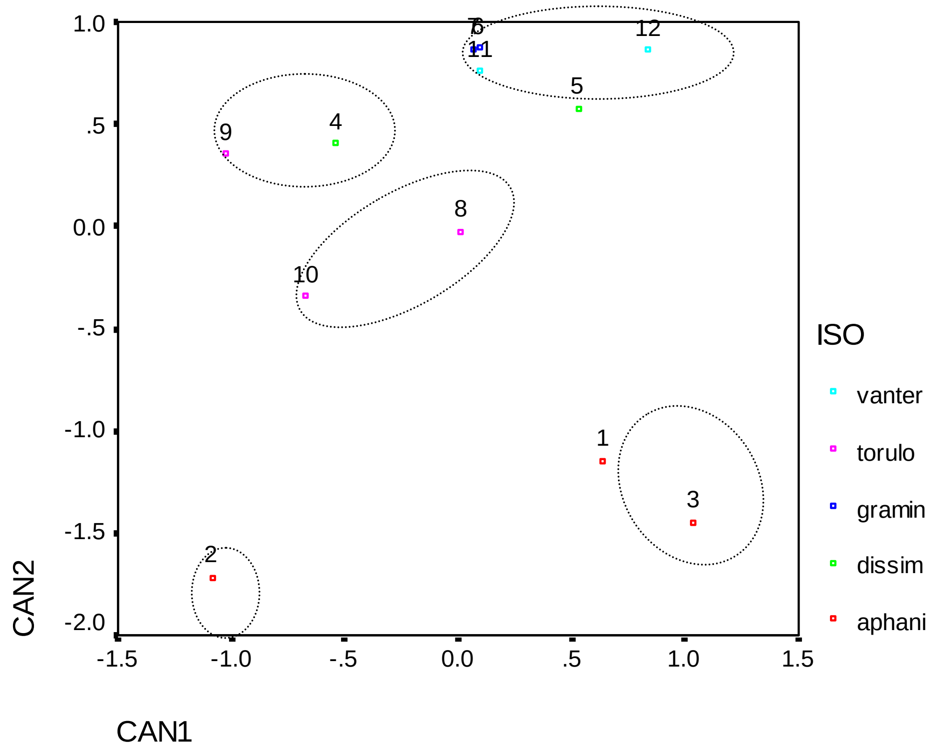

Figure (3.12): A plot of 10 isolates with lobulate sporangia of Pythium species, on two canonical variates 1 (horizontal), 2 (vertical); character used are: oogonia diameter, oospore diameter, oospore wall thickness.

```
                    Rescaled Distance Cluster Combine

    C A S E      0         5        10        15        20        25
  Label     Num  +---------+---------+---------+---------+---------+

  gramin1     6   ⇩×⇩⇩⇩⬂
  gramin2     7   ⇩⬃   ▫⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⬂
  aphani3     3   ⇩⇩⇩⇩⇩⬃
▫⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⬂
  vanter1    11   ⇩×⇩⇩⇩⬂                         ⬄
⬄
  vanter2    12   ⇩⬃   ▫⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⬃
⬄
  aphani1     1   ⇩⇩⇩×⇩⬃                                              ⬄
  aphani2     2   ⇩⇩⇩⬃                                                ⬄
  torulo2     9   ⇩⬂                                                  ⬄
  torulo3    10   ⇩⇳⇩⇩⇩⇩⇩⬂
⬄
  dissim1     4   ⇩▫
▫⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⇩⬃
  torulo1     8   ⇩⬃     ⬄
  dissim2     5   ⇩⇩⇩⇩⇩⇩⇩⬃
```

Figure (3.13): Dendrogram for lobulate sporangia isolates of Pythium species, characters used are: oogonia diameter, oospore diameter, and oospore wall thickness.

Table (3.7): Cluster groups for lobulate sporangia Pythium isolates

	cluster 1	cluster 2	Cluster3	cluster 4	cluster 5	cluster 6
	P.vanterpoolii (1,2)	*P.aphanidermatum* (3)	*P.dissimile* (1)	*P.dissimile* (2)	*P.graminicola* (1,2)	*P.aphanidermatum* (1,2)
			P.torulosum (1-3)			
Range of oogonia	18.2-19.3	22.64	13.4-16.3	11.4	26-26.2	22.88-25.88
Range of oospore	18.2-19.3	19.49	13.4-15.5	11.4	26-26.2	20.16-22.72
Range of oospore wall thickness	2.5-2.8	1.78	1-1.2	1	2.5	2.56-3.23

3.2.7 Canonical variate analysis using means of isolates for plerotic oospore species

Four groups of isolates (Table A.8.4) can be seen from the plots of means of isolates of all *Pythium* species on canonical variates 1 (horizontal) which explained 58.4% and 2 (vertical) (Figure 3.14) which explained 34.4% of the variance.

And from the constructed dendrogram (Figure 3.15) four groups of isolates (Table 3.8) can be seen. *Pythium graminicola* was the only species that could be easily segregated on the basis of their oogonial and oospore dimensions, having significantly larger oogonia and oospores.

Inter and intra specific variations in the species studied were large enough to mask the differences between species and therefor one species could be separated on these basis (*P. graminicola* which has the largest oogonia and oospore). Based on studied criteria only one species was separated from the other.

Since dimensions are comparable in groups 1, 3, 4; consist separation of species basis of their oospore and oogonial dimensions alone are obviously difficult.

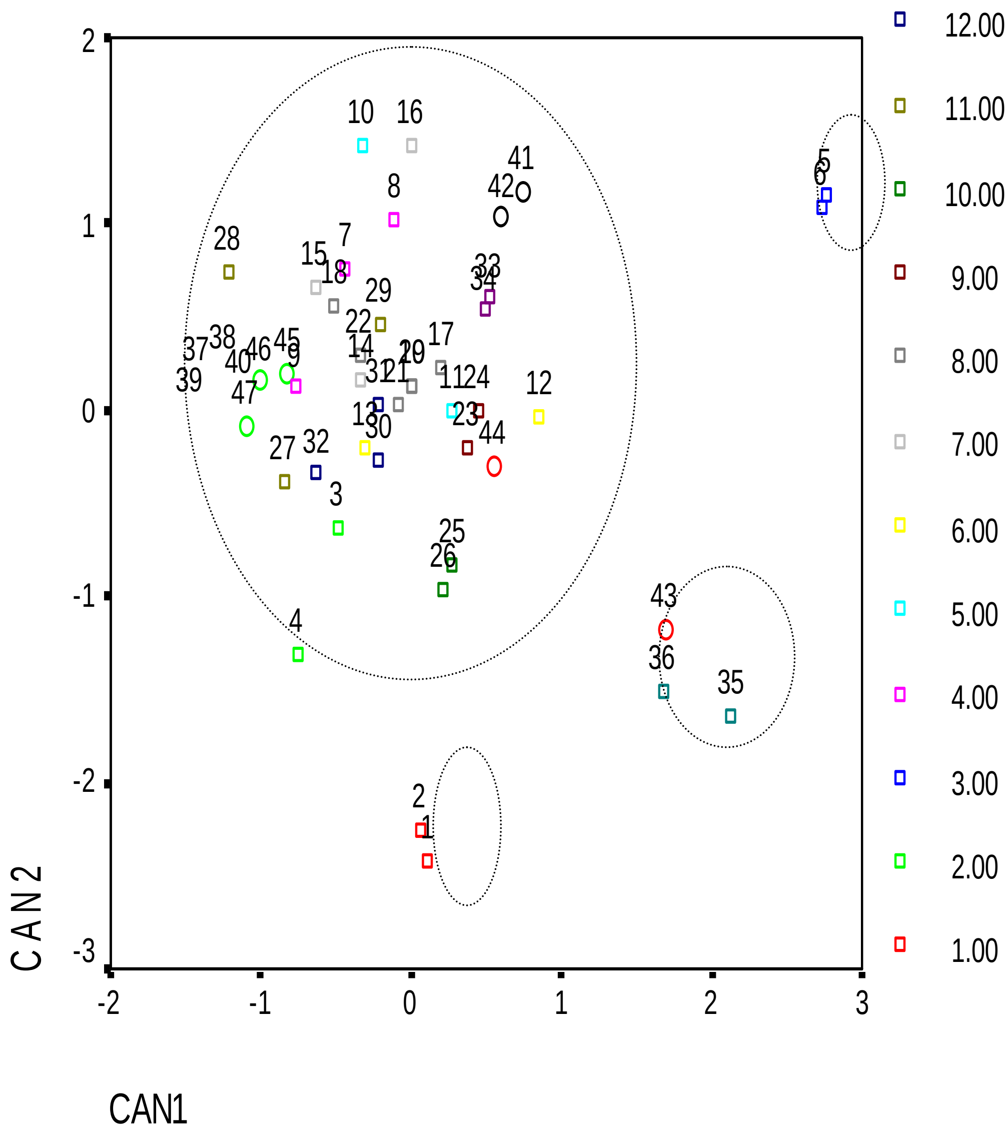

Figure (3.14): A plot of 47 isolates of plerotic oospore species, on two canonical variates 1 (horizontal), 2 (vertical); character used are: oogonia diameter, oospore diameter, oospore wall thickness.

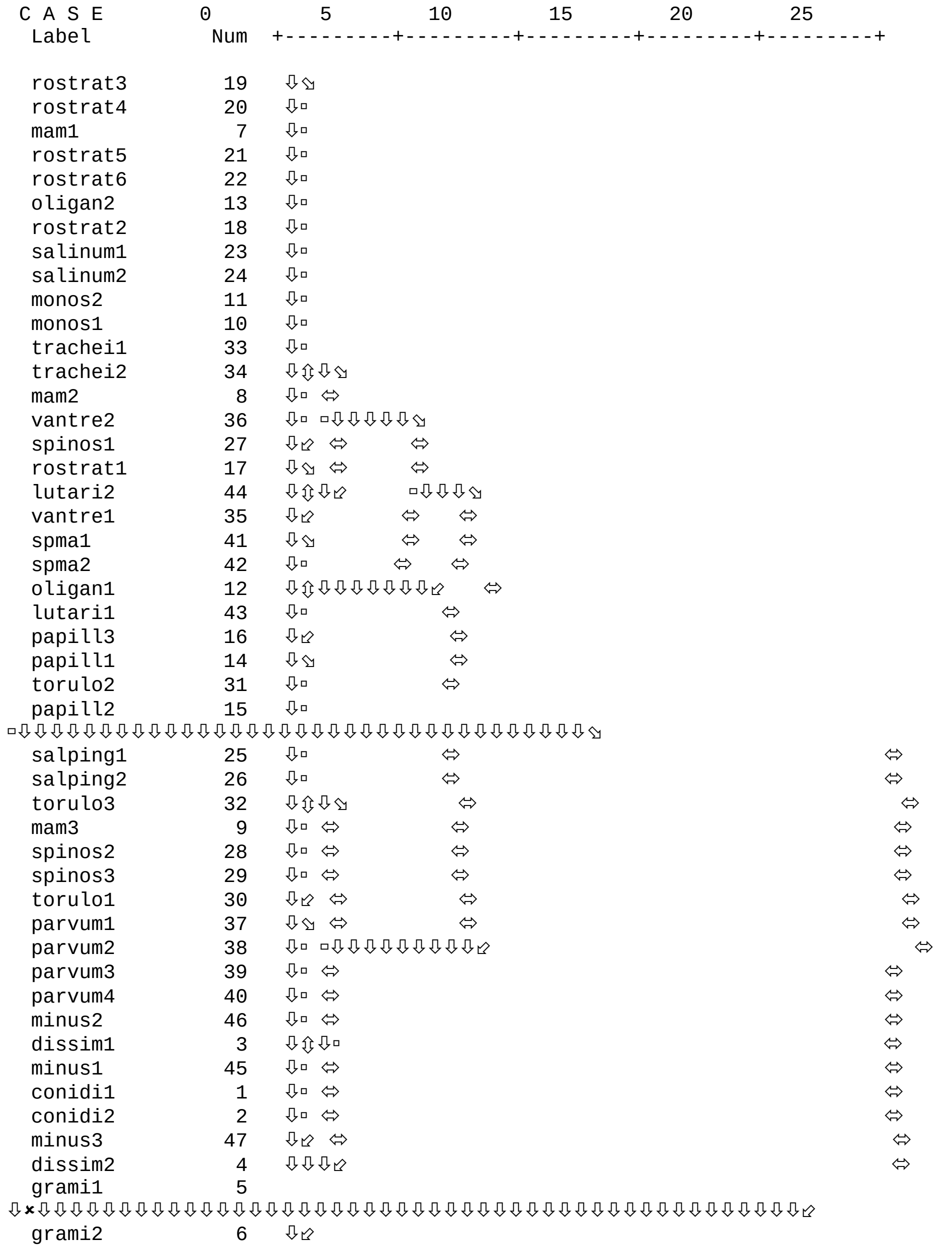

Figure (3.15): Dendrogram for isolates of plerotic oospore species, characters used are: oogonia diameter, oospore diameter, and oospore wall thickness.

Table (3.8): Cluster groups for plerotic oospore species

	cluster 1	cluster 2	cluster 3	cluster 4
	P.conidiophorum (1,2)	*P.graminicola* (1,2)	*P.lutarium* (2)	*P.lutarium* (1)
	P.dissimile (1,2)		*P.mamillatum* (1,2)	*P.oligandrum* (1)
	P.mamillatum (3)		*P.monospermum* (1,2)	*P.papillatum* (3)

	P.minus (1-3)		*P.oligandrum* (2)	*P.sp(ma2153)* (1,2)
	P.papillatum (1,2)		*P.rostratum* (1-6)	
	P.parvum (1-4)		*P.salinum* (1,2)	
	P.salpingophorum (1,2)		*P.spinosum* (1)	
	P.spinosum (2,3)		*P.tracheiphilum* (1,2)	
	P.torulosum (1-3)		*P.vantrepoolii* (1,2)	
Range of oogonia	11.4-16.8	26-26.2	16.8-21.2	21.9-24.3
Range of oospore	11.4-16.2	26-26.2	15.2-19.3	20.2-21.1
Range of oospore wall thickness	0.5-1.9	2.5	0.6-2.8	1.3-2.7

3.2.8 Canonical variate analysis using means of isolates for nearly plerotic oospore species

Six groups of isolates can be seen from the constructed dendrogram (Figure 3.17). No *Pythium* species segregated on the basis of their oogonial and oospore dimensions, because it having nearly measurement of oogonia and oospores.

Four groups of isolates (Table A.9.4) can also be seen from the plots of means of isolates of all *Pythium* species on canonical variates 1 (horizontal) which explained 68.8% and 2 (vertical) (Figure 3.16) which explained 31.2% of the variance.

Based on studied criteria no species were separated from the other. Inter specific variations in the species studied were large enough to mask the differences between species.

However, two isolates of *Pythium* species although formed a group. One isolate of *Pythium ultimum var ultimum* and the other isolate of *Pythium aphanidermatum* because *Pythium aphanidermatum* has significantly larger oogonia and oospores between the other isolates of the same species and because *Pythium ultimum var ultimum* has significantly smaller oogonia and oospores between the other isolates of the same species. Intra specific variations in this species studied were large enough to mask the differences between isolates of *Pythium ultimum var ultimum* and *Pythium aphanidermatum.* Groups 1 consist of species which are obviously difficult to separate on the basis of their oospore and oogonial dimensions alone.

