STUDIES ON CHARACTERIZATION AND VARIABILITY OF PHYTOPHTHORA SPECIES PATHOGENIC TO BLACK PEPPER *(PIPER NIGRUM)*

Thesis submitted to University of Calicut
In partial fulfillment
For the award of the
degree of Doctor of Philosophy in Botany

By

VIJAYA, P

INDIAN INSTITUTE OF SPICES RESEARCH
(Indian Council of Agricultural Research)
Marikunnu P.O, Calicut - 673012, Kerala

2008

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DECLARATION

I hereby declare that this thesis entitled 'Studies on characterization and Variability of Phytophthora species pathogenic to black pepper (Piper nigrum)', submitted by me for the award of the degree of Doctor of Philosophy in Botany of the University of Calicut, contains the results of bonafide research work done by me at Indian Institute of Spices Research, Calicut, under the guidance of Dr. Y.R Sarma (Former Director, Indian Institute of Spices Research). This thesis or part of it has not been submitted to any other university for the award of any other degree or diploma. All sources of help received by me during the course of this study have been duly acknowledged.

Place: Calicut *(Vijaya, P)*

Date: March 2008

CERTIFICATE

*I hereby certify that the thesis entitled '**Studies on characterization and Variability of Phytophthora species pathogenic to black pepper (Piper nigrum)',** submitted by **Vijaya, P,** for the award of the degree of **Doctor of Philosophy in Botany** of the **University of Calicut**, contains the results of bonafide research work done by her during 1999-2008 at Indian Institute of Spices Research, Calicut, under my supervision and guidance. No part of this thesis has been submitted to any other university for the award of any other degree or diploma. All sources of help received by her during the course of this investigation have been duly acknowledged. Certified that she has also passed the required qualifying examination.*

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SYMBOLS AND ABBREVIATIONS

ANOVA	Analysis of variance
ACP	Acid phosphatase
AFLP	Amplified fragment length polymorphism
cm	Centimeter
bp	Base pairs
CA	Carrot agar
CAT	Catalase
CMA	Corn meal agar
DIA	Diaphorase
CTAB	Cetyltrimethyl ammonium bromide
DMRT	Duncan multiple range test
DNA	Deoxyribo Nucleic Acid
DNTP	Dinucleotide triphosphate
EDTA	Disodium Ethylene Diamine Tetra Acetate
ETS	Elecctrophoretic types
g	Gram
G6PDH	Glucose 6 Phosphate dehydrogenase
GYP	Glucose yeast peptone
Hae III	Hemophilus aegyptius (restriction endonuclease)
Hinf I	Haemophilus influenzae (restriction endonuclease)
IDH	Isocitrate dehydrogenase
Isozymes	Isoenzymes
ITS	Internal transcribed spacer regions
µg	Micro gram
µM	Micro mole
MDH	Malate dehydrogenase
ME	Malic enzyme
MF4	Morphological form 4

Msp1	Moraxella spp. (restriction endonuclease)
mg	Milli gram
mt DNA	Mitochondrial DNA
mM	Milli mole
mm	Millimeter
PAGE	Polyacrylamide gel electrophoresis
PCAP	*Phytophthora capsici* specific primer
PCR	Polymerase Chain Reaction
PDA	Potato dextrose agar
PGI	Phospho gluco isomerase
PINF	*Phytophthora infestans* specific primer
PVPH	Pimaricin, vancomycin, pentachloro nitrobenzene, Hymexazol (*Phytophthora* specific medium)
RAPD	Random Amplification of Polymorphic DNA
rDNA	Ribosomal DNA
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribo Nucleic Acid
SOD	Superoxide dis mutase
SPSS	Social science programme for statistical analysis
Taq I	Thermus aquaticus (restriction endonuclease)
Taq pol	Tag polymerase
TAE	Tris Acetic acid EDTA
TE	Tris EDTA
TEMED	Tetramethylene diamine
Tris	Tris (hydroxymethyl) amino ethane
UPGMA	Unweighed pair group method

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INTRODUCTION

INTRODUCTION

Black pepper (*Piper nigrum* L.) is one of the most important spice crops widely used in cooking and medicine. Though India had monopoly in black pepper production in the world, its position dwindled in recent times. Foot rot disease of black pepper caused by *Phytophthora capsici* (Tsao and Alizadeh, 1988) and slow decline caused by nematodal fungal complex (Nambiar and Sarma, 1977) are the two major production constraints in India.

World pepper production reached 305170 tonnes in the year 2005. This was 29658 tonnes less than the projected 334828 tonnes for 2004, against the production of 342625 tonnes in 2003 (Directorate of Arecanut and Spice Development, Calicut, Kerala, India).

In India, the incidence of root disease was reported as early as in 1902 (Menon, 1949 and Barber, 1902, 1903, 1905). Butler (1906, 1918) investigated the disease at Wynad district of Kerala. The first authentic record of the wilt of vines due to *Phytophthora* species in India was in 1966 (Samraj and Jose, 1966) from Kerala, though the disease was noticed as early as (1929) in Karnataka (Venkata Rao, 1929). Now the disease is prevalent in Kerala, Karnataka, and Tamil Nadu state and recently in Assam state (Sarkar et al.1985).

Phytophthora capsici was first described by Leonian (1922) as the causal agent of the blight of *Capsicum annum* L. (Chili pepper) in New Mexico, United States. In India *Phytophthora capsici* is now known to infect many plant species like black pepper, betel vine, cocoa, and bell pepper (Sarma et al. 2002). *Phytophthora capsici* has been redescribed to accommodate a wider range of biotypes and to include the '*Phytophthora palmivora*' MF4 from cocoa and black pepper (Tsao and Alizadeh, 1988; Tsao, 1991). *Phytophthora* infecting black pepper from India was identified as '*Phytophthora palmivora*' MF4 (Sarma et al. 1982). Later Tsao and Alizadeh (1988) merged '*Phytophthora palmivora*' MF4 with *Phytophthora capsici*.

Black pepper is grown in humid tropics of Kerala and Karnataka states both as a pure crop and also as a mixed crop in high density multispecies cropping systems (HDMC) like coconut – black pepper or arecanut – black pepper or coffee – black pepper or cardamom – black pepper. Especially in the HDMC systems more than one *Phytophthora* species are involved, where black pepper becomes a major component. In addition to *Phytophthora capsici*, *Phytophthora parasitica* was reported from Thailand (Tsao 1991). Whether there are more than these two species involved in the disease and their biology and also the variability of *Phytophthora* species needs to be studied. It is important to find out whether any races of *Phytophthora capsici* exist. Information on biology, the variability of *Phytophthora* species affecting black pepper is lacking at present. The present investigation has been undertaken to clarify some of these points, which are important in futuristic disease management programmes.

The variability of the isolates in relation to media, temperature and pH were studied to find out the physiological parameters in grouping of these isolates. The biology of the isolates was investigated by studying the sporangial morphology and their relationship to different temperature, pH and media.

Pathogenic variability has been studied in detail to find out whether there are any biotypes present among the isolates. The comparative virulence of the isolates on different hosts has been studied to understand variation in virulence pattern, and whether it has any correlation with the morphology as well as the molecular characters.

Isozyme study of Oudemans and Coffey (1991 b) demonstrated that *P. capsici* had a very high degree of genetic variability; they concluded that *Phytophthora capsici* could be separated into three sub groups, CAP 1, CAP 2 and CAP 3. The first sub group CAP 1 containing isolates primarily from *Capsicum*, *Lycopersicum* and cucurbits, as well as from cocoa and *Piper nigrum*; CAP 2 having isolates from *Piper nigrum*, cocoa,

Hevea, *Macadamia*, *Carica* and *Solanum melongena* and CAP 3 containing isolates only from cocoa from Brazil. Mchau and Coffey (1995) based on their study resolved the species into two major subgroups, Cap A consisting of isolates which were previously placed in CAP 1 subgroup, and Cap B consisting of those previously placed in CAP 2 and CAP 3. Aragaki and Uchida (2001) based on their studies separated *Phytophthora capsici* into two taxa, *Phytophthora capsici* (Cap A isolates) and *P. tropicalis* sp.nov. (Cap B).

Whether the *P. capsici* isolates from black pepper in India can be grouped based on the categorisation of Oudemans and Coffey (1991b) or Cap A, Cap B grouping of Mchau and Coffey (1995) or *P. capsici* and *P. tropicalis* sp.nov. based on the study of Aragaki and Uchida (2001). For the development of disease management schedules to prevent the outbreak of *Phytophthora* epidemics in black pepper plantations, detailed studies of *Phytophthora* of black pepper as mentioned earlier have been under taken in the present programme.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

1. Black pepper an important spice crop

India is known as the Land of Spices. Among the spices grown in India, black pepper is most important both for export as well as for internal consumption. Black pepper known as the 'king of spices', originated from Western Ghats of India is the most important and most widely used spice in the world. The black pepper of commerce is the dried, mature fruits (called berries) of the tropical, perennial climbing plant *Piper nigrum* L. It belongs to the family *Piperaceae*. Black pepper is a woody climber, grown in the south western region of India, comprising the states of Kerala, parts of Karnataka, and Tamil Nadu, the entire region once known as Malabar, a name now used restrictively to mean only the northern parts of Kerala. It is grown as a pure crop trained on live tree supports of *Erythrina indica* or *Garuga pinnata* and also as a mixed crop in coffee plantations and also on coconut and arecanut trunks.

This spice with its characteristic pungency and flavour is an ingredient in many food preparations. For the common Indians, pepper is spice as well as a medicine. White pepper of commerce is also a product from the same pepper plant, produced by removing the pericarp from the ripe pepper fruits. In India black pepper is grown over an area 225327 hectares producing about 72465 tonnes per annum. The productivity of black pepper in India is the lowest (322 Kg/ ha) in 2007 (Directorate of Arecanut and Spice Development, Calicut, Kerala, India). Crop loss caused by *Phytophthora* is one of the major production constraints in black pepper.

India is major producer, consumer and exporter of black pepper in the world. Kerala state accounts for 95 % of the country's area and production. The state of Karnataka and Tamil Nadu contribute for the remaining. Small quantities of pepper is

also produced in the states of Goa, Andhrapradesh, Orissa and Assam. Recently Andaman and Nicobar islands and North Eastern states are recognized as a very potential production centre. Globally the major pepper growing countries are India, Indonesia, and Brazil, Malaysia, Srilanka, Vietnam and China, which come under international pepper community (IPC). Malagasy republic, South east Asian countries, South pacific islands, Latin America, and African countries are the other countries where pepper is grown to a small extent (Ravindran, 2000).

Foot rot disease of black pepper is a major production constraint in India. *Phytophthora capsici* (‘*P. palmivora*’ MF4) is considered as the causative organism of foot rot of black pepper (Tsao, 1991). Samraj and Jose (1966) reported the *Phytophthora* wilt of black pepper in Kerala adopting the identification of Muller (1936) as *Phytophthora palmivora* var. *piperina.*

2. Foot rot disease of black pepper

Though India had monopoly on the black pepper production in the world, its position dwindled in recent times and the hectare per production is the lowest. The disease problems in black pepper had been reviewed earlier (Nambiar, 1977; Abicheeran and Mathew, 1984) and the numbers of disease since then increased in recent years. However foot rot (quick wilt) and slow decline (slow wilt) are most important because of their severity and consequent crop loss. The terminology of these two diseases was changed to *Phytophthora* foot rot and slow decline diseases respectively since 1988 (Nair and Sarma, 1988).

The term quick wilt was vague to describe foot rot earlier because of quick death of black pepper vines due to *Phytophthora*. Foot rot is the most destructive disease prevalent in all pepper growing tracts of India and takes a heavy toll of the crop not only in India but all countries were pepper is grown. The losses are so heavy that farmers get disheartened and abandons the crop. The disease is known in Kerala, Karnataka, and

Tamil Nadu states and recently in Assam state (Sarkar et al. 1985).

Crop loss surveys for foot rot conducted for three years (1982 - 84) in Calicut and two years (1985 - 86) in Kannur districts of Kerala has shown that the foot rot incidence of 3.7 and 9.4 % caused vine deaths of about 188947 and 106425 amounting to an annual loss of 119 and 905 metric tones black pepper in Calicut and Kannur districts of Kerala respectively (Balakrishnan et al. 1986, Anandaraj et al. 1988). The survey also indicated that many farmers were not aware about the symptoms and details of the disease and control measures to be undertaken against the disease. Very few farmers adopted (less than 10 %) preventive measures. The overall loss due to foot rot in major pepper growing countries of the world, when estimated at 3 - 5 % loss of the total planted area, would amount to US $ 4.5-7.5 million per annum (de Waard, 1979). The studies on the disease were intensified with the establishment of All India Co-ordinated project on Cashew and Spice.

Detailed symptomatology and epidemiology of the disease have been well worked out (Sarma and Nambiar, 1982; Sarma et al. 1991).

Epidemiology of *Phytophthora* foot rot of black pepper

The detailed epidemiological studies established clearly the aerial and soil phases of the disease. The fungus infected all parts of black pepper (Anandaraj et al. 1991).

Aerial phase

Infection on the leaves start as water soaked lesions and rapidly expands into large brown spots with a fimbriate margin. The leaf spots may remain uniformly dark or they may show concentric zonation with a grayish centre. Tender leaves are more susceptible than mature leaves. Infection on the spikes resulted in spike shedding.

Infection is noticed on tender to woody stems as dark wet spots and later rotting sets in causing die back symptoms. Foliar infection leads to varying degrees of defoliation depending on the severity of the disease. Rarely does it lead to death of the vine (Ramachandran et al. 1986).

Soil Phase

Collar and root infection are fatal and the infected vine succumbs in 10 - 20 days and hence the often locally used term 'quick wilt'. Collar and root infection go unnoticed until foliar yellowing is noticed. Infection start as wet slimy dark patch on the collar (foot) and rotting occurs as the disease progresses. Vascular discolourations of the affected stem beyond the point of infection have been reported. The collar infection progresses upwards and downwards. The collar infected vine show foliar yellowing, flaccidity of leaves, defoliation and breaking of stem at the nodal region and spike shedding (Sarma et al. 1988).

Even though infection progresses from collar to root region exclusive root infections are also noticed. The foliar symptoms will be similar to collar infection. The studies carried out recently under simulated field conditions clearly established that feeder root infection lead to collar rot and subsequent death of the vine. The collar rot is possibly the culmination of cumulative root infection (Anandaraj et al. 1991). (**Plate 1).**

3. Taxonomy of *Phytophthora*

Confusion and controversy existed regarding the identity and nomenclature of the *Phytophthora* species pathogenic on *Piper* species. The first description of the disease and its causal fungus (1936) in the Dutch Indies was reported as *Phytophthora palmivora* var. *piperina* (Muller, 1936).

Prior to 1979, isolates from various parts of the world had been identified as *Phytophthora colocasiae* Raciborski in Malysia (Thompson, 1929) and in India (Thomas
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004a08b80b20f91600040000002d01000003000000000
and Ramakrishnan, 1948), *Phytophthora palmivora* Butl. in Sarawak, Malaysia (Holliday and Mowat, 1963; Turner, 1969) and in Brazil and Puerto Rico (Alconero et al. 1972), and *Phytophthora parasitica* var. *piperina* in India (Dastur, 1935). Black pepper isolates from Jamaica, *Phytophthora hibernalis* (Leather, 1967).

Careful examination of a large number of *Phytophthora* isolates from black pepper worldwide revealed that the great majority of these isolates produced conspicuous long pedicellate caducous sporangia and were identifiable as the so called '*Phytophthora palmivora'* MF4. A few other isolates were identifiable as *Phytophthora parasitica* (*Phytophthora nicotianae*). No isolates from black pepper so far examined were identifiable as typical *Phytophthora palmivora* (*P. palmivora sensu. stricto*). The so called '*P. palmivora*' MF4 were recently renamed as *P. capsici*, after the latter was accurately redescribed to include in the revised description the correct characters, the newly recognized features and the range of natural variability, which were missing in the previous description of *P. capsici* in taxonomic literature. *Phytophthora capsici* sensu lato encompasses all isolates formerly called '*P. palmivora*' MF4, and should hence forth be used for naming all isolates of this type from black pepper and other tropical hosts around the world (Tsao, 1991).

Phytophthora capsici Leonian first described from diseased pepper (*Capsicum*

annum. L) characterized by papillate ovoid sporangia averaging 60 X 36 µm, brown oospore 25-35 µm in diameter and mycelium (Leonian, 1922). The typical strain, or *Piper* form of *P. palmivora* later turned out to be the same or similar to the Thai isolates possessing the long pedicellate caducous sporangia, which occur also in many other non *Piper* tropical hosts such as *Cacao*, *Macadamia*, Rubber, *Sechium*, *Spondias*, Vanilla and others (Alizadesh and Tsao, 1985 a).

The (1976) Rothamsted Cocoa *Phytophthora* workshop (Brasier and Griffin, 1979; Griffin, 1977) contributed greatly to the clarification of the nomenclatural controversy in the *Phytophthora palmivora* complex, when the designation of '*Phytophthora palmivora*' MF4 (Morphological form 4) was given to this group of atypical or *Piper* form isolates with the long pedicellate caducous sporangia. Leonian (1934) questioned the type concept. According to him "the accident of a first discovery and description determines the typical strain, while a subsequent discovery and observation of another strain, which may sharply differ from the original, determines the atypical strain". He also stated that "there is no such things as typical or atypical, but merely variability of living things". Some species of *Phytophthora* were considered stable, while others showed a high degree of morphological variability. Identification of the exact strain causing the disease is important. As far as the classification of *Phytophthora* is concerned, it is imperfect due to high degree of variability seen among them. It had a genetic system more similar to that of higher organisms rather than to that of most other fungi. Variability was classified as morphological, physiological and pathogenic variability.

Brasier and Griffin (1979) described the morphology, physiology and cytology of MF4 differs that greatly from the MF3 (= *P. megakarya*) and MF1. MF1 equates typical *P. palmivora* or *P. palmivora sensu stricto.* In order to resolve the controversy and to answer convincingly the questions whether the so called '*P. palmivora*'MF4 isolates were indeed the same as *P. capsici*, it was indeed necessary for a comparative study on

the morphology, reproductive physiology and electrophoretic protein patterns and isozyme patterns of a large number of isolates. The study completed by Alizadeh (1983) in 25 authentic or typical isolates of *P. capsici* from 5 hosts and 29 isolates identifiable as '*P. palmivora*' MF4 from 7 other hosts were compared. The results of this four year study showed that most of the '*P. palmivora*' MF4 isolates resembled *P. capsici* isolates, but some differed from *P. capsici* in several aspects either quantitatively or qualitatively (Alizadeh, 1983; Alizadeh and Tsao, 1985 a; 1985 b). They include: a) higher percentage of distinct umbellate ontogeny of sporangiophore in continuous light, b) higher percentage of pyriform sporangia, greater sporangium pedicel length, c) higher percentage of sporangia with wider septum or double septum, or no or few round based sporangia in continuous light, formation of chlamydospores and inability to grow at 35 ^{0}C. None of these features were reported in the existing *P. capsici* species described by Leonian (1922), Tucker (1931), Waterhouse (1963), Novotel nova (1974); Newhook et al. (1978), Krober (1985), and Stamps (1985). Variability in the size and shape of the sex organs was found to be extremely great among the isolates within the MF4 group and *Phytophthora capsici*. But sex organ morphology was of little diagnostic value and was not useful for identifying and separating these two *Phytophthora* groups. Electrophoretic studies showed similarities in major banding patterns of buffer soluble bulk proteins of *Phytophthora capsici* and MF4 isolates. Malate dehydrogenase isozyme patterns for two groups were identical. The esterase profiles of all *P. capsici* isolates and the majority of MF4 isolates were similar, but five MF4 isolates exhibited a fast migrating band which was not detected in any of the *P. capsici* isolates in other MF4 isolates (Alizadeh, 1983). Despite the variability many of the stable and easily recognizable characters were common to all isolates of both *P. capsici* and '*P. palmivora*' MF4. The electrophoretic data had further contributed to the resolution of the taxonomic status and nomenclature of '*P. palmivora*' MF4 isolates (Alizadeh, 1983; Tsao and Alizadeh, 1988).

The revised description of *P. capsici*, accepted from Alizadeh's (1983) dissertation. It was based on five isolates of *P. capsici* (including Leonian's type isolate)

and five isolates hitherto known as '*P. palmivora*' MF4, all ten isolates had been deposited in the American Type culture collections at Rockville, Maryland USA. Tsao and Alizadeh (1988) in the proceedings of the tenth international Cocoa Research Conference, the cocoa producers alliance of Lagos, Nigeria; published the revised description.

Over time taxonomists had changed the classification position of *Pythium* and *Phytophthora*. Now it was established that these two fungal like groups of the family Pythiaceae belongs to the kingdom Chromista (Dick 1995 a and b), excluded from the traditional “true fungi” of the kingdom Myceteae. They accordingly are believed to be more closely related to diatoms and brown or golden algae than to fungi. Evidences for this included heterokont flagellation of zoospores, predominantly cellulosic cell walls, tubular cristae in the mitochondria and diploid somatic phase (Gunderson et al. 1987). It was considered as a non photosynthetic parasitic alga. The ITS based phylogeny supported the evolution of *Phytophthora* via a *Pythium* like ancestor whose nature is still unclear. Higher genetic divergence occurs within *Pythium* than *Phytophthora*. Moreover *Phytophthora* is proved to be a monophyletic group where as *Pythium* is a polyphyletic one, suggesting that *Phytophthora* is a relatively recently evolved genus having not yet radiated into many forms as compared to *Pythium* (Villa et al. 2006)

4. Morphology and Biology of *Phytophthora*

Basic studies on the biology of *P. capsici* of black pepper were lacking . To a greater extent biology of *P. palmivora* of cocoa had been well documented (Gregory, 1983)

4.1. Morphology of *Phytophthora*

Identification of a species has been based more on morphology than on any other criterion. The morphology of an individual is the ultimate expression of its growth processes, final display of all its complex relationships with its normal habitat. The extensive variability in the sporangia and sex organs in *Phytophthora* had been reviewed by several workers. Leonian (1934) studied morphological variability in *P. parasitica* var. *rhei* and found high degree of variability in the sporangial size of three variants.

Brasier and Griffin (1979) studied morphological difference seen among the various isolates of *P. megakarya.* The characters like sporangial shape, chlamydospore shape and oogonial shape were highly variable.

4.1.1. Morphology of cultures of *Phytophthora*

Tsao and Alizadeh (1988) observed a wide range of variation in morphological characters of black pepper isolates clearly indicated that even among single species, great variation was noticed depending upon the climate and other conditions. In species such as *P. cinnamomi*, certain isolates appear to remain consistent in morphology. However, when different isolates from many regions of world were observed, there were many variants from the original type species. Zentmyer et al. (1976) showed that colony diameter of 187 isolates from the different regions of the world varied from 47.5 - 82.5 mm (4 days). Shepherd and Pratt (1974) studied 361 isolates of *P. cinnamomi* from Australia and noted considerable variation in growth rate on corn meal agar of single zoospore progeny. Dantanarayana et al. (1984) studied the cultural and morphological characters of *Phytophthora* isolates from rubber and cocoa, *P. heveae*, *P. arecae*, *P. meadii*, *P. botryosa*, *P. megakarya*, *P. palmivora* and '*P. palmivora* MF4' on lima bean agar. *P. meadii* differed from *P. palmivora* in pedicel length, sporangial and sporangiophore morphology, chlamydospores frequency, size and colour, cytological and in oospore morphology and size. *P. meadii* isolates from small cardamom showed three distinct types of colony morphology. They were uniform cotton wool like aerial mycelium over entire colony with vague lobbed pattern, stellate or petalloid pattern and lobed pattern (Suseela Bhai and Sarma, 2005).

4.1.2. Sporangial morphology of *Phytophthora*

Manohara and Sato (1992) studied the morphology and physiology of 43 *Phytophthora* isolates from black pepper (*Piper nigrum*) in Indonesia. Potato dextrose

agar, corn meal agar, oatmeal agar and carrot agar were used to observe colony morphology. Out of 43 isolate 42 had ellipsoid sporangia, which were markedly papillate, tapered at the base and caducous with long pedicel. The remaining one isolate had spherical sporangium which were markedly papillate and caducous.

The term caducity describes the capability of sporangia to become readily detachable from the mycelium. Waterhouse (1974 b) reviewed the status of caducity in the genus, explaining that in some species, sporangia were shed in dry air and in water (only *P. infestans* and *P. phaseoli*) and in others only in water (*P. palmivora* and 17 other species). Kaosiri et al (1978) reported as stalk length as a stable character for *P. palmivora* isolates from cocoa. Al-Hedaithy and Tsao (1979 a; 1979 b) showed the importance of using a uniform technique to determine caducity. They reported that the age of the culture greatly affected the degree of caducity, because sporangia get separated from the mycelium only when mature. Although the percentage of caducous sporangia was low in some species and often varied on different media, the length of the pedicel was always uniform.

Some sporangia of *P. cinnamomi*, which produced non - caducous sporangia, often become detached accidentally but the length of the stalk of these sporangia was always variable. Caducous species could be divided into those with short pedicels (< 5 µm); those with pedicels of intermediate length (5 - 20 µm) and those with long pedicels (> 20 µm) (Saleh et al. 1979).