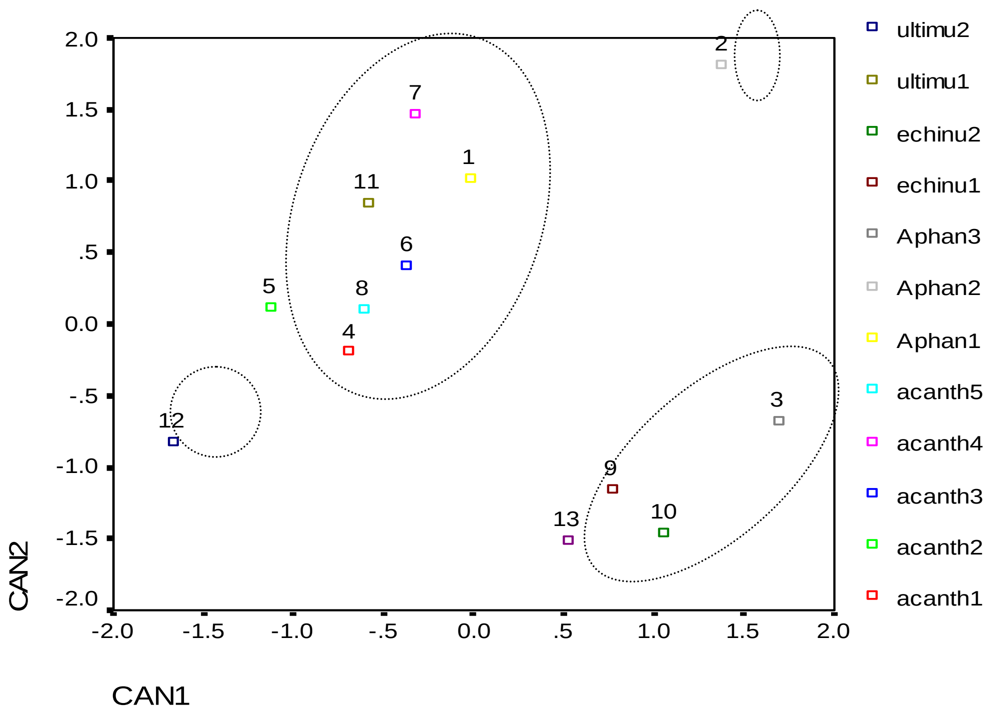

Figure (3.16): A plot of 13 isolates of nearly plerotic oospore species, on two canonical variates 1 (horizontal), 2 (vertical); character used are: oogonia diameter, oospore diameter, oospore wall thickness.

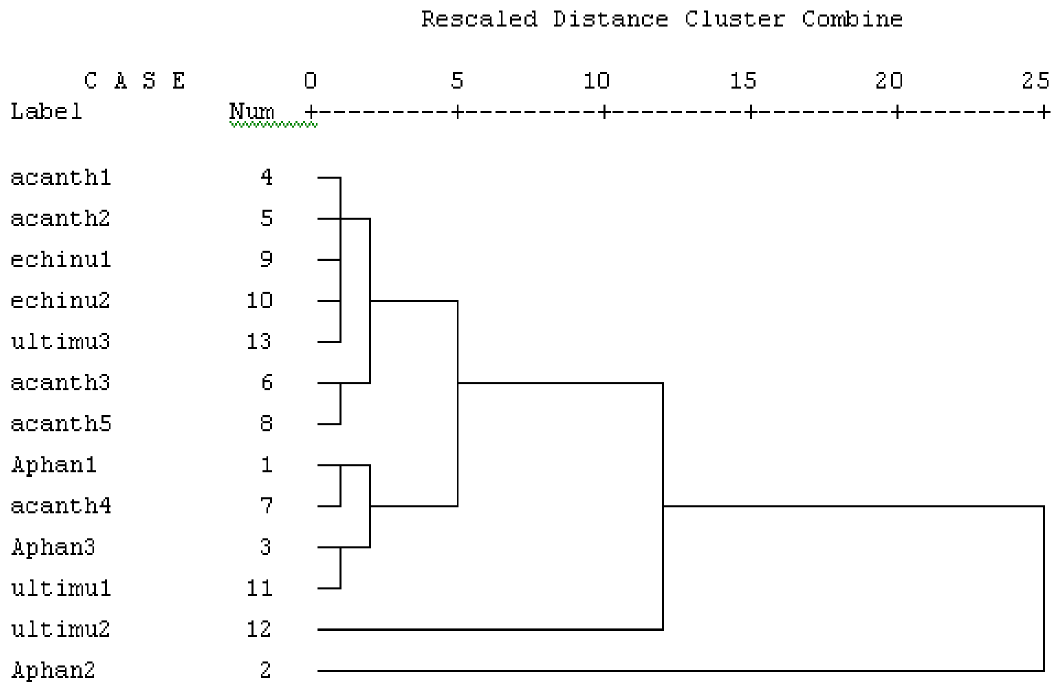

Figure (3.17): Dendrogram for isolates of nearly plerotic oospore species, characters used are: oogonia diameter, oospore diameter, and oospore wall thickness

Table (3.9): Cluster groups for nearly plerotic Pythium isolates

	cluster 1	cluster 2	cluster 3	Cluster 4	Cluster 5	Cluster 6
	P.acanthicum (1,2)	*P.aphanidermatum* (2)	*P.ultimum var ultimum* (2)	*P.aphanidermatum* (1)	*P.acanthicum* (3,5)	*P.ultimum var ultimum* (1)
	P.ultimum var ultimum (3)			*P.acanthicum* (4)		*P.aphanidermatum* (3)
	P.echinulatum (1,2)					
Range of oogonia	20.5-22.64	25.88	18.16	22.88-23.13	20.56-21.36	21.93-22.64
Range of oospore	18.3-19.49	22.72	16.44	20.16-20.44	17.45-18.24	19.49-19.52
Range of oospore wall thickness	1.1-1.78	3.23	1.1	2.56-2.8	2.1-2.3	1.78-2.34

3.2.9 Canonical variate analysis using means of isolates for a plerotic oospore species

Ten groups of isolates (Table A.10.4) can be seen from the plots of means of isolates of all *Pythium* species on canonical variates 1 (horizontal) which explained 84.8 % and 2 (vertical) (Figure 3.18)which explained 10.8 % of the variance.

From the constructed dendrogram (Figure 3.19) four groups of isolates (Table 3.10) can be seen. No *Pythium* species were segregated on the basis of their oogonial and oospore dimensions because they have comparable oogonia and oospores diameters.

Based on studied criteria no species separated from the other. Inter specific variations in the species studied weren't large enough to mask the differences between species.

However, one isolate of *Pythium tenue* formed a group on the basis used could be separated by the differences in its sporangia and other morphological characters, because it has significantly smaller oogonia and oospores between the other isolates of the other species. Intra specific variations in this species studied were large enough to mask the differences between isolates of *Pythium tenue*. Groups 1-3 consist of species which are obviously difficult to separate on the basis of their oospore and oogonial dimensions alone, because these dimentions are verey near values together.

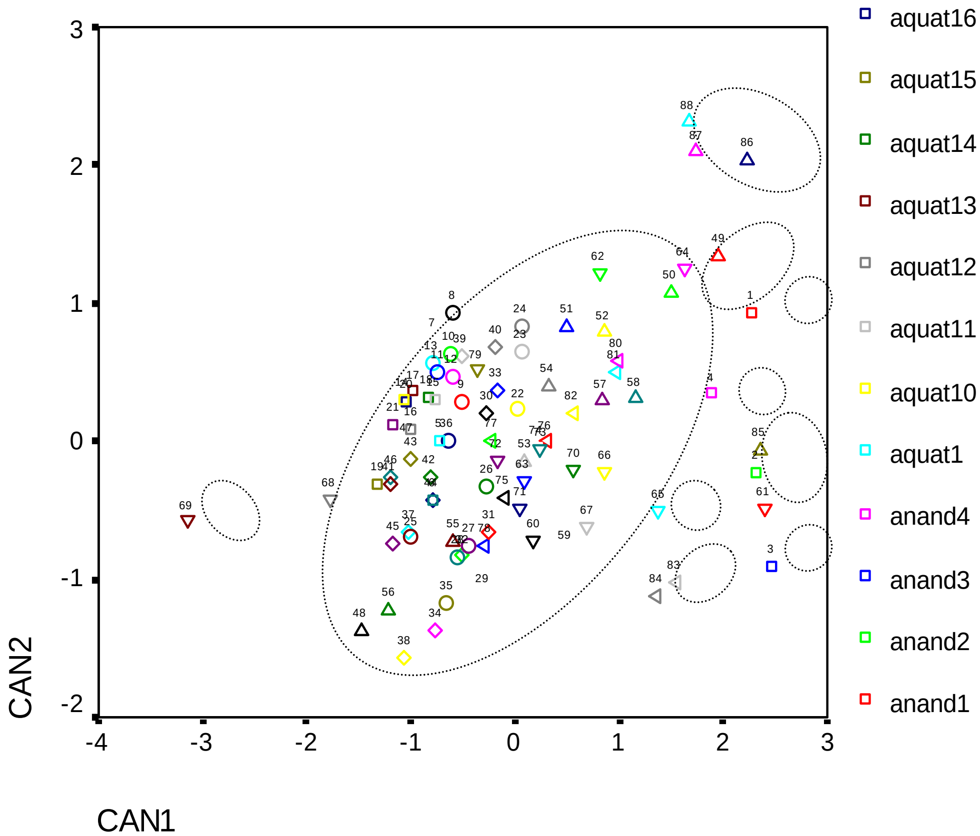

Figure (3.18): A plot of 88 isolates of a plerotic oospore species, on two canonical variates 1 (horizontal), 2 (vertical); character used are: oogonia diameter, oospore diameter, oospore wall thickness.

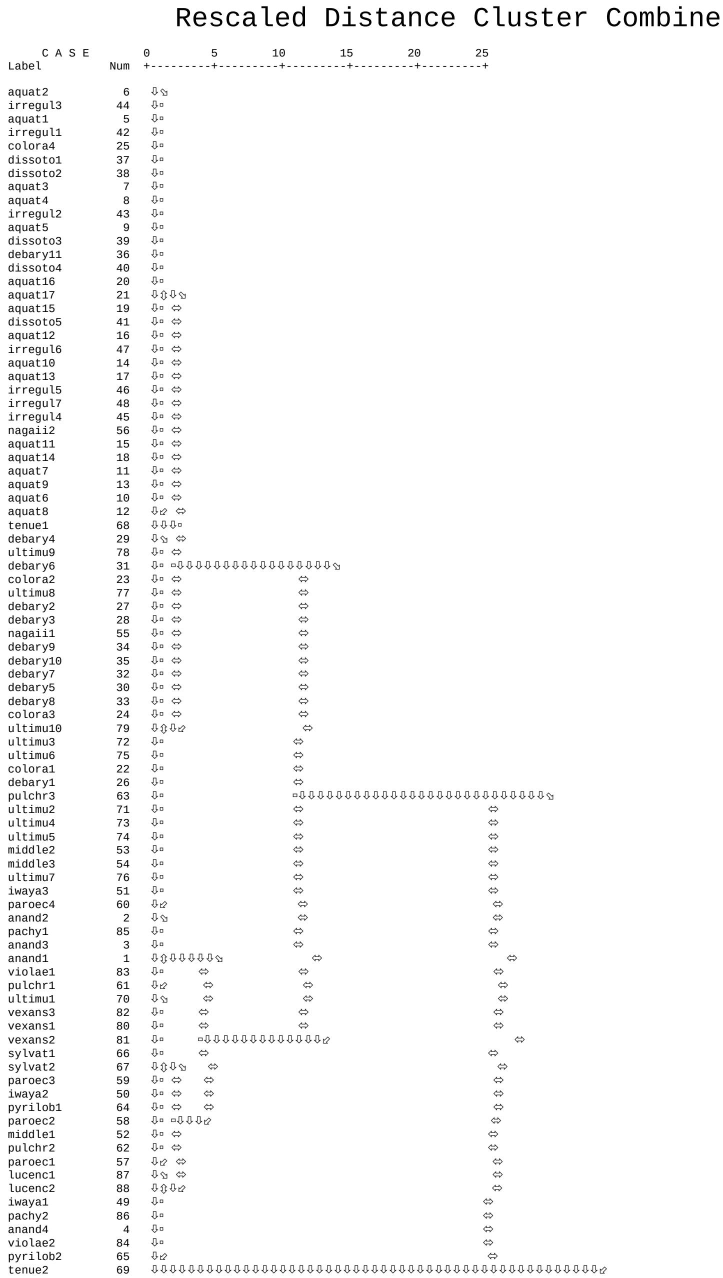

Figure (3.19): Dendrogram for isolates of a plerotic oospore species, characters used are: oogonia diameter, oospore diameter, and oospore wall thickness.

Table 3.10 Cluster groups for a plerotic oospore species

	cluster 1	cluster 2	cluster 3	cluster 4
	P.anandrum (1-3)	*P.anandrum* (4)	*P.aquatile* (1-17)	*P. tenue* (2)
	P. pachycaule (1)	*P iwayami* (1,2)	*P. coloratum* (1-4)	
	P. pulchrum (1)	*P. lucens* (1,2)	*P.debaryanum* (1-11)	
	P. violae (1)	*P middletoni* (1)	*P.dissotocum* (1-5)	
		P. pachycaule (2)	*P. irregular* (1-7)	
		P.paroecandrum (1-3)	*P. iwayami* (3)	
		P. pulchrum (2)	*P. middletonii* (2,3)	
		P. pyrilobum (1,2)	*P. nagaii* (1,2)	
		P. sylvaticum (1,2)	*P paroecandrum* (4)	
		P. ultimum var. sporangiiferum (1)	*P. pulchrum* (3)	
		P. vexans (1-3)	*P. tenue* (1)	
		P. violae (2)	*P.ultimum var. sporangiiferum* (2-10)	
Range of oogonia	26.4-28.4	22.2-28.5	15.9-26	11.8
Range of oospore	22-24.3	18.3-23.8	12.7-26	9.2
Range of oospore wall thickness	0.8-1.7	0.9-2.3	0.5-2.5	0.9

Chapter four

Discussion

Discussion

The distribution of means of oospore diameter, and oospore wall index is remarkably uniform, with a few outlying species at the lower and upper extremes of each variable. Findings on diameter and wall index of oospore represented to plots figures 3.1 and 3.2 lead one to conclude that the distribution of both variables is discontinuities between isolate means in few isolates and extremely narrow in others, these findings were in agreement with the result of MØller & Hockenthull, (2001) who also found that the parameter correlations both within and between isolates are generally high between original parameters. The use of any of these variables, on its own, is not considered as an acceptable tool for species or isolates separation without introducing arbitrary cut-off points, with some isolates falling on either side of the cut-off value. That was the reason behind considering all variables together in the current study.

Correlations between pairs of variables within (upper triangle and between lower triangle) for means of species isolates show high correlation between the oogonium and oospore diameters and moderate correlation between these diameters and wall thickness. Thus large oogonium diameter tends to go with a large oospore diameter. This is in agreement with the findings of Shahzad *et al*. (1992) who also reported high correlation between the later parameters and moderate correlation between these parameters and wall thickness.

Correlations between measured parameters and their derived indices are low within isolates and generally low between isolates, although higher than those found by Shahzad *et al* (1992) who also reported that correlation between the original variables and derived index were much smaller and correlations between measured parameters and their derived indices were low within isolates and generally low between isolates. Thus we have constructed indices which do not depend on the overall size of the oogonia, but are measuring different, independent, characteristics. There are many pairs of species or isolates that could not be distinguished by looking only at the oospore diameter but which have widely differing values of one or other of the indices.

According to canonical variate plots and cluster analysis some *Pythium* species were separated on the basis of their oogonial and oospore dimensions, having significantly larger oogonia and oospores like *Pythium (P.graminicola, P.conidiophorum* and *P.anandrum)* or comparable oogonia and oospores dimentions like *Pythium (P.graminicola* and *P.vanterpoolii*). Such inter-specific variation in diameters seems to be valuable in differentiating some *Pythium* species.

Intra-specific variation among the studied *Pythium* species isolates on the basis of sporangial characteristics and other morphological characters were found. These variations were clear for *Pythium* (*P.dissimile*, *P.tenue*, *P.ultimum var ultimum, P.coloratum, P.papillatum* and *P.aphanidermatum).*

The findings of other species without any inter or intra specific variation is most likely due to the fact that they have very simillar dimension for both oogonia and oospore. For such species one can not relay on oogonia and oospore diameter and further morphological studies are needed for the separation and classification of these species.

In general, biometric data (oogonia diameter, oospore diameter and oospore wall thickness) used in the current study showed limited inter and intra specific variation in certain isolates and failed to show variations for some other isolates, thus such morphological criteria seems to have poor and limited power in differenting *Pytium* isolates. The use of other morphological characteristics like ooplast diameter, antheridial attachment, zoospore cyst seems to be essential for more accurate for differentiation and separation of *Pytium* species. It is important to note that multivariate analysis was also of a limited value in the separation and differentiation of *Pytium* species. We belive that further use of molecular tools will be of great value for separation of *Pytium* species isolated from this region (Hall, 2008).

References

Ali-Shtayeh, M. S. (1982). **Phenology of *Pythium* in cultivated and uncultivated soils in the reading area**. Unpublished Ph. D. Thesis of the University of Reading.

Ali-Shtayeh, M. S. (1986). **Seasonal variations in population levels of Pythium in irrigated and non-irrigated fields in the West Bank of Jordan and the Gaza Strip**. *Transactions of the British Mycological Society 87*:503-509.

Ali-Shtayeh, M. S., Len, L. H, C., & Dick, M. W. (1986). **An improved method and medium for quantitative estimates of populations of *Pythium* species from soil**. *Transactions of the British Mycological society 86*, 39-47.

Allen, T.W., A. Martinez, and L.L. Burpee. 2004. *Pythium* blight of turfgrass. The Plant Health Instructor. DOI:10.1094/PHI-I-2004-0929-01.

Bagnall, R. (2007). **Control of Pythium wilt and root rot of hydroponically grown lettuce by means of chemical treatment of the nutrient solution.** University of Pretoria, Pretoria, South Africa

Brasier, C. M. (1991). **Current questions in Phytophthora systematics : the role of the population approach**. 104-128 in: Phytophthora. J.A. Lucas, R.C. Shattoch, D.S. Shaw and L.R. Cooke, eds. Cambridge, U.K. 492 pp.

Bruehl, G.W. (1987). **Soil borne Plant Pathogens**. Macmillan Publishing Company, London. p. 326

Cook, R. J., L. S. Thomashow, D. M. Weller, D. Fujimoto, M. Mazzola, G. Bangera, and D. Kim. (1995). **Molecular mechanisms of defense**

by rhizobacteria against root disease. *Proceedings of National Academy of Science 92*:4197-4201

Dick, M. W., (1990). **Keys to *Pythium***. Reading, U.K.: the author.

Elad, Y., and I. Chet. (1987). Possible role of competition for nutrients in biocontrol of *Pythium* damping-off by bacteria. *Phytopathology 77:*190-195.

Erwin, C. D. & Ribeiro, K. O. (1996). ***Phytophthora* Diseases Worldwide**. The American Phytopathological Society.

Evans, N., McRoberts, N., Hitchcock, D., and Marshall, G. (1997). **Identification of the determinants of host resistance and pathogenicity in interactions between *Alternaria linicola* Groves & Skolvo and *Linum usitatissimum* L accessions using multivariate analyses**. *Annuals of Applied Biology. 130*:537-547.

Golinski, P., Kacmarek, Z., Kiecana, I., Wisniewska, H., Kaptur, P., Kostecki, M., and Chelkowski, J. (2002). **Fusarium head blight of common Polish winter wheat cultivars—Comparison of effects of *Fusarium avenaceum* and *Fusarium culmorum* on yield components**. *J. Phytopathol. 150*:135-141.

Hall, R. (2008). **Molecular approaches to taxonomy of fungi**. *Botanical Review35*: 285-304**.**

Hair, J. F., Jr., Anderson, R. E., Tatham, R. L., and Black, W. C. (1998). **Multivariate Data Analysis**. 5th ed. Prentice Hall, Englewood Cliffs, NJ.

Handelsman, J., and E. V. Stabb. (1996). **Bio-control of soil borne plant pathogens**. *Plant Cell 8:*1855-1869

Hendrix F. F. and Campbell W. A. (1973). ***Pythium* as plant pathogens**. *Annuals Review of Phytopathology 11*, 77-98.

Hendrix F. F. and Campbell W. A. (1974). **Taxonomy of *Pythium sylvaticum* and related species.** *Mycologia 66*: 1049-1052.

Ho, C. L. (1975). **Population studies in the genus *Pythium***. PhD thesis, university of reading, Department of Botany.

James, F. C., and McCulloch, C. E. (1990). **Multivariate analysis in ecology and systematics: Panacea or pandora's box**. *Annuals Review of Ecological Systems. 21*:129-166.

Jarvis, W.R. (1992). **Managing diseases in greenhouse crops**. APS Press, St. Paul, Minn.

Jeffries, P. (1995). **Biology and ecology of mycoparasitism**. *Canadian Journal of Botany 73:*S1284-S1290.

Kageyama K., Ohyama A., and Hyakumachi M. (1997). **Detection of *Pythium ultimum* using polymerase chain reaction with species-specific primers**. *Plant Disease 81*,1155-1160.

Kenkel, N. C., Derksen, D. A., Thomas, A. G., and Watson, P. R. (2002). **Multivariate analysis in weed science research**. *Weed Science Society of America Journal. 50*:281-292.

Kerbs, Ch. J., (1998). **Ecological methodology**. By Addison-Welsy Educational Publishers. Inc. Publeshed under the Benjamin/cummings imprint, 2nd ld., 393-400.

Korzeniowski, A. (1987). **On logarithmic Sobolev constant for diffusion semigroups**. *Journal of functional analysis71:* 363-370.

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

Martin, F. N., and J. E. Loper. (1999). **Soil borne plant diseases caused by *Pythium* spp.: ecology, epidemiology, and prospects for biological control**. *Critical Review of Plant Science 18:*111-181.

Matthews, V. D. (1931). **Studies on the genus *Pythium***. Univ. N. Carol. Press, Chapel Hill, 136 pp.

Middelton, J. T. (1943). **The taxonomy, host range and geographic distribution of the genus *Pythium***. *Memoirs torrey botanical club 20*: 1-171.

MØller, K. & Hockenhull, J. (2001). **Biometry Oospores and Intraspicific Variation of Four Pythium species**. Department of Plant Biology. *Mycological research 105:* 1206-1215.

Owen-Going, T.N.; Sutton, J.C.; Grodzinski, B. (2003). **Relationships of *Pythium* isolates and sweet pepper plants in single-plant hydroponic units**. *Canadian Journal of Plant Pathology, 25*:155-167.

Plaats-Niterink AJ van der. (1981). **Monograph of the genus *Pythium***. *Studies in Mycology(Baarn) 21*:1–244.

Plant clinic, cornell education (2007). http://plantclinic.cornell.edu /FactSheets/pythium/phythium_cycle_big.jpg

Ruttledge, T. R., and E. B. Nelson. (1997). **Extracted fatty acids from *Gossypium hirsutum* stimulatory to the seed-rotting fungus, *Pythium ultimum***. *Phytochemistry 46:*77-82.

Schlosser, I., Kranz, J., and Bonman, J. M. (2000). **Characterization of plant type and epidemiological development in the pathosystem "upland rice/rice blast" (*Pyricularia grisea*) by means of multivariate statistical methods**. *Journal of Plant Dissease. 107*: 12-32.

Schwartz, H. F., and Gent, D. H. (2005). **Pythium Root Rot**. Retrieved from World Wide Web. http://highplainsipm.org/ HpIPM Search/ Docs/ Pythium Root Rot-Saf flower. Pdf.

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

Sideris, C. P. (1932). **Taxonomic studies in the family of *Pythiaceae*.2.** *Pythium. Mycologia 24*: 14-61.

Stanghellini, M. E. (1974). **Spore germination, growth, and survival of Pythium in soil**. *Proceedings of American Phytopathololological Society. 1*:211-214.

Stanghellini, M. E., and Hancock, J. G. (1970). **Aquantitative method for isolation of *Pythium ultimum* from the soil**. *Phytopathology 60:*551-552.

Stanghellini, M. E., and Hancock, J. G. (1971). **The sporangium of *Pythium ultimum* as a survival structure in soil**. *Phytopathology 61:*157-164.

Stevens, J. (1992). **Applied Multivariate Statistics for the Social Sciences**. Lawrence Erlbaum, Hillsdale, NJ.

Tedla, T., and M. E. Stanghellini. (1992). **Bacterial population dynamics and interactions with *Pythium aphanidermatum* in intact rhizosphere soil**. *Phytopathology 82*:652-656.

Van Dijk, E and Nelson, E. B (2000). **Fatty Acid Competition as a Mechanism by which Enterobacter cloacae Suppresses Pythium ultimum Sporangium Germination and Damping-Off**. *Applied Environmental Microbiology (12):* 5340–5347.

Van Dijk, K., and E. B. Nelson. (1998). **Inactivation of seed exudate stimulants of *Pythium ultimum* sporangium germination by biocontrol strains of Enterobacter cloacae and other seed-associated bacteria**. *Soil Biology and Biochemistry. 30*:183-19.

Van Loon, L. C., P. M. Bakker, and C. M. J. Pieterse. (1998). **Systemic resistance induced by rhizosphere bacteria.** *Annuals Review of Phytopathology 36:*453-483.

Waterhouse, G. M. (1967). **Key to *Pythium* pringsheim**. *Mycol. Pap 109*: 1-15.

Waterhouse, G. M. (1973). **Peronosporales. In G. C. Ainsworth, F. K. Sparrow, and A. S. Sussman (eds), The Fungi**. Volume *IVB*: 165-183. New York and London: Academic Press.

Whipps, J. M. (1997). **Developments in the biological control of soil-borne plant pathogens**. *Advanced Botanical Research 26*:1-134.

Young-nian, Y. U., & Guo-zhong, M. A. (1989). **The Genus *Pythium* in Chaina**. *Mycosystema 2:*1-10.

Appendix

Table A.1 Isolate means for four directly measured criteria and two derived indexes for oogonia from each of 147 isolate

species	isolate #	oogonia	oospore	oospore wall thickness	sporangia	oospore wall index	aplerotic index
P.acanthicum Drechler	AFCC1059	20.6	19.3	1.4	27.7	37.51	82.23757
	AFCC 2230	20.5	19.4	1.5	24.2	39.59	84.75076
	AFCC 192	21.36	18.24	2.3	1.24	58.18	62.26884
	AFCC 171	23.13	20.44	2.8	1.06	61.73	69.0106
	AFCC 181	20.56	17.45	2.1	1.2	56.22	61.13881
P.anandrum Drechler	AFCC 2027	27.4	22.2	1.7	27.7	39.27	53.18721
	AFCC 704	26.7	23.3	1.5	28.1	33.87	66.45597
	AFCC 806	26.6	24.3	1.4	27.9	30.74	76.23842
	AFCC 2227	25.3	21.7	1.7	31.1	40.02	63.0983
P.aphanidermatum Edson	BERC-fc1	22.88	20.16	2.56	2.32	58.48	68.40747
	BERC-fc3	25.88	22.72	3.23	3.14	63.34	67.66003
	BERC-fc4	22.64	19.49	1.78	3.16	45.4	63.79788
P.aquatile Höhnk	AFCC 756	19.4	15.1	1.1	.	37.65	47.15477
	AFCC 817	19	15.3	1	.	34.31	52.21719
	AFCC 728	18.9	14.3	1.4	.	47.99	43.31344
	AFCC 1054	18.8	14.6	1.6	.	52.39	46.83656
	AFCC 809	18.6	15.4	1.5	.	47.8	56.75751
	AFCC 2010	18.3	14.8	1.6	.	51.85	52.8971
	AFCC 2020	18.2	14.6	1.5	.	49.84	51.62314

species	isolate #	oogonia	oospore	oospore wall thickness	sporangia	oospore wall index	aplerotic index
	AFCC 2138	18.1	15	1.6	.	51.32	56.91648
	AFCC 1032	18.1	14.4	1.5	.	50.38	50.35606
	AFCC 865	17.8	13.9	1.3	.	25.52	47.61945
	AFCC 2072	17.8	14.7	1.5	.	49.58	56.32381
	AFCC 897	17.7	14.3	1.3	.	45.23	52.73371
	AFCC 725	17.7	14.1	1.4	.	48.53	50.55191
	AFCC 2111	17.6	14.5	1.5	.	50.11	55.91985
	AFCC 770	17.5	13.8	1	.	37.48	49.03691
	AFCC 864	17.4	14	1.4	.	48.8	52.08784
	AFCC 667	17.2	13.8	1.3	.	46.54	51.64781
P.coloratum Vaartaja	AFCC 804	20.7	16.9	1.4	.	41.92	54.41881
	AFCC 2362	20	16.6	1.7	.	49.72	57.1787
	AFCC 1109	19.3	16.4	1.9	.	54.65	61.35636
	AFCC 1002	18.9	15	0.8	.	28.71	49.9906
P.conidiophorum Jok1	AFCC 5679	13.2	12.5	1.9	21.6	66.28	84.91966
	AFCC 2538	13	12.5	1.8	21.9	63.91	88.89964
P.debaryanum Hesse	AFCC 701	20.8	16.6	1	23.6	31.96	50.83166
	AFCC 818	19.6	16.5	1	.	32.13	59.66005
	AFCC 2746	19.6	16.3	0.9	24.8	29.61	57.51678
	AFCC 773	19.5	17.2	1	.	30.98	68.62487
	AFCC 727	19.2	16.1	1.5	23.8	46.13	58.96224

species	isolate #	oogonia	oospore	oospore wall thickness	sporangia	oospore wall index	aplerotic index
	AFCC 805	18.9	16.9	1.3	.	39.42	71.49484
	AFCC 772	18.8	16.4	1.1	.	35.09	66.38317
	AFCC 858	18.8	16.2	1.7	23.8	50.67	63.98402
	AFCC 816	18.6	16.2	0.8	.	26.8	66.07029
	AFCC 807	18.3	16.3	1	.	32.48	70.66584
	AFCC 861	18.2	15.3	1.4	24.4	45.47	59.41008
P.dissimile Vaartaja	AFCC 2799	13.4	13.4	1	.	38.43	100
	AFCC 2590	11.4	11.4	1	.	43.94	100
P.dissotocum Drechler	AFCC 788	18.9	14.9	0.8	.	28.88	48.99744
	AFCC 896	18.8	15.6	0.5	.	18.02	57.13474
	AFCC 2073	18.6	15.1	1.6	.	51.05	53.50471
	AFCC 2193	18.3	15.9	1.9	.	55.93	65.59007
	AFCC 894	17.4	14.1	1.1	.	39.88	53.212
P.echinulatum Matthews	AFCC 2592	20.9	18.6	1.2	24.3	33.93	70.48553
	AFCC 2245	20.8	18.3	1.1	26.8	31.9	68.10253
P.graminicola Subramaniam	AFCC 2567	26.2	26.2	2.5	.	47.02	100
	AFCC 7756	26	26	2.5	.	47.31	100
P.irregulare Buisman	AFCC 726	19.2	15.1	1	23.8	34.7	48.64376
	AFCC 672	19.1	14.5	0.94	27	34.07	43.7526