Carrot and V8 CaCO3 media both were natural media and not greatly different in nutrient levels, influenced in varying degrees the percent sporangium detachment in different *Phytophthora* species. Other media that differ in nutrient levels showed an effect on the degree of caducity of these *Phytophthora* species (Saleh et al. 1979).

The percentage of detached sporangia with solid agar plate method was in most cases (with the exception of ‘*P. palmivora*’ MF3), not significantly different from those with mycelial mat in water method. The mycelial mat in agar method was originally devised to handle *Phytophthora* isolates, which do not produce abundant sporangia on solid agar media. The shortest length of long pedicels of *P. palmivora* was obtained from the mycelial mat in water method, while from agar disc in water and solid agar plate methods were longer and longest respectively (Saleh et al. 1979).

Failure to use sporangium caducity and pedicel length as diagnostic characters had resulted in occasional misidentification of the *Phytophthora* isolates. For example some isolates from *Pipe*r species were thought to possess persistent sporangia, (Holliday and Mowat, 1963; Waterhouse, 1974 a), but actually had caducous sporangia with long pedicels (Tsao, 1977; Tsao and Tummakate, 1977). Baker (1936) described strain ‘c’ of *P*. *palmivora* that he had obtained from the black pod of cocoa in Trinidad had sporangia with much longer pedicels, than the typical *P*. *palmivora* isolates. Baker suggested that the strain ‘c’ might be in the *arecae*, *meadii* group. Strain ‘c’ also showed elongate sporangia with an L / B ratio of 2.2.

Kunimoto et al. (1976) referred an isolate of *Phytophthora* from *Macadamia* in Hawaii, previously identified as *P. nicotianae var. parasitica* (Hunter et al. 1971) to *Phytophthora capsici*. This isolate had elongated sporangia and long pedicels, similar to the isolates from Brazil and Central America (Zentmyer et al.1977). Four morphological forms within the *P. palmivora* complex (designated MF1, MF2, MF3, MF4) were separated on the basis of sporangial stalk, cultural and chromosome types. Their characteristics and geographical distribution were described.

4.1.3. Chlamydospore morphology of *Phytophthora*

Some workers used the absence of chlamydospores as one of the major criteria for separating or distinguishing *P. capsici* Leonian from other morphologically similar species (Newhook et al. 1978; Satour and Butler, 1968; Tucker, 1931; Waterhouse, 1963). Kaosiri (1978) used the absence of chlamydospores as one of the five criteria to designate certain cocoa isolates of '*P. palmivora*' MF4 as *P. capsici*. When in addition to other characteristics of the species, these isolates did not produce chlamydospores. The study done by Alizadeh and Tsao (1985 b) showed that 6 out of 29 '*P. palmivora*' MF4 examined formed abundant chlamydospores in agar media. The mean chlamydospore diameter of six isolates ranged from 27 to 29 µm, viability of chlamydospores ranged from 92 - 97 %. Post growth incubation temperature greatly affected chlamydospores production, with 15 ^{0}C being the optimum. Like majority of the '*P. palmivora*' MF4 isolates examined none of the 25 *P. capsici* isolates tested produced chlamydospores. If the '*P. palmivora*' MF4 is indeed *P. capsici* as considered by some workers, their data constitute the first report of chlamydospores formation in this species and the species description of *P. capsici* should be revised. It also suggests that the absence of chlamydospores can no longer be viewed as important criteria for merging '*P. palmivora*' MF4 with *P. capsici* or for distinguishing it from other morphologically similar species (Alizadeh and Tsao, 1985 b). Addition of β sitosterol to V8 agar at 10-40 µm /ml stimulated chlamydospores production in culture incubated in the light but not in those incubated in dark. In dark growth was stimulated by β sitosterol at 20 - 40 µg / ml; in the light however no stimulation was detected with sterol up to 200 µg / ml (Englander and Turbitt, 1979).

In Hawaii, *Phytophthora* isolates from diseased *Macadamia integrifolia*, macadamia soil, were identified as *P. capsici* (Kunimoto et al. 1976). The absences of

chlamydospores along with other morphological characters were the criteria adopted to separate them from *P. palmivora* (Butl) and *P.nicotianae* Breda. De Hann var. *parasitica* (Dast) Waterhouse = *P. parasitica* Dast). Most *P. capsici* and all *P. palmivora* isolates using the V8 juice agar submerged culture method produced numerous chlamydospores (Tsao, 1971).

Phytophthora palmivora produced abundant thin walled chlamydospores. Using the submerged culture method described by Tsao (1971) for *P. parasitica*. In naturally infected papaya fruits as many as 90 % of the chlamydospores had thick walls (Kadooka and Ko, 1973). So they tested various fruit juice media in *P. palmivora* to stimulate thick walled chlamydospores. Chlamydospore production was poor if incubation temperature was 24 ^{0}C or above. Formation of sporangia was prevented due to reduced aeration in submerged cultures (Tsao, 1971). Tsao (1969, and 1971) reported that types of asexual spores (sporangia and chlamydospores) produced by *P. parasitica* were determined to a great extent by environmental factors.

> Alizadeh and Tsao (1985 b) noted much variation in chlamydospore production among the 25 isolates of *P. capsici.* Based on difference in chlamydospore production, pedicel length, and L/B ratio and sporangial ontogeny. Uchida and Aragaki (1989) believed that some *P. capsici* isolates in Hawaii might be sufficiently distinct to warrant a separate species designation as *P. tropicalis*.

4.1.4. Oospore morphology of *Phytophthora*

The heterothallic species contain two mating types designated as A1 and A2. Oospores form after contact of the mycelia of A1 and A2 mating types. Isolates of each type are bisexual and self-incompatible. Relative degrees of maleness and femaleness occur within *P. infestans* (Gallegly and Galindo, 1958). Thus some isolates are strongly male will form more antheridia than oogonia; some isolates are intermediate in sexuality. The A2 mating type was not always stable, and occasionally self-fertile isolates arise

from A2 type, some examples of this phenomenon are cited below. Some isolates of certain species were neuter and did not produce oospores alone or when paired with A1 and A2 mating types (Ko, 1981). Nearly all heterothallic species formed oospores when paired with the opposite mating types of most other species (Savage et al. 1968).

The different mechanisms that are operative for the production of heterothallic oospores include direct hybridization, stimulation by aging and exotic substances, and stimulation of the opposite mating type, presumably by the water soluble α1 or α2 hormone like substances described by Ko (1978). Oospores are the most persistent of all propagules produced by *Phytophthora* (Weste, 1983). Elliot (1983) and Ribeiro (1983) reviewed oospore production.

Several factors influence oospore production. Oospore production was optimal in darkness and reduced in light (Harnish, 1965). Continuous darkness or darkness altered by light was required for sexual reproduction (Klisiewicz, 1970). No normal oospores were produced in any of the clarified liquid culture media, although oospore formation was observed in semi-synthetic media containing agar, used as a control, and in non-clarified juice liquid media. Antheridia and oogonia formed in some clarified juice media and synthetic media with sterol or lecithin, the oogonia, without oospores, frequently germinated, behaving like vegetative structures (Ansani and Matsuoka, 1988). The conditions leading to the formation of oospores by species of *Phytophthora* appears to be inhibited by continuous bright light, but not by continuous light of low intensity, or total darkness. Generally oospores were more abundant in total darkness than in reduced light (Wayne and Harnish, 1965).

Cornmeal agar supported the production of lowest number of gametangia and the smallest percentage of normal oospores. For taxonomic purpose media should be used which support normal maturation and germination of oospores and facilitate

observations. A single set of conditions cannot suffice for all species and the conditions used must be described in detail (Zentmyer et al. 1979).

The role of oospores in heterothallic species was not well understood, but evidences strongly indicated that the crossing of A1 and A2 mating type isolates could be a source of new races or biotypes when A1 and A2 mating types coexist in nature, for example, *Phytophthora infestans* on potato in the Toluca region of Mexico (Galindo and Gallegly, 1960). Recombination of genetic factors in the progeny resulted in production of different races or more virulent biotypes on potato (Spielman et al. 1989; 1990). The recent introduction of the A2 mating types into Europe had not induced perceptible changes in the development of new pathological races of *P. infestans* in some areas (Fry et al. 1991), but in Poland new biotypes had been found (Sujkowski et al. 1994). The frequency of A2 mating type was more than A1 mating type in India (Sarma, 2002).

4.2. Physiological variability of *Phytophthora*

Temperature and nutrition are extremely important factors that affect the size of various structures and mycelial growth (Zentmyer et al. 1979). Alizadeh and Tsao (1985 a) studied effect of light on sporangia formation. On carrot agar plates, sporulation was enhanced in continuous light, whereas no sporangia formed in the dark. Sporangia formed in the dark when the agar-disc-in-water technique was employed. Umbellate arrangement of sporangia was observed in cultures incubated in the light and was considered a light-induced response. In the dark, sporangium arrangement was irregular and many sporangia were round-based, wider, and with low length to breadth ratios. These sporangia were not easily dislodged from the vegetative mycelium. The importance of the effect of light on pedicel length, L/B ratio, caducity, and other morphological features of sporangia in the taxonomy of these fungi was also reported (Alizadeh and Tsao, 1985 a). The growth of *Phytophthora nicotianae* increased with the increase of temperature up to 30 ^{0}C and then showed a decline. Maximum growth was

recorded at 30 ^{0}C, which was closely followed by growth at 28 ^{0}C. No growth was recorded at 10 ^{0}C and 37 ^{0}C and was very poor at 36 ^{0}C and 15 ^{0}C (Jain et al. 1980).

Zentmyer et al. (1976) studied the growth of 187 isolates of *P. cinnamomi* (20 A1 and 167 A2 mating type) from 24 countries and 59 hosts were compared at 25 ^{0}C on potato dextrose agar. The frequency distribution obtained for these isolates closely approximated a normal curve with 90 isolates below the mean and 97 above. Colony diameters after four days of growth ranged from 41-85 mm. Nutrition affected the growth and temperature response. Some isolates grew rapidly on one medium and slowly on another medium at 25 ^{0}C. Thus the cardinal temperatures for *P. cinnamomi* ranged from minimum 5 - 16 ^{0}C, optimum 20 - 32.5 ^{0}C and maximum 30 - 36 ^{0}C.

The temperature requirements for growth of the L, S and MF4 types on CA in darkness were examined using 1 ^{0} steps on growth /no growth basis to determine the upper and lower limits and comparative growth rate studies at 2 ^{0} steps over 22 - 32 ^{0}C to determine the optima. The S type was attributed to *P. palmivora*. The L type was described as *P. megakarya* Sp. Nov. The results revealed striking differences between the three types. The optimum temperature (24 - 26 ^{0}C) for the L type was considerably lower than for the S and MF4 types (28 - 30 ^{0}C) (Brasier and Griffin, 1979).

Thomas, (1939) stated that *Phytophthora* from *P. betle* grew at temperature range of 15 - 33 ^{0}C with an optimum at 30 ^{0} C. Hussain and Ahmed, (1961) found maximum zoospore production and liberation in the fungus at 21 - 23 ^{0}C. Tiwari, (1973) reported 30 ^{0}C as the optimum temperature for growth and sporangium production. Lower temperature favoured long periods of motility of zoospore but their germination was found over a wide range with an optimum between 20 - 25 ^{0}C. For growth and sporulation pH 7 - 8 was favourable. According to Tiwari, 1973, sufficient aeration was also essential for sporulation. Light played an important role in the mode of germination of sporangia. Sporangia developed in continuous light germinated directly where as those

that developed in complete darkness germinated indirectly. The mobility of zoospore was also reduced considerably in continuous light.

Zentmyer et al. (1979) showed that oospores from pairing an A1 and A2 isolate of *P. cinnamomi* from Avocado and from the A2 isolate incubated in Avocado root extract formed at temperatures between 12 and 30 ^{0}C with optimal production at 15 - 21 ^{0}C for the isolate from the paired culture and at 18 - 24 ^{0}C for the A2 homothallic isolate produced in root extract. Although temperature did not markedly affect size of oogonia and oospore, nutrients provided by different media did. The oospore from root extract and those paired on a synthetic medium (Ribeiro et al. 1975) were consistently larger than those paired on carrot agar, corn meal agar, clear V8 juice agar, or clear V8 juice broth. It was due to the formation of more oospores on the natural media than on the synthetic medium.

Soil moisture, temperature, pH, light and soil aeration influence the growth and sporulation of *P. capsici*, of which the first two components were more common to act as main deterrents in pepper plantations. Kasim and Prayitno (1979) found that the pathogen reached its optimum growth and sporulation at 25.5 ^{0}C, with a pH range of 6 - 7 and under continuous light. Soil with good aeration supported poor sporulation of the pathogen. Kueh and Kew (1982) found that optimum pH and field capacity of soil for growth of *P. palmivora* ranged between 6.5 - 7 and 24 - 25 % respectively.

Phytophthora nicotianae grew in a wide range of pH but it preferred acidic medium, optimum pH 6.1 (Jain et al. 1980). Lily and Burnett, (1951) observed that most of the fungi grew satisfactorily between pH 5 and 7, but optimum generally lay on acidic side. Cameron and Milbrath (1965) found that most of the *Phytophthora* species studied grew best on a buffered medium at pH 4.5 - 5.5.

Sato, (1994) reported that washing of sporangia of *P. infestans* in distilled water

caused loss of power of indirect germination, and the power was recovered by suspending them in water solution containing appropriate concentration of inorganic salts.

Abundant production of sporangia by *P. citrophthora* and *P. parasitica* occurred between 20 and 30 ^{0}C (Matheron and Matejka, 1992). Pfender et al. (1977) reported that indirect germination of sporangia was greater at 48 hr than at 24 hr at all temperatures, and was greater at 16 ^{0}C than at any other temperatures tested.

4.3. Pathogenicity of *Phytophthora*

Pathogenicity is the ability of a pathogen to cause disease. But the virulence of the isolates determines the severity of the disease. Variation in pathogenicity among isolates within a species has long been recognized. This variation is important as far as plant pathologists and the plant breeders are concerned. Either the type of pathogenic race or degree of aggressiveness may vary. Aggressiveness is the capacity of a pathogen to invade and grow in its host plant and reproduce on or in it. Schick (1932) first noted races of *P. infestans* after the introduction of resistant hybrid potato cultivars.

Malcolmson (1970) studied about how new and complex races originated from the functionally asexual *P. infestans*. She concluded that mixtures of races grown together on a susceptible host combine genetically to produce new races that had the sum of the parental specificities. Physiological races are common in *P. megasperma.* Hildebrand (1959) noted different levels of virulence among isolates. Faris (1985) tested virulence in six isolates of *P. megasperma* f. sp. *medicaginis* and found that there were 2 levels of virulence among the isolates. In *P. cinnamomi,* there is no evidence for physiological races. But, several reports indicate varying degree of virulence of isolates on different hosts.

Zhang et al. (2003) evaluated pathogenic variability in greenhouse studies using

five tobacco cultivars that had different levels of resistance to tobacco black shank organism; *Phytophthora parasitica* var. *nicotianae* populations. They were genotypically and phenotypically variable, but no distinct genotypic differences were identified among populations from the seven locations. Kapsa in (2002) conducted comparative studies on the pathogenicity of potato leaf- and stem-isolates *P. infestans* on potato. Generally, the stem isolates were more pathogenic than the leaf isolates. Under the optimum temperature of 18 ^{0}C, the inoculation effectiveness of stem and leaf isolates were similar, although incubation time was shorter by 1.5 days for stem compared to leaf isolates. Significant differences were observed between the size of lesions and sporulation in leaves caused by stem and leaf isolates. Under less favourable conditions for development of late blight on leaves (25 ^{0}C), the stem isolates were more pathogenic than leaf isolates.

Lebreton et al. (1999) conducted a preliminary trial with four isolates (two each from potato and tomato) showed that lesion appearance and development were similar for each isolate in detached leaflets and in whole plant tests in growth cabinets. Isolates collected from tomato were more pathogenic to tomato than isolates collected from potato. This was particularly the case for isolates belonging to the A2 mating type. Isolates originating from potato had higher infection efficiency and a higher sporulation capacity on this host, but they induced lesions that generally spread more slowly than those caused by isolates from tomato.

Sujkowski, (1986) conducted a 4 year study, the mean lesion area (MLA) developed on potato leaflets by *P. infestans* varied with cultivars and isolates and also with the time of year. It peaked in spring, with a lower peak in the autumn. A similar periodicity was found in sporulation density of *P. infestans* isolates in vitro, but the largest MLA was accompanied by the least sporulation and vice versa. It was concluded that *P. infestans* exhibits seasonal variations in pathogenicity.

Kennedy et al. (1986) developed system for scoring the virulence of *P. fragariae* isolates based on a scale of root rot from 0 (no symptoms) to 5 (76-100 % roots rotted) on a series of strawberry cultivars. Single-zoospore isolates (32) from one field site were compared by subjecting their root rot scores to cluster analysis and grouped them into two major clusters equivalent to physiologic races. Apart from differences in virulence between the sub-clusters there was some evidence for differences in aggressiveness between isolates within sub-clusters. Faris et al.1983 tested 26 isolates individually on three resistant and three susceptible lucerne cultivars showed a wide range of virulence, with resistant cultivars being generally less affected by all isolates. Seven isolates differentiated as belonging to a highly pathogenic pathotype were morphologically characterized by having small oogonia. The remaining less pathogenic isolates had large oogonia.

Aldwinckle et al (1975) observed differential interaction between apple cultivars and 15 widely distributed isolates of *P. cactorum* in a twig inoculation test. Fifteen widely distributed isolates of *Phytophthora cactorum* were placed in four groups according to their interactions with six test cultivars. Turner (1973) did inoculation experiments of rooted, single-node cuttings of five standard clones, 24 isolates of *Phytophthora palmivora* from black pepper varied in their mean reaction and in their reaction to individual isolates, damage in the latter case ranging from less than 10% to over 75% root necrosis. The most resistant variety was the Indian cultivar Balancotta. There was no apparent relationship between pathogenicity of the isolates and their geographical origin.

Dodan and Shyam (1994) found that considerable variation in cultural and pathogenic behaviour was shown by 26 isolates of buckeye rot pathogen collected from different tomato growing areas of Himachal Pradesh, India. No correlation was found between linear growth, size of sporangia, chlamydospores, and oospores and pathogenic behaviour of these isolates. The isolates could be grouped into 18 strains on the basis of

their aggressiveness patterns and non-differential host-isolate interactions.

Lima et al. (1993) evaluated the virulence of ten *P. drechsleri* isolates (8 from cassava, 1 from beet and 1 from safflower) and pathogenicity of 2 *P. capsici* isolates (from sweet pepper (*Capsicum*) and squash) were tested on leaves, roots and stems of cassava plants. The isolates of *P. drechsleri* and *P. capsici* were pathogenic to all parts of the plant evaluated.

Pereira et al. (1987) studied the pathogenicity of *P. capsici*, *P. palmivora* isolates from Hevea, and cocoa was evaluated in a *Hevea* nursery. *Hevea* isolates were more virulent on young shoots, and did not differ from the cocoa isolates when inoculated on stems. Maximum infection of *P. capsici* on young shoots occurred at 105 zoospores/ml, but maximum infection of *P. palmivora* required higher concentration. A mixture of 104 zoospores/ml of each species resulted in 100 % infection.

Yang et al. (1989) evaluated 108 isolates of *P. capsici* obtained from diseased *Capsicum annum* plants in 9 major growing areas during 1986 - 87 could be grouped into 4 types based on colony morphology. Differences in growth rate and sporangial production were not related to colony type. In greenhouse tests, the level of resistance to *P. capsici* varied with cultivar, and 19 representative isolates selected from the 108 also differed in pathogenicity on 10 cultivars. Virulence was not related to geographical origin but isolates from severely infected fields tended to be more virulent. No correlation was apparent between in vitro characteristics and disease-inducing ability of the isolates. Specific interactions between cultivar and isolate were not found. In a field test with 15 major cultivars and 9 isolates selected on their performance in the greenhouse tests, isolate and cultivar were the major sources of pathogenic variation. Interactions between cultivar and isolate were not apparent. The absence of interactions in both greenhouse and field experiments indicates that pathogenic specificity in *P. capsici* operates

horizontally rather than vertically. These results suggest the importance of selecting isolates in resistance screening.

In India work on the isolation and identification of physiological races of *Phytophthora infestans* was taken up in 1958. Up to 1965, races 0 and I were predominant in North- western hills and 0 and 4 in eastern hills (Dutt, 1965). Gradually more complex races appeared since 1966 in Eastern hills and by 1971 in Shimla hills. From 1978 onwards the frequency of simple races had declined and those of complex races increased (Dutt et al. 1973). The complex races of pathogen started appearing in 1968 in the hills and 1982 in plains coinciding with the large-scale cultivation of resistant varieties. Since 1985 only complex races having 7 - 10 virulence genes are prevalent and the simple races like 0 and 1 had become almost extinct (Phadtare and Sharma, 1970; Phadtare and Pushkarnath, 1968). According to Bhattacharya et al 1990 the races had also shown increased rate of sporulation on resistant cultivars. Khanna et al. (1977, 1982) identified some high spectrum races of *P. infestans* in Khasi hills. Sokhi et al (1993) reported 11 pathotypes in *P. infestans* on potato and three pathotypes on tomato in the plains of Punjab (Mehrotra and Aggarwal; 2001).

The investigation on the occurrence of physiologic races of the fungus made during 1960 - 63 showed the predominance of race 0 of the fungus in the northern states together with few race 1. In the eastern hills 10 races were recorded. Blighted leaves of potato cultivar Local and Kasigaro were collected and identification of races was done (Phukan, and Baurah, 1991).

In assessing potato clones as potential cultivars a measure of their field resistance to late blight is desirable. Identification of the races of *Phytophthora infestans* was made according to its reaction in 7 days on detached leaflets from the differential host plants for *Solanum desimum* series of R genes maintained at the Scottish plant breeding station. The differentials were possessing R genes 1, 2, 3 and 4 alone or in combination, R 10 and

R 11.Of the 330 isolates tested all but four were identified as race 1, 2, 3, 4, 10 and 11 and this race was obtained from all the plots. The remaining four isolates, taken from the same plot were race 3,10 and 11 (Malcolmson, 1979).

Singh and Dubey (2005) studied seven differential hosts of pigeon pea distinguished population of *P. dreschsleri* f. sp. *cajani* isolated from Delhi (pp-1), Bihar (pp-8), U.P (pp-2, 3, 4, 5 and 9) and Uttaranchal (pp-6 and 7) into four variable groups as least aggressive (group IPP-1,2 and 3), virulent (group Ii, Pp-4 and5), moderately virulent group (group IV, pp-8 and 9). The studies showed the existence of four races of the pathogen in North India.

4.4. Cross infectivity studies of *Phytophthora* species on different plantation crops

In view of the occurrence of different species of *Phytophthora* in multi-storied cropping system, it has been opined that the possibilities of development of interspecific hybrids are high and may result in the development of new strains of *Phytophthora* with high virulence (Sastry, 1982; Santhakumari, 1987). Based on the cross inoculation studies, sporangial ontogeny and morphology, it was reported that arecanut, rubber, cocoa, coconut, and small cardamom serve as the collateral hosts for quick wilt of black pepper. They also considered that *Phytophthora* of these hosts belongs to *P. palmivora* (Manmohandas, 1982; Manmohandas and Abicheeran, 1985). The information on positive cross inoculation of different *P. palmivora* on pepper, though important, was not of epidemiological significance unless the same strains were isolated from the infected pepper tissues. The pepper isolates examined so far by the authors were distinct from the *Phytophthora* isolates from rubber, cocoa, palmyra and cardamom (Sarma and Nambiar, 1982).

Cross inoculation studies conducted with six isolates of *Phytophthora* from six different hosts like cocoa, pepper, arecanut, coconut, rubber and cardamom were positive

and the symptom produced on the same hosts by different isolates was more or less identical (Mammootty et al. 1988). Similar successful inoculation experiments with two or Coleman, 1910; Loh, 1970 and Radha and Joseph (1974) reported more of these hosts and isolates. However Tucker (1927), Holliday and Mowat, (1963) and Chandramohan et al. (1979) reported negative results. Studies with the isolates of *Phytophthora* from small cardamom and *P. palmivora* from coconut and rubber revealed that the isolate could cross infect the hosts. The results of cross inoculation studies indicate that capsule rot of cardamom may also be caused by *P. palmivora* or by its strain or variety (Radha and Joseph, 1974). A species of *Phytophthora* isolated from coconut also induced rotting of cardamom capsule (Nambiar and Sarma, 1976).

Thankamma (1983) studied *P. nicotianae* var. *nicotianae* isolates from pomegranate, *Piper nigrum* and *Hibiscus rosa-sinensis* and *P. meadii* from brinjal (aubergine) and *Artocarpus hirsuta* were pathogenic to rubber. Isolates of *P. heveae* from mango and guava did not infect rubber. Muniz et al. (2003) investigated the pathogenicity of three isolates of *Phytophthora nicotianae* on tomato in the laboratory. The pathogen was inoculated by wounding of the hosts and evaluation of pathogenicity was done 4 and 6 days after inoculation. The isolates were pathogenic to all tested hosts.