species	isolate #	oogonia	oospore	oospore wall thickness	sporangia	oospore wall index	aplerotic index
	AFCC 2087	19	15.3	1	23.5	34.31	52.21719
	AFCC 631	18.3	14.6	0.8	26.9	29.41	50.78147
	AFCC 713	18	14.1	1	24.9	36.8	48.0662
	AFCC 2019	17.9	14.3	1.2	22.6	42.37	50.98577
	AFCC 2088	17.3	14.3	0.6	23.7	23.12	56.47676
P.iwayami S.Ito	AFCC 747	25.6	21	2	22.1	46.95	55.19986
	AFCC 2732	24.7	20	1.8	28.8	44.86	53.08834
	AFCC 2651	21.2	17.6	1.8	24.2	49.67	57.2177
P.lucens Ali-Shtayeh	AFCC 2454	26.6	19.8	1.9	24.7	47.23	41.24304
	AFCC 8241	26.2	19.4	2	20.9	49.98	40.59769
P.lutarium Ali-Shtayeh	AFCC 2361	21.9	20.2	2.7	.	60.67	78.47327
	AFCC 893	21.2	18.9	1.9	.	49	70.8562
P.mamillatum Meurs	AFCC 2014	18.4	17.1	0.9	20.3	28.37	80.2666
	AFCC 2239	18.4	17.9	0.9	18.6	27.24	92.06735
	AFCC 2241	16.8	15.2	0.9	22.4	31.49	74.06328
P.middletonii Sparrow	AFCC 760	23.7	18.6	1.5	35.2	41	48.33857
	AFCC 758	21.3	17.4	1.2	36.6	35.93	54.51411
	AFCC 761	20.9	17.5	1.6	32.8	45.44	58.70503
P.minor Ali-Shtayeh	AFCC 2368	13.9	13.9	0.6	23.3	23.73	100

species	isolate #	oogonia	oospore	oospore wall thickness	sporangia	oospore wall index	aplerotic index
	AFCC 2660	13.3	13.3	0.5	19.1	20.9	100
	AFCC 2787	12.6	12.6	0.5	25.4	21.97	100
P.monospermum Pringsh	AFCC 2555	17.6	17.6	0.6	.	19.09	100
	AFCC 779	16.8	16.8	1.3	.	39.61	100
P.nagaii S.Ito& Tokunaga	AFCC 2390	19.2	16.1	1	24.2	32.83	58.96224
	AFCC 2729	17.9	14.9	0.7	25	25.62	57.67661
P.oligandrum Drechsler	AFCC 738	22.4	20.2	2	27.4	48.42	73.33479
	AFCC 6139	19.2	16.7	1.4	27.1	42.34	65.80301
P.pachycaule Ali-Shtayeh	AFCC 650	27.1	23.3	1.5	.	33.87	63.55649
	AFCC 572	26.1	21.1	2.3	.	52.18	52.8355
P.papillatum Matthews	AFCC 3021	15.3	15.3	0.9	.	31.3	100
	AFCC 3023	15.3	15.3	0.6	.	21.73	100
P.paroecandrum Drechsler	AFCC 708	24.8	19	1.1	22.7	30.87	44.96823
	AFCC 2012	23.9	19.8	1.5	21.1	38.92	56.85935
	AFCC 2308	22.9	19	0.9	25.7	25.81	57.11555
	AFCC 775	19.7	18.1	1.4	22.4	39.6	77.55987
P.parvum Ali-Shtayeh	AFCC 2259	14.9	13.3	0.5	17.8	20.9	71.12072

species	isolate #	oogonia	oospore	oospore wall thickness	sporangia	oospore wall index	aplerotic index
	AFCC 1094	14.5	13.5	0.5	17.6	20.62	80.70442
	AFCC 871	14.3	12.8	0.5	16.5	21.65	71.71695
	AFCC 651	13.5	13.1	0.5	16.8	21.2	91.37188
P.pulchrum Minden	AFCC 2247	28.5	23.8	1.1	33.9	25.25	58.23664
	AFCC 2522	24.2	18.1	1.5	27.4	41.94	41.8398
	AFCC 4001	21.5	17.5	1.1	23.8	33.17	53.92607
P.pyrilobum Vaartaja	AFCC 2458	24.4	20.2	2	.	48.42	56.73939
	AFCC 2853	24.3	21.1	1.3	.	32.6	65.46792
P.rostratum Butler	AFCC 2121	20.4	18.5	1.5	25.7	41.19	74.5804
	AFCC 2089	18.9	17	1	21.3	31.3	72.7715
	AFCC 2248	18.4	17.2	1.3	24.1	38.84	81.68304
	AFCC 673	18.4	17.2	1.3	21.1	38.84	81.68304
	AFCC 2023	18.3	16.9	1.3	24.4	39.42	78.76021
	AFCC 581	18.2	16.7	1.1	21.8	34.54	77.25654
P.salinum Höhnk	AFCC 2387	17.8	17.2	1.5	16.8	43.73	90.22468
	AFCC 3343	17.3	17.3	1.4	16	41.12	100
P.salpingophorum Drechsler	AFCC 1093	15.3	15.3	1.5	22	48.04	100
	AFCC 2231	14.9	14.9	1.5	24.2	49.06	100
P.spinosum Sawada	AFCC 2344	18.2	15.2	1.2	25.3	40.28	58.25277
	AFCC 8496	16.8	15.1	0.5	25.5	18.58	72.6111

species	isolate #	oogonia	oospore	oospore wall thickness	sporangia	oospore wall index	aplerotic index
	AFCC 2424	16.2	16.2	0.9	25.9	29.77	100
P.sp (ma2153)	AFCC 2153	22.6	21.3	1.5	28.8	36.58	83.71697
	AFCC 2199	22.5	20.9	1.5	28.6	37.18	80.14774
P.sylvaticum Campbell & Hendrix	AFCC 2470	23.1	19.5	1.3	26	34.9	60.15447
	AFCC 688	22.9	19.4	1.1	26.8	30.31	60.79932
P.tenue Gobi	AFCC 771	15.9	12.7	1	.	40.19	50.95887
	AFCC 384	11.8	9.2	0.9	.	47.96	47.39336
P.torulosum Coker & Patterson	AFCC 869	16.3	15.5	1.2	.	39.63	85.9869
	AFCC 2232	15.4	15.4	1	.	34.12	100
	AFCC 2092	15.3	14.3	1	.	36.36	81.64579
P.tracheiphilum Matta	AFCC 2420	18.6	18.6	1.3	23.6	36.35	100
	AFCC 2402	18.4	18.4	1.3	23.2	36.68	100
P. ultimum var. sporangiiferum Drechsler	AFCC 2197	22.2	18.7	1.3	26.6	36.18	59.76761
	AFCC 2222	21.6	17.6	1	25.3	30.36	54.09744
	AFCC 2130	21.4	16.7	1	26.8	31.8	47.52346
	AFCC 2221	21.4	17.7	1.3	27.1	37.91	56.58202
	AFCC 682	21.3	17.6	1.3	.	38.09	56.4156
	AFCC 707	21.2	17.1	1	.	31.14	52.47842

species	isolate #	oogonia	oospore	oospore wall thickness	sporangia	oospore wall index	aplerotic index
	AFCC 850	20.7	17.8	1.5	.	42.52	63.58416
	AFCC 2018	20	16.4	1.3	.	40.42	55.1368
	AFCC 733	19.6	16.9	1.1	.	34.19	64.105
	AFCC 683	19.4	15.6	1.5	20.7	47.31	51.99584
P.ultimum var. ultimum Trow	AFCC 291	21.93	19.52	2.34	1.42	56.06	70.52183
	AFCC 141	18.16	16.44	1.1	1.32	35.01	74.19214
	AFCC 181	20.16	18.27	0.86	1.42	25.67	74.42932
P.vantrepoolii V. Kouyeas & H. Kouyeas	AFCC 2172	19.3	19.3	2.8	.	64.23	100
	AFCC 2213	18.2	18.2	2.5	.	61.85	100
P.vexans de Bary	AFCC 2253	22.8	19.1	1.7	16.2	44.46	58.78893
	AFCC 2149	22.6	19.1	1.7	.	44.46	60.36355
	AFCC 2082	22.3	18.3	1.4	15.3	39.24	55.26354
P.violae Chesters & Hickman	AFCC 2160	26.4	22	0.8	28.9	20.27	57.87037
	AFCC 3020	25.2	21.6	0.9	28.3	22.97	62.97376

AFCC**:** An_Najah Fungal Culture Collection; BERC-fc: Biodiversity Environmental Research Center- fungal collection.

Table A.1.1 Names of all Pythium isolates depend on numbers

Species	Nmbers
P.aphanidermatum1	1
P.aphanidermatum2	2
P.aphanidermatum3	3
P.acanthicum1	4
P.acanthicum2	5
P.acanthicum3	6
P.acanthicum4	7
P.acanthicum5	8
P.anandrum1	9
P.anandrum2	10
P.anandrum3	11
P.anandrum4	12
P.aquatile1	13
P.aquatile2	14
P.aquatile3	15
P.aquatile4	16
P.aquatile5	17
P.aquatile6	18
P.aquatile7	19
P.aquatile8	20
P.aquatile9	21
P.aquatile10	22
P.aquatile11	23
P.aquatile12	24
P.aquatile13	25
P.aquatile14	26
P.aquatile15	27
P.aquatile16	28
P.aquatile17	29
P.coloratum1	30
P.coloratum2	31
P.coloratum3	32
P.coloratum4	33
P.conidiophorum1	34
P.conidiophorum2	35
P.debaryanum1	36
P.debaryanum2	37
P.debaryanum3	38

Species	Nmbers
P.debaryanum4	39
P.debaryanum5	40
P.debaryanum6	41
P.debaryanum7	42
P.debaryanum8	43
P.debaryanum9	44
P.debaryanum10	45
P.debaryanum11	46
P.dissimile1	47
P.dissimile2	48
P.dissotocum1	49
P.dissotocum2	50
P.dissotocum3	51
P.dissotocum4	52
P.dissotocum5	53
P.echinulatum1	54
P.echinulatum2	55
P.graminicola1	56
P.graminicola2	57
P.irregulare1	58
P.irregulare2	59
P.irregulare3	60
P.irregulare4	61
P.irregulare5	62
P.irregulare6	63
P.irregulare7	64
P.iwayami1	65
P.iwayami2	66
P.iwayami3	67
P.mamillatum1	68
P.mamillatum2	69
P.mamillatum3	70
P.middletonii1	71
P.middletonii2	72
P.middletonii3	73
P.monospermum1	74
P.monospermum2	75
P.nagaii1	76
P.nagaii2	77

Species	Nmbers
P.oligandrum1	78
P.oligandrum2	79
P.papillatum1	80
P.papillatum2	81
P.paroecandrum1	82
P.paroecandrum2	83
P.paroecandrum3	84
P.paroecandrum4	85
P.pulchrum1	86
P.pulchrum2	87
P.pulchrum3	88
P.pyrilobum1	89
P.pyrilobum2	90
P.rostratum1	91
P.rostratum2	92
P.rostratum3	93
P.rostratum4	94
P.rostratum5	95
P.rostratum6	96
P.salinum1	97
P.salinum2	98
P.salpingophorum1	99
P.salpingophorum2	100
P.spinosum1	101
P.spinosum2	102
P.spinosum3	103
P.sylvaticum1	104
P.sylvaticum2	105
P.tenue1	106
P.tenue2	107
P.torulosum1	108
P.torulosum2	109
P.torulosum3	110
P.tracheiphilum1	111
P.tracheiphilum2	112
P.ultimum var ultimum1	113
P.ultimum var ultimum2	114
P.ultimum var ultimum3	115
P. ultimum var.	116

Species	Nmbers
sporangiiferum1	
P. ultimum var. sporangiifer	117
P. ultimum var. sporangiiferum3	118
P. ultimum var. sporangiiferum4	119
P. ultimum var. sporangiiferum5	120
P. ultimum var. sporangiiferum6	121
P. ultimum var. sporangiiferum7	122
P. ultimum var. sporangiiferum8	123
P. ultimum var. sporangiiferum9	124
P. ultimum var. sporangiiferum10	125
P.vantrepoolii1	126
P.vantrepoolii2	127
P.vexans1	128
P.vexans2	129
P.vexans3	130
P.violae1	131
P.violae2	132
P.pachycaule1	133
P.pachycaule2	134
P.parvum1	135
P.parvum2	136
P.parvum3	137
P.parvum4	138
P.sp (ma2153)1	139
P.sp (ma2153)2	140
P.lutarium1	141
P.lutarium2	142
P.minus1	143
P.minus2	144
P.minus3	145
P.lucens1	146
P.lucens2	147

Table A.1.2 Cluster Membership for all isolates of the genus Pythium

Case	6 Clusters
1:Ap1	1
2:Ap2	2
3:Ap3	1
4:ac1	1
5:ac2	1
6:ac3	1
7:ac4	1
8:ac5	1
9:an1	2
10:an2	2
11:an3	2
12:an4	2
13:aq1	3
14:aq2	3
15:aq3	3
16:aq4	3
17:aq5	3
18:aq6	3
19:aq7	3
20:aq8	3
21:aq9	3
22:aq10	3
23:aq11	3
24:aq12	3
25:aq13	3
26:aq14	3
27:aq15	3
28:aq16	3
29:aq17	3
30:co1	1
31:co2	3
32:co3	3
33:co4	3
34:con1	4
35:con2	4
36:de1	1

Case	6 Clusters
37:de2	3
38:de3	3
39:de4	3
40:de5	3
41:de6	3
42:de7	3
43:de8	3
44:de9	3
45:de10	3
46:de11	3
47:di1	4
48:di2	5
49:diso1	3
50:diso2	3
51:diso3	3
52:diso4	3
53:diso5	3
54:ech1	1
55:ech2	1
56:gr1	6
57:gr2	6
58:ir1	3
59:ir2	3
60:ir3	3
61:ir4	3
62:ir5	3
63:ir6	3
64:ir7	3
65:iw1	2
66:iw2	1
67:iw3	1
68:ma1	3
69:ma2	3
70:ma3	4
71:mid1	1
72:mid2	1
73:mid3	1

Case	6 Clusters
74:mo1	3
75:mo2	3
76:nag1	3
77:nag2	3
78:ol1	1
79:ol2	3
80:pa1	4
81:pa2	4
82:par1	1
83:par2	1
84:par3	1
85:par4	1
86:pu1	2
87:pu2	1
88:pu3	1
89:py1	1
90:py2	2
91:ro1	1
92:ro2	3
93:ro3	3
94:ro4	3
95:ro5	3
96:ro6	3
97:sa1	3
98:sa2	3
99:sal1	4
100:sal2	4
101:sp1	3
102:sp2	4
103:sp3	4
104:sy1	1
105:sy2	1
106:te1	4
107:te2	5
108:to1	4
109:to2	4
110:to3	4