Ann et al. (2002) investigated *Phytophthora* species in the fields of *Piper betle* and *Piper longum* in Taiwan from 1978 to 1996. *Phytophthora parasitica* (*Phytophthora nicotianae* var. *parasitica*) was detected in all of the investigated fields before 1988, whereas only *P. capsici*, but not *P. parasitica*, was isolated from the diseased tissues of pepper in the fields from 1990 - 1996. All tested isolates of *P. capsici* from *P. betle* and *P. longum* were pathogenic to sweet pepper and vice versa.

Thomidis et al. (2002) studied the pathogenicity and virulence of 11 *Phytophthora* sp. isolated from various host plants were examined on an apple (*Malus domestica* (*M. pumila*) (MM 106) and a pear (O.H.F. 333) rootstock. Only *P. cactorum*

and *P. citricola* isolates were pathogenic to these rootstocks. The isolates of *P. cactorum* were the most aggressive.

Thomidis (2001) studied the relative virulence of *Phytophthora cactorum* and *P. syringae* originating from almond trees, and of *P. citrophthora* originating from citrus, to apple, pear, peach, cherry and plum rootstocks. All tested *Phytophthora* isolates showed little virulence to pear rootstocks causing only minor crown rot symptoms and no virulence at all to apple rootstocks. In contrast, they were highly virulent to stone fruit rootstocks, causing crown rot disease. The non-pathogenicity of these isolates to pome rootstocks could be interpreted as strict host specificity. Hantula et al. (2000) did pathogenicity experiments with strawberry and *Betula pendula* proved that strains show a tendency towards host specialization; strains tended to be more virulent on host than non-host plants.

Azvedo and Silva (1986) inoculated detached fruits of *Capsicum annum*, tomato, cucumber, melon and squash cultivars with an isolate of *P. capsici* obtained from *Cucurbita moschata* hybrid, all the fruits were susceptible. Abundant fructification of the fungus was observed on tomato and *Capsicum*, from which the fungus was reisolated.

Tsuchiya et al. (1986) studied the pathogenicity of 17 isolates of this stem rot pathogen from *Phaseolus radiatus* var. *aurea* (*Vigna radiata*) and 4 from cowpea was compared, the former isolates were virulent to *V. radiata* but not to cowpea, while the latter were virulent to cowpea but not *V. radiata*. In addition, from differences in pathogenicity to 6 cultivars of *V. radiata*, 3 races were recognized within *Phytophthora vignae* f. sp. *adzukicola*.

Steekelenburg (1980) showed, *P. capsici*, to be the pathogen causing root and crown rot of glasshouse *Capsicum annum* plants in the Netherlands (1977). It was pathogenic on inoculation to *Capsicum*, tomato and occasionally eggplant, but not to

Xanthi tobacco. Of these plants, only tomato was infected by *P. nicotianae* var. *nicotianae*. Both fungi induced identical symptoms. Dipping of *Capsicum* or tomato roots in a suspension of *P. capsici* caused more infection than pouring the suspension on the stem base. Tomato cultivars resistant to *P. nicotianae* var. *nicotianae* were not resistant to *P. capsici*. A method was described for distinguishing soil isolates of the 2 pathogens. Both infected green fruits of tomato and *Capsicum* used as bait. *P.capsici* survived in moist soil without the host for at least 15 months (Steekelenburg, 1980).

Bonnet et al. (1978) cross-inoculated sixteen isolates of *P. nicotianae* var. *parasitica* from carnation, tomato and citrus were inoculated into carnation cuttings, tomato seedlings and plants, and citrus leaves. Symptom expression depended on the isolate, inoculum level, cultivar variety and physiological state or age of the plant. On fresh carnation cuttings symptoms appeared with all isolates, but when cuttings were kept for 7 days in a rooting medium, rotting was severe only with those from carnation. Tomato isolates produced serious attacks on tomato seedlings and stems of mature plants. Isolates seemed more capable of overcoming the resistance mechanism of homologous plants.

When cucumber and squash were planted in soil infested with the 2 fungi, cucumber was attacked very severely by *P. melonis* and moderately by *P. capsici*, while squash was severely infected by *P. capsici* but not at all by *P. melonis*. All 7 squash varieties tested were susceptible to *P. capsici*. The results indicated that although grafting on squash was highly effective for control of the disease on cucumber when it was caused by *P. melonis*, it was not necessarily so when it was caused by *P. capsici* (Teramoto, 1974).

Ten black pepper isolates of '*P. palmivora*' MF4 had a higher virulence rating on five cultivars visually Uddakare, Bilimalligesara, Doddagya, and Panniyur 1 of *P. nigrum* than on other hosts tested. The *P. nigrum* cultivar Panniyur-1 was the least

susceptible to the various *Phytophthora species* as well as to the isolates of '*P. palmivora*' MF4 from various *P. nigrum* growing localities. The var. Doddgya was highly susceptible (Sastry, 1982; Sastry and Hegde, 1987 a). (**Table 1)**.

Table: 1. *Phytophthora* infections in plantation crops

Sl No:	***Phytophthora* species**	**Source of isolates**	**Pathogenicity reaction on respective hosts**	**Reaction on other hosts**	**Reference**
1	'*P. palmivora*' MF4	Black pepper	Roots of *P.betle*, *P. longum*, and *P. attenuatum* infected.	Cocoa pods, tender rubber leaves, castor leaves, capsule of cardamom were infected.	Sarma and Nambiar 1982
2	*Phytophthora* sp.	Coconut	Produced rotting	Produced rotting on cardamom capsule	Nambiar and Sarma 1976
3	*P. capsici* and *P. parasitica*	*P. betle* and *P. longum*	Pathogenic to *P. betle* and *P. longum*.	Pathogenic to sweet pepper**.**	Ann et al. 2002.
4	*P. palmivora*	Cocoa, rubber and black pepper.	All isolates produced lesion on cocoa seedlings. Black pepper isolates produced smaller lesion on cocoa. All were pathogenic to rubber. Only pepper isolates were	Rubber and cocoa isolates were not pathogenic to black pepper	Resnik et al. 1980

	P. meadii	Small cardamom	pathogenic black pepper.		Suseela Bhai, 1998.
5	*P. meadii* ‘*P. palmivora*’ MF4	Arecanut, cocoa, rubber and small cardamom. Black pepper	All of them were pathogenic on the respective hosts. Pathogenic to black pepper	*P. meadii* from arecanut, cocoa and rubber isolates were pathogenic to all the hosts tested. Cardamom isolates were pathogenic to all the hosts tested except arecanut. Black pepper isolates were pathogenic to all the hosts except cardamom.	Sastry, 1982; Sastry and Hegde, 1987a.
6	*P. palmivora*, *P. capsici*, and *P. citrophthora*	Cocoa	Pathogenic to cocoa		Chowdappa and Chandramohan, 1997
7	*Phytophthora* spp. from six different hosts	Black pepper, arecanut, coconut, rubber, cocoa and cardamom.	All of them were pathogenic to the respective hosts.	Positive cross infectivity reactions were obtained on all the tested hosts.	Mammooty et al. 1988.

8	*P. nicotianae* var. *nicotianae*	Pomegranate, *P. nigrum* and *Hibiscus rosa sinensis.* Small cardamom	Pathogenic to the respective hosts and produced brownish to black lesions. .	Pathogenic to rubber. .	Thankamma, 1983. Nair, 1979
9	*P. arecae*	Arecanut	Pathogenic to arecanut	Pathogenic to potato tubers, apple fruits, young tomato and brinjal seedlings.	Koti Reddy and Anandaraj, 1980
10	*P. palmivora* *'P. palmivora'* *MF4* *P. nicotianae* var. *nicotianae* *P. meadii*	Cocoa, coconut Black pepper Small cardamom Arecanut	Pathogenic to the respective hosts.		Santhakumari, 1987

4.5. Molecular characterization of *Phytophthora*

The *Phytophthora* genetic system presents a wide variety of mechanisms for the expression of variation. Until 1970's the variability in *Phytophthora* study was based on the morphological and cultural, physiological and pathogenicity characters. But with the development of new technologies now it is possible to study the variability at the genetic level. There are some of the technologies used for the identification of variability like protein electrophoresis (Kaosiri and Zentmyer, 1980), Isozyme pattern (Clare and

Zentmyer, 1966), molecular characterization using Internal Transcribed Spacer regions of rDNA gene repeates (Gray et al. 1984) and other molecular methods.

4.5.1. Protein electrophoresis of *Phytophthora*

Proteins are gene products; they are the direct reflection of the functional portion of the fungal genome. The protein composition of an isolate can be visualized as a series of discrete bands on a gel after separation by electrophoresis and staining with suitable reagents. Valid species would differ in their protein make up and such differences can be detected by the rate at which the protein move in a gel aided by an electric current. Electrophoretic measurement of protein differences can be much less difficult and time consuming. Starch and acrylamide gels have been used in slab and tubular electrophoresis to produce the patterns. This helped in the identification of morphospecies within *P. palmivora* on cocoa, separation of biological species within *P. megasperma.* Buffer soluble, non-specific protein and enzyme patterns from polyacrylamide disc gel electrophoresis were compared for three morphological forms (MF) and one "other type" of *Phytophthora palmivora* from cocoa and for the culture of *P. capsici* and *P. cinnamomi.* Comparisons of the total protein patterns indicated that each MF produced consistent major bands distinctly different from those of others (Kaosiri and Zentmyer, 1980).

Latorre and Perez, (1995) compared isolates of *Phytophthora* from Kiwi fruit using electrophoretic patterns of total soluble proteins. All the 33 *P. cryptogea* isolates from Californa formed a homogeneous group based on electrophoretic patterns of undissociated proteins, although a few unique bands were present in Chilean isolate. Protein profiles of Chliean kiwifruit isolates were similar to some of the Californian *P. cryptogea* isolates. However they differed from four *P. cryptogea* isolates from fruit crops in Newyork, which formed a separate homogenous group.

By polyacrylamide gel electrophoresis the intraspecific variation in banding patterns among isolates identified as *P. cactorum*, *P. cambivora*, and *P. syringae* was less than that among isolates identified as *P. megasperma*, *P. cryptogae*, and *P. drechsleri*. When native proteins were analysed the number of distinct subgroups distinguished were two in *P. cactorum*, two in *P. syringae*, one in *P. cambivora*, two in *P. drechsleri*, three in *P. cryptogea* and six in *P. megasperma*. When dissociated proteins were analysed, *P. cactorum*, *P. syringae*, and *P. cambivora* each formed single distinct groups; *P. cryptogea* and *P. drechsleri* each formed two subgroups, one of which was common to isolates of both species (Beilenin et al.1988).

Within *P. megasperma* their protein banding patterns distinguished six major groups of isolates. The groups generally comprised isolates from related hosts .The isolates with similar protein patterns had similar colony morphology. The patterns of total proteins provided a reproducible and sensitive fingerprint for various isolates. Most isolates had unique protein patterns, but they were readily placed in one of the six protein groups. Isolates with similar protein patterns often came from widely separated geographical origin, and occasionally from widely differing hosts (Hansen et al. 1986). Clare and Zentmyer (1966) used gel electrophoresis of mycelial proteins to differentiate *P. cinnamomi*, *P. citrophthora* and *P. palmivora*, by starch gel electrophoresis. Gill and Powell (1968) delimited *P. cactorum*, *P. fragariae*, and *P. sojae* by polyacrylamide disc gel electrophoresis and further demonstrated the usefulness of the technique for diagnostic purposes at the species level rather than at race level (Gill and Powell, 1968).

Chowdappa and Chandramohan (1995) examined the electrophoretic protein patterns of three species of *Phytophthora* associated with black pod disease of cocoa, which were compared on a single polyacrylamide gel; the isolates of same species were readily distinguished both quantitatively and by visual similarity in banding patterns and quantitatively by calculating similarity coefficients. Similarity coefficients were generally

much higher between isolates of different species. The dendrograms obtained with arithmetic averaging cluster analysis revealed that all isolates of different species of *Phytophthora capsici* were highly homogenous and formed a single cluster. The isolates of *P. citrophthora* were resolved into two electrophoretic types, which were clustered into two distinct subgroups. Thus the results revealed that polyacrylamide gel electrophoresis can be successfully used in distinguishing species of *Phytophthora* on cocoa. Using protein banding pattern alone Hamm and Hansen (1983) distinguished *Phytophthora pseudosugae* as a new species of *Phytophthora* from *P. cactorum*. It had been used along with other conventional criteria for precise identification of *P. citricola* from avocado (Zentmyer et al. 1974), *P. palmivora* from milkweed (Feitchtenberger et al.1984), *P. drechsleri* from cucumber (Ho et al. 1984) and *P. cinnamomi* from Taiwan (Ann and Ko, 1985).

At least two distinct groups of *P. megasperma* could be distinguished based on protein patterns (Faris et al.1986). Erwin and Ribeiro (1996) have given in a tabular form references on use of protein patterns to differentiate species of *Phytophthora*. According to them protein patterns provides confirmatory evidences in the identification process and supplement morphology data that might not be definitive.

A modification of the gel electrophoretic method described by Gill and Zentmyer (1978) was used to compare the soluble protein pattern bands of four isolates from milkweed vine using disc gel electrophoresis of hyphal proteins. Great variability among single zoospore cultures was observed only with milkweed vine isolates. Single zoospore cultures of all other isolates of *Phytophthora* from milkweed vine and ten isolates of *Phytophthora citrophthora* from citrus did not exhibit significant variation (Feitchtenberger et al.1984) used protein profiling to differentiate many species of *Phytophthora*. Gel electrophoresis can be used as an adjunct to the taxonomic characters in identification of *Phytophthora* species wherever doubt exists (Aggarwal et al. 2001).

4.5.2. Isozyme characterization of *Phytophthora* species

Isozymes are defined as a variant form of an enzyme, which share the same catalytic function. Using the polymorphic forms of the enzymes, the variability can be detected. Specific enzymes separated by electrophoresis in suitable medium, usually starch or acrylamide gels, were cytochemically stained. Difference in the electrophoretic mobility is noted. The individual protein cannot be identified readily on total protein gels. Isozyme analysis overcomes this limitation by revealing the isomeric forms of one enzyme system at a time. Isozymes are ideal tools for molecular identification, because they are easily assayed, stable, co dominant (which is important in a diploid organism like *P. infestans*) and generally under simple genetic control. Allozymes are isozymes whose variant forms are coded at the same locus. Allozymes are therefore allelic to each other, and are ideal for population investigations, because the best analytical methods depend on knowledge of allelic frequencies and distributions (Erwin and Ribeiro, 1996). Erwin (1983) cites several examples were morphological criteria have produced confusing and doubtful species designations; including the *P. megasperma* complex and varieties of *P. parasitica* (*P. nicotianae*). One approach that has been used in *Phytophthora* systematics is the comparison of the electrophoretic patterns generated from total native and denatured soluble proteins. One major drawback with protein electrophoresis is that comparison is based on a large number of unidentified protein bands and quantitative analysis can be difficult. Isozyme analysis using starch gels has a major advantage over total protein gel electrophoresis in that comparisons are made based on a number of specific proteins, which interpreted separately. Isozyme phenotype is consistent with a diploid organism and this has already been demonstrated in *Phytophthora* for certain enzymes (Shattock et al.1986; Spielman et al. 1989). This approach can be used to quantify genetic diversity within species and genetic distance between species (Nei 1987). Nygaard et al. 1989, pointed out that isozyme banding patterns are less complex than total protein patterns and can be differentiated and

interpreted more readily. By use of isozyme techniques (Old et al. 1984; 1988) noted that the genetic diversity of many isolates of *P. cinnamomi* from various regions of Australia was relatively low; however, the isozyme patterns the A1 and A2 mating types differed.

To investigate the level of intraspecific variation present in a *Phytophthora* species, isolates were collected from a large range of host plants and geographical locations as possible. For each isolate, the data, expressed in the form of electophoretic mobilities of several enzyme loci were compared with those from all other isolates and the comparison was expressed in a distance matrix, such as Rogers (1972) genetic distance as modified by Wright (1978). The genetic distance ranges from zero to one, with zero indicating complex identity and complete dissimilarity. Isolates can be grouped into electrophoretic types (ETS) with identical multilocus phenotypes. An unweighed pair grouping with means averaging (UPGMA) cluster analysis (Sneath and Sokai, 1973) can be used to calculate the divergence among ETS of each species, and presented in the form of a dendrogram. In cases were more than one cluster is found per species, each one is treated as a subgroup. Using the cophenetic correlation can test the dendrogram; to assess the accuracy with which the dendrogram reflects the original distance matrix. Interspecific comparisons are made using representative isolates from each species.

From isozyme analysis, some species commonly regarded as morphologically closely related, such as *P. parasitica* and *P. palmivora*, or *P. cambivora* and *P. cinnamomi* were clearly differentiated as distinct genetic entities. Interspecific comparison using this techinique has also revealed instances where two species were nonspecific, such as *P. palmivora* and *P. arecae* as well as *P. capsici* (CAP 1) and *P. mexicana*. Many of the genetic relationships uncovered by isozyme analysis would not have been predicted on the basis of morphology alone. For example, *P. meadii* and *Phytophthora botryosa*, which are closely related by isozyme analysis are considered quite distinct based on morphological considerations (Dantanarayana et al. 1984) but may be the result of fairly recent speciation events. Also, *P. capsici* and *P. citrophthora*,

which are also related genetically, can be distinguished easily on morphological grounds (Zentmyer, 1988). Interspecific distances between unrelated species were very high and corresponded to genomic level distances reported for other group of organism (Ayala, 1983). *Phytophthora* species in group II and group III (Waterhouse, 1963) consists of several distinct and genetically divergent lines (Mills et al, 1991; Oudeman, 1990; Oudeman and Coffey, 1991a). Unfortunately a complete phylogenetic construction was not possible since the genetic distances obtained using isozymes were too great. Recent studies revealed that *P. capsici* and *P. citrophthora* and *P. megakarya* are complex species (Forster et al, 1990; Oudeman, 1990) clustering into subgroups, which may represent distinct biological species that could reflect recent speciation events.

Oudeman and Coffey (1991a, b, c) showed distinct differentiation of *P. cactorum*, *P. cambivora* and *P. cinnamomi*, *P .megakarya* and regarded as heterogenous species (Hansen and Hamm, 1983; Hansen, 1987, 1991; Hansen et al. 1986). Several biotypes were readily differentiated by use of isozymes (Nygaard et al. 1989). Isozyme analysis of 12-papillate species in-group II (Oudeman and Coffey, 1991 a, b, c) confirmed the validity of *P. botryosa*, *P. heveae*, *P. katsurae*, *P. meadii*, *P. palmivora* and *P. parasitica* (= *P. nicotianae*) as distinct species. The tobacco isolate of *P. parasitica* designated as *Phytophthora parasitica* var. *nicotianae* sensu Tucker (1931) could not be distinguished from isolates from other hosts, Oudemans and Coffey (1991c) concluded that maintaining both *P. nicotianae* var. nicotianae and *P. nicotianae* var. *parasitica* (sensu Waterhouse, 1963 should be discontinued. Brasier and Hansen (1992) also noted this. Previous reports based on morphology (Tsao and Sisemore; 1978) supported deletion of two varieties of *P. nicotianae*. Following the recent report by (Hall, 1993), the two varieties of *P. nicotianae* can be deleted from future keys.

Isolates of *P. capsici*, including those previously identified as '*P. palmivora*' MF4 separated into three subgroups CAP 1, CAP 2 and CAP 3. Those in CAP 1 were widely distributed geographical locations on a range of hosts including *Capsicum* species,

tomato, cucurbits, as well as cacao and black pepper and it contained the greatest amount of intraspecific diversity. A single representative of *P. mexicana* also clustered with this subgroup and as such is considered. Conspecific to *P. capsici* CAP 2 were found primarily on black pepper and from Hawaii on *Macadamia integrifolia* as well as other hosts. In black pepper from India and Indonesia, both CAP 1 and CAP 2 were found. However the evidences from isozyme suggest that the two groups are genetically isolated from one another. All representation of CAP 3 was derived from diseased cacao in Brazil and this subgroup was monomorphic for all loci examined (Oudeman and Coffey, 1991b). Intraspecific comparison revealed relationship, which would have been predicted, based on morphological comparisons alone. *P. meadii* and *P. botryosa* clustered together indicating a very close genetic relatedness. *P. katsurae* and *P. heveae* also formed a single cluster. *P. capsici* and *P. citrophthora* also formed a cluster indicating that they were genetically related. Finally the results of this isozyme analysis suggest that the papillate species of group II of Waterhouse (1963) scheme may form at least six distinct evolutionary lines (Oudeman and Coffey, 1991 b).

Mchau and Coffey (1994) extended the study of *P. capsici* with more isolates and found that isolates clustered in two subgroups. Cap A and Cap B. The isolates described by *P. tropicalis* fell within both CAP 2 and CAP 3. Protein and isozyme patterns from the type cultures of *P. capsici* and *P. cinnamomi* were strikingly different from each other and from those of most of the cacao isolates; some similarities were noted between MF4 and *P. capsici*. These results and other morphological studies indicate that the three morphological forms of *P. palmivora* are sufficiently different to be classified as three distinct species (Kaosiri and Zentmyer, 1980).

Mchau and Coffey, (1995) studied the morphological, physiological and isoenzyme data for 113 isolates of *P. capsici*. Isoenzyme analysis of 18 loci from 15 enzymes revealed 2 distinct subgroups, Cap A and Cap B. Both subgroups contained isolates from widely distributed geographical locations and from a range of hosts. There

was considerable morphological and physiological diversity amongst the isolates, but also significant differences between the two-isoenzyme subgroups. The majorities of isolates in Cap A possessed rounded sporangia and exhibited irregular sporangial ontogeny. Most Cap B isolates produced ellipsoid-lanceolate sporangia and 50 % had an umbellate ontogeny. Chlamydospore forming isolates occurred exclusively in the Cap B subpopulation. A minority of Cap A or Cap B isolates exhibited sporangial traits more typical of the other isoenzyme subgroup. Consequently, use of morphological characters alone would not always allow correct assignment of an isolate to its isoenzyme subgroup. *P. capsici* is re-defined based on the wide variation in characters found in this worldwide population sample. A single isolate of *P. mexicana* was placed in isoenzyme subgroup Cap A, though it differed morphologically from the *P. capsici* isolates in producing non-caducous sporangia.

Nygaard et al. 1989, studied the intraspecific isozyme diversity was presented among isolates of *P. megasperma* that were recovered from ten host plant species and from many geographical origins; these isolates represented many morphological types and included the three formae specialis of *P. megasperma* (f. sp. *glycinea*, f. sp. *medicaginis*, f.sp.*trifolii*). *P. megasperma* is divided into at least six intraspecific groups. Reproducible isozyme diversity was evident within *P. megasperma*; expanded isolates of *P. megasperma* from many geographical origin and hosts. Based on MDH, PGI, G6PDH, DIA and ACP isozyme pattern each isolate could be described by one of nine different isozyme patterns.

Oudemans and Coffey, 1991a studied 162 isolates representing world wide source of *Phytophthora cambivora*, *P. cinnamomi* and *P. cactorum* were compared using 18 enzyme loci separated by starch gel electrophoresis. Based on isozyme analysis, the three species were clearly separated and each was further subdivided into electriphoretic types (ETS). Mills et al. 1991 in the combined results of isozyme and mitochondrial DNA analysis indicate that there were at least 7 distinct molecular species represented by 123

isolates of *P. cryptogea* and *P. dreschsleri*. Forster and Coffey, (1991) described the isolates previously described, as *P. cryptogea* and *P. drechsleri* should not be merged into one species.

The type isolate of *P. capsici* was classified with subgroup Cap A and shared the same ETs, as did the majority of other green pepper (*Capsicum annum*) isolates from New Mexico. Subgroup Cap B was composed of 9 ETs, which were variable at the ACO, PGI, LDH2, MDH1, IDH1and G6PDH loci. Isolates from this subgroup were previously resolved into two separate subgroups CAP2 and CAP3 (Oudeman and Coffey; 1991b). Cluster analysis using the UPGMA single linkage technique defined four clusters and pathogenicity to tobacco and tomato. Separation of the species into two varieties on the basis of morphological or pathological characters is not supported (Hall 1993).

Mills et al. (1991) utilized isozyme analysis (Oudeman and Coffey, 1991 a, b) to study the relationship of 123 isolates from various regions of the world. Surprisingly isozyme analysis delineated 10 distinct groups on the basis of numerical analysis of 24 putative enzyme loci. The electrophoretic patterns were not associated with previously identified species on the basis of morphology or temperature tolerance. A parallel study of Mt. DNA, RFLPs yielded data that led to similar conclusions.

The intra specific variability among five isolates of black pepper (*Piper nigrum*) foot rot pathogen *P. capsici* in its nutritional preferences and isozyme profiles was studied. The isozyme profiles of esterase, acid phosphatase, and superoxide dis mutase and catalase systems, the similarity between the isolates ranged between 25 % to 44.7 % (Shamina Azeez and Sarma, 2006).

4.5.3. Molecular characterization using Internal Transcribed Spacer regions of rDNA gene repeates

Molecular tools including protein profiles (Brasier et al. 1993) and isozymes (Oudeman and Coffey, 1991 a,b, c; Latorre and Perez, 1995) has been useful in resolving differences between isolates and species but in recent years perhaps the most important have been the technological advances in molecular biology allowing examination of polymorphism at the DNA level. The ribosomal RNA gene repeat (rDNA) has been used extensively to compare and relate taxa at many levels, from kingdoms (Gray, et al. 1984) to genera (Berbee and Taylor, 1992) and species (Lee and Taylor, 1992; Briard et al.1995), studying the *Pythiaceae*, found little variation in the DNA sequence of 28S RNA gene of 15 *Phytophthora* species, in contrast to *Pythium*, 8 species of which could be assigned to four distinct groups. The internal transcribed spacer regions (ITS 1 and ITS 2), which lie between the 18 s and 28 s genes lack a functional role (Nues et al, 1994), which is thought to explain the higher levels of sequence variation within them. Studies of ITS region from rusts (Zambino and Szabo, 1993) *Colletotrichum* (Sherriff et al. 1994) and Alternaria (Jasalavich et al. 1995) have shown sequence variation at the interspecific level but generally low levels of intraspecific variation within well defined species. Lee and Taylor (1992) published the ITS 1 and ITS 2 sequences of the tropical *Phytophthora* species *P. palmivora*, *P. megakarya*, *P. capsici*, *P. citrophthora* and *P. cinnamomi* and showed excellent resolution at the species level. Trout, et al. in 1997 developed a rapid and accurate method for specific detection of *P. infestans* by the construction of a new primer PINF. The PINF primer will provide a valuable tool for the detection of *P. infestans* in potatoes and tomatoes.

The internal transcribed spacer regions (ITS 1 and ITS 2) of the ribosomal gene repeat from the *Phytophthora* species were amplified using the polymerase chain reaction and sequenced. Sequences from *P. cambivora, P. cinnamomi, P. citricola, P. cryptogea, P. drechsleri, P. fragariae* var. *frageriae, P. frageriae* var. *rubi, P. megasperma* var.

megasperma and *P. nicotianae* were compared with published sequences and phylogenetic trees were produced. The resultant grouping of species generally agreed with grouping established using classical morphological criteria, primarily sporangial morphology. Among species with non-papillate sporangia two clads were evident, one consisting of *P. fragariae, P. cambivora* and *P. cinnamomi* and the other *P. megasperma, P. drechsleri* and *P. cryptogea*. With improved technology, rapid automatic sequencing of PCR amplified ITS regions is now possible and yields detailed information of relationship within the genus as well as allowing the design of species specific PCR primers for diagnostic purposes (Cook and Duncan, 1997).

The ITS sequences of the nine species those already published (Lee and Taylor, 1992; Cook et al. 1996) confirms their utility in identifying species, determining natural groupings of species within the genus and gaining an understanding of their evolution. A clear grouping of species according to ITS sequence emergence was evident and it matched, to some degree, the classification based on type of papilla. However, a separation of semi-papillate and papillate species was not evident and the papillate and semipapillate species found within groups I-IV (Waterhouse, 1970) was all grouped in the same clad, distinct from the clad consisting of the non papillate species from groups V-VI. Papilla type therefore may be a sound criterion for classifying *Phytophthora* species.

Phylogenies based on ITS sequence suggest that antheridial attachment and homothallism and heterothallism cannot be taken as indicators of close phylogenetic relationships. Homo and heterothallic species were spread throughout the genus and paragynous and amphigynous species were placed in the same clad. Forster et al. (2000) reached a similar conclusion. The lack of correlation in the ITS tree suggests that antheridial attachment and sexuality are under relatively simple genetic control or have evolved more than once and that their significance had been overplayed (Forster et al. 2000).

Papillate and semipapillate species group I species were tightly clustered. Only a few well conserved; differences in ITS sequences distinguished the morphologically similar *P. idaei, P. cactorum* and *P. pseudotsugae* from each other, randomly amplified polymorphic DNA could be used to separate them. (Cook et al.1996). Another distinct group of papillate species consisted of *P. capsici, P. citricola*, and *P.citrophthora*. The close relationship between *P. capsici* and *P. citrophthora* has been shown by isozyme (Oudeman and Coffey 1991b) and ITS sequence analysis (Lee and Taylor, 1992). Isozyme and mt DNA variation amongst many isolates of *P. citricola* resolved many genetic subgroups that varied little in morphology and indicated a close relationship with *P. capsici* and *P. citrophthora* (Oudeman et al. 1994). The inclusion of *P. citricola*, a semipapillate group III species, serves to indicate that the division between semi- and papillate species is not phylogenetically significant. From this study it appears that affinities within the group are unclear as *P. capsici* grouped with *P. citrophthora* in the ITS1 tree and with *P. citricola* in the ITS 2 tree.

Low level of intraspecific ITS variation within *P. citrophthora* was noted by Lee and Taylor (1992), resulting in a difficulty in separating *P. capsici* and *P. citrophthora*. Although the situation appears to be clarified in analysis of ITS 1 sequences (Forster et al. 1995) further studies on this group of this group of species is justified in order to define the species boundaries more clearly or if necessary, remove them. Two other group II species, *P. megakarya* and *P. palmivora*, formed a distinct cluster separate from *P. citrophthora*, *P. capsici* and *P. cinnamomi* (Lee and Taylor, 1992). These two species still grouped together in this study, although the position of their group was slightly different between ITS 1 and ITS 2 trees.

There was a clear division between the relatively tight cluster of *P. fragariae* var. *rubi, P. fragariae* var. *fragariae, P. cambivora, P. cinnamomi*, and other species. The isolates of *P. cryptogea* and *P. drechsleri* grouped closely together but the *Phytophthora*

megasperma sequence was markedly different and this species was placed in an intermediate position between the papillate and non- papillate groups (Lee and Taylor, 1992).

P. fragariae has two varieties, one causes red rot of strawberry (Hickman, 1940), and the other Raspberry root rot. The latter was variously identified as *P. erythrseptica* sensu lato (Converse and Schwartz, 1968) and *P. megasperma* (Duncan et al. 1987), and only recently described as *P. fragariae* var. *rubi* (Wilcox et al. 1993) ITS sequence of the two varieties accord with other molecular evidences based on ribosomal RFLPs (Stammler et al.1993) and mitochondrial DNA analysis (Forster and Coffey, 1992) and confirms their varietal status.

Ristaino et al. (1998) had developed a PCR procedure to amplify DNA for quick identification of the economically important species from each of the six taxonomic groups in the plant pathogen genus *Phytophthora.* Restriction digests with Msp1 separated *P. capsici* from *P. citricola* and separated *P. cactorum* from *P. infestans* and *P. mirabilis*. Restriction digests with *Hae* III separated *P. citrophthora* from *P. cryptogea*, *P. cinnamomi* from *P. frageriae* and *P. megasperma* on peach, *P. palmivora* from *P. citrophthora*, and *P. megasperma* on raspberry from *P. sojae*. *P. infestans* and *P. mirabilis* digests were identical and *P. cryptogea* and *P. erythroseptica* digests were identical with all restriction enzymes tested. A unique DNA sequence from ITS region I in *P. capsici* was used to develop a primer called PCAP. The PCAP primer was used in PCRs with ITS 1 and amplified only isolates of *P. capsici*, *P. citricola*, and *P. citrophthora* and not 13 other species in the genus. Restriction digests with Msp I separated *P. capsici* from other two species.

Phytophthora palmivora isolates from cocoa and coconut, and *P. capsici* isolates of cocoa, black pepper and bell pepper collected from different localities of Karnataka and Kerala, India, were examined at the molecular level using the ITS1 and ITS2 primers

to amplify the internal transcribed spacer (ITS) regions of rDNA gene repeat and by amplified fragment length polymorphism (AFLP) analysis. An isolate of *P. capsici* (P 575) from cocoa in Mexico was also included for comparison. ITS-restriction fragment length polymorphism revealed consistent polymorphisms that are correlated to morphological description. The AFLP fingerprints of *P. palmivora* from cocoa and coconut showed similar patterns but amongst the isolates of *P. capsici*, four AFLP fingerprint groups were evident that were distinct from the *P. palmivora* type. Thus, ITS regions can be used, as taxonomic markers for the identification of *Phytophthora* associated with black pod disease of cocoa and AFLP fingerprints are useful for assessing intra-specific population variation (Chowdappa et al. 2003 a).

A molecular technique based on the internal transcribed spacer (ITS) region of ribosomal DNA was developed for the rapid identification of *Phytophthora* species. DNA was isolated from cultures of *P. capsici* from cocoa in Indonesia; *P. nicotianae* and *P. arecae* (*P. palmivora*) from coconut in Indonesia; *P. meadii* from rubber in Sri Lanka, Malaysia and India; and *P. meadii* from arecanut in India. The ITS region from the mycelial extracts was amplified by polymerase chain reaction (PCR) using primers ITS 1 and ITS 4. The amplified product was digested with the restriction enzymes Hinf I, Msp I, Hae III and Rsa I. Amplification with ITS 1 and ITS 4 yielded A PCR product of 860 bp for *P. capsici*, 900 bp for *P. arecae* and 920 bp for *P. nicotianae*. The ITS - restriction fragment length polymorphism patterns of *P. arecae*, *P. capsici*, *P. meadii* and *P. nicotianae* significantly varied. The isolates of the same species, however, showed identical banding patterns. The results were almost similar irrespective of the enzyme used. This method can be used as a taxonomic marker for pathogen identification and disease diagnosis (Chowdappa et al. 2003 b).

The internal transcribed spacer regions (ITS 1 and ITS 2) of the ribosomal RNA gene repeat from *Phytophthora boehmeriae* and *P. capsici* were amplified using the polymerase chain reaction and sequenced. The size of ITS 1 in *P. boehmeriae* is 206 bp

ITS2 is 453 bp. In *P. capsici* the total length of ITS 1 is 174 bp and ITS 2 is 432 bp. The ITS sequences in two *P. boehmeriae* strains are same. The homology of ITS 1 between *P. boehmeriae* and *P. capsici* is about 70.9 %. The ITS2 homology in the two *Phytophthora* species is about 70.9 % (Wang et al. 2000).

Two non-papillate species of *Phytophthora* are the principal cause of pistachio gummosis in Iran. Their previous description as *P. megasperma* and *P. drechsleri* was re-examined in the light of RFLPs and sequence comparisons of internal transcribed spacer (ITS) regions of rDNA. Both taxa were more closely related to a clad comprising *P. sojae*, *P. cajani*, *P. vignae*, *P. melonis* and *P. sinensis* than to the unrelated *P. megasperma* or *P. drechsleri*. The *P. megasperma*-like isolates from pistachio differed from all the above taxa in morphology, ITS sequence and amplified fragment length polymorphism (AFLP) patterns, and are described as a new species, *P. pistaciae* (Mirabolfathy et al. 2002).

Phylogenetic relationships among *Phytophthora* species were investigated by sequence analysis of the internal transcribed spacer region I of the ribosomal DNA repeat unit. The extensive collection of isolates included taxa from all 6 morphological groups recognized by Waterhouse (1963) including molecular groups previously identified using isoenzymes and mitochondrial DNA restriction fragment length polymorphisms. Similar to previous studies, the inferred relationships indicated that molecular groups of *P. cryptogea*, *P. drechsleri*-like and *P. megasperma*-like taxa are polyphyletic. This study provides evidence that the morphological characters used in *Phytophthora* taxonomy are of limited value for deducing phylogenetic relationships because they exhibit convergent evolution (Forster et al. 2000).

MATERIALS AND METHODS

MATERIALS AND METHODS

1. Source of *Phytophthora* isolates

Phytophthora isolates from black pepper (173 Nos:) stored at National Repository of *Phytophthora* at IISR, Calicut from different localities of Kerala, Karnataka, Tamil Nadu and Andhrapradesh had been utilised for the present investigation. Sixty isolates were selected based on their morphological characters for the investigation in detail. Out of these sixty isolates thirty two were from leaves, six from stem, nine from root, ten from soil, and one each from berry, spike and collar. (**Table : 2**)

2. Isolation of *Phytophthora*

The pathogen *Phytophthora* was isolated from infected plant parts and cultures were maintained on carrot agar for different experiments. For isolation selective medium (PVPH) was used. The antibiotic solution consisted of pimaricin, vancomycin, PCNB and hymexazole was prepared in sterile double distilled water and incorporated into carrot agar medium. Media and methods of preparation are given in Appendix. 1.

PVPH medium (Tsao and Ocana, 1969)

Pimaricin	10 ppm (10µg/ ml)
Vancomycin HCl	200 ppm (1.7g/ 100ml)
Penta chloro nitrobenzene (PCNB) 75%	100ppm (146.3mg/ 100ml)
Hymexazol (purity 99.4%)	50 ppm
Sterile distilled water	100 ml

Preparation

All antibiotics dissolved in 100 ml sterile double distilled water and stored in amber coloured bottle in refrigerator as stock solution for further use. Antibiotic solution (PVPH) 10 ml was added to 90 ml molten carrot agar medium and distributed into petriplates. This was done in laminar flow under sterile condition. Since pimaricin is photosensitive antibiotic solution preparation and incorporation to CA was done without the presence of direct light.

2.1. Isolation procedure

Diseased plant parts were thoroughly washed in tap water. Small pieces were cut from the advancing margins of infected tissue and surface sterilized with 0.01 % mercuric chloride for one minute. Later the pieces were washed in sterile water taken in petriplates. Later the material was blotted well on sterile filter paper strips and plated on selective medium (PVPH). The plates were incubated in dark at 24 ± 1 ^{0}C for one or two days. The growing mycelium from the infected pieces was transferred to carrot agar medium.

2.2. Isolation from soil

Baiting technique with *Albizzia* leaves as adopted earlier (Anandaraj and Sarma, 1990) was used for isolation from soil. About 100g of infected soil was suspended in 500ml water and stirred well. The suspension was taken in 90 cm petriplates. Later *Albizzia* leaves were placed within the petriplates. The infected leaves were washed in water and later passed through sterile distilled water and the leaves were plated on selective medium for isolation.

2.3. Maintenance of cultures

The cultures were purified and stored in carrot agar slopes at 10 ± 1 ^{0}C in dark in BOD incubator until required.

Table: 2, Source of *Phytophthora* isolates infecting black pepper, used in the present investigation

Sl. No	Isolate No.	Place of collection	State	Plant part/soil
1	96-8	Sirsi	Karnataka	Leaf
2	97-50	Sakleshpur	Karnataka	Stem
3	97-51	Mercara	Karnataka	Leaf
4	97-52	Taravana, Wayanad	Kerala	Leaf
5	98-7	Sakleshpur	Karnataka	Root
6	98-135	Sirsi	Karnataka	Root
7	98-157	Sirsi	Karnataka	Root
8	98-177	Siddhapur	Karnataka	Root
9	99-139	Meenangadi, Wayanad	Kerala	Leaf
10	98-24	Chelavoor, Calicut	Kerala	Leaf
11	98-76	Sontikkopa	Karnataka	Leaf
12	98-131	Kunnamangalam, Calicut	Kerala	Stem
13	98-140	Sirsi	Karnataka	Soil
14	99-158	Valara, Idukki	Kerala	Leaf
15	99-106	Sugandhagiri, Wayanad	Kerala	Leaf
16	98-49	Madikeri	Karnataka	Berry
17	98-81	Malappuram	Kerala	Leaf
18	98-144	Sirsi	Karnataka	Soil
19	98-167	Chethalli	Karnataka	Root
20	98-172	Sirsi	Karnataka	Leaf
21	98-198	Manjachola, Calicut	Kerala	Soil
22	99-103	Chelavoor, Calicut	Kerala	Leaf
23	99-104	Chelavoor, Calicut	Kerala	Leaf
24	99-125	Pollibetta	Karnataka	Root
25	99-144	Pollibetta	Karnataka	Leaf
26	99-166	Peruvannamuzhi, Calicut	Kerala	Leaf
27	99-167	Naikkunnu, Idukki	Kerala	Soil
28	97-45c	Gudallur	Tami Nadu	Leaf
29	98-67	Valnoor, Kodagu	Karnataka	Root

30	98-127	Kunnamangalam, Calicut	Kerala	Stem
31	98-162	Puttannamane	Karnataka	Soil
32	98-181	Sirsi	Karnataka	Soil
33	98-182	Isloor	Karnataka	Leaf
34	98-166	Sirsi	Karnataka	Leaf
35	99-162	Puttannamane	Karnataka	Soil
36	98-174	Isloor	Karnataka	Leaf
37	99-145	Sughandhagiri, Wayanad	Kerala	Spike
38	98-87	Kunnamangalam, Calicut	Kerala	Leaf
39	99-91	Thamarassery, Calicut	Kerala	Leaf
40	99-124	Gudallur	Tamil Nadu	Stem
41	96-5	Mettur	Tamil Nadu	Soil
42	96-13	Kozhikode	Kerala	Leaf
43	98-60	Valnoor, Kodagu	Karnataka	Leaf
44	98-145	Puttannamane	Karnataka	Root
45	00-18	Peruvannamuzhi, Calicut	Kerala	Leaf
46	00-40	Kasaragod	Kerala	Leaf
47	96-4	Kumily, Idukki	Kerala	Soil
48	97-11a	Pulpally, Wayanad	Kerala	Leaf
49	96-11	Sirsi	Karnataka	Leaf
50	97-55	Valparai	Tamil Nadu	Root
51	98-17	Pulpally, Wayanad	Kerala	Leaf
52	98-86	Medical college, Calicut	Kerala	Leaf
53	98-90	Chethalli	Karnataka	Stem
54	98-192	Sirsi	Karnataka	Soil
55	98-1	Thamarassery, Calicut	Kerala	Soil
56	99-127	KAU, Trichur	Kerala	Leaf
57	99-186	Belur	Karnataka	Leaf
58	99-188	Gunalanka	Andhrapradesh	Leaf
59	98-95	Adivarum, Calicut	Kerala	Stem
60	98-75	Wayanad	Kerala	Collar

3. Biology and morphology of *Phytophthora*

The biology of *Phytophthora* species include the morphological, and physiological studies. The morphological studies include colony morphology, sporangial ontogeny and sporangial morphology, chlamydospore morphology and oospore morphology.

3.1 Colony morphology of *Phytophthora*

For culture studies sterile glass petriplates of 90 cm were used through out. Three day old cultures grown on carrot agar medium were used as source of inoculum and 4 mm discs were cut from actively growing edges. The disc was inoculated in the centre of carrot agar medium taken in 90 cm petriplates and incubated in dark at 24 ± 1 ^{0}C. After the second day onwards they were examined for colony characters against a black background. Care was taken to avoid exposure to light before examination. Colony characters and radial growth for each isolates was measured at 48 and 72 hours of incubation by marking on the reverse side with a marker pen. Three replicates were maintained for each isolate. Growth rate was expressed as mm / day.

3.2. Sporangial morphology of *Phytophthora*

Sporangial morphology was studied according to agar disc in water method (Al hedaithy and Tsao, 1979a). Three day old cultures grown in carrot agar medium in dark were used. Discs of 4 mm size were cut from the actively growing edges were placed in petriplate containing 4 ml sterile distilled water. They were kept under continuous fluorescent light for 48 hours. Observations on ontogeny and sporangial characters were recorded using microscope. Sporangia were mounted either in distilled water or in lactophenol - cotton blue. Measurements were taken from these prepared slides in 40x magnification of a microscope.

3.2.1. Sporangium pedicel length and caducity of *Phytophthora*

Pedicel length of sporangia was determined in sterile distilled water. The isolates were inoculated to 15 ml carrot agar plates (90 cm) and incubated in dark at 25 ^{0}C for 48 hrs. From the growing edges 4 mm discs were cut and kept in a sterile petriplate and about 4 ml distilled water was added, in such a way that the water level should be just

below the disc surface. The petriplates were kept under continuous fluorescent light in a sporulation chamber for 48 hrs. After 48 hrs the cultures were observed for sporulation. Slides were prepared by tapping the sporulating discs on a clean glass slide. Stained with lactophenol cotton blue and covered with a cover slip and pedicel length was measured using a compound microscope.

Caducity was determined by sporangium harvest method. The sporangia bearing discs after 48 hrs incubation in sporulation chamber were taken, sporangia bearing discs were transferred to a test tube containing 4 ml of sterile distilled water (1disc/ tube) and shaken for 1 minute to dislodge the sporangia. This water was poured into a counting dish and the number of sporangia was counted in 4X magnification. These discs after dislodging the sporangia were pressed against a glass slide using a cover slip. The number of attached sporangia in three discs was counted. The total number of sporangia per 4m.m diameter disc was calculated by multiplying the average number of sporangia per microscopic field by the total number of sporangia in 4mm disc. The percentage caducity was calculated using the formula below.

Percentage caducity = No. of detached sporangia X 100 / Total no. of sporangia

3.2.2. Calculation of the area of the microscopic field

The diameter of the microscopic field was measured using a stage micrometer and ocular micrometer.

The diameter of the microscopic field in 10x X 15x magnification = 142 ocular divisions.

In 10x X 15x magnification 1 OD = 10 µm

1 µm = 10^{-6} m, ie. 10 µm = 0.01mm

142 OD = 1.42 mm

Therefore the diameter of a 10X x15X mf =1.42 mm

Radius = 0.71 mm

Area of the circular microscopic field = πr^2 = 3.14 x 0.71 x 0.71

= 1.582874 mm^2

Area of a 4 mm disc = πr^2 = 3.14 x 2 x 2 = 12.56 mm^2

No of mf in 4 mm disc = Area of 4 mm diameter disc / Area of microscopic field

= 12.56/ 1.582874 = 7.9435 ie Approximately 8 microscopic fields per 4 mm diameter disc.

The number of sporangia per microscopic field (average number of three microscopic fields) multiplied by 7.9435 gave the total number of sporangia per disc.

3.2.3. Slide culture technique (Dantanarayana et al.1984)

Sporangial morphology and sporangial ontogeny was studied by slide culture technique.

A wide mouthed 50 ml conical flask containing about 50 ml of CA was sterilized and the agar kept molten over a low flame. A sterile glass slide was then dipped in the agar so that half of its length was covered. The agar on lower surface was removed and slides placed resting on sterile glass rod in a petridish moist chamber. Inoculum was placed on one corner of the coated slide surface and incubated for 5 - 7 days. A drop of cotton blue in lacto phenol was added and a cover slip placed over the slide surface. Superfluous agar was removed. Best results were obtained after excess moisture was allowed to evaporate for 7-14 days, by which time the cover slip was firmly in position. A sealant was applied after cleaning the slide with alcohol. The slides were then examined for sporangial morphology and sporangial ontogeny under microscope. Photographs were taken using VIDEO PRO 32 computer program in 15x X 20x magnification.

3.3. Chlamydospore production of *Phytophthora*

All the 60 isolates were tested for chlamydospores. Carrot agar slants were taken and inoculated with 4 mm culture discs taken from the advancing margins of 48 hrs old

cultures. These slants were kept in light for five days and allowed to grow, and then the slants were kept in a BOD incubator set at 15 ^{0}C. They were taken after two months of incubation, a bit of mycelium was taken and observed under the microscope for chlamydospores. The chlamydospore producers and non-producers were categorized. The chlamydospore diameter was measured for 50 chlamydospores from three replicate slants and three slides were made. The average of 50 chlamydospores was calculated. Chlamydospores were induced in the cultures, failed to produce by the above method, by growing the cultures in β sitosterol added carrot agar slants in the same conditions. After induction also they were observed for chlamydospore production. Photographs were taken in 15x X 20x magnification.

3.4. Mating type determination of *Phytophthora*

All the 60 isolates were tested for mating type by growing the isolates together with known mating types of A1 and A2 (VC-6 and 98-12) isolates. The medium used for mating type study was clarified carrot agar supplemented with β sitosterol (30 mg / litre). Pour a thin film of medium, about 5 ml per plate. From the actively growing edges of the 48 hr old test isolate 4 mm agar plugs were taken and placed approximately 30 mm away from the known isolate. The test isolate was also paired with the same to test whether it was homothallic. The plates were incubated in dark below 25 ^{0}C. After 15-20 days, oospore production was observed under a microscope. Slides were made from the three replicates. Oogonia, antheridia and oospore dimensions were measured for fifty numbers per isolate. Five slides were prepared for each isolate. The mean was taken and recorded. Photographs were taken in 15x X 20x magnification.

4. Physiology of *Phytophthora*

The physiological studies include the effect of temperature, pH and media of *Phytophthora* species infecting black pepper.

4.1 Effect of temperature on *Phytophthora*

The effect of temperature on growth, sporulation, zoospore liberation and zoospore germination of *Phytophthora* species infecting black pepper was studied. In order to determine the growth temperatures of *Phytophthora* isolates different temperatures were tested ranging from 9-36 ^{0}C and four different temperatures were selected for further study. They were 10 ^{0}C, 20 ^{0}C, 28 ^{0}C and 35 ^{0}C. Inoculum discs (4 mm) from cultures grown in dark for 48 hrs in carrot agar at 25 ^{0}C were plated in the centre of carrot agar plates (90 cm). Three replicates were kept for each isolate. After inoculation they were maintained in the BOD incubators set at 10 ^{0}C, 20^{0}C, 28^{0}C and 35 ^{0}C respectively. The radial growth was taken after 48 and 72 hr intervals as mentioned earlier and recorded. The average of three replicates was calculated.