Case	6 Clusters
111:tr1	3
112:tr2	3
113:ul1	1
114:ul2	3
115:ul3	1
116:ulv1	1
117:ulv2	1
118:ulv3	1
119:ulv4	1
120:ulv5	1
121:ulv6	1
122:ulv7	1
123:ulv8	3
124:ulv9	3
125:ulv10	3
126:va1	1
127:va2	3
128:ve1	1
129:ve2	1
130:ve3	1
131:vi1	2
132:vi2	2
133:pac1	2
134:pac2	2
135:par1	4
136:par2	4
137:par3	4
138:par4	4
139:sp1	1
140:sp2	1
141:luta1	1
142:luta2	1
143:min1	4
144:min2	4
145:min3	4
146:lu1	2
147:lu2	2

Table A.1.3 Eigen values for 147 isolates

Function	Eigen value	% of Variance	Cumulative %	Canonical Correlation
1	14.342	63.5	63.5	.967
2	6.198	27.5	91.0	.928
3	2.036	9.0	100.0	.819

Table A.1.4 Clusters for all isolates species produced by canonical variate analysis

Cluster number	Pythium species isolates
Cluster 1	*P.acanthicum* (3,5), *P.aphanidermatum*(3), *P.aquatile* (1-17), *P.coloratum* (1,2,4), *P.debaryanum* (1-11), *P.dissotocum* (1-5), *P.echinulatum* (1,2), *P.irregulare1*(1-7), *P.iwayami* (3), *P.mamillatum* (1,3), *P.middletonii* (2,3), *P.nagaii* (1,2) *P.oligandrum* (2), *P.paroecandrum* (2-4), *P.pulchrum* (3), *P.pyrilobum* (2), *P.rostratum* (1,2,4-6), *P.spinosum* (1,2), *P.sylvaticum* (1,2), *P.tenue* (1), *P. ultimum var. sporangiiferum* (1-10), *P.ultimum var ultimum* (2,3), *P.vexans* (1-3).
Cluster 2	*P.conidiophorum* (1,2), *P.dissimile* (1,2), *P.mamillatum* (2), *P.minus* (1-3), *P.monospermum* (1,2), *P.papillatum* (1,2), *P.parvum* (4), *P.salinum* (1,2), *P.salpingophorum* (1,2), *P.spinosum* (3), *P.torulosum* (1-3), *P.tracheiphilum* (1,2) .
Cluster 3	*P.lucens* (1, 2).
Cluster 4	*P.paroecandrum* (1), *P.pulchrum* (2).
Cluster 5	*P.acanthicum* (4), *P.anandrum* (3), *P.aphanidermatum* (1, 2), *P.lutarium* (1), *P.oligandrum* (1), *P.sp (ma2153)* (1, 2), *P .ultimum var ultimum* (1).
Cluster 6	*P.anandrum* (1, 2, 4), *P.iwayami* (1,2) , *P.pachycaule* (1,2), *P.pulchrum* (1), *P.pyrilobum* (1), *P.violae* (1,2).
Cluster 7	*parvum* (1-3).
Cluster 8	.*vantrepoolii* (1,2).
Cluster 9	.*middletonii* (1).
Cluster 10	*graminicola* (1, 2).
Cluster 11	*acanthicum* (1, 2).
Cluster 12	*P.tenue* (2).

Table A.2 Means and standard deviation for four directly measured criteria and two derived indexes for oogonia from each of 41 speacies

species	oogonium	oospore	oospore wall tkickness	sporangia	# of isolates	aplerotic index	
P.acanthicum	21.23	18.966	2.02	11.08	5	71.88131	nearly plerotic
P.anandrum	26.5	22.9	1.575	28.7	4	64.74498	aplerotic
P.aphanidermatum	23.8	20.79	2.523333333	2.8733333	3	66.62179	nearly plerotic
P.aquatile	18.1235294	15.32352941	1.382352941	.	17	51.41139	aplerotic
P.coloratum	19.725	16.225	1.45	.	4	55.73612	aplerotic
P.conidiophorum	13.1	12.5	1.85	21.75	2	86.90965	plerotic
P.debaryanum	19.1181818	16.36363636	1.154545455	24.08	11	63.05489	aplerotic
P.dissimile	12.4	12.4	1	.	2	100	plerotic
P.dissotocum	18.4	15.12	1.18	.	5	55.68779	aplerotic
P.echinulatum	20.85	18.45	1.15	25.55	2	69.29403	nearly plerotic
P.graminicola	26.1	26.1	2.5	.	2	100	plerotic
P.irregulare	18.4	14.6	0.934285714	24.628571	7	50.13197	aplerotic
P.iwayami	23.8333333	19.53333333	1.866666667	25.033333	3	55.16863	aplerotic
P.lucens	26.4	19.6	1.95	22.8	2	40.92036	aplerotic
P.lutarium	21.55	19.55	2.3	.	2	74.66473	plerotic
P.mamillatum	17.8666667	16.73333333	0.9	20.433333	3	82.13241	plerotic
P.middletonii	21.9666667	17.83333333	1.433333333	34.866667	3	53.85257	aplerotic
P.minus	13.2666667	13.26666667	0.533333333	22.6	3	100	plerotic
P.monospermum	17.2	17.2	0.95	.	2	100	plerotic
P.nagaii	18.55	15.5	0.85	24.6	2	58.31942	aplerotic

species	oogonium	oospore	oospore wall tkickness	sporangia	# of isolates	aplerotic index	
P.oligandrum	20.8	18.45	1.7	27.25	2	69.5689	plerotic
P.pachycaule	26.6	22.2	1..9	.	2	58.196	aplerotic
P.papillatum	15.3	15.3	0.75	.	2	100	plerotic
P.paroecandrum	22.825	18.975	1.225	22.975	4	59.12575	aplerotic
P.parvum	14.3	13.175	0..5	17.175	4	78.724849	plerotic
P.pulchrum	24.7333333	19.8	1.233333333	28.366667	3	51.33417	aplerotic
P.pyrilobum	24.35	20.65	1.65	.	2	61.10365	aplerotic
P.rostratum	18.7666667	17.25	1.25	23.066667	6	77.78912	plerotic
P.salinum	17.55	17.25	1.45	16.4	2	95.11234	plerotic
P.salpingophorum	15.1	15.1	1.5	23.1	2	100	plerotic
P.spinosum	17.0666667	15.5	0.866666667	25.566667	3	76.95462	plerotic
P.sp (ma2153)	22.55	21.1	1..5	28.7	2	81.93236	plerotic
P.sylvaticum	23	19.45	1.2	26.4	2	60.4769	aplerotic
P.tenue	13.85	10.95	0.95	.	2	49.17611	aplerotic
P.torulosum	15.6666667	15.06666667	1.066666667	.	3	89.2109	plerotic
P.tracheiphilum	18.5	18.5	1.3	23.4	2	100	plerotic
P. ultimum var. sporangiiferum	20.88	17.21	1..23	25.3	10	56.16864	aplerotic
P.ultimum var ultimum	20.0833333	18.07666667	1.433333333	1.3866667	3	73.04776	nearly plerotic
P.vantrepoolii	18.75	18.75	2.65	.	2	100	plerotic
P.vexans	22.5666667	18.83333333	1.6	15.75	3	58.13867	aplerotic
P.violae	25.8	21.8	0.85	28.6	2	60.42207	aplerotic

Table A.2.1 Cluster membership for means of Pythium species

Cluster Membership				
Case	5 Clusters	4 Clusters	3 Clusters	2 Clusters
1:aph	1	1	1	1
2:aca	2	2	2	2
3:ana	1	1	1	1
4:aqu	3	2	2	2
5:col	3	2	2	2
6:con	4	3	3	2
7:deb	3	2	2	2
8:dis	4	3	3	2
9:disso	3	2	2	2
10:ech	2	2	2	2
11:gra	5	4	1	1
12:irr	3	2	2	2
13:iwa	1	1	1	1
14:mam	3	2	2	2
15:mid	2	2	2	2
16:mon	3	2	2	2
17:nag	3	2	2	2
18:oli	2	2	2	2
19:pap	4	3	3	2
20:par	2	2	2	2
21:pul	1	1	1	1
22:pyr	1	1	1	1
23:ros	3	2	2	2
24:sal	3	2	2	2
25:salpi	4	3	3	2
26:spi	3	2	2	2
27:syl	2	2	2	2
28:ten	4	3	3	2
29:tor	4	3	3	2
30:tra	3	2	2	2
31:ult	2	2	2	2
32:ultvar	2	2	2	2
33:van	3	2	2	2
34:vex	2	2	2	2
35:vio	1	1	1	1
36:pac	1	1	1	1
37:par	4	3	3	2

Cluster Membership				
Case	5 Clusters	4 Clusters	3 Clusters	2 Clusters
38:sp	1	1	1	1
39:lut	2	2	2	2
40:min	4	3	3	2
41:luc	1	1	1	1

Table A.3 Classifying of Pythium species

Type of sporangia	Smooth oogonia and globose or subglobose sporangia.	Spiny oogonia and globose sporangia and/or hyphal swellings	Filamentous (non-swollen) sporangia	Lobulate sporangia
1	*P.conidiophorum*	*P.acanthicum*	*P.aquatile*	*P.aphanidermatum*
2	*P.debaryanum*	*P.anandrum*	*P.coloratum*	*P.dissimile*
3	*P.irregulare*	*P.echinulatum*	*P.dissitocum*	*P.graminicola*
4	*P.iwayamai*	*P.mamillatum*	*P.lutarium*	*P.torulosum*
5	*P.lucens*	*P.oligandrum*	*P.pachycaule*	*P.vanterpoolii*
6	*P.middletonii*	*P.spinosum*	*P.papillatum*	
7	*P.minor*		*P.tenue*	
8	*P.monospermum*			
9	*P.nagaii*			
10	*P.pareocandrum*			
11	*P.parvum*			
12	*P.pulchrum*			
13	*P.pyrilobum*			
14	*P.rostratum*			
15	*P.salinum*			
16	*P.salpingophorum*			
17	*P.sp.(ma2153)*			
18	*P.sylvaticum*			
19	*P.tracheiphilum*			
20	*P. ultimum var. sporangiiferum*			
21	*P.ultimum var ultimum*			
22	*P.vexans*			
23	*P.violae*			

Table A.4 Isolate means for four directly measured criteria and two derived indexes for oogonia from each 71isolates of Smooth oogonia and globose or subglobose sporangia Pythium species

Pythium species	Isolate #	oogonia	oospore	oospore wall thickness	sporangia	oospore wall index	aplerotic index
P.Conidiophorum	5679	13.2	12.5	1.9	21.6	66.28	84.91966
	2538	13	12.5	1.8	21.9	63.91	88.89964
P.debaryanum	701	20.8	16.6	1	23.6	31.96	50.83166
	818	19.6	16.5	1	.	32.13	59.66005
	2746	19.6	16.3	0.9	24.8	29.61	57.51678
	773	19.5	17.2	1	.	30.98	68.62487
	727	19.2	16.1	1.5	23.8	46.13	58.96224
	805	18.9	16.9	1.3	.	39.42	71.49484
	772	18.8	16.4	1.1	.	35.09	66.38317
	858	18.8	16.2	1.7	23.8	50.67	63.98402
	816	18.6	16.2	0.8	.	26.8	66.07029
	807	18.3	16.3	1	.	32.48	70.66584
	861	18.2	15.3	1.4	24.4	45.47	59.41008
P.irregulare	726	19.2	15.1	1	23.8	34.7	48.64376
	672	19.1	14.5	0.94	27	34.07	43.7526
	2087	19	15.3	1	23.5	34.31	52.21719
	631	18.3	14.6	0.8	26.9	29.41	50.78147
	713	18	14.1	1	24.9	36.8	48.0662
	2019	17.9	14.3	1.2	22.6	42.37	50.98577
	2088	17.3	14.3	0.6	23.7	23.12	56.47676
P.iwayamai	747	25.6	21	2	22.1	46.95	55.19986
	2732	24.7	20	1.8	28.8	44.86	53.08834
	2651	21.2	17.6	1.8	24.2	49.67	57.2177
P.middletonii	760	23.7	18.6	1.5	35.2	41	48.33857
	758	21.3	17.4	1.2	36.6	35.93	54.51411
	761	20.9	17.5	1.6	32.8	45.44	58.70503
P.nagaii	2390	19.2	16.1	1	24.2	32.83	58.96224
	2729	17.9	14.9	0.7	25	25.62	57.67661
P.pareocandrum	708	24.8	19	1.1	22.7	30.87	44.96823
	2012	23.9	19.8	1.5	21.1	38.92	56.85935
	2308	22.9	19	0.9	25.7	25.81	57.11555
	775	19.7	18.1	1.4	22.4	39.6	77.55987
P.pulchrum	2247	28.5	23.8	1.1	33.9	25.25	58.23664
	2522	24.2	18.1	1.5	27.4	41.94	41.8398
	4001	21.5	17.5	1.1	23.8	33.17	53.92607
P.pyrilobum	2458	24.4	20.2	2	.	48.42	56.73939
	2853	24.3	21.1	1.3	.	32.6	65.46792
P.rostratum	2121	20.4	18.5	1.5	25.7	41.19	74.5804
	2089	18.9	17	1	21.3	31.3	72.7715
	2248	18.4	17.2	1.3	24.1	38.84	81.68304
	673	18.4	17.2	1.3	21.1	38.84	81.68304

Pythium species	Isolate #	oogonia	oospore	oospore wall thickness	sporangia	oospore wall index	aplerotic index
	2023	18.3	16.9	1.3	24.4	39.42	78.76021
	581	18.2	16.7	1.1	21.8	34.54	77.25654
P.salinum	2387	17.8	17.2	1.5	16.8	43.73	90.22468
	3343	17.3	17.3	1.4	16	41.12	100
P.salpingophorum	1093	15.3	15.3	1.5	22	48.04	100
	2231	14.9	14.9	1.5	24.2	49.06	100
P.sp.(ma2153)	2153	22.6	21.3	1.5	28.8	36.58	83.71697
	2199	22.5	20.9	1.5	28.6	37.18	80.14774
P.sylvaticum	0	23.1	19.5	1.3	26	34.9	60.15447
	0	22.9	19.4	1.1	26.8	30.31	60.79932
P.tracheiphilum	2420	18.6	18.6	1.3	23.6	36.35	100
	2402	18.4	18.4	1.3	23.2	36.68	100
P. ultimum var. sporangiiferum	2197	22.2	18.7	1.3	26.6	36.18	59.76761
	2222	21.6	17.6	1	25.3	30.36	54.09744
	2130	21.4	16.7	1	26.8	31.8	47.52346
	2221	21.4	17.7	1.3	27.1	37.91	56.58202
	682	21.3	17.6	1.3	.	38.09	56.4156
	707	21.2	17.1	1	.	31.14	52.47842
	850	20.7	17.8	1.5	.	42.52	63.58416
	2018	20	16.4	1.3	.	40.42	55.1368
	733	19.6	16.9	1.1	.	34.19	64.105
	683	19.4	15.6	1.5	20.7	47.31	51.99584
P.ultimum var ultimum	291	21.93	19.52	2.34	1.42	56.06	70.52183
	141	18.16	16.44	1.1	1.32	35.01	74.19214
	181	20.16	18.27	0.86	1.42	25.67	74.42932
P.vexans	2253	22.8	19.1	1.7	16.2	44.46	58.78893
	2149	22.6	19.1	1.7	.	44.46	60.36355
	2082	22.3	18.3	1.4	15.3	39.24	55.26354
P.violae	2160	26.4	22	0.8	28.9	20.27	57.87037
	3020	25.2	21.6	0.9	28.3	22.97	62.97376