From the advancing margin of the cultures grown in different temperatures discs were cut and placed in sterile petriplate. After adding 4 ml sterile distilled water they were kept in sporulation chamber under continuous fluorescent light. After 48 hrs of incubation they were observed under inverted microscope. The number of sporangia produced per microscopic field was counted and recorded.

For zoospore liberation (indirect germination) of sporangia the sporulating culture discs were taken and kept in the freezer at 4 ^{0}C for 10 minutes and after that the plates were moved into lab temperature for thirty minutes. Later the discs were observed for observed for zoospore release under inverted microscope. The number of sporangia with indirect germination (zoospores liberated) was counted against the number of sporangia without zoospore liberation in three microscopic fields for three replicates and the average was calculated.

The zoospores were taken with a pipette and mounted on cavity slides with a drop of water and the germination was observed after 30 minutes. The number of zoospores germinated was counted in three microscopic fields from three replicates and the average was calculated.

4.2. Effect pH on *Phytophthora*

Variability of *Phytophthora* isolates with different pH was studied. The mycelial growth, sporulation, zoospore liberation (indirect germination) and zoospore germination was studied as mentioned earlier. The mycelial growth of *Phytophthora* isolates at different pH, like pH 2, pH 5, pH 7 and pH 9 was studied. For this study GYP broth was used. The cultures grown in carrot agar plates kept at 25 ^{0}C for 48 hrs were used. Three 4 mm discs were inoculated into 25 ml GYP medium kept in 100 ml flasks, whose pH was adjusted with NaOH or HCl. The cultures were kept at 25 ^{0}C for ten days. For each isolate three replicates were kept. After 10 days the cultures were harvested in funnels lined with Whatman No.1 preweighed filter paper fixed in the flask by vacuum filtering using vacuum flask with side arm. The harvested mycelium was dried overnight by keeping in an oven kept at 65 ^{0}C. The mycelial weight was obtained by substracting the initial weight of the filter paper from the dried filter paper and the average of three replicates was calculated.

Phytophthora cultures grown in carrot agar medium at 25 ^{0}C for 48 hrs old were taken and from the advancing margins discs were cut and placed in sterile petriplates. To each petriplate 4ml pH adjusted Petri's medium was added . Sporulation, zoospore liberation (indirect germination) and zoospore germination were studied.

4.3. Effect media on *Phytophthora*

Growth of *Phytophthora* isolates from black pepper was studied in different media. The colony diameter, sporulation, zoospore liberation (indirect germination) and zoospore germination were studied. In order to ascertain the growth behaviour of black pepper *Phytophthora* isolates different solid media were evaluated viz, carrot agar, potato dextrose agar, corn meal agar and papaya dextrose agar. For this study *Phytophthora* cultures grown in carrot agar at 25 ^{0}C for 48 hrs were used. From the advancing margins discs (4 mm) were cut and placed in the centre of the petriplate containing different solid media. After that the plates were kept in dark at 25 ^{0}C. After 48 and 72 hrs the radial growth was measured and recorded. For each isolate three replicates were maintained, the average of three replicates were calculated and recorded. The same cultures were used for studying sporulation, zoospore liberation and zoospore germination. The details of media and preparation are given in appendix 1.

5. Pathogenicity of *Phytophthora* species

Pathogenicity of *Phytophthora* on nine different *Piper* species and on stem and roots of Sreekara plants were carried out.

5.1. Pathogenicity of *Phytophthora* species on differentials of *Piper* species

Nine different species of *Piper* were grown in the nursery for pathogenicity studies. The species includes *Piper nigrum* (Sreekara), *Piper betle*, *Piper longum*, *Piper colubrinum*, *Piper arboreum*, *Piper ornatum*, *Piper hapnium*, *Piper chaba* and *Piper attenuatum*. The pathogenicity studies were conducted in green house conditions. Single zoospore isolates of *Phytophthora* passed through the host recently were used for pathogenicity studies. *Phytophthora* isolates were grown in carrot agar medium for 48 hrs in dark at 25^{0}C were taken and five discs were cut from the growing edges and kept in

sterile petriplate. About 4 ml sterile distilled water was poured and kept it for sporulation under continuous fluorescent light. Zoospore suspension was prepared as mentioned earlier. The number of zoospores per ml was maintained at 2 x 10^4 spores per ml. Each leaf was inoculated with 50 µl zoospore suspension. A cotton swab was kept at the place of inoculation and it was kept wet every day. After 48 and 72 hrs lesion diameter was taken at right angles to each other. The young leaves, third leaf from the tip, with uniform maturity were taken and washed well. They were kept in plastic box, lined with wet filter paper. The leaves were inoculated with zoospore suspension. They were kept at green house conditions at temperature of 20 ^{0}C and relative humidity of 90 % (Cassia Siamia Technologies, Hyderabad). For each isolate five replicates were kept and mean lesion diameter was calculated. The virulence rating is measured as 0 – no infection, 1 -15 mm, less virulent, 16 - 24 mm, moderately virulent and a > 25 mm highly virulent.

5.2. Pathogenicity of *Phytophthora* species by stem inoculation on Sreekara variety of black pepper

For this study 25 isolates were selected based on their morphology and mycelial discs were used as inoculum. *Phytophthora* isolates were grown in carrot agar medium for 48 hrs at 25 ^{0}C under dark. Inoculum discs were cut from the margins of the agar culture. The black pepper selection that was highly susceptible to *P. capsici* (Sreekara) was raised as single node cuttings in polybags (18 X 16cm) containing potting mixture (3:1:1-soil, sand and farm yard manure). Four-month-old Sreekara plants were taken to the green house conditions of 20 ^{0}C temperature and relative humidity of 90 %. A small prick was made on the third internodal region from the top of the plant, a mycelial plug was placed and covered with a little wet cotton swab. The region of inoculation was wrapped in a piece of cellophane tape. The plants were watered regularly and the cotton swab was maintained throughout. The plants were kept in greenhouse condition throughout to experiment. Pathogenic reaction was measured as per the rating given below in five replicates and mean was calculated and recorded. The pathogenicity reaction was measured as 0- no infection, < 25 % less virulent, 25 - 50 % moderately

virulent and 50- 100 % highly virulent.

5.3. Pathogenicity of *Phytophthora* black pepper by root inoculation on Sreekara variety of black pepper

Four-month-old nursery grown plants were taken to green house. They were inoculated with zoospore suspension prepared from 48 hr old mycelial discs, kept in water. The concentration of zoospore suspension was maintained as 2 x 10^4 zoospores / ml. 50 ml zoospore suspension was poured in each bag and stirred the soil gently through the sides to ensure uniform distribution of inoculum. The inoculated plants were well watered and kept in the green house throughout the study. The plants inoculated after fourteen days of inoculation were uprooted and observed for infection. The root system of the inoculated plants were carefully removed and washed. The root rot was visually scored. The rate of infection was compared with the control and recorded. The pathogenicity reaction was measured as 0- no infection, < 25 % less virulent, 25-50 % moderately virulent and 50- 100 % highly virulent.

6. Cross infectivity studies of *Phytophthora* species on different plantation crops

Phytophthora isolates of rubber, cocoa, cardamom, arecanut, black pepper, betel vine and coconut were used in the study. The isolates from rubber, cardamom and arecanut were *P.meadii*, and from cocoa and coconut were *P. palmivora*. The black pepper isolates were *P.capsici and* 5 betle vine isolates were *P. parasitica* and one was *P. capsici.* Six representative isolates of each crop was selected and grown in carrot agar medium in dark for 48 hrs, at 25^{0}C. The culture discs from growing edges were inoculated to arecanut, rubber leaves, pepper leaves, betel vine leaves, cocoa pods and coconut.

Bunches of young green areca nuts were collected and inoculated with culture discs. Pinpricks were made in the middle portion of the areca nut and culture discs were

placed and piece of wet cotton was kept to keep moisture. They were kept in plastic boxes containing wet filter paper. The readings were recorded after 96 hrs. Five replicates were kept for each isolates. The average of 5 replicates was calculated. Rubber leaves, third from the tip of uniform maturity were taken and the petioles were inoculated with 4mm culture discs. They were placed in plastic boxes containing wet filter paper. The reading were taken after 72 hrs and recorded. The average of replicates was taken.

In pepper the third leaves from the tip of uniform maturity were inoculated with 4 mm disc and the readings were taken after 72 hrs and recorded. In betel vine the third leaves from the tip with uniform maturity were collected and placed in plastic boxes containing filter paper and inoculated with 4 mm culture discs. The lesion diameter was taken after 72 hrs. The average of five replicates was calculated.

Green nearly mature and unripe cocoa pods were taken and with a cork borer a 3 mm disc is cut from the pod and a mycelial disc was inserted and wet cotton was placed. The reading was taken after 96 hrs for five replicates and the average was calculated and recorded.

Young coconuts were collected and inoculated with 4mm culture discs and the lesion diameter was taken after 96 hrs. The average of five replicates were calculated and recorded. The virulence rating is calculated as 0 – no infection, 1 -15 mm, less virulent, 16-24 mm, moderately virulent and a >25 mm highly virulent.

7. Study of *Phytophthora capsici* isolated from other hosts

P.capsici isolates from different hosts like betel vine, *P. chaba*, *Capsicum*, cocoa and *Bauhinia purpurea* were selected for the study.

7.1. Morphology of *Phytophthora capsici* isolated from other hosts

The morphological characters were studied as mentioned earlier by growing the isolates on carrot agar plates. After 48 hours incubation in dark the colony morphology was observed. Discs were cut from the advancing margins and they were put in sterile petriplates and about 4 ml water was poured into the petriplate and kept under continuous light for sporulation. After two days of incubation they were taken and observed for sporulation. The ontogeny, sporangial characters pedicel length and caducity were determined.

7. 2. Pathogenicity of *P.capsici* isolated from other hosts

Pathogenicity of *P.capsici* isolated from different hosts like betel vine, *P.chaba*, *Capsicum*, cocoa and *Bauhinia purpurea* was studied by root inoculation and leaf inoculation experiment.

7.2.1. Root inoculation of *P.capsici* isolated from other hosts

The isolates from different hosts were grown in carrot agar plates and after 48 hrs incubation in dark, twenty discs were cut from each plate and kept in petri plates, 4 ml sterile distilled water was added and kept under continuous light for sporulation. Zoospore suspension was prepared from these cultures after 48 hours incubation. From each plate 10 ml zoospore suspension is made and diluted to 50 ml. The concentration of zoospore suspension was maintained as $2x10^4$ zoospores / ml. The rooted cuttings were inoculated with each isolate. Plants inoculated with typical *P.capsici* isolates from black pepper were kept for comparison. An absolute control of uninoculated plant for comparison was also maintained. The pathogenicity reaction was measured as 0- no infection, < 25 % less virulent, 25-50 % moderately virulent and 50- 100 % highly virulent.

7.2.2. Pathogenicity *P.capsici* isolated from other hosts on black pepper leaves

Pathogenicity of *P. capsici* isolated from other hosts on black pepper leaves was also tested. For this study all the isolates were grown on CA plates and mycelial discs cut from 48 hr old cultures were inoculated on young pepper leaves, kept in a moistened plastic box. Wet cotton wad was kept on the mycelial discs. They were kept for three days, the lesion development was observed and measured at 24, 48 and 72 hrs intervals. The virulence rating is calculated as 0 – no infection, 1 -15 mm, less virulent, 16-24 mm, moderately virulent and a >25 mm highly virulent.

8. Polyacrylamide gel electrophoresis (SDS PAGE) of *Phytophthora* species infecting black pepper

Polyacrylamide gel electrophoresis (SDS PAGE) of soluble mycelial proteins of selected isolates was carried out. For this study 17 isolates were selected from each morphological group. The isolates used in the study were 99-103, 98-60, 97-55, 98-24, 98-67, 99-91, 99-124, 99-139, 97-50, 98-1, 99-127, 98-86, 98-192, 98-90, 98-95, 98-75, 99-188. The isolates were grown in modified GYP medium for 10 days in 150 ml medium in roux bottles.

8.1. Extraction of soluble proteins

From an actively growing three day old *Phytophthora* culture grown on CA, ten discs were taken and put in sterilized GYP medium and incubated at 24 ± 1 ^{0}C for ten days in dark. After 10 days growth in dark the mycelium was harvested using Buckner funnel and side arm flask. The mycelium was air-dried. Then freeze dry at –20 ^{0}C over night. The mycelium was ground with extraction buffer (phosphate buffer) in liquid nitrogen. The extract was centrifuged at 10000 rpm. The solution was taken and kept at –

20 ^{0}C.

Buffer soluble proteins were extracted by grinding 2g, damp dried mycelium with a pestle and mortar containing acid washed sand (0.5g) and 1 ml of 0.1M-phosphate buffer (pH 7). The mixture was centrifuged at 12000g for 40 min. The resultant clear supernatant liquid from the fungal extract was decanted and immediately used for electrophoresis. All the operations were carried out at 4 ^{0}C.

8.2. Protein estimation

Protein conc. was determined by the method of Lowry et al (1951). Bovine serum albumine (BSA) is used as standard.

8.3. Electrophoretic separation of native proteins

Electrophoresis of native proteins preparations was carried out on a discontinuous system using 2.5 % stacking gel and 7.5 % separating gel in a vertical slab of 30x20x10cm size. Gels were fixed stained and destained according Bielenin et al. (1988). Electrophoresis buffer was tris glycine buffer (pH 8.3). Sample loading buffer was mixed with soluble protein preparations in the ratio of 1:1 and aliquots containing 80µg of proteins of which *Phytophthora* isolates were placed into well of the gel. Electrophoresis was performed at 60V for stacking gel and 120 V for separating gel at 4 ^{0}C.The proteins were visualized by staining the gels for 7 hr with coomassie brilliant blue G in water: methanol: perchloric acid (15: 1: 4) mixture and destained with several change of mixture of, water: methanol: acetic acid (7:2:1). The details of reagents are given in appendix 1.

The bands visually observed and compared. For cluster analysis unweighed pair

grouping with arithematic averaging (UPGMA) was used to construct dendrogram from similarity coefficients.

9. Isozyme analysis of *Phytophthora* species infecting black pepper

9.1.Single Zoospore isolation

To limit variability of the isolate it was obtained from a single germinated cyst (a zoospore that has lost the flagella) or hyphal tips to make sure that only one biotype was isolated. After passing through the host the cultures were reisolated using PVPH amended carrot agar. They were subcultured in carrot agar medium and incubated under dark for two days. Then 4 mm discs were cut from actively growing edges and kept for sporulation under continuous fluorescent light. After two days of incubation they were observed for sporulation. Cold shock was given for 10 minutes and after half hour zoospore liberation started. From this spore suspension one ml was taken and serially diluted and plated in carrot agar or plane agar. After 24 or 36 hrs germinating cysts could be observed under a microscope. With a sharp needle the germinating spores were transferred to CA.

9. 2. Fungal culture

All the sixty isolates were used for the isozyme analysis. Single zoospore cultures of all the isolates were routinely maintained on carrot agar plates at 25 ^{0}C. Mycelial discs taken from the margins of actively growing 3 day old cultures were used for mass multiplication in liquid culture. The isolates were mass multiplied in GYP (Glucose yeast peptone) broth as modified by Hall *et al.* (1969), which included all the ingredients of GYP except yeast extract and peptone. Cultures were grown in Roux bottles containing 150 ml broth for 10 days under diffuse light at 24 ± 1 ^{0}C. The mycelial mats were harvested under vacuum and stored at -85 ^{0}C until use.

9.3. Enzyme extraction

The frozen mycelia were extracted in 0.1 M sodium phosphate buffers, pH 7.5 (0.5 g / ml) and the extracts stored in small aliquots at –85 ^{0}C until used for the electrophoretic runs. A small aliquot was saved for protein estimation by Lowry's method (Lowry, 1951). The details of reagents are given in appendix 1.

9.4. Electrophoretic resolution of enzymes

The enzymes were separated by native polyacrylamide gel electrophoresis. The isoforms of the different enzymes were separated by non-dissociating, discontinuous buffer system. Thus, A discontinuous buffer system was used for electrophoresis in which the stacking and separating gels were prepared. Different enzymes were employed for the characterization of black pepper isolates. Six enzymes; namely catalase (CAT), superoxide dismutase (SOD), malate dehydrogenase (MDH), malic enzyme (ME), diaphorase (DIA) and isocitrate dehydrogenase (IDH) were used to analyze black pepper isolates. Aconitase and esterase did not show consistent and clear banding patterns and hence was not included in the study.

9.5. Sample preparation and electrophoresis

Aliquots of samples equivalent to 100 μg protein were mixed with 4X sample buffer in the ratio 3:1 and loaded in different wells of the polyacrylamide gel. The samples were run in 10% gels as described above. Electrophoresis was carried out in a Hoefer mighty small vertical electrophoresis unit at a constant current of 2mA per well at 4^{0}C. Internal standards were used in all gels to compare isolates resolved in different gels. These consisted of isolates, which showed maximum number of bands for a particular enzyme.

9.6. Activity staining

The gels were stained according to the recipes given by Oudemans and Coffey (1991a) with some modification for ME and MDH as described by Soltis *et al.*, (1983).

9.7. Staining procedure

The gels were incubated in the stain solution in dark at room temperature till the bands appeared. Immersing the gels in 7 % acetic acid stopped the reaction. The stain solutions were kept in dark to avoid photo-oxidation.

9.8. Data analysis

The isozyme banding patterns were recorded according to their relative mobilities. The isozyme data was analyzed by studying the presence or absence of a band of particular Rf value in each locus. The raw data was then used for cluster analysis using a computer program, NTSys. Isolates with the same banding pattern for all the enzyme loci studied, were grouped into electrophoretic types (ETs). Dendrograms were constructed based on UPGMA (Unbiased Paired Group of Arithmetic Averages) cluster analysis and conclusions drawn.

10. Molecular characterization using internal transcribed spacer regions of ribosomal DNA of *Phytophthora*

From the sixty isolates studied eighteen isolates were selected based on morphological characters for ITS, rDNA analysis. The isolates were 97-50, 99-139, 98-166, 99-103, 98-67, 99-91, 99-124, 98-24, 99-158, 00-40, 98-60, 97-55, 99-167, 98-87,

98-1, 99-186, 98-86, 98-192, collected from different geographical regions and different plant parts.

10.1. Culturing

Single zoospore isolates of *Phytophthora* was used in the study. The cultures were grown on carrot agar medium and after 48 hrs the mycelial discs were aseptically transferred into 25 ml glucose yeast peptone broth (GYP) taken in conical flasks

10.2. DNA extraction

To extract DNA isolates were grown in Glucose Yeast Peptone broth (GYP) for two days at 24 ± 1^0C. Freeze-dried mycelia were ground using a mini grinder with sodium dodecyl sulphate extraction buffer (200 nM Tris HCl, 250 nM NaCl, 25 mM EDTA, and 5% SDS). Extraction with phenol/ chloroform / isoamyl alcohol (25: 24:1 Vol / Vol) was then carried out. Precipitation was carried out with cold isopropanol and the pellet was washed with 70% ethanol and resuspended in sterile double distilled water. The concentration of DNA was measured spectrophotometrically at 260 nm. The details of reagents are given in appendix 1.

10.3. ITS-RFLP of rDNA

PCR amplfication of ITS regions of rDNA was carried out using primer pair ITS4 5’ TCCTCCGCTTATTGATATGC 3’ And ITS6 5’GAAGGTGAAGTCGTAACAAGG 3’.

The PCR mixture contained

DNA	1.0µl (50ng)
Taq pol(1U)	0.5µl

Reaction buffer (1X)	2.5µl
$MgCl_2$ (2mM)	3.0µl
dNTP (100µM)	2.0 µl
Primers ITS4 and ITS6	1.25µl +1.25µl (each primer)
Water to makeup	25µl

The PCR machine was programmed as follows.

Initial denaturation at 94°C for 3 minutes, 40 cycles of denaturation at 94°C for 1 minute, followed by annealing at 55°C for 1 minute and polymerization at 72°C for 2 minutes and final extention at 72° C for 10 minutes.

Restriction digestion of the amplified product was done.

Once the reaction was over, out of 25 µl, 5 µl was used to check out the presence of the amplified product of 862 bp by agarose gel electrophoresis of 1.4 %. Once the presence of the product was confirmed, out of the remaining 20 µl was separated into 10 µl each, and the first 10µl was subjected to Msp restriction digestion by adding

0.5 µl of restriction enzyme (Msp I or Taq I)

0.2 µl of restriction enhancer (BSA),

Add 2 µl of 10 X restriction buffer and 7.3 µl of sterile distilled water, and making up the total volume of 20 µl.

This was subjected to incubation at 37°C for 2 hrs.

The same was done with the left out 10 µl of the PCR product but with the change in the restriction enzyme, i.e instead of Msp I, Taq I (restriction enzyme) was used and incubated at 65°C for 2 hrs.

And the restricted product of Msp I and Taq I was electrophoresed in 2.4% agarose gel, and documented.

10.4. Data analysis

The gel pictures were analyzed using the UPGMA cluster analysis using the NTSYS computer programme.

RESULTS

RESULTS

RESULTS

1. Collection and isolation of *Phytophthora* species infecting black pepper

Phytophthora isolates were collected from various localities of south India like Kerala, Karnataka, Tamil Nadu and Andhrapradesh, where black pepper is cultivated. *Phytophthora* species were isolated adopting selective medium (PVPH) from various infected plant parts like leaf, stem, collar, spike, root and rhizosphere soil of the affected plants. 60 isolates were used for characterization of which 32 were from leaf, 7 from stem, and 9 from root, each from collar and berry and 10 from soil. (**Table: 2**)

2. Identity of *Phytophthora* species infecting black pepper

A detailed study of sixty *Phytophthora* isolates was carried out. The black pepper isolates were identified as *Phytophthora capsici* Leonian emend. A. Alizadeh and P.H. Tsao (51 isolates), *Phytophthora meadii* McRae (3 isolates), *Phytophthora palmivora* E. Butler (2 isolates), and *Phytophthora parasitica* Dastur (4 isolates). Based on caducity the isolates were broadly classified into caducous and non-caducous. The caducous isolates were classified into three groups based on pedicel length. They were, with short pedicels, < 5 µm (*P. palmivora*), intermediate pedicels, 5-19 µm (*P. meadii*) and with long pedicels > 20µm (*P. capsici*). The *P. capsici* isolates were further divided into four groups based on colony morphology, growth rate, pedicel length and LB ratio. The *Phytophthora parasitica* isolates were non caducous.

P. capsici **(Isolate Nos: 1-51)**

The *P. capsici* isolates were further grouped into four based on morphological characters like colony morphology, sporangial morphology, sporangial ontogeny, pedicel length, caducity, chlamydospore production and oospore production.

Group I (Isolate Nos: 1-9):

There were nine *P. capsici* isolates with caducous, long pedicels and pedicel length range was 31-94 µm. They had chrysanthemum like colony, pure white and appressed mycelium. The isolates were 96-8, 97-50, 97-51, 97-52, 98-7, 98-135, 98-157, 98-177, 99-139.

Group II (Isolate Nos: 9-15):

This group consists of six *P. capsici* isolates with caducous, long pedicels and pedicel length range was 65-150 µm. They had white colony with slow growth. The isolates were 98-24, 98-76, 98-131, 98-140, 99-158, and 99-106.

Group III (Isolate Nos: 16-40):

This group consists of twenty five *P. capsici* isolates with caducous, long pedicels and pedicel length range was 38.7-192 µm. Their colony morphology was varied. This group consists of isolates with chrysanthemum like pattern, stellate pattern and floral pattern. The isolates were 98-49, 98-81, 98-144, 98-167, 98-172, 98-198, 99-103, 99-104, 99-125, 99-144, 99-166, 99-167, 97-45c, 98-67, 98-127, 98-162, 98-181, 98-182, 98-166, 99-162, 98-174, 99-145, 98-87, 99-91, and 99-124.

Group IV (Isolate Nos: 41-51):

This group consists of eleven *P. capsici* isolates with caducous, long pedicels and pedicel length range was 70-167 µm. They had fast growth and uniform cotton wool like aerial mycelium. The isolates were 96-5, 96-13, 98-60, 98-145, 00-18, 00-40, 96-4, 97-11a, 96-11, 97-55, and 98-17.

P. meadii **(Isolate Nos: 52-54)**

There were three *P. meadii* isolates with pedicel length range of 10-14 µm; they were 98-86, 98-90, and 98-192. The isolates 98-86 and 98-90 had white cottony colony with faded patterns and 98-192 had floral pattern.

P. palmivora **(Isolate Nos: 55&56)**

There were two *P. palmivora* isolates, 98-1 and 99-127 with very small pedicels. The pedicel length range was 4-5 µm. They had white cottony colony with faded patterns.

P. parasitica **(Isolate Nos: 57-60)**

There were four non-caducous *P. parasitica* isolates. The isolates were 99-186, 99-188, 98-75 and 98-95. They had white fluffy colony and fast growth.

3. Colony morphology and growth rate of *Phytophthora* species infecting black pepper on carrot agar

The colony morphology of the isolates showed variation in growth pattern as well as rate of growth. (**Fig. 1 and Plate: 2**).

Fig: 1, Radial growth of *Phytophthora* species infecting black pepper on carrot agar

Colony characters ***P. capsici* (Isolate Nos: 1-51)**

Out of the sixty isolates studied 51 belong to *P. capsici*. They had five types of colony morphology; chrysanthemum like pattern, white cottony, stellate, floral pattern and uniform cotton wool like aerial mycelium. The group I consists of nine isolates with chrysanthemum like pattern. The aerial mycelium was very less for these isolates; mean radial growth range was 30-37 mm after 72 hrs in carrot agar medium. The group II consists of six white cottony isolates; their mean radial growth range was 22-30 mm after 72 hrs. The group III consists of isolates with chrysanthemum like pattern, stellate pattern and floral pattern and their mean radial growth range from 26-40 mm after 72 hrs intervals. The group IV consists of isolates with uniform cotton wool like aerial mycelium and had mean radial growth range of 32-38 mm in 72 hrs (**Table 3)**.

Table: 3, Colony morphology and growth rate of *Phytophthora capsici* infecting black pepper on carrot agar

Sl. No.	**Isolate no.**	**Colony pattern**	**Radial growth in mm**	
			48 hrs	**72 -**
1	96-8	Chrysanthemum like pattern	25	32

2	97-50	,,	24	32
3	97-51	,,	23	30
4	97-52	,,	24	30
5	98-7	,,	21	31
6	98-135	,,	20	32
7	98-157	,,	27	37
8	98-177	,,	21	31
9	99-139	,,	20	30
10	98-24	White cottony	22	30
11	98-76	,,	18	26
12	98-131	,,	18	25
13	98-140	,,	18	25
14	99-158	,,	20	25
15	99-106	,,	16	22
16	98-49	Chrysanthemum like pattern	30	40
17	98-81	,,	27	37
18	98-144	,,	29	46
19	98-167	,,	28	38
20	98-172	,,	22	33
21	98-198	,,	21	32
22	99-103	,,	26	34
23	99-104	,,	23	39
24	99-125	,,	24	36
25	99-144	,,	26	36
26	99-166	,,	27	39
27	99-167	,,	28	38
28	97-45c	Stellate pattern	23	30
29	98-67	,,	22	30
30	98-127	,,	20	29
31	98-162	,,	22	33
32	98-181	,,	24	31
33	98-182	,,	27	37
34	98-166	,,	25	33
35	99-162	,,	22	33
36	98-174	Floral pattern	28	40
37	99-145	,,	23	31
38	98-87	,,	21	35
39	99-91	,,	21	34
40	99-124	,,	20	26
41	96-5	ike aerial mycelium	25	36

42	96-13	,,	25	35
43	98-60	,,	22	35
44	98-145	,,	23	34
45	00-18	,,	25	34
46	00-40	,,	25	35
47	96-4	,,	25	33
48	97-11a	,,	24	34
49	96-11	,,	25	32
50	97-55	,,	27	38
51	98-17	,,	24	33

Statistical analysis of colony morphology and growth rate of *Phytophthora capsici* infecting black pepper on carrot agar

Growth of *Phytophthora capsici* isolates on carrot agar medium was analyzed statistically. There was significant variation among the four groups of *Phytophthora capsici* isolates. The mean value was minimum for group II isolates. It was maximum for group III, IV isolates (**Table 4)**.

Table: 4, ANOVA and DMRT of growth of *Phytophthora capsici* infecting black pepper on carrot agar

Growth	Sum of Squares	df	Mean Square	F	GROUP	N	Mean value
Between Groups	1824.748	3	608.249	19.030	2.00	36	22.2500a
Within Groups	9652.611	302	31.962		1.00	54	27.2778b
Total	11477.359	305			4.00	72	29.1944bc
					3.00	144	29.9583c

2) Colony characters of *P. meadii* (Isolate Nos: 52-54)

There were three *P. meadii* isolates. Two of them had white cottony faded pattern and the isolate 98-192 had floral pattern. Mean radial growth range from 26-37 mm. (**Table 5)**.

Table: 5, Colony morphology and growth rate of *Phytophthora meadii* infecting black pepper on carrot agar

Sl. No.	Isolate no.	Colony pattern	Radial growth in mm
			72 hrs
52	98-86	White cottony with faded pattern	26
53	98-90	,,	28
54	98-192	Floral pattern	37

3) Colony characters of *P. palmivora* (Isolate Nos: 55&56)

There were two *P. palmivora* isolates with white cottony faded pattern and their mean radial growth range from 24-30 mm. (**Table 6)**.

Table: 6, Colony morphology and growth rate of *Phytophthora palmivora* infecting black pepper on carrot agar

Sl. No.	**Isolate no.**	**Colony pattern**	**Radial growth in mm**	
			48 -	**72 hrs**
55	98-1	White cottony with faded pattern	18	24
56	99-127	,,	21	30

4) Colony characters of *P. parasitica* (Isolate Nos: 57-60)

There were four *P. parasitica* isolates with white fluffy and without any prominent growth pattern and their mean radial growth range from 30-35 mm after 72 hrs interval (**Table 7)**.

Table: 7, Colony morphology and growth rate of *Phytophthora parasitica* infecting black pepper on carrot agar

Sl. No.	**Isolate no.**	**Colony pattern**	**Radial growth in mm**	
			48 hrs	**72 hrs**
57	99-186	White fluffy	23	32
58	99188	,,	23	32
59	98-95	,,	20	35
60	98-75	,,	23	32

3. Sporangial ontogeny and sporangial morphology of *Phytophthora* species infecting black pepper

Sporangial ontogeny and sporangial morphology of *Phytophthora* species showed variation among themselves and between different species (**Fig. 2**, **Plate 3, 4 and 5).**

Sporangial ontogeny and sporangial morphology of *P. capsici*

1) *P. capsici* (Isolate Nos: 1-51)

Umbellate ontogeny was the common one and out of 51 *P. capsici* isolates studied 50 isolates had umbellate ontogeny. The isolate 98-87 had irregular ontogeny.

Group I (Isolate Nos: 1-9):

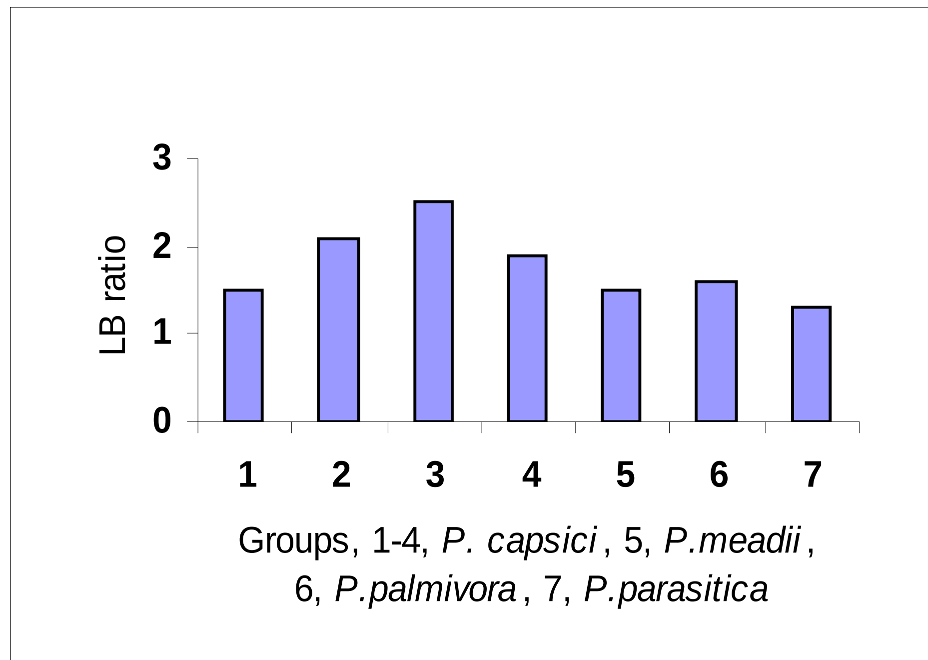
Sporangia were ovoid in shape. They were borne in umbels. Each umbel contains an average of 8-12 sporangia. The size of the sporangia was found to be uniform. The shape was also stable to this group of isolates. The nine isolates of first group were (96-8, 97-50, 97-51, 97-51, 98-7, 98-135, 98-157, 98-177, and 99-139). Their sporangial length range from (39-45.5) µm and sporangial breadth range was (23.6-30) µm. Their L/B ratio was 1.4-1.6. Prominent papilla was present and some times more than one papilla was present.

Group II (Isolate Nos: 9-15):

The isolates were umbellate. The number of sporangia per umbels was more than that of the first group. Each umbel contains an average of 14-20 sporangia. The sporangia were ovoid to

obovoid and fusiform in shape. The sporangial breadth was less compared to the first group. The sporangia were papillate. This group contains six isolates. They

Fig: 2, Sporangial LB ratio of *Phytophthora* species infecting black pepper



were (98-24, 98-76, 98-131, 98-140, 99-158 and 99-106). These isolates had a sporangial length range of (35-59.5) µm and sporangial breadth range was (18.8-27.3) µm. Their L/B ratio was 1.8-2.3. They had higher L/B ratio than first group.

Group III (Isolate Nos: 16-40):

The isolates had umbellate ontogeny. Each umbel contains an average of 14-18 sporangia. This group consists of twenty five isolates (98-49, 98-81, 98-144, 98-167, 98-172, 98-198, 99-103,

99-104, 99-125, 99-144, 99-166, 99-167, 97-45c, 98-67, 98-127, 98-162, 98-181, 98-182, 98-166, 99-162, 98-174 and 99-145). The sporangial shape was somewhat varied in this group. The elongated sporangia showed conspicuous papilla or inconspicuous papilla. The shape ranges from ovoid, obovoid, obpyriform, or with distorted shapes. Their sporangial length ranges from 42-74.6 µm and sporangial breadth range was 18-34 µm and L/B ratio was 2-3.5. This group had the higher L/B ratio than the first two groups. The isolate 98-87 with floral colony pattern had irregular sporangial ontogeny. They were poorly caducous and produce very long pedicelled sporangia. They produce sporangia with distorted shapes. Mean sporangial length was 70.2 µm and mean breadth was 26 µm and L/B ratio was 3.

Group IV (Isolate Nos: 41-51):

This group consists of 96-5, 96-13, 98-60, 98-145, 00-18, 00-40, 96-4, 97-11a, 96-11, 97-55, and 98-17. They were with uniform cotton wool like aerial mycelium. They had umbellate ontogeny and each umbel contains an average of 14-20 sporangia. They had papillate ovoid sporangia. Their mean length range was 37.8-46.5 µm and mean breadth range was 31-35 µm and their LB ratio range was 1.6-2.9 (**Table: 8**)

Table: 8, Sporangial ontogeny and sporangial morphology of *Phytophthora capsici* infecting black pepper

Sl. no	Isolate No.	Ontogeny	Mean length µm	Mean breadth µm	Mean L/B
1	96-8	Umbellate	42.8±2.2	25.8±1.2	1.56

2	97-50	Umbellate	39.2±2	25±2.2	1.5
3	97-51	Umbellate	39.2±1.9	24.9±2	1.5
4	97-52	Umbellate	39±1.9	25.2±2	1.5
5	98-7	Umbellate	40±2.8	23.6±1.3	1.5
6	98-135	Umbellate	43.6±1.8	28.3±0.9	1.5
7	98-157	Umbellate	45.5±2	30±12	1.45
8	98-177	Umbellate	40.7±2.8	23.6±1.3	1.5
9	99-139	Umbellate	39.8±1.7	24.2±1.3	1.5
10	98-24	Umbellate	35±3.8	20±2	1.8
11	98-76	Umbellate	59.5±3	27.3±2.1	2.1
12	98-131	Umbellate	48.6±3.6	22.9±2	2.2
13	98-140	Umbellate	46.5±2.9	18.8±1	2.3
14	99-158	Umbellate	52.5±3.3	26±1.7	2.0
15	99-106	Umbellate	48±4	23±1.7	2.1
16	98-49	Umbellate	63.5±3.9	29±1.4	2.9
17	98-81	Umbellate	55.5±3	27.3±2.1	2.4
18	98-144	Umbellate	48.8±4	21.5±1.4	2.2
19	98-167	Umbellate	50±4.2	20.8±1.4	2.7
20	98-172	Umbellate	60.5±3.3	26.4±1.7	2.5
21	98-198	Umbellate	47±3.6	20.8±2.1	2.2
22	99-103	Umbellate	60.6±4.7	25.6±1.2	2.5
23	99-104	Umbellate	52.8±5.6	22.2±2	2.3
24	99-125	Umbellate	60.3±5.7	23.4-1.2	3.0
25	99-144	Umbellate	67.2±3.1	23±1	2.4
26	99-166	Umbellate	54.6±5.5	21.5±1.8	2.5
27	99-167	Umbellate	57.2±6.8	23.6±2	2.5
28	97-45c	Umbellate	45.9±3.6	22.2±2	2.0
29	98-67	Umbellate	74.6±2.7	34.8±2.3	2.2
30	98-127	Umbellate	68.5±3	25.7±1.8	3.1
31	98-162	Umbellate	74.5±3.9	28.9±2.2	3.5
32	98-181	Umbellate	45.4±3.1	20.9±1.2	2.3
33	98-182	Umbellate	43±2.3	24.7±1.8	2.4
34	98-166	Umbellate	42.2±3.2	24.5±2	2.4
35	99-162	Umbellate	53±4.9	21.5±2	2.4
36	98-174	Umbellate	50.4±3.7	18.3±2.6	2.5

37	99-145	Umbellate	47.3±4.8	20.8±2.5	2.4
38	98-87	Irregular	70.2±9.4	26±1.7	3.0
39	99-91	Umbellate	43±2	22.4±0.9	2.0
40	99-124	Umbellate	42.7±2.4	21.1±1	1.9
41	96-5	Umbellate	45±3.3	21.6±1.7	2.0
42	96-13	Umbellate	37.8±2	24.6±1.2	1.6
43	98-60	Umbellate	41.6±1.5	21.5±1	2.0
44	98-145	Umbellate	43.2±3.5	21.2±1.3	2.0
45	00-18	Umbellate	46.5±2.7	22.6±1.5	2.1
46	00-40	Umbellate	44.9±2.9	23.6±1.7	2.0
47	96-4	Umbellate	45.5±3.5	37.8±1.3	2.0
48	97-11a	Umbellate	40.9±2.2	21±1.2	1.8
49	96-11	Umbellate	44.9±2.4	27.5±1.8	1.6
50	97-55	Umbellate	42.7±3.3	20.7±1.8	1.6
51	98-17	Umbellate	39.8±3.8	20.5±1.5	2.0

Statistical analysis of LB ratio of sporangia of *Phytophthora capsici* infecting black pepper

LB ratio of *Phytophthora capsici* isolates was analyzed statistically. There was significant variation between the different groups of *P. capsici* isolates. The mean value was minimum for group I and group III had maximum LB ratio. The LB ratio of group II and III were similar (**Table: 9)**

Table 9: Statistical analysis of LB ratio of sporangia of *Phytophthora capsici* infecting black pepper

LB ratio	Sum of Squares	df	Mean Square	F	Group	N	Mean value
Between Groups	7.737	3	2.579	33.584	1.00	9	1.5011a
Within Groups	3.609	47	7.680		4.00	12	1.8833b

Total	11.347	50			2.00	6	2.0833bc
					3.00	24	2.5083c

1) **Sporangial ontogeny and Morphology of *P. meadii* (Isolate Nos: 52-54)**

The three *P. meadii* isolates were 98-86, 98-90, and 98-192. Two isolates (98-86 and 98-90) had sympodial ontogeny and the isolate 98-192 had irregular ontogeny, papillate and caducous sporangia with intermediate pedicels. The sporangial shape varies from globose to ovoid. Their mean length range from 46-49.4 µm and mean breadth range was 28.3-33.5 µm and the range of LB ratio was 1.45- 1.6 (**Table: 10).**

Table: 10, Sporangial ontogeny and sporangial morphology of *Phytophthora meadii* infecting black pepper

Sl. no	**Isolate No.**	**Ontogeny**	**Mean length µm**	**Mean breadth µm**	**Mean L/B**
52	98-86	Sympodial	46±3.5	32.9±2.5	1.46
53	98-90	Sympodial	45.9±3.4	28.3±1.3	1.6
54	98-192	Irregular	49.4±1.2	33.5±1.3	1.45

2) **Sporangial ontogeny and sporangial morphology of *P. palmivora* (Isolate Nos: 55&56)**

There were two *P. palmivora* isolates, 98-1 and 99-127. They had sympodial ontogeny and caducous sporangia with small pedicels. They were papillate. The shape of the sporangia was globose or ovoid. Their sporangial length range was 45.2-45.5 µm and sporangial breadth range was 25.7-27 µm. Their LB ratio was 1.6-1.65 (**Table : 11).**

Table: 11, **Sporangial ontogeny and sporangial morphology of *Phytophthora palmivora* infecting black pepper**

Sl. no	Isolate No.	Ontogeny	Mean length µm	Mean breadth µm	Mean L/B
55	98-1	Sympodial	45.5±1.8	25.7±1.6	1.6
56	99-127	Sympodial	45.2±0.8	27±1	1.6

3) Sporangial ontogeny and sporangial morphology of *P. parasitica* (Isolate Nos:57-60)

There were four *P. parasitica* isolates 99-186, 99-188, 98-95 and 98-75. They had sympodial ontogeny and globose sporangia. . Mean length range was 32-5 - 46.8 µm and mean breadth range was 30-33.3 µm and LB ratio range was 1.2-1.5 (**Table: 12).**

Table: 12, Sporangial ontogeny and sporangial morphology of *Phytophthora parasitica* infecting black pepper

Sl. no	Isolate No.	Ontogeny	Mean length µm	Mean breadth µm	Mean L/B
57	99-186	Sympodial	32.5±2.2	31±1.8	1.25
58	99188	Sympodial	33.2±2	30.1±1.3	1.2
59	98-95	Sympodial	46.8±2.7	33.6±1.8	1.3
60	98-75	Sympodial	49.2±2	23.4±3.2	1.2

Fig: 3, Caducity of *Phytophthora* species infecting black pepper

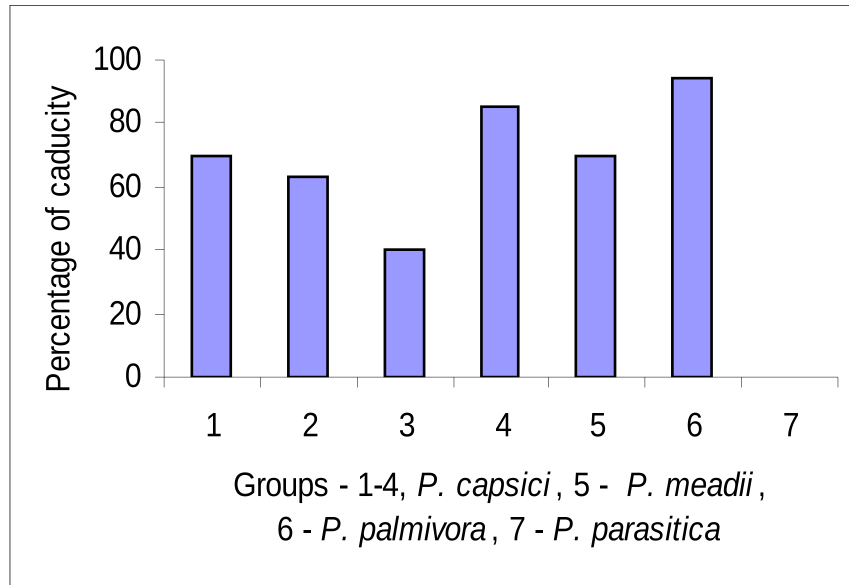
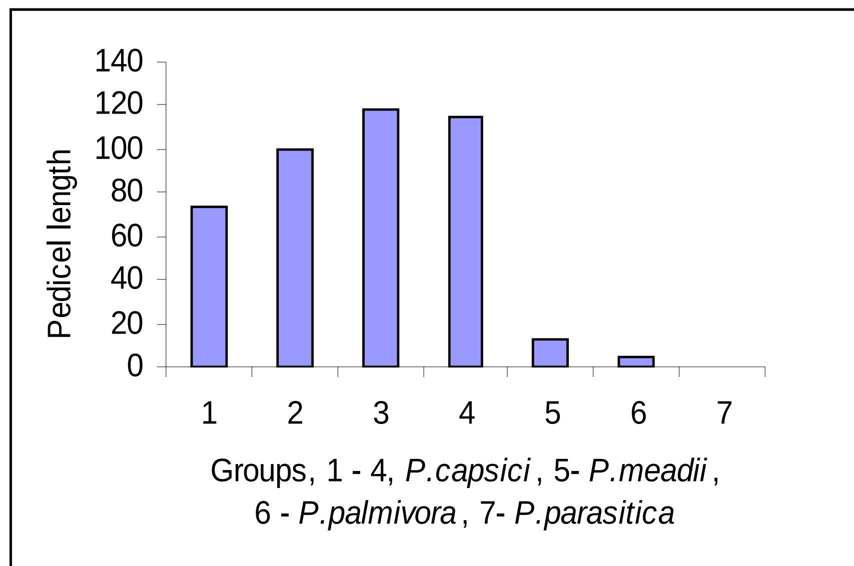


Fig: 4, Pedicel length of *Phytophthora* species infecting black pepper



3.2. Caducity and pedicel length of *Phytophthora* species infecting black pepper

The caducity and pedicel length of the isolates vary very much. There were caducous and non caducous species of *Phytophthora*. When cold shock was given sporulating discs in water for 10 minutes zoospore liberation (indirect germination) was possible. Sometimes direct germination was seen in *Phytophthora* (**Fig. 3, 4 and Plates: 6, 7**, **8** and **9)**.

1) Caducity and pedicel length of *P. capsici* (Isolate Nos: 1-51)

The isolates of the group I and II had 51 - 75 % caducity. The pedicel length range was 31-94 µm for group I and 65 - 150 µm for group II. The isolates of group III showed caducity of 25 - 50 % and pedicel length range was 10.5-13.7 µm. The isolates of group IV were found to be highly caducous 76 - 100 %. Their pedicel length range was 70 - 167 µm (**Table: 13).**

Table: 13, Caducity and pedicel length of *Phytophthora capsici* infecting black pepper

Sl. No.	Isolate no.	Caducity	Mean pedicel length µm	Range of pedicel length µm	Percentage of caducity
1	96-8	Caducous	94	78-147	+++
2	97-50	Caducous	59	40-78.6	+++
3	97-51	Caducous	58.5	39-78	+++
4	97-52	Caducous	59.7	42-79	+++
5	98-7	Caducous	93	50-117	+++
6	98-135	Caducous	31	20-78	+++
7	98-157	Caducous	84	39-110	+++
8	98-177	Caducous	92	52-117	+++
9	99-	Caducous	92	46-156	+++

	139				
10	98-24	Caducous	88	31-103	+++
11	98-76	Caducous	65	39-91	+++
12	98-131	Caducous	90.3	57.2-169	+++
13	98-140	Caducous	127	78-247	+++
14	99-158	Caducous	80	60-112	+++
15	99-106	Caducous	150	72-299	+++
16	98-49	Caducous	71	28-135	++
17	98-81	Caducous	85	39-156	++
18	98-144	Caducous	131	78-156	++
19	98-167	Caducous	132.6	57.2-160	++
20	98-172	Caducous	112	78-182	++
21	98-198	Caducous	38.7	25.9-67.3	++
22	99-103	Caducous	134	104-208	++
23	99-104	Caducous	112	70-182	++
24	99-125	Caducous	107	39-156	++
25	99-144	Caducous	138	46-156	++
26	99-166	Caducous	117	65-169	++
27	99-167	Caducous	125.3	78-182	++

28	97-45c	Caducous	86.2	37.2-150.8	++
29	98-67	Caducous	113	65-161.2	++
30	98-127	Caducous	78	39-117	++
31	98-162	Caducous	116.2	65-168	++
32	98-181	Caducous	109	52-208	++
33	98-182	Caducous	117	65-169	++
34	98-166	Caducous	110.5	39-182	++
35	99-162	Caducous	137.8	85.8-100	++
36	98-174	Caducous	121.6	78-195	++
37	99-145	Caducous	105	41.3-156	++
38	98-87	Caducous	192	180-226	+
39	99-91	Caducous	121	44-182	+
40	99-124	Caducous	136	93.6-195	+
41	96-5	Caducous	89	15-207	++++
42	96-13	Caducous	70.2	18.2-130	++++
43	98-60	Caducous	139	104-169	++++
44	98-145	Caducous	144	78-260	++++
45	00-18	Caducous	128	78-208	++++
46	00-40	Caducous	167	78-260	++++
47	96-4	Caducous	123	18-181	++++
48	97-11a	Caducous	131	62-221	++++
49	96-11	Caducous	108	64-150	++++
50	97-55	Caducous	78	44-116	++++
51	98-17	Caducous	85	36-134	++++

Percentage of caducity, + -- 25, ++ -- 26 -50, +++ -- 51 –75, ++++-- 76 – 100

Statistical Analysis of Caducity of *Phytophthora capsici* infecting black pepper

Caducity of *Phytophthora capsici* isolates was analyzed statistically. There was significant variation between different groups of *P. capsici*. The group IV had maximum caducity and group III had minimum caducity. The groups I and II, were similar in caducity. (**Table: 14)**.