Table A.4.1 Names of Smooth oogonia and globose or subglobose sporangia Pythium isolates depend on numbers

species	Numbers
P.Conidiophorum1	1
P.Conidiophorum2	2
P.debaryanum1	3
P.debaryanum2	4
P.debaryanum3	5
P.debaryanum4	6
P.debaryanum5	7
P.debaryanum6	8

P.debaryanum7	9
P.debaryanum8	10
P.debaryanum9	11
P.debaryanum10	12
P.debaryanum11	13
P.irregulare1	14
P.irregulare2	15
P.irregulare3	16
P.irregulare4	17
P.irregulare5	18
P.irregulare6	19
P.irregulare7	20
P.iwayamai1	21
P.iwayamai2	22
P.iwayamai3	23
P.middletonii1	24
P.middletonii2	25
P.middletonii3	26
P.nagaii1	27
P.nagaii2	28
P.pareocandrum1	29
P.pareocandrum2	30
P.pareocandrum3	31
P.pareocandrum4	32
P.pulchrum1	33
P.pulchrum2	34
P.pulchrum3	35
P.pyrilobum1	36
P.pyrilobum2	37
P.rostratum1	38
P.rostratum2	39
P.rostratum3	40
P.rostratum4	41
P.rostratum5	42
P.rostratum6	43
P.salinum1	44
P.salinum2	45
P.salpingophorum1	46
P.salpingophorum2	47
P.sylvaticum1	48

P.sylvaticum2	49
P.tracheiphilum1	50
P.tracheiphilum2	51
P.ultimum var ultimum1	52
P.ultimum var ultimum2	53
P.ultimum var ultimum3	54
P. ultimum var. sporangiiferum1	55
P. ultimum var. sporangiiferum2	56
P. ultimum var. sporangiiferum3	57
P. ultimum var. sporangiiferum4	58
P. ultimum var. sporangiiferum5	59
P. ultimum var. sporangiiferum6	60
P. ultimum var. sporangiiferum7	61
P. ultimum var. sporangiiferum8	62
P. ultimum var. sporangiiferum9	63
P. ultimum var. sporangiiferum10	64
P.vexans1	65
P.vexans2	66
P.vexans3	67
P.violae1	68
P.violae2	69
P.sp.(ma2153)1	70
P.sp.(ma2153)2	71

Table A.4.2 Cluster membership for Smooth oogonia and globose or subglobose Pythium isolates

2 Clusters	3 Clusters	4 Clusters	5 Clusters	Case
1	1	1	1	1:conidi1
1	1	1	1	2:conidi2
2	2	2	2	3:debary1
2	2	2	2	4:debary2
2	2	2	2	5:debary3
2	2	2	2	6:debary4

2	2	3	3	7:debary5
2	2	2	2	8:debary6
2	2	2	2	9:debary7
2	2	3	3	10:debary8
2	3	4	4	11:debary9
2	2	2	2	12:debary10
2	2	3	3	13:debary11
2	2	2	2	14:irregu1
2	2	2	2	15:irregu2
2	2	2	2	16:irregu3
2	2	2	2	17:irregu4
2	2	2	2	18:irregu5
2	2	3	3	19:irregu6
2	3	4	4	20:irregu7
2	2	3	3	21:iwayam1
2	2	3	3	22:iwayam2
2	2	3	3	23:iwayam3
2	2	3	3	24:middle1
2	2	2	2	25:middle2
2	2	3	3	26:middle3
2	2	2	2	27:nagaii1
2	3	4	4	28:nagaii2
2	2	2	2	29:pareoc1
2	2	2	2	30:pareoc2
2	3	4	4	31:pareoc3
2	2	2	2	32:pareoc4
2	3	4	4	33:pulchr1
2	2	3	3	34:pulchr2
2	2	2	2	35:pulchr3
2	2	3	3	36:pyrilo1
2	2	2	2	37:pyrilo2
2	2	2	2	38:rostra1
2	2	2	2	39:rostra2
2	2	2	2	40:rostra3
2	2	2	2	41:rostra4
2	2	2	2	42:rostra5
2	2	2	2	43:rostra6
2	2	3	3	44:salinu1
2	2	2	2	45:salinu2
2	2	3	3	46:salpin1
2	2	3	3	47:salpin2
2	2	2	2	48:sylvat1
2	2	2	2	49:sylvat2
2	2	2	2	50:trache1
2	2	2	2	51:trache2
2	2	3	5	52:ultimu1
2	2	2	2	53:ultimu2
2	3	4	4	54:ultimu3
2	2	2	2	55:ultimu4
2	2	2	2	56:ultimu5
2	2	2	2	57:ultimu6
2	2	2	2	58:ultimu7
2	2	2	2	59:ultimu8
2	2	2	2	60:ultimu9
2	2	2	2	61:ultimu10
2	2	2	2	62:ultimu11

2	2	2	2	63:ultimu12
2	2	3	3	64:ultimu13
2	2	3	3	65:vexans1
2	2	3	3	66:vexans2
2	2	2	2	67:vexans3
2	3	4	4	68:violae1
2	3	4	4	69:violae2
2	2	2	2	70:spma1
2	2	2	2	71:spma2

Table A.4.3 Eigen values for 71 isolates of Smooth oogonia and globose or subglobose Pythium species

Canonical Correlation	Cumulative %	% of Variance	Eigen value	Function
.929	52.8	52.8	6.300	1
.881	81.7	28.9	3.452	2
.757	93.0	11.2	1.341	3
.676	100.0	7.0	.839	4

Table A.4.4 Clusters for Smooth oogonia and globose or subglobose Pythium species produced by canonical variate analysis

Cluster number	Pythium species isolates
Cluster 1	*P.debaryanum* (1-11), *P.irregulare* (1-6), *P.iwayamai* (1-3), *P.middletonii* (1-3), *P.nagaii* (1, 2), *P.pareocandrum* (1-4), *P.pulchrum* (2, 3), *P.pyrilobum* (1), *P.rostratum* (1-6), *P.sylvaticum* (1, 2), *P. ultimum var. sporangiiferum* (1-10), *P.ultimum var ultimum* (1-3), *P.vexans* (1-3).
Cluster 2	*n* (2), *P.violae* (1, 2).
Cluster 3	*P.salinum* (1, 2), *P.tracheiphilum* (1, 2).
Cluster 4	*P.sp.(ma2153)* (1, 2).
Cluster 5	*P.salpingophorum* (1, 2).
Cluster 6	*P.Conidiophorum* (1, 2).
Cluster 7	*P.irregulare* (7).
Cluster 8	*P.pulchrum* (1).

Table A.5 Isolate means for four directly measured criteria and two derived indexes for oogonia from each 18 isolates of Spiny oogonia and globose sporangia and/or hyphal swellings Pytium species

Pythium species	Isolates #	oogonia	oospore	oospore wall thickness	sporangia	oospore wall index	aplerotic index
P.acanthicum	1059	20.6	19.3	1.4	27.7	37.51	82.23757
	2230	20.5	19.4	1.5	24.2	39.59	84.75076
	192	21.36	18.24	2.3	1.24	58.18	62.26884
	171	23.13	20.44	2.8	1.06	61.73	69.0106
	181	20.56	17.45	2.1	1.2	56.22	61.13881
P.anandrum	2027	27.4	22.2	1.7	27.7	39.27	53.18721
	704	26.7	23.3	1.5	28.1	33.87	66.45597
	806	26.6	24.3	1.4	27.9	30.74	76.23842
	2227	25.3	21.7	1.7	31.1	40.02	63.0983
P.echinulatum	2592	20.9	18.6	1.2	24.3	33.93	70.48553
	2245	20.8	18.3	1.1	26.8	31.9	68.10253
P.mamillatum	2014	18.4	17.1	0.9	20.3	28.37	80.2666
	2239	18.4	17.9	0.9	18.6	27.24	92.06735
P.oligandrum	738	22.4	20.2	2	27.4	48.42	73.33479
	6139	19.2	16.7	1.4	27.1	42.34	65.80301
P.spinosum	2344	18.2	15.2	1.2	25.3	40.28	58.25277
	8496	16.8	15.1	0.5	25.5	18.58	72.6111
	2424	16.2	16.2	0.9	25.9	29.77	100

Table A.5.1 Names of Spiny oogonia and globose sporangia and/or hyphal swellings Pythium isolates depend on numbers

species	Numbers
P.acanthicum1	1
P.acanthicum2	2
P.acanthicum3	3
P.acanthicum4	4
P.acanthicum5	5
P.anandrum1	6
P.anandrum2	7
P.anandrum3	8
P.anandrum4	9
P.echinulatum1	10
P.echinulatum2	11
P.mamillatum1	12
P.mamillatum2	13
P.oligandrum1	14
P.oligandrum2	15

P.spinosum1	16
P.spinosum2	17
P.spinosum3	18

Table A.5.2 Cluster membership for Spiny oogonia and globose sporangia and/or hyphal swellings Pythium isolates

Case	3 Clusters
1:acanth1	1
2:acanth2	1
3:acanth3	1
4:acanth4	1
5:acanth5	1
6:anand1	2
7:anand2	2
8:anand3	2
9:anand4	2
10:echinu1	1
11:echinu2	1
12:mamil1	3
13:mamil2	3
14:oligan1	1
15:oligan2	3
16:spino1	3
17:spino2	3
18:spino3	3

Table A.5.3 Eigen values for 18 isolates of Spiny oogonia and globose sporangia and/or hyphal swellings Pythium species

Function	Eigen value	% of Variance	Cumulative %	Canonical Correlation
1	19.174	89.6	89.6	.975
2	2.103	9.8	99.4	.823
3	.122	.6	100.0	.329

Table A.5.4 Clusters for Spiny oogonia and globose sporangia and/or hyphal swellings Pythium species produced by canonical variate analysis

Cluster number	Pythium species isolates
Cluster 1	*P.acanthicum* (1, 2), *P.echinulatum* (1, 2), *P.mamillatum* (1, 2), *P.oligandrum* (2), *P.spinosum* (1, 3).
Cluster 2	*P.acanthicum* (3, 5), *P.oligandrum* (1).
Cluster 3	*P.anandrum* (1-3).
Cluster 4	*P.anandrum* (4).
Cluster 5	*P.acanthicum*(4).
Cluster 6	*P.spinosum* (2).

Table A.6 Isolate means for four directly measured criteria and two derived indexes for oogonia from each 32 isolates of filamentous (non-swollen) sporangia Pytium species

Pythium species		oogonia	oospore	oospore wall thickness	sporangia	oospore wall index	aplerotic index
P.aquatile	756	19.4	15.1	1.1	.	37.65	47.15477
	817	19	15.3	1	.	34.31	52.21719
	728	18.9	14.3	1.4	.	47.99	43.31344
	1054	18.8	14.6	1.6	.	52.39	46.83656
	809	18.6	15.4	1.5	.	47.8	56.75751
	2010	18.3	14.8	1.6	.	51.85	52.8971
	2020	18.2	14.6	1.5	.	49.84	51.62314
	2138	18.1	15	1.6	.	51.32	56.91648
	1032	18.1	14.4	1.5	.	50.38	50.35606
	865	17.8	13.9	1.3	.	25.52	47.61945
	2072	17.8	14.7	1.5	.	49.58	56.32381
	897	17.7	14.3	1.3	.	45.23	52.73371
	725	17.7	14.1	1.4	.	48.53	50.55191
	2111	17.6	14.5	1.5	.	50.11	55.91985
	770	17.5	13.8	1	.	37.48	49.03691
	864	17.4	14	1.4	.	48.8	52.08784
	667	17.2	13.8	1.3	.	46.54	51.64781
P.coloratum	804	20.7	16.9	1.4	.	41.92	54.41881
	2362	20	16.6	1.7	.	49.72	57.1787
	1109	19.3	16.4	1.9	.	54.65	61.35636
	1002	18.9	15	0.8	.	28.71	49.9906
P.dissitocum	788	18.9	14.9	0.8	.	28.88	48.99744

Pythium species		oogonia	oospore	oospore wall thickness	sporangia	oospore wall index	aplerotic index
	896	18.8	15.6	0.5	.	18.02	57.13474
	2073	18.6	15.1	1.6	.	51.05	53.50471
	2193	18.3	15.9	1.9	.	55.93	65.59007
	894	17.4	14.1	1.1	.	39.88	53.212
P.monospermum	2555	17.6	17.6	0.6	.	19.09	100
	779	16.8	16.8	1.3	.	39.61	100
P.papillatum	3021	15.3	15.3	0.9	.	31.3	100
	3023	15.3	15.3	0.6	.	21.73	100
P.tenue	771	15.9	12.7	1	.	40.19	50.95887
	384	11.8	9.2	0.9	.	47.96	47.39336

Table A.6.1 Names of filamentous (non-swollen) sporangia Pythium isolates depend on numbers

species	Numbers
P.aquatile1	1
P.aquatile2	2
P.aquatile3	3
P.aquatile4	4
P.aquatile5	5
P.aquatile6	6
P.aquatile7	7
P.aquatile8	8
P.aquatile9	9
P.aquatile10	10
P.aquatile11	11
P.aquatile12	12
P.aquatile13	13
P.aquatile14	14
P.aquatile15	15
P.aquatile16	16
P.aquatile17	17
P.coloratum1	18
P.coloratum2	19
P.coloratum3	20
P.coloratum4	21
P.dissitocum1	22
P.dissitocum2	23
P.dissitocum3	24
P.dissitocum4	25
P.dissitocum5	26
P.monospermum1	27
P.monospermum2	28
P.papillatum1	29
P.papillatum2	30
P.tenue1	31
P.tenue2	32

Table A.6.2 Cluster membership for filamentous (non-swollen) sporangia Pythium isolates

2 Clusters	3 Clusters	4 Clusters	5 Clusters	Case
1	1	1	1	1:Paquat1
1	1	1	1	2:Paquat2
1	2	2	2	3:Paquat3
1	2	2	2	4:Paquat4
1	2	2	2	5:Paquat5
1	2	2	2	6:Paquat6
1	2	2	2	7:Paquat7
1	2	2	2	8:Paquat8
1	2	2	2	9:Paquat9
2	3	3	3	10:Paquat10
1	2	2	2	11:Paquat11
1	2	2	2	12:Paquat12
1	2	2	2	13:Paquat13
1	2	2	2	14:Paquat14
1	1	1	1	15:Paquat15
1	2	2	2	16:Paquat16
1	2	2	2	17:Paquat17
1	1	1	1	18:Pcolora1
1	2	2	2	19:Pcolora2
1	2	2	2	20:Pcolora3
2	3	3	3	21:Pcolora4
2	3	3	3	22:Pdissit1
2	3	4	4	23:Pdissit2
1	2	2	2	24:Pdissit3
1	2	2	2	25:Pdissit4
1	1	1	1	26:Pdissit5
2	3	4	4	27:Pmonosp1
1	1	1	1	28:Pmonosp2
2	3	3	3	29:Ppapill1
2	3	4	4	30:Ppapill2
1	1	1	1	31:Ptenue1
1	2	2	5	32:Ptenue2

Table A.6.3 Eigen values for 32 isolates of filamentous (non-swollen) sporangia Pythium species

Canonical Correlation	Cumulative %	% of Variance	Eigen value	Function
.966	81.4	81.4	13.817	1
.870	99.7	18.3	3.108	2
.196	99.9	.2	.040	3
.136	100.0	.1	.019	4

Table A.6.4 Clusters for filamentous (non-swollen) sporangia Pythium species produced by canonical variate analysis

Cluster number	Pythium species isolates
Cluster 1	*P.aquatile* (3, 4).
Cluster 2	*P.coloratum* (1-3).
Cluster 3	*P.dissitocum* (2).
Cluster 4	*P.monospermum* (1).
Cluster 5	*P.monospermum* (2).
Cluster 6	*P.papillatum* (1, 2).
Cluster 7	*P.aquatile* (1, 2, 5-17), *P.coloratum* (4), *P.dissitocum* (1, 3-5).
Cluster 8	*P.tenue* (1).
Cluster 9	*P.tenue* (2).