Table: 14, ANOVA and DMRT of Caducity of *Phytophthora capsici* infecting black pepper

Caducity	Sum of Squares	df	Mean Square	F	Group	N	Mean value
Between Groups	14726.406	3	4908.802	92.131	3.00	25	46.4000a
Within Groups	2504.182	47	53.280		1.00	9	75.0000b
Total	17230.588	50			2.00	6	75.0000b
					4.00	11	85.7273c

Statistical analysis of pedicel length of *Phytophthora capsici* infecting black pepper

Pedicel length of *Phytophthora capsici* isolates was analyzed statistically. There was significant variation between different groups of *P. capsici* isolates. The pedicel length was minimum for group I. The group III had maximum and was similar to group IV. (**Table: 15).**

Table: 15, ANOVA and DMRT of Pedicel length of *Phytophthora capsici* infecting black pepper

Pedicel length	Sum of Squares	df	Mean Square	F	Group	N	Mean value
Between Groups	13110.992	3	4370.331	4.895	1.00	9	73.6889a
Within Groups	42858.711	48	892.890		2.00	6	100.0500ab
Total	55969.703	51			4.00	12	106.0583b
					3.00	25	117.8640b

2) Caducity and pedicel length of *P. meadii* isolates (Isolate Nos: 52-54)

The three *P. meadii* isolates showed caducity of 51-75 % and their pedicel length range was 10.5-13.7 µm. (**Table: 16**).

Table: 16, Caducity and pedicel length of *Phytophthora meadii* infecting black pepper

Sl. No.	Isolate no.	Caducity	Mean pedicel length µm	Range of pedicel length µm	Percentage of caducity
52	98-86	Caducous	10.5	5.2-13	+++
53	98-90	Caducous	13.7	5.2-26	+++
54	98-192	Caducous	13.7	18-22	+++

Percentage of caducity, + -- 25, ++ -- 26 -50, +++ -- 51 –75, ++++-- 76 – 100

3) Caducity and pedicel length of *P.palmivora* (Isolate Nos: 55&56)

The *P. palmivora* isolates showed caducity of 76-100% and pedicel length range was 4.5-5.2 µm. (**Table: 17).**

Table: 17, Caducity and pedicel length of *Phytophthora palmivora* infecting black pepper

Sl. No.	Isolate no.	Caducity	Mean pedicel length µm	Range of pedicel length µm	Percentage of caducity
55	98-1	Caducous	4.5	3.9-5.2	++++
56	99-127	Caducous	5.2	3.9-5.2	++++

Percentage of caducity, + -- 25, ++ -- 26 -50, +++ -- 51 –75, ++++-- 76 – 100

4. Caducity and pedicel length of *P. parasitica* (Isolate Nos: 57-60)

All the isolates of *P. parasitica* tested are non-caducous. (**Table: 18).**

Table: 18, Caducity and pedicel length of *Phytophthora parasitica* infecting black pepper

Sl.No.	**Isolate no.**	**Caducity**
57	99-186	Non caducous
58	99188	Non caducous
59	98-95	Non caducous
60	98-75	Non caducous

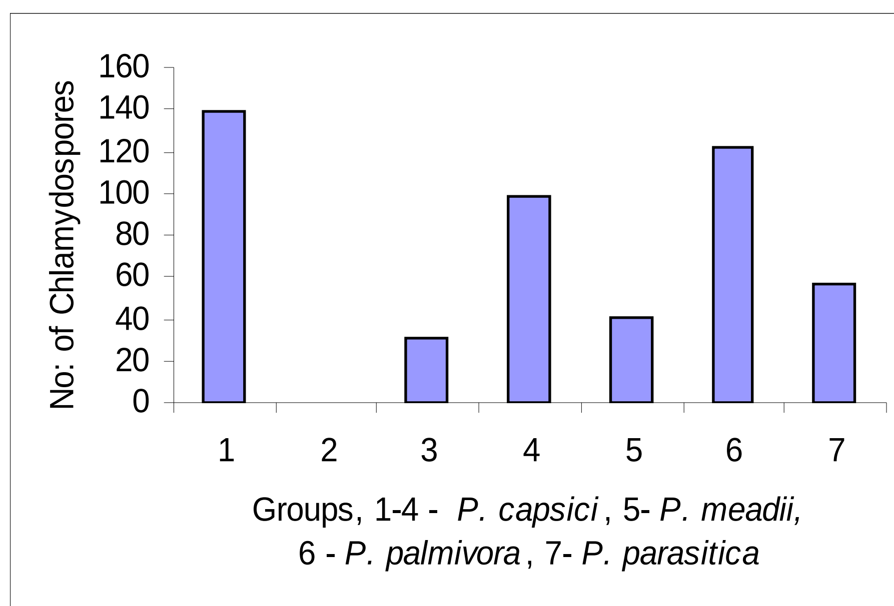
5. Chlamydospore production of *Phytophthora* species infecting black pepper

All the sixty isolates were studied for chlamydospore production. The isolates can be broadly classified into Chlamydospore producers and chlamydospore non-producers. (**Fig. 5 and** Plate**: 10).**

1. Chlamydospore production in *P. capsici* (Isolate Nos: 1-51)

All the isolates of group I, and group IV produced chlamydospores. Among group III isolates only seven isolates were producing chlamydospores and 18 isolates were not producing chlamydospores. All isolates of group II were not producing chlamydospores. Chlamydospores produced after induction was smaller and vacuolated. They had 23-25 μm diameters and an average of 26-46 chlamydospores were produced per microscopic

Fig: 5, Chlamydospore production of *Phytophthora* species infecting black pepper



field. The isolates of group II were not producing chlamydospores even after induction. Chlamydospores were induced in four group III isolates (98-167, 99-125, 99-167, and 99-145). (**Table: 19).**

Table: 19, Chlamydospore production of *Phytophthora capsici* infecting black pepper

Sl.no	Isolate no.	Natural	Induced	Chlamydospore diameter in μm	No of chlamydospores / 3 microscopic fields
1	96-8	+	+	35	135
2	97-50	+	+	33	142
3	97-51	+	+	35	140
4	97-52	+	+	35	142
5	98-7	+	+	34	167

6	98-135	+	+	33	153
7	98-157	+	+	34	137
8	98-177	+	+	35	133
9	99-139	+	+	38	94
10	98-24	-	-	No	No
11	98-76	-	-	No	No
12	98-131	-	-	No	No
13	98-140	-	-	No	No
14	99-158	-	-	No	No
15	99-106	-	-	No	No
16	98-49	-	-	No	No
17	98-81	-	-	No	No
18	98-144	+	+	24	31.5
19	98-167	-	+	24.5	46.8
20	98-172	-	-	No	No
21	98-198	-	-	No	No
22	99-103	-	-	No	No
23	99-104	-	-	No	No
24	99-125	-	+	23.5	38
25	99-144	-	-	No	No
26	99-166	-	-	No	No
27	99-167	-	+	25	26
28	97-45c	-	-	No	No
29	98-67	-	-	No	No
30	98-127	-	-	No	No
31	98-162	-	-	No	No
32	98-181	-	-	No	No
33	98-182	-	-	No	No
34	98-166	-	-	No	No
35	99-162	-	-	No	No
36	98-174	-	-	No	No
37	99-145	-	+	24	36
38	98-87	-	-	No	No
39	99-91	+	+	28	102
40	99-124	+	+	28.6	79
41	96-5	+	+	29	61.6
42	96-13	+	+	32.6	66
43	98-60	+	+	33.5	80
44	98-145	+	+	28.3	97.6
45	00-18	+	+	33	95

46	00-40	+	+	30	80
47	96-4	+	+	33	81
48	97-11a	+	+	32	106.6
49	96-11	+	+	28	131
50	97-55	+	+	28.6	130
51	98-17	+	+	30	122

Statistical Analysis of Chlamydospore production of *Phytophthora capsici* infecting black pepper

Chlamydospore production of *Phytophthora capsici* isolates was analyzed statistically. There was significant variation between different groups of *P. capsici*. Group II did not produce chlamydospores. Most of the group III also did not produce chlamydospores. Groups I and II produced chlamydospores. (**Table: 20).**

Table: 20, ANOVA and DMRT of Chlamydospore production of *Phytophthora capsici* infecting black pepper

Chlamydospore production	Sum of Squares	df	Mean Square	F	Group	N	Mean value
Between Groups	426865.234	3	142288.411	226.276	2.00	18	.0000a
Within Groups	93695.237	149	628.827		3.00	75	14.3733b
Total	520560.471	152			4.00	33	97.7576c
					1.00	27	138.7037d

2) Chlamydospore production in *P. meadii* (Isolate Nos: 52-54)

The isolates 98-86 and 98-90 were producing chlamydospores and 98-192 was not producing chlamydospores. (**Table: 21).**

Table: 21, Chlamydospore production of *Phytophthora meadii* infecting black pepper

Sl.no	Isolate no.	Natural	Induced	Chlamydospore diameter in µm	No of chlamydospores / 3 microscopic fields
52	98-86	+	+	27	76
53	98-90	+	+	27.5	60
54	98-192	-	-	No	No

3) Chlamydospore production in *P. palmivora* (Isolate No: 55&56)

The *P. palmivora* isolates produced chlamydospores. (**Table: 22).**

Table: 22, Chlamydospore production of *Phytophthora palmivora* infecting black pepper

Sl.no	Isolate no.	Natural	Induced	Chlamydospore diameter in µm	No of chlamydospores / 3 microscopic fields
55	98-1	+	+	28.6	120
56	99-127	+	+	30	123

4) Chlamydospore production in *P. parasitica* (Isolate Nos:57-60)

All the *P. parasitica* isolates produced chlamydospores. (**Table: 23).**

Table: 23, Chlamydospore production of *Phytophthora parasitica* infecting black pepper

Sl.no	Isolate no.	Natural	Induced	Chlamydospore diameter in µm	No of chlamydospore / 3 microscopic fields
57	99-186	+	+	28.6	60
58	99-188	+	+	32.5	61.6
59	98-95	+	+	33.5	48
60	98-75	+	+	33	48

6. Mating type determination of *Phytophthora* species infecting black pepper

The isolates were studied for their compatibility type. They were A1, A2, neuter or sterile in mating type. Sterile or neuter isolates did not produce oospores with A1 or A2 standards. (**Plate: 11).**

1) Mating type of *P. capsici* (Isolate Nos:1-51)

The 51 *P. capsici* isolates were grouped into 40 A1, 3 A2 and 8 neuter. Three A2 isolates were obtained from *P. capsici*. They were a leaf isolate (96-11) obtained from Sirsi, (Karnataka), a root isolate (97-55) obtained from Valparai (Tamil Nadu); and another leaf isolate (98-17) obtained from Pulpally (Wynad). Their mean oospore diameter was 26.7- 32.6 µm and antheridial length range was 12.5-13.2 µm and mean antheridial breadth was 12.4-14.5 µm. Among the *P. capsici* isolates there were eight sterile isolates (neuter). They were 96-5, 98-60, 98-145, 00-18, 00-40, 96-4, and 97-11a.

Oospore diameter did not vary much among the isolates. All of them produce amphigynous antheridia. The oogonia were spherical in shape with thick walls. The amphigynous antheridia were commonly barrel shaped and attached as collar to the oogonium sometimes they were not attached and so were usually more spherical.

(**Table: 24)**. Morphology of *Phytophthora capsici* infecting black pepper at a glance is given in (**Table: 28)**

Table: 24, Mating type determination of *Phytophthora capsici* infecting black pepper

Sl.No.	Isolate no.	Mating type	Oogonial diameter μm	Mean oospore diameter μm	Mean antheridial length μm	Mean antheridial breadth μm
1	96-8	A1	24	23.8	15.6	13.8
2	97-50	A1	25	24.0	15.2	13.2
3	97-51	A1	24	23.5	15.2	13.2
4	97-52	A1	25	24.0	15.0	13.2
5	98-7	A1	24	23.0	15.0	13.0
6	98-135	A1	21	19.5	13.7	13.3
7	98-157	A1	24	23.5	15.2	13.2
8	98-177	A1	22	20.4	17.4	14.3
9	99-139	A1	27	26.7	13.6	13.6
10	98-24	A1	31	30.0	10.0	12.0
11	98-76	A1	22	20.9	16.9	16.9
12	98-131	A1	29	28.5	13.5	14.0
13	98-140	A1	25	24.0	19.0	15.3
14	99-158	A1	25	24.2	15.6	14.3
15	99-106	A1	23	22.2	15.8	12.2
16	98-49	A1	27	26.0	14.3	13.0
17	98-81	A1	32	30.0	13.0	16.2
18	98-144	A1	25	24.0	19.0	15.3
19	98-167	A1	28	27.9	15.2	13.2

20	98-172	A1	26	25.0	15.9	13.6
21	98-198	A1	32	30.0	14.3	12.9
22	99-103	A1	27	26.0	16.6	14.3
23	99-104	A1	25	24.2	15.3	13.5
24	99-125	A1	20	18.6	16.9	13.3
25	99-144	A1	29	27.5	16.3	14.5
26	99-166	A1	25	24.0	14.5	13.2
27	99-167	A1	28	27.5	14.0	13.3
28	97-45c	A1	29	25.0	14.2	13.5
29	98-67	A1	32	31.2	11.7	14.3
30	98-127	A1	32	30.8	14.0	14.2
31	98-162	A1	31	29.0	15.0	16.0
32	98-181	A1	25	25.0	13.0	13.2
33	98-182	A1	26	25.5	10.8	16.0
34	98-166	A1	27	26.0	14.0	15.0
35	99-162	A1	28	26.2	13.4	13.3
36	98-174	A1	28	28.0	13.3	13.3
37	99-145	A1	29	28.3	13.3	13.2
38	98-87	A1	27	26.8	15.6	13.6
39	99-91	A1	22	21.0	18.2	14.8
40	99-124	A1	29	27.0	14.0	13.6
41	96-5	Neuter	0	0	0	0
42	96-13	,,	0	0	0	0
43	98-60	,,	0	0	0	0
44	98-145	,,	0	0	0	0
45	00-18	,,	0	0	0	0
46	00-40	,,	0	0	0	0
47	96-4	,,	0	0	0	0
48	97-11a	,,	0	0	0	0
49	96-11	A2	29	27.5	13.1	12.4
50	97-55	A2	33	32.6	13.2	13.2
51	98-17	A2	28	26.7	12.5	14.5

2) Mating type of *P. meadii* (Isolate Nos: 52-54)

Out of the three *P. meadii* isolates two were neuter, 98-86 and 98-90 and 98-192 was A1 isolate. It has a mean oospore diameter of 22 µm and antheridial

breadth was 13.2 µm and antheridial length 16.25 µm. (**Table: 25**).

Table: 25, Mating type determination of *Phytophthora meadii* infecting black pepper

Sl.No.	Isolate no.	Mating type	Oogonial diameter µm	Mean oospore diameter µm	Mean antheridial length µm	Mean antheridial breadth µm
52	98-86	Neuter	0	0	0	0
53	98-90	,,	0	0	0	0
54	98-192	A1	24	22.0	16.25	13.2

4) Mating type of *P. palmivora* (Isolate Nos:55&56)

Two of the *P. palmivora* isolates had A2 mating type. Their mean oospore diameter range was 23.8-29.6 µm, mean antheridial length was 15.2-16 µm, mean antheridial breadth range was 13.5-14.3 µm. (**Table : 26**).

Table: 26, Mating type determination of *Phytophthora palmivora* infecting black pepper

Sl.No.	Isolate no.	Mating type	Mean oogonial diameter µm	Mean oospore diameter µm	Mean antheridial length µm	Mean antheridial breadth µm
55	98-1	A2	26	23.8	15.2	13.5
56	99-127	A2	31	29.6	16.0	14.3

5. Mating type of *P. parasitica* (Isolate Nos: 57-60)

Out of the four *P. parasitica* isolates three A1 isolates showed mean oospore diameter range of 23.4-26.4 µm, antheridial length 14.3-16.5 µm and mean antheridial breadth range of 13.5-14.3 µm. The *P. parasitica* isolate 98-95 was neuter**.** (**Table: 27)**.

Table: 27, Mating type determination of *Phytophthora parasitica* infecting black pepper

Sl.No.	**Isolate no.**	**Mating type**	**Oogonial diameter µm**	**Mean oospore diameter µm**	**Mean antheridial length µm**	**Mean antheridial breadth µm**
57	99-186	A1	24	23.4	14.3	14.3
58	99188	A1	27	26.4	16.5	13.5
59	98-95	Neuter	0	0	0	0
60	98-75	A1	29	27.3	14	14.3

Table: 28, Morphological groups of *Phytophthora capsici* infecting black pepper at a glance

Groups	Isolates	Ontogeny	Sporangial shape	LB ratio	Pedicel length	Caducity	Chlamydospore production	Mating type	Races
1	1-9 (Table 1)	Umbellate	Ovoid	1.4 - 1.6	31-94 µm	51-75 %	Produced	A1	R1(7) R2(2)
2	10-15	Umbellate	Ovoid to obovoid and fusiform	1.8 - 2.3	65-150 µm	51-75 %	Not produced	A1	R1(6)
3	16-40	Umbellate	Ovoid to obovoid, obpyriform and distorted shapes	2 - 3.5	38.7-192 µm	25-50 %	Produced (7) Not produced (18)	A1	R2(23) R4 (1) R5(1)
4	41-51	Umbellate	Ovoid to obovoid and fusiform	1.6 - 2.9	70.2-167 µm	76-100 %	Produced	A2(3) Neuter (8)	R3 (11)

R1- Race 1, R2 –Race 2, R3 – Race 3, R4 –Race 4 and R 5 –Race 5.

7. Biology of *Phytophthora* species infecting black pepper

The variation in the biology of *Phytophthora* species was done. The effect of temperature, pH, and media was studied.

7.1. Effect of temperature on *Phytophthora* species infecting black pepper

The reaction of the *Phytophthora* species varied in relation to temperature. Mycelial growth, sporulation, zoospore liberation (indirect germination) and zoospore germination of different species varied very much. (**Fig. 6 and** Plate**: 12)**.

7.1.1. Effect of temperature on growth of *Phytophthora* species infecting black pepper on carrot agar

***P. capsici* (Isolate Nos: 1-51)**

The isolates were grouped into four based on their morphology. Considerable variation was obtained at the four different temperatures. Growth at 48 and 72 hrs intervals were measured radially. 10 ^{0}C was unfavourable for growth and very little growth was noticed at this temperature. 20 ^{0}C and 28 ^{0}C were favourable for growth. 28 ^{0}C was the optimum temperature for growth. 35 ^{0}C was found to be unfavorable for growth. Some isolates cannot grow at this temperature. For all the isolates maximum radial growth was obtained at 28 ^{0}C and at 72 hrs time. The isolates of group I, II and III, grew at 35 ^{0}C. The isolates of group IV were not growing at 35 ^{0}C. (**Table: 29).**

Table: 29, Effect of temperature on growth of *Phytophthora capsici* infecting black pepper on carrot agar

Sl.no	Isolate No.	Radial growth in mm			
		10 ^{0}C		20 ^{0}C	
		48 hr	**72 hr**	**48 hr**	**72 hr**
1	96-8	2.6	3.3	19.0	25.0
2	97-50	2.6	5.6	13.3	19.6
3	97-51	4.6	9.3	13.3	21.6
4	97-52	4.6	8.3	14.0	19.6
5	98-7	4.6	7.0	15.0	22.3
6	98-135	4.6	8.3	12.0	17.6
7	98-157	5.3	8.3	20.0	25.0
8	98-177	5.0	9.6	14.6	20.0
9	99-139	5.6	10	11.3	18.0
10	98-24	1.6	3.3	9.6	13.6
11	98-76	1.6	3.6	9.0	13.0
12	98-131	0.0	4.6	9.0	11.0
13	98-140	3.6	5.3	11.0	16.6
14	99-158	2.3	4.3	5.6	11.0
15	99-106	3.0	4.3	10.6	16.3
16	98-49	4.2	10.2	11.6	19.2
17	98-81	4.6	5.3	15.0	22.0
18	98-144	0.0	6.0	20.3	27.6
19	98-167	4.3	9.0	11.3	21.0
20	98-172	3.6	7.4	12.3	20.2
21	98-198	0.0	0.0	21.6	26.2
22	99-103	4.5	7.3	10.5	14.2
23	99-104	4.3	8.0	10.3	12.3
24	99-125	6.2	9.0	15.2	23.5
25	99-144	5.3	11.0	14.5	21.0
26	99-166	4.3	10.0	12.0	16.3
27	99-167	4.3	12.0	11.0	20.3
28	97-45c	4.2	9.0	19.3	26.0
29	98-67	2.6	3.6	11.0	15.6
30	98-127	4.3	4.6	10.3	15.3
31	98-162	5.0	9.6	12.3	18.6
32	98-181	2.3	4.3	10.3	15.3
33	98-182	1.6	3.3	11.3	16.0

34	98-166	6.0	10.0	11.6	16.6	
35	99-162	3.0	4.2	11.0	15.6	
36	98-174	6.0	8.3	12.0	20.3	
37	99-145	4.0	10.0	8.6	16.3	
38	98-87	5.3	6.6	11.0	19.3	
39	99-91	4.6	10.0	13.0	21.3	
40	99-124	7.0	9.6	13.0	17.0	
41	96-5	1.6	2.5	20.2	26.2	
42	96-13	0.0	2.5	19.0	25.0	
43	98-60	1.3	4.2	21.0	27.0	
44	98-145	2.2	2.5	21.0	26.3	
45	00-18	1.3	2.6	20.0	25.0	
46	00-40	1.6	2.6	19.6	25.0	
47	96-4	1.6	2.3	20.3	26.0	
48	97-11a	4.6	8.3	18.0	22.0	
49	96-11	0.0	3.8	26.0	30.3	
50	97-55	3.6	5.3	17.3	25.3	
51	98-17	1.6	3.5	18.0	25.0	

Statistical Analysis of the effect of temperature on growth infecting black pepper on carrot agar of *Phytophthora capsici*

The effect of temperature on growth of *Phytophthora capsici* isolates at different temperature was analyzed statistically. There was significant variation between different groups of *P. capsici* isolates. Minimum growth was showed by group II and maximum growth was showed by group I isolates. Growth was similar in isolates of group I, III and IV isolates. (**Table: 30)**.

Table: 30, ANOVA and DMRT of the effect of temperature on growth *Phytophthora capsici* infecting black pepper on carrot agar

Temperature and growth	Sum of Squares	df	Mean Square	F	Group	N	Mean value
Between Groups	2960.283	3	986.761	10.382	2.00	144	10.6285a
Within Groups	115954.183	1220	95.044		4.00	288	14.3924b
Total	118914.465	1223			3.00	576	15.5226b
					1.00	216	15.5509b

***P. meadii* (Isolate Nos: 52-54):**

Growth was negligible at 10 ^{0}C and 35 ^{0}C**.** Optimum growth was at 28 ^{0}C. *P .meadii* isolates grew well at 20 ^{0}C and 28 ^{0}C. (**Table: 31)**.

Table 31, Effect of temperature in growth on carrot agar of *Phytophthora meadii* infecting black pepper

Sl. no	Isolate No.	Radial growth in mm			
		10 ^{0}C		20 ^{0}C	
		48 hr	72 hr	48 hr	72 hr
52	98-86	2.3	6.0	9.0	13.3
53	98-90	2.3	4.6	9.2	15.6
54	98-192	2.3	2.6	12.3	20.3

***P. palmivora* (Isolate Nos: 55&56):**

Optimum mycelial growth occurred at **28** ^{0}C. No growth at 10^{0}C and negligible growth at 35 ^{0}C. (**Table: 32)**.

Table 32, Effect of temperature on growth of *Phytophthora palmivora* infecting black pepper on carrot agar

Sl. no	Isolate No.	Radial growth in mm			
		10 ^{0}C		20 ^{0}C	
		48 hr	72 hr	48 hr	72 hr
55	98-1	0.0	0.0	17.3	21.0
56	99-127	0.0	0.0	17.6	20.0

***P. parasitica* (Isolate Nos: 57-60):**

All the *P. parasitica* isolates grew well at 35 ^{0}C. Minimum growth was at 10 ^{0}C. Optimum growth occurred at 28 ^{0}C. (**Table: 33).**

Table 33, Effect of temperature on growth of *Phytophthora parasitica* infecting black pepper on carrot agar

Sl. no	Isolate No.	Radial growth in mm			
		10 ^{0}C		20 ^{0}C	
		48 hr	72 hr	48 hr	72 hr
57	99-186	2.6	4.3	14.0	19.6
58	99188	2.6	3.6	13.3	19.0
59	98-95	2.3	3.3	17.6	23.3
60	98-75	4.3	4.3	11.0	18.0

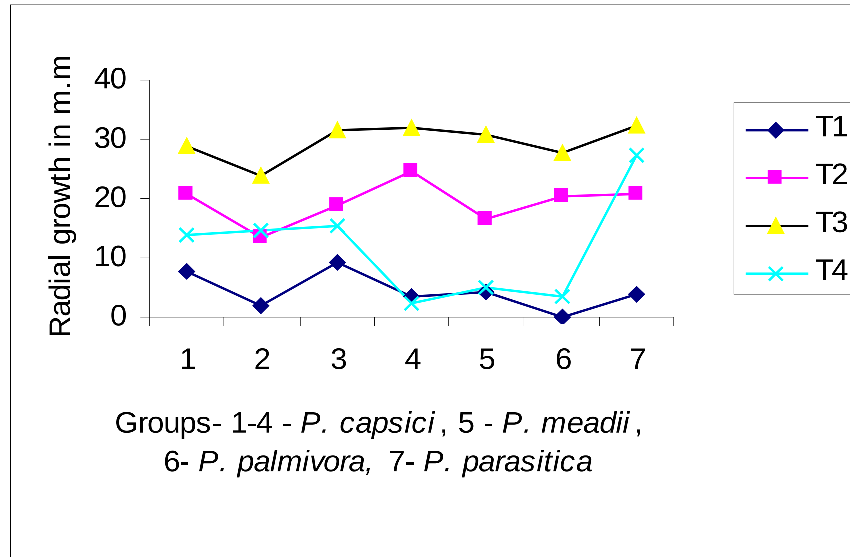
7.1.2. Effect of temperature on sporulation of *Phytophthora* species infecting black pepper

Sporulation of the 60 isolates was studied at 4 different temperatures, and it was found that maximum sporulation was at 20 ^{0}C followed by 28 ^{0}C. But minimum sporulation was at 35 ^{0}C (**Fig.7).**

***P.capsici* (Isolate Nos: 1-51)**

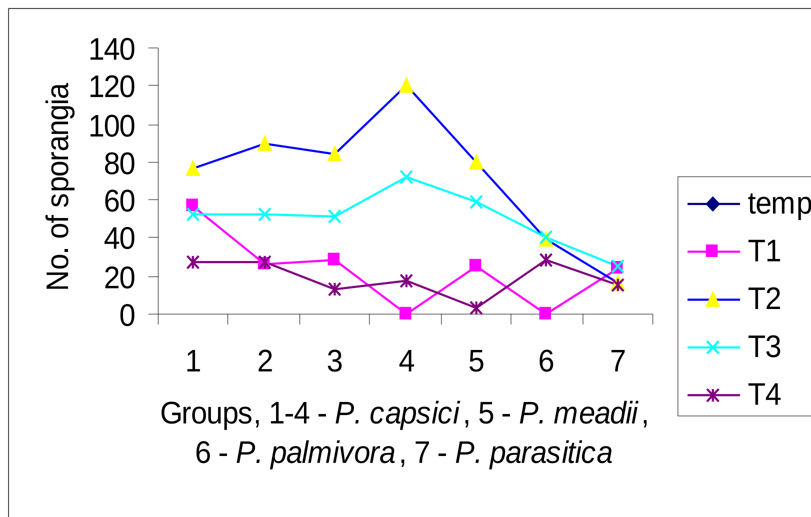
The group I isolates sporulated at all the four temperatures. And maximum sporulation was at 20 ^{0}C. At 35 ^{0}C sporulation was minimum. The isolates of group II and group I were similar in sporulation. But the isolate 98-131 did not sporulate at 10 ^{0}C and 99-158 at 35 ^{0}C. The isolates of group III showed the same sporulation tendency. The isolate 98-144 did not sporulate at 10 ^{0}C and 99-103 at 35 ^{0}C. The group IV isolates showed a different tendency that none of them sporulated at 10 ^{0}C and their sporulation was less at 35 ^{0}C. (**Table: 34)**.

Fig: 6, Effect of temperature on growth of *Phytophthora* species infecting black pepper



T1 - 10^{0}C, T2 - 20^{0}C, T3 - 28^{0}C, T4 - 35^{0}C

Fig: 7, Effect of temperature on sporulation of *Phytophthora* species infecting black pepper



T1 - 10^{0}C, T2 - 20^{0}C, T3 - 28^{0}C, T4 - 35^0

Table: 34, **Effect of temperature on sporulation of *Phytophthora capsici* infecting black pepper**

Sl.No	Isolate No.	Mean no. of sporangia produced / 3 microscopic fields			
		10 ^{0}C	**20 ^{0}C**	**28 ^{0}C**	**35 ^{0}C**
1	96-8	46.0	66	50	20.6
2	97-50	46.3	68.3	54.6	20
3	97-51	47.3	58.3	54.3	24.3
4	97-52	46.6	62	42	25.3
5	98-7	56.3	82	45.3	26
6	98-135	86.3	81	62.6	32.6
7	98-157	74.3	98	72.3	32
8	98-177	60.3	94.6	63.3	30
9	99-139	44.6	82.3	24	34.3
10	98-24	24.3	70	47.3	30
11	98-76	46.3	84.3	47.6	27
12	98-131	0	116.6	79.3	32.6
13	98-140	46.3	52.3	27.6	31
14	99-158	20	112	47	0
15	99-106	20	105	63.6	42.3
16	98-49	39.3	64	49	15
17	98-81	51	179.3	50.3	20
18	98-144	0	144.3	60.6	20
19	98-167	84	133.6	34	18.6
20	98-172	60.3	155	33.3	11.6
21	98-198	48.3	157.6	45	17.6
22	99-103	78.3	195	46.6	0
23	99-104	51.3	115	54.3	16.6
24	99-125	55.6	64.3	42.3	11
25	99-144	56	70	61	19
26	99-166	49	72.3	45	16
27	99-167	49.6	78.3	48.6	24
28	97-45c	36	61.6	54	15
29	98-67	21	66	21	0
30	98-127	16.3	58.6	47.3	12.6
31	98-162	15	34.6	44.6	7
32	98-181	16	41.3	45	11
33	98-182	36	68.6	41.6	19.3

34	98-166	31	88.3	65.3	21
35	99-162	24.3	75	40	20.6
36	98-174	21	97	57.6	22.6
37	99-145	73.3	112.3	81.6	22
38	98-87	10.6	18	34	20.6
39	99-91	0	95.6	55.3	0
40	99-124	0	66.6	57.6	0
41	96-5	0	114	62	18.6
42	96-13	0	126.3	60.6	16
43	98-60	0	161.6	61	15
44	98-145	0	112.6	71	17
45	00-18	0	113.3	81	20
46	00-40	0	123.6	76	21
47	96-4	0	123	66.6	21.3
48	97-11a	0	120	76.6	18.6
49	96-11	0	121.6	86.6	16.6
50	97-55	0	103	81.3	12.6
51	98-17	0	105.6	70	10.6

Statistical Analysis of the effect of temperature on sporulation of *Phytophthora capsici* infecting black pepper

Temperature and sporulation of *Phytophthora capsici* isolates at different temperatures was analyzed statistically. Isolates of group II had minimum sporulation. Group I had maximum sporulation and it was almost similar to groups IV, II and II. **(Table: 35)**.

Table: 35, ANOVA and DMRT of the effect of temperature on sporulation of *Phytophthora capsici* infecting black pepper

Temperature and sporulation	**Sum of Squares**	**df**	**Mean Square**	**F**	**Group**	**N**	**Mean value**
Between Groups	2366.437	3	788.812	.535	2.00	72	48.8750a
Within Groups	896374.208	608	1474.300		3.00	288	48.8889a
Total	898740.645	611			4.00	144	50.7778a
					1.00	10	54.1667a

						8	

***P. meadii* (Isolate Nos: 52-54):**

Among the *P. meadii* isolates 98-86 and 98-192 did not sporulate at 35 ^{0}C and the isolate 98-90 sporulated but very few sporangia were produced. (**Table: 36)**.

Table: 36, Effect of temperature on sporulation of *Phytophthora meadii* infecting black pepper

Sl.No	Isolate No.	Mean no. of sporangia produced /3 microscopic fields			
		10 ^{0}C	**20 ^{0}C**	**28 ^{0}C**	**35 ^{0}C**
52	98-86	23.3	63.6	78.3	0
53	98-90	33.3	109	67.3	9
54	98-192	20	67	31.6	0

***P. palmivora* (Isolate Nos: 55&56):**

The *P. palmivora* isolates did not sporulate at 10 ^{0}C. Maximum sporulation occurred at 20 ^{0}C. (**Table: 37)**.

Table: 37, Effect of temperature on sporulation of *Phytophthora palmivora* infecting black pepper

Sl.No	Isolate No.	Mean no. of sporangia produced / 3 microscopic fields			
		10 ^{0}C	**20 ^{0}C**	**28 ^{0}C**	**35 ^{0}C**
55	98-1	0	66.3	27.6	33.6
56	99-127	0	75	53.3	23

***P. parasitica* (Isolate Nos: 57-60):**

The *P. parasitica* isolates sporulated at all the four selected temperatures. The isolate 98-75 did not sporulate at 35 ^{0}C. (**Table: 38)**.

Table: 38, Effect of temperature on sporulation of *Phytophthora parasitica* infecting black pepper

Sl.No	Isolate No.	Mean no. of sporangia produced/ 3 microscopic fields			
		10 ^{0}C	**20 ^{0}C**	**28 ^{0}C**	**35 ^{0}C**
57	99-186	29.7	163	27.6	16.3
58	99188	20	163	23.3	14.6
59	98-95	22	183	24.6	15.6
60	98-75	36	86.3	66.6	0

7.1.3. Effect of temperature on indirect germination (zoospore liberation) of *Phytophthora* species infecting black pepper

Zoospore release was maximum in the temperature 20 ^{0}C followed by 28 ^{0}C and at 35 ^{0}C no zoospores released. 10 ^{0}C was also not a favourable temperature and minimum number of zoospores was released at this temperature (**Fig. 8).**

P. capsici **(Isolate Nos: 1-51):**

The group I isolates had maximum zoospore liberation at 20 ^{0}C. Minimum zoospore liberation was at 10 ^{0}C and no zoospore liberation was at 35 ^{0}C. The group II showed similar trend but 98-131 did not liberate zoospores at 10 ^{0}C. Among the group III isolates 98-144 was not releasing zoospores at 10 ^{0}C. The isolates of group IV were not liberating their zoospores at 10 ^{0}C and 35 ^{0}C. (**Table: 39).**

Table: 39, Effect of temperature on indirect germination (zoospore liberation) of *Phytophthora capsici* infecting black pepper

Sl. No.	Isolate No.	Mean no. of sporangia with indirect germination/ 3 microscopic fields			
		10 ^{0}C	**20 ^{0}C**	**28 ^{0}C**	**35 ^{0}C**
1	96-8	13.3	54.6	71	0
2	97-50	15	81.6	75	0
3	97-51	13.3	99.3	91.6	0
4	97-52	16.3	101.6	90	0
5	98-7	11.6	81.6	73.3	0
6	98-135	12.6	96	86.6	0
7	98-157	16.6	100	88.3	0
8	98-177	16.6	81.6	68.3	0
9	99-139	9.3	75	56.6	0
10	98-24	5.3	61.6	55	0
11	98-76	5	61.6	49.3	0
12	98-131	0	51.6	41.6	0
13	98-140	5.3	40	33.3	0
14	99-158	2.3	53.3	44.3	0
15	99-106	7.6	47.6	43.3	0
16	98-49	9.3	55	48.3	0
17	98-81	8.3	63.3	53.3	0
18	98-144	0	45	40	0
19	98-167	5	51.6	48.6	0
20	98-172	6	61.6	51	0
21	98-198	7	65	54.3	0
22	99-103	7.6	80	70	0
23	99-104	7	80	65	0
24	99-125	11.6	81.6	73.3	0
25	99-144	7.6	58.3	53.3	0
26	99-166	6	75	63.3	0
27	99-167	5.6	88.3	78.3	0
28	97-45c	5	83.3	76.6	0
29	98-67	6	83.3	73.3	0

30	98-127	5	86	80	0
31	98-162	4.3	86	69.3	0
32	98-181	5.6	92.6	76.6	0
33	98-182	5	98.3	63.3	0
34	98-166	4.6	95.6	86	0
35	99-162	5.3	86.6	78	0
36	98-174	5.6	91.6	85.3	0
37	99-145	7	100	87	0
38	98-87	7.3	83.3	82.6	0
39	99-91	0	35	32	0
40	99-124	0	36.6	33.3	0
41	96-5	0	45	38.3	0
42	96-13	0	31.6	28.3	0
43	98-60	0	46.6	37.6	0
44	98-145	0	40	36.6	0
45	00-18	0	41.6	35	0
46	00-40	0	50	43.3	0
47	96-4	0	42.6	39.3	0
48	97-11a	0	38.3	34	0
49	96-11	0	39	35	0
50	97-55	0	39.3	29.3	0
51	98-17	0	31.6	27.6	0

Statistical Analysis of the effect of temperature on indirect germination (zoospore liberation) of *Phytophthora capsici* infecting black pepper

Temperature and zoospore liberation of *Phytophthora capsici* isolates at different temperatures was analyzed statistically. There was significant variation between different groups of *P. capsici* isolates. Group IV had minimum zoospore liberation. Group I had maximum zoospore liberation. **(Table: 40)**

Table: 40, ANOVA and DMRT of the effect of temperature on indirect germination (zoospore liberation) of *Phytophthora capsici* infecting black pepper

Temperature and zoospore	**Sum of Square**	**df**	**Mean Square**	**F**	**Group**	**N**	**Mean value**

liberation	s						
Between Groups	50995.545	3	16998.515	15.529	4.00	144	18.8056a
Within Groups	665518.455	608	1094.603		2.00	72	25.6528a
Total	716514.000	611			3.00	288	37.0382b
					1.00	108	44.1667b

P. meadii **(Isolate Nos: 52-54):**

The *P. meadii* isolates liberated zoospores at 10 ^{0}C, 20 ^{0}C, and 28 ^{0}C. They did not liberate zoospores at 35 ^{0}C. (**Table: 41).**

Table: 41, Effect of temperature on indirect germination (zoospore liberation) of *Phytophthora meadii* infecting black pepper

Sl. No.	Isolate No.	Mean no. of sporangia with indirect germination/ 3 microscopic fields			
		10 ^{0}C	**20 ^{0}C**	**28 ^{0}C**	**35 ^{0}C**
52	98-86	6.3	40	38	0
53	98-90	7.3	41.6	38.6	0
54	98-192	8.3	27.6	22.6	0

P. palmivora **(Isolate Nos: 55&56):**

P. palmivora isolates liberated zoospores at 20 ^{0}C, and 28 ^{0}C. No zoospore liberation was at 10 ^{0}C and 35 ^{0}C. Maximum zoospore liberation occurred at 20 ^{0}C. (**Table: 42).**

Table: 42, Effect of temperature on indirect germination (zoospore liberation) of *Phytophthora palmivora* infecting black pepper

Sl. No.	Isolate No.	Mean no. of sporangia with indirect germination/ 3 microscopic fields

		10 ^{0}C	**20 ^{0}C**	**28 ^{0}C**	**35 ^{0}C**
55	98-1	0	113.3	90	0
56	99-127	0	118.3	107.3	0

P. parasitica **(Isolate Nos: 57-60):**

The *P. parasitica* isolates liberated zoospores at 10 ^{0}C, 20 ^{0}C, and 28 ^{0}C. Maximum zoospore liberation was at 20 ^{0}C. (**Table: 43).**

Table: 43, Effect of temperature on indirect germination (zoospore liberation) of *Phytophthora parasitica* infecting black pepper

Sl. No.	Isolate No.	Mean no. of sporangia with indirect germination/ 3 microscopic fields			
		10 ^{0}C	**20 ^{0}C**	**28 ^{0}C**	**35 ^{0}C**
57	99-186	7.6	31.6	31	0
58	99188	4.5	34.3	29.3	0
59	98-95	5	34.3	28.3	0
60	98-75	5	71.6	62.6	0

7.1.4. Effect of temperature on zoospore germination of *Phytophthora* species infecting black pepper

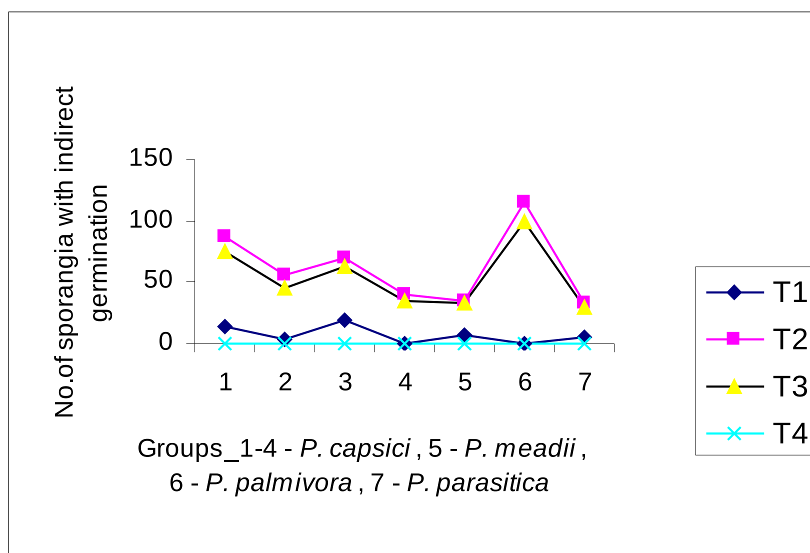
All the 60 isolates were studied for zoospore germination. 20 ^{0}C was favourable for zoospore germination followed by 28 ^{0}C (**Fig. 9).**

***P. capsici* (Isolate Nos: 1-51):**

Zoospore germination was not possible at 10 ^{0}C and 35 ^{0}C. All the groups showed similar trend in zoospore germination. The group I had maximum zoospore germination at 20 ^{0}C. Zoospore germination was less in group II while comparing with the group I. Group III was similar to the group I. The zoospore germination was less in the group IV while comparing with other groups of *P. capsici* (**Table : 44).**

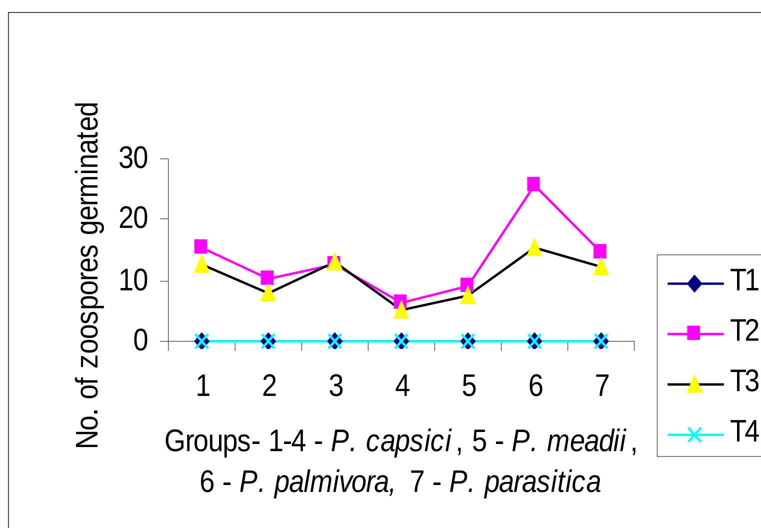
Fig: 8, Effect of temperature on indirect germination (zoospore liberation)

of *Phytophthora* species infecting black pepper



T1 - 10^{0}C, T2 - 20^{0}C, T3 - 28^{0}C, T4 - 35^{0}C

Fig: 9, Effect of temperature on zoospore germination of *Phytophthora* species infecting black pepper



T1 - 10^{0}C, T2 - 20^{0}C, T3 - 28^{0}C, T4 - 35^{0}C

Table: 44, Effect of temperature on zoospore germination of *Phytophthora capsici* infecting black pepper

Sl no.	Isolate No.	Mean no. of zoospores germinated / 3 microscopic fields			
		10 ^{0}C	**20 ^{0}C**	**28 ^{0}C**	35 **^{0}C**
1	96-8	0	15	12.3	0
2	97-50	0	17.6	15	0
3	97-51	0	14.6	12	0
4	97-52	0	15.6	12	0
5	98-7	0	13.3	11	0
6	98-135	0	12.3	10	0
7	98-157	0	14	11.6	0
8	98-177	0	18.3	14	0
9	99-139	0	12.6	12.6	0
10	98-24	0	10	8	0
11	98-76	0	8.3	7	0
12	98-131	0	9	7.6	0
13	98-140	0	7.6	6.3	0
14	99-158	0	9	6.6	0
15	99-106	0	17	11.6	0
16	98-49	0	21.3	15.6	0
17	98-81	0	17.6	14	0
18	98-144	0	16	14	0
19	98-167	0	17.6	16.3	0
20	98-172	0	19	14.3	0
21	98-198	0	19.3	15.3	0
22	99-103	0	22.3	16	0
23	99-104	0	21	15.6	0
24	99-125	0	16.3	11	0
25	99-144	0	16.6	12	0
26	99-166	0	17.6	12.6	0
27	99-167	0	20	13.3	0
28	97-45c	0	19.3	13.3	0
29	98-67	0	18.6	12.3	0
30	98-127	0	17	13.6	0
31	98-162	0	16.3	13.3	0
32	98-181	0	20	15.3	0
33	98-182	0	18	16	0
34	98-166	0	18	16	0

35	99-162	0	20.3	11.6	0
36	98-174	0	14.3	15	0
37	99-145	0	11.6	12.3	0
38	98-87	0	11.6	9	0
39	99-91	0	10	6.3	0
40	99-124	0	10.3	9	0
41	96-5	0	6.3	5.3	0
42	96-13	0	6.3	5.3	0
43	98-60	0	5.3	5	0
44	98-145	0	6	4	0
45	00-18	0	6	5.6	0
46	00-40	0	6.6	4.3	0
47	96-4	0	6.3	5.3	0
48	97-11a	0	5.3	4.3	0
49	96-11	0	6	5.3	0
50	97-55	0	6.6	5.3	0
51	98-17	0	7.3	6.6	0

Statistical Analysis of the effect of temperature on zoospore germination of *Phytophthora capsici* infecting black pepper

Temperature and zoospore germination of *Phytophthora capsici* isolates at different temperatures was analyzed statistically. Minimum germination was in group IV and it was similar to group II. Maximum germination was in group III (**Table: 45).**

Table: 45, ANOVA and DMRT of the effect of temperature on zoospore germination of *Phytophthora capsici* infecting black pepper

Temperature and zoospore germination	Sum of Squares	df	Mean Square	F	Group	N	Mean value
Between Groups	2467.662	3	822.554	17.361	4.00	144	3.0069a
Within Groups	28806.809	608	47.380		2.00	72	4.5139a
Total	31274.471	611			1.00	108	6.9722b
					3.00	288	7.7951b

***P. meadii* (Isolate Nos: 52-54):**

The zoospore germination was less while comparing with the *P. capsici* isolates. No germination occurred at 10 ^{0}C and 35 ^{0}C. Maximum germination was at 20 ^{0}C and germination was possible at 28 ^{0}C. (**Table: 46).**

Table: 46, Effect of temperature on zoospore germination of *Phytophthora meadii* infecting black pepper

Sl no.	Isolate No.	Mean no. of zoospores germinated / 3 microscopic fields			
		10 ^{0}C	**20 ^{0}C**	28 ^{0}C	35 ^{0}C
52	98-86	0	10.3	9	0
53	98-90	0	9.6	8.3	0
54	98-192	0	7.6	5.3	0

***P. palmivora* (Isolate Nos: 55&56):**

Zoospore germination was high while comparing with *P. capsici*. No zoospore germination occurred at 10 ^{0}C and 35 ^{0}C. (**Table: 47).**

Table: 47, Effect of temperature on zoospore germination of *Phytophthora palmivora* infecting black pepper

Sl no.	Isolate No.	Mean no. of zoospores germinated / 3 microscopic fields			
		10 ^{0}C	**20 ^{0}C**	**28 ^{0}C**	**35 ^{0}C**
55	98-1	0	24.3	14.3	0
56	99-127	0	27	16.3	0

***P. parasitica* (Isolate Nos: 57-60):**

No zoospore germination occurred at 10 ^{0}C and 35 ^{0}C. Maximum zoospore germination occurred at 20 ^{0}C. Zoospore germination was less while comparing with *P. capsici*. (**Table: 48).**

Table: 48, Effect of temperature on zoospore germination of *Phytophthora parasitica* infecting black pepper

Sl no.	Isolate No.	Mean no. of zoospores germinated / 3 microscopic fields			
		10 ^{0}C	**20 ^{0}C**	**28 ^{0}C**	**35 ^{0}C**
57	99-186	0	14.6	12.3	0
58	99-188	0	11	11.3	0
59	98-95	0	14.3	12.6	0
60	98-75	0	12	12	0

7.2. Effect of pH on *Phytophthora* species infecting black pepper

The variation in the growth, sporulation, and zoospore