Table A.7 Isolate means for four directly measured criteria and two derived indexes for oogonia from each 12 isolates of lobulate sporangia Pytium species

Pythium species	Isolate#	oogonia	oospore	oospore wall thickness	sporangia	oospore wall index	aplerotic index
P.aphanidermatum	1	22.88	20.16	2.56	2.32	58.48	68.40747
	3	25.88	22.72	3.23	3.14	63.34	67.66003
	4	22.64	19.49	1.78	3.16	45.4	63.79788
P.dissimile	2799	13.4	13.4	1	.	38.43	100
	2590	11.4	11.4	1	.	43.94	100
P.graminicola	2567	26.2	26.2	2.5	.	47.02	100
	7756	26	26	2.5	.	47.31	100
P.torulosum	869	16.3	15.5	1.2	.	39.63	85.9869
	2232	15.4	15.4	1	.	34.12	100
	2092	15.3	14.3	1	.	36.36	81.64579
P.vanterpoolii	2172	19.3	19.3	2.8	.	64.23	100
	2213	18.2	18.2	2.5	.	61.85	100

Table A.7.1 Names of lobulate sporangia Pythium isolates depend on numbers

species	numbers
P.aphanidermatum1	1
P.aphanidermatum2	2
P.aphanidermatum3	3
P.dissimile1	4
P.dissimile2	5
P.graminicola1	6
P.graminicola2	7

P.torulosum1	8
P.torulosum2	9
P.torulosum3	10
P.vanterpoolii1	11
P.vanterpoolii2	12

Table A.7.2 Cluster membership for lobulate sporangia Pythium isolates

2 Clusters	3 Clusters	4 Clusters	5 Clusters	Case
1	1	1	1	1:aphani1
1	1	1	1	2:aphani2
1	2	2	2	3:aphani3
2	3	3	3	4:dissim1
2	3	4	4	5:dissim2
1	2	2	5	6:gramin1
1	2	2	5	7:gramin2
2	3	3	3	8:torulo1
2	3	3	3	9:torulo2
2	3	3	3	10:torulo3
1	1	1	1	11:vanter1
1	1	1	1	12:vanter2

Table A.7.3 Eigen values for 12 isolates of lobulate sporangia Pythium species

Canonical Correlation	Cumulative %	% of Variance	Eigen value	Function
.995	76.9	76.9	106.202	1
.981	95.5	18.6	25.727	2
.927	100.0	4.4	6.139	3
.192	100.0	.0	.038	4

Table A.7.4 Clusters for lobulate sporangia Pythium species produced by canonical variate analysis

Cluster number	Pythium species isolates
Cluster 1	*P.aphanidermatum* (1, 3).
Cluster 2	*P.dissimile* (2), *P.graminicola* (1, 2), *P.vanterpoolii* (1, 2).
Cluster 3	*P.dissimile* (1), *P.torulosum* (2).
Cluster 4	*P.torulosum* (1, 3).
Cluster 5	*P.aphanidermatum* (2).

Table A.8 Isolate means for four directly measured criteria and two derived indexes for oogonia from each 47 isolates of plerotic oospore species

Pythium species	isolate #	oogonia	oospore	oospore wall thickness		sporangia	oospore wall index	aplerotic index		
P.conidiophorum	5679	13.2	12.5	1.9	0.6	21.6	66.28	84.91966	86.90965	plerotic
	2538	13	12.5	1.8	0.7	21.9	63.91	88.89964		
P.dissimile	2799	13.4	13.4	1	0.1	.	38.43	100	100	plerotic
	2590	11.4	11.4	1	0.1	.	43.94	100		
P.graminicola	2567	26.2	26.2	2.5	0.2	.	47.02	100	100	plerotic
	7756	26	26	2.5	0.2	.	47.31	100		
P.lutarium	2361	21.9	20.2	2.7	0.6	.	60.67	78.47327	74.66473	plerotic
	893	21.2	18.9	1.9	0.7	.	49	70.8562		
P.mamillatum	2014	18.4	17.1	0.9	0.2	20.3	28.37	80.2666	82.13241	plerotic
	2239	18.4	17.9	0.9	0.2	18.6	27.24	92.06735		
	2241	16.8	15.2	0.9	0.2	22.4	31.49	74.06328		
P.minus	2368	13.9	13.9	0.6	0.2	23.3	23.73	100	100	plerotic
	2660	13.3	13.3	0.5	0.2	19.1	20.9	100		
	2787	12.6	12.6	0.5	0	25.4	21.97	100		
P.monospermum	2555	17.6	17.6	0.6	0.2	.	19.09	100	100	plerotic
	779	16.8	16.8	1.3	0.3	.	39.61	100		
P.oligandrum	738	22.4	20.2	2	0.3	27.4	48.42	73.33479	69.5689	plerotic
	6139	19.2	16.7	1.4	0.2	27.1	42.34	65.80301		
P.papillatum	3021	15.3	15.3	0.9	0.2	.	31.3	100	100	plerotic
	3023	15.3	15.3	0.6	0.2	.	21.73	100		
	2853	24.3	21.1	1.3	0.4	.	32.6	65.46792		
P.parvum	2259	14.9	13.3	0.5	0	17.8	20.9	71.12072	78.72849	plerotic
	1094	14.5	13.5	0.5	0	17.6	20.62	80.70442		
	871	14.3	12.8	0.5	0	16.5	21.65	71.71695		
	651	13.5	13.1	0.5	0	16.8	21.2	91.37188		

Pythium species	isolate #	oogonia	oospore	oospore wall thickness		sporangia	oospore wall index	aplerotic index		
P.rostratum	2121	20.4	18.5	1.5	0.3	25.7	41.19	74.5804	77.78912	plerotic
	2089	18.9	17	1	0.2	21.3	31.3	72.7715		
	2248	18.4	17.2	1.3	0.3	24.1	38.84	81.68304		
	673	18.4	17.2	1.3	0	21.1	38.84	81.68304		
	2023	18.3	16.9	1.3	0.3	24.4	39.42	78.76021		
	581	18.2	16.7	1.1	0.2	21.8	34.54	77.25654		
P.salinum	2387	17.8	17.2	1.5	0.3	16.8	43.73	90.22468	95.11234	plerotic
	3343	17.3	17.3	1.4	0.3	16	41.12	100		
P.salpingophorum	1093	15.3	15.3	1.5	0.2	22	48.04	100	100	plerotic
	2231	14.9	14.9	1.5	0.2	24.2	49.06	100		
P.spinosum	2344	18.2	15.2	1.2	0.3	25.3	40.28	58.25277	76.95462	plerotic
	8496	16.8	15.1	0.5	0.1	25.5	18.58	72.6111		
	2424	16.2	16.2	0.9	0.2	25.9	29.77	100		
P.sp.(ma2153)	2153	22.6	21.3	1.5	0.3	28.8	36.58	83.71697	81.93236	plerotic
	2199	22.5	20.9	1.5	0.3	28.6	37.18	80.14774		
P.torulosum	869	16.3	15.5	1.2	0.3	.	39.63	85.9869	89.2109	plerotic
	2232	15.4	15.4	1	0	.	34.12	100		
	2092	15.3	14.3	1	0.1	.	36.36	81.64579		
P.tracheiphilum	2420	18.6	18.6	1.3	0.3	23.6	36.35	100	100	plerotic
	2402	18.4	18.4	1.3	0.3	23.2	36.68	100		
P.vantrepoolii	2172	19.3	19.3	2.8	0.6	.	64.23	100	100	plerotic
	2213	18.2	18.2	2.5	0.3	.	61.85	100		

Table A.8.1 Names of plerotic oospore speices depend on numbers

species	numbers
conidiophorum1	1
conidiophorum2	2
dissimile1	3
dissimile2	4
graminicola1	5
graminicola2	6
mamillatum1	7
mamillatum2	8
mamillatum3	9
monospermum1	10
monospermum2	11
oligandrum1	12
oligandrum2	13
papillatum1	14
papillatum2	15
papillatum3	16
rostratum1	17
rostratum2	18
rostratum3	19
rostratum4	20
rostratum5	21
rostratum6	22
salinum1	23
salinum2	24
salpingophorum1	25
salpingophorum2	26
spinosum1	27
spinosum2	28
spinosum3	29
torulosum1	30
torulosum2	31
torulosum3	32
tracheiphilum1	33
tracheiphilum2	34

species	numbers
vantrepoolii1	35
vantrepoolii2	36
parvum1	37
parvum2	38
parvum3	39
parvum4	40
sp.(ma2153)1	41
sp.(ma2153)2	42
lutarium1	43
lutarium2	44
minus1	45
minus2	46
minus3	47

Table A.8.2 Cluster membership for plerotic oospore speices

Case	4 Clusters
1:conidi1	1
2:conidi2	1
3:dissim1	1
4:dissim2	1
5:grami1	2
6:grami2	2
7:mam1	3
8:mam2	3
9:mam3	1
10:monos1	3
11:monos2	3
12:oligan1	4
13:oligan2	3
14:papill1	1
15:papill2	1
16:papill3	4
17:rostrat1	3
18:rostrat2	3
19:rostrat3	3

Case	4 Clusters
20:rostrat4	3
21:rostrat5	3
22:rostrat6	3
23:salinum1	3
24:salinum2	3
25:salping1	1
26:salping2	1
27:spinos1	3
28:spinos2	1
29:spinos3	1
30:torulo1	1
31:torulo2	1
32:torulo3	1
33:trachei1	3
34:trachei2	3
35:vantre1	3
36:vantre2	3
37:parvum1	1
38:parvum2	1
39:parvum3	1
40:parvum4	1
41:spma1	4
42:spma2	4
43:lutari1	4
44:lutari2	3
45:minus1	1
46:minus2	1
47:minus3	1

Table A.8.3 Eigen values for 47 isolates of plerotic oospore species

Function	Eigen value	% of Variance	Cumulative %	Canonical Correlation
1	13.207	58.4	58.4	.964
2	7.791	34.4	92.8	.941
3	1.636	7.2	100.0	.788

Table A.8.4 Clusters for plerotic oospore species produced by canonical variate analysis

Cluster number	Pythium species isolates
Cluster 1	*P.conidiophorum* (1, 2).
Cluster 2	*P.graminicola* (1, 2).
Cluster 3	*P. lutarium* (1), *P.vantrepoolii* (1, 2).
Cluster 4	*P.dissimile* (1,2), *P.lutarium* (2), *P. mamillatum* (1-3), *P.minus* (1-3), *P.monospermum* (1,2), *P.oligandrum* (1,2), *P. papillatum* (1-3), *P.parvum* (1-4), *P.rostratum* (1-6), *P.salinum* (1,2), *P.salpingophorum* (1,2), *P.spinosum* (1-3), *P.sp.(ma2153)* (1,2), *P.torulosum* (1-3), *P.tracheiphilum* (1,2).

Table A.9 Isolate means for four directly measured criteria and two derived indexes for oogonia from each 13 isolates of nearly plerotic oospore species

Pythium species	isolate #	oogonia	oospore	oospore wall thickness		sporangia	oospore wall index	aplerotic index	
P.acanthicum	1059	20.6	19.3	1.4	27.7	37.51	82.23757	71.88131	nearly plerotic
	2230	20.5	19.4	1.5	24.2	39.59	84.75076		
	192	21.36	18.24	2.3	1.24	58.18	62.26884		
	171	23.13	20.44	2.8	1.06	61.73	69.0106		
	181	20.56	17.45	2.1	1.2	56.22	61.13881		
P.aphanidermatum	1	22.88	20.16	2.56	2.32	58.48	68.40747	66.62179	nearly plerotic
	3	25.88	22.72	3.23	3.14	63.34	67.66003		
	4	22.64	19.49	1.78	3.16	45.4	63.79788		
P.echinulatum	2592	20.9	18.6	1.2	24.3	33.93	70.48553	69.29403	nearly plerotic
	2245	20.8	18.3	1.1	26.8	31.9	68.10253		
P.ultimum var ultimum	291	21.93	19.52	2.34	1.42	56.06	70.52183	73.04776	nearly plerotic
	141	18.16	16.44	1.1	1.32	35.01	74.19214		
	181	20.16	18.27	0.86	1.42	25.67	74.42932		

Table A.9.1 Names of nearly plerotic oospore species depend on numbers

species	numbers
P.aphanidermatum1	1
P.aphanidermatum2	2
P.aphanidermatum3	3
P.acanthicum1	4
P.acanthicum2	5
P.acanthicum3	6
P.acanthicum4	7
P.acanthicum5	8
P.echinulatum 1	9
P.echinulatum 2	10
P.ultimum var ultimum1	11
P.ultimum Va rultimum2	12
P. ultimum var ultimum3	13

Table A.9.2 Cluster membership for nearly plerotic oospore species

Case	3 Clusters
1:Aphan1	1
2:Aphan2	2
3:Aphan3	1
4:acanth1	1
5:acanth2	1
6:acanth3	1
7:acanth4	1
8:acanth5	1
9:echinu1	1
10:echinu2	1
11:ultimu1	1
12:ultimu2	3
13:ultimu3	1

Table A.9.3 Eigen values for 13 isolates of nearly plerotic Pythium species

Function	Eigen value	% of Variance	Cumulative %	Canonical Correlation
1	1.784	68.8	68.8	.801
2	.808	31.2	100.0	.669
3	.000	.0	100.0	.021

Table A.9.4 Clusters for nearly plerotic oospore species produced by canonical variate analysis

Cluster number	Pythium species isolates
Cluster 1	*P.aphanidermatum* (3), *P.echinu* (1, 2), *P.ultimum var ultimum* (3).
Cluster 2	*P.acanthicum* (1-5), *P.aphanidermatum* (1), *P.ultimum var ultimum* (1).
Cluster 3	*P.aphanidermatum* (2).
Cluster 4	*P.ultimum var ultimum* (2).

Table A.10 Isolate means for four directly measured criteria and two derived indexes for oogonia from each 88 isolates of aplerotic oospore species

Pythium species	isolate #	oogonia	oospore	oospore wall thickness		sporangia	oospore wall index	aplerotic index		
P.anandrum	2027	27.4	22.2	1.7	0.3	27.7	39.27	53.18721	64.74498	aplerotic
	704	26.7	23.3	1.5	0.2	28.1	33.87	66.45597		
	806	26.6	24.3	1.4	0.2	27.9	30.74	76.23842		
	2227	25.3	21.7	1.7	0.8	31.1	40.02	63.0983		
P.aquatile	756	19.4	15.1	1.1	0.2	.	37.65	47.15477	51.41139	aplerotic
	817	19	15.3	1	0.3	.	34.31	52.21719		
	728	18.9	14.3	1.4	0.3	.	47.99	43.31344		
	1054	18.8	14.6	1.6	0.2	.	52.39	46.83656		
	809	18.6	15.4	1.5	0	.	47.8	56.75751		
	2010	18.3	14.8	1.6	0.3	.	51.85	52.8971		
	2020	18.2	14.6	1.5	0.1	.	49.84	51.62314		
	2138	18.1	15	1.6	0.2	.	51.32	56.91648		
	1032	18.1	14.4	1.5	0	.	50.38	50.35606		
	865	17.8	13.9	1.3	0.4	.	25.52	47.61945		
	2072	17.8	14.7	1.5	0.4	.	49.58	56.32381		
	897	17.7	14.3	1.3	0.4	.	45.23	52.73371		
	725	17.7	14.1	1.4	0.3	.	48.53	50.55191		
	2111	17.6	14.5	1.5	0.4	.	50.11	55.91985		
	770	17.5	13.8	1	0	.	37.48	49.03691		
	864	17.4	14	1.4	0.3	.	48.8	52.08784		
	667	17.2	13.8	1.3	0.4	.	46.54	51.64781		
P.coloratum	804	20.7	16.9	1.4	0.2	.	41.92	54.41881	55.73612	aplerotic
	2362	20	16.6	1.7	0.5	.	49.72	57.1787		
	1109	19.3	16.4	1.9	0.5	.	54.65	61.35636		
	1002	18.9	15	0.8	0.3	.	28.71	49.9906		

Pythium species	isolate #	oogonia	oospore	oospore wall thickness		sporangia	oospore wall index	aplerotic index		
P.debaryanum	701	20.8	16.6	1	0.2	23.6	31.96	50.83166	63.05489	aplerotic
	818	19.6	16.5	1	0.4	.	32.13	59.66005		
	2746	19.6	16.3	0.9	0.2	24.8	29.61	57.51678		
	773	19.5	17.2	1	0.3	.	30.98	68.62487		
	727	19.2	16.1	1.5	0.2	23.8	46.13	58.96224		
	805	18.9	16.9	1.3	0.3	.	39.42	71.49484		
	772	18.8	16.4	1.1	0.2	.	35.09	66.38317		
	858	18.8	16.2	1.7	0.3	23.8	50.67	63.98402		
	816	18.6	16.2	0.8	0.4	.	26.8	66.07029		
	807	18.3	16.3	1	0.2	.	32.48	70.66584		
	861	18.2	15.3	1.4	0.3	24.4	45.47	59.41008		
P.dissotocum	788	18.9	14.9	0.8	0.3	.	28.88	48.99744	55.68779	aplerotic
	896	18.8	15.6	0.5	0.1	.	18.02	57.13474		
	2073	18.6	15.1	1.6	0.3	.	51.05	53.50471		
	2193	18.3	15.9	1.9	0.4	.	55.93	65.59007		
	894	17.4	14.1	1.1	0.3	.	39.88	53.212		
P.irregulare	726	19.2	15.1	1	0.1	23.8	34.7	48.64376	50.13197	aplerotic
	672	19.1	14.5	0.94	0	27	34.07	43.7526		
	2087	19	15.3	1	0.4	23.5	34.31	52.21719		
	631	18.3	14.6	0.8	0.2	26.9	29.41	50.78147		
	713	18	14.1	1	0.1	24.9	36.8	48.0662		
	2019	17.9	14.3	1.2	0.3	22.6	42.37	50.98577		
	2088	17.3	14.3	0.6	0.2	23.7	23.12	56.47676		
P.iwayami	747	25.6	21	2	0.5	22.1	46.95	55.19986	55.16863	aplerotic
	2732	24.7	20	1.8	0.4	28.8	44.86	53.08834		
	2651	21.2	17.6	1.8	0.3	24.2	49.67	57.2177		
P.pachycaule	650	27.1	23.3	1.5	0.6	.	33.87	63.55649	58.196	aplerotic
	572	26.1	21.1	2.3	0.3	.	52.18	52.8355		

Pythium species	isolate #	oogonia	oospore	oospore wall thickness		sporangia	oospore wall index	aplerotic index		
P.middletonii	760	23.7	18.6	1.5	0.2	35.2	41	48.33857	53.85257	aplerotic
	758	21.3	17.4	1.2	0.3	36.6	35.93	54.51411		
	761	20.9	17.5	1.6	0.3	32.8	45.44	58.70503		
P.nagaii	2390	19.2	16.1	1	0.2	24.2	32.83	58.96224	58.31942	aplerotic
	2729	17.9	14.9	0.7	0.6	25	25.62	57.67661		
P.lucens	2454	26.6	19.8	1.9	0.2	24.7	47.23	41.24304	40.92036	aplerotic
	8241	26.2	19.4	2	0.3	20.9	49.98	40.59769		
P.paroecandrum	708	24.8	19	1.1	0.2	22.7	30.87	44.96823	59.12575	aplerotic
	2012	23.9	19.8	1.5	0.3	21.1	38.92	56.85935		
	2308	22.9	19	0.9	0.4	25.7	25.81	57.11555		
	775	19.7	18.1	1.4	0.3	22.4	39.6	77.55987		
P.pulchrum	2247	28.5	23.8	1.1	0.4	33.9	25.25	58.23664	51.33417	aplerotic
	2522	24.2	18.1	1.5	0.1	27.4	41.94	41.8398		
	4001	21.5	17.5	1.1	0.2	23.8	33.17	53.92607		
P.pyrilobum	2458	24.4	20.2	2	0.4	.	48.42	56.73939	61.10365	aplerotic
	2853	24.3	21.1	1.3	0.4	.	32.6	65.46792		
P.sylvaticum	2470	23.1	19.5	1.3	0.4	26	34.9	60.15447	60.4769	aplerotic
	688	22.9	19.4	1.1	0.4	26.8	30.31	60.79932		
P.tenue	771	15.9	12.7	1	0.2	.	40.19	50.95887	49.17611	aplerotic
	384	11.8	9.2	0.9	0.2	.	47.96	47.39336		
P. ultimum var. sporangiiferum	2197	22.2	18.7	1.3	0.3	26.6	36.18	59.76761	56.16864	aplerotic
	2222	21.6	17.6	1	0.2	25.3	30.36	54.09744		
	2130	21.4	16.7	1	0.2	26.8	31.8	47.52346		
	2221	21.4	17.7	1.3	0.3	27.1	37.91	56.58202		
	682	21.3	17.6	1.3	0.3	.	38.09	56.4156		
	707	21.2	17.1	1	0.3	.	31.14	52.47842		
	850	20.7	17.8	1.5	0	.	42.52	63.58416		

Pythium species	isolate #	oogonia	oospore	oospore wall thickness		sporangia	oospore wall index	aplerotic index		
	2018	20	16.4	1.3	0.3	.	40.42	55.1368		
	733	19.6	16.9	1.1	0.2	.	34.19	64.105		
	683	19.4	15.6	1.5	0.1	20.7	47.31	51.99584		
P.vexans	2253	22.8	19.1	1.7	0.4	16.2	44.46	58.78893	58.13867	aplerotic
	2149	22.6	19.1	1.7	0.3	.	44.46	60.36355		
	2082	22.3	18.3	1.4	0.2	15.3	39.24	55.26354		
P.violae	2160	26.4	22	0.8	0.3	28.9	20.27	57.87037	60.42207	aplerotic
	3020	25.2	21.6	0.9	0.3	28.3	22.97	62.97376		

Table A.10.1 Names of aplerotic oospore species depend on numbers

species	Numbers
P.anandrum1	1
P.anandrum2	2
P.anandrum3	3
P.anandrum4	4
P.aquatile1	5
P.aquatile2	6
P.aquatile3	7
P.aquatile4	8
P.aquatile5	9
P.aquatile6	10
P.aquatile7	11
P.aquatile8	12
P.aquatile9	13
P.aquatile10	14
P.aquatile11	15
P.aquatile12	16
P.aquatile13	17
P.aquatile14	18
P.aquatile15	19
P.aquatile16	20
P.aquatile17	21
P.coloratum1	22
P.coloratum2	23
P.coloratum3	24
P.coloratum4	25
P.debaryanum1	26
P.debaryanum2	27
P.debaryanum3	28
P.debaryanum4	29
P.debaryanum5	30
P.debaryanum6	31
P.debaryanum7	32
P.debaryanum8	33
P.debaryanum9	34
P.debaryanum10	35
P.debaryanum11	36
P.dissotocum1	37

species	Numbers
P.dissotocum2	38
P.dissotocum3	39
P.dissotocum4	40
P.dissotocum5	41
P.irregulare1	42
P.irregulare2	43
P.irregulare3	44
P.irregulare4	45
P.irregulare5	46
P.irregulare6	47
P.irregulare7	48
P.iwayami1	49
P.iwayami2	50
P.iwayami3	51
P.pachycaule1	52
P.pachycaule2	53
P.middletonii1	54
P.middletonii2	55
P.middletonii3	56
P.nagaii1	57
P.nagaii2	58
P.lucens1	59
P.lucens2	60
P.paroecandrum1	61
P.paroecandrum2	62
P.paroecandrum3	63
P.paroecandrum4	64
P.pulchrum1	65
P.pulchrum2	66
P.pulchrum3	67
P.pyrilobum1	68
P.pyrilobum2	69
P.sylvaticum1	70
P.sylvaticum2	71
P.tenue1	72
P.tenue2	73
P. ultimum var. sporangiiferum1	74
P. ultimum var. sporangiiferum2	75
P. ultimum var. sporangiiferum3	76

species	Numbers
P. ultimum var. sporangiiferum4	77
P. ultimum var. sporangiiferum5	78
P. ultimum var. sporangiiferum6	79
*P. ultimum var. sporangiiferum*7	80
P. ultimum var. sporangiiferum8	81
P. ultimum var. sporangiiferum9	82
P. ultimum var. sporangiiferum10	83
P.vexans1	84
P.vexans2	85
P.vexans3	86
P.violae1	87
P.violae2	88

Table A.10.2 Cluster membership for aplerotic oospore species

Case	4 Clusters
1:anand1	1
2:anand2	1
3:anand3	1
4:anand4	2
5:aquat1	3
6:aquat2	3
7:aquat3	3
8:aquat4	3
9:aquat5	3
10:aquat6	3
11:aquat7	3
12:aquat8	3
13:aquat9	3
14:aquat10	3
15:aquat11	3
16:aquat12	3
17:aquat13	3
18:aquat14	3
19:aquat15	3
20:aquat16	3
21:aquat17	3
22:colora1	3
23:colora2	3

Case	4 Clusters
24:colora3	3
25:colora4	3
26:debary1	3
27:debary2	3
28:debary3	3
29:debary4	3
30:debary5	3
31:debary6	3
32:debary7	3
33:debary8	3
34:debary9	3
35:debary10	3
36:debary11	3
37:dissoto1	3
38:dissoto2	3
39:dissoto3	3
40:dissoto4	3
41:dissoto5	3
42:irregul1	3
43:irregul2	3
44:irregul3	3
45:irregul4	3
46:irregul5	3
47:irregul6	3
48:irregul7	3
49:iwaya1	2
50:iwaya2	2
51:iwaya3	3
52:middle1	2
53:middle2	3
54:middle3	3
55:nagaii1	3
56:nagaii2	3
57:paroec1	2
58:paroec2	2
59:paroec3	2
60:paroec4	3
61:pulchr1	1
62:pulchr2	2

Case	4 Clusters
63:pulchr3	3
64:pyrilob1	2
65:pyrilob2	2
66:sylvat1	2
67:sylvat2	2
68:tenue1	3
69:tenue2	4
70:ultimu1	2
71:ultimu2	3
72:ultimu3	3
73:ultimu4	3
74:ultimu5	3
75:ultimu6	3
76:ultimu7	3
77:ultimu8	3
78:ultimu9	3
79:ultimu10	3
80:vexans1	2
81:vexans2	2
82:vexans3	2
83:violae1	1
84:violae2	2
85:pachy1	1
86:pachy2	2
87:lucenc1	2
88:lucenc2	2

Table A.10.3 Eigen values for 88 isolates of aplerotic oospore species

Canonical Correlation	Cumulative %	% of Variance	Eigen value	Function
.954	84.8	84.8	10.214	1
.751	95.6	10.8	1.297	2
.590	100.0	4.4	.534	3

Table A.10.4 Clusters for a plerotic oospore species produced by canonical variate analysis

Cluster number	Pythium species isolates
Cluster 1	*P.aquatile* (1-17), *P.coloratum* (1-4), *P.debaryanum* (1-11), *P.dissotocum* (1-5), *P.irregulare* (1-7), *P.iwayami* (3), *P.middletonii* (1-3), *P.nagaii* (1,2), *P.paroecandrum (1-4)*, *P.pulchrum* (2,3), *P.sylvaticum* (1,2), *P.tenue* (1), *P. ultimum var. sporangiiferum* (1-10), *P.vexans*(1-3).
Cluster 2	*P.lucens* (1, 2), *P.pachycaule* (2).
Cluster 3	*P.iwayami* (1, 2), *P.pyrilobum* (1).
Cluster 4	*P.anandrum*(2), *P.pulchrum* (1), *P.pyrilobum* (2).
Cluster 5	*P.violae* (1, 2).
Cluster 6	*P.tenue* (2).
Cluster 7	*P.pyrilobum* (2).
Cluster 8	*P.anandrum* (1).
Cluster 9	*P.anandrum* (3)*0.*
Cluster 10	*P.anandrum* (4).

جامعة النجاح الوطنية
كلية الدراسات العليا

استخدام تحليل التباين متعدد المتغيرات للمساعدة في فصل فطر البيثيم

إعداد
هديل رشيد ظاهر الظاهر

إشراف
أ. د. محمد سليم اشتية

قدمت هذه الأطروحة استكمالا لمتطلبات درجة الماجستير في العلوم البيئية بكليـــة الدراســـات العليا في جامعة النجاح الوطنية في نابلس, فلسطين.
2008

استخدام تحليل التباين متعدد المتغيرات للمساعدة في عملية فصل فطر البيثيم

إعداد

هديل رشيد ظاهر الظاهر

اشراف

أ. د. محمد سليم أشتية

الملخص

استعملت في هذه الدراسة مئة وسبع وأربعون عزلة فطرية تابعة لواحد وأربعين نوع من انواع فطر البيثيم . تم عزل هذه العينات من التربة في منطقة في الضفة الغربية وقطاع غزة باستخدام (VP3) كوسط انتقائي وتقنية محلول التربة السطحية في الصحون البلاستيكية .

استخدمت العزلات لاختبار مدى متانة الصفات البيومترية في عملية فصل وتصنيف أنواع فطر البيثيم . استخدم لهذا الغرض الصفات المورفولوجية المعتمدة على (sporangia and oogonia) و اقطار (oogonium and oospore) وسمك جدار (oospore) ومعامل جدار (oospore) و معامل (aplerotic) .

أظهرت الدراسة أهمية استخدام البينات البيومترية في الفصل بين أنواع البيثيم , وأظهرت الدراسة أيضاً وجود اختلافات واضحة بين أنواع الفطر وداخلها الأمر الذي أمكن من استخدام هذه الاختلافات للفصل بين الأنواع.

أوضحت الدراسة بأن استخدام تحليل التباين متعدد المتغيرات يعتبر تقنية ذات أهمية تصنيفية للفصل بين أنواع الفطر *Pythium*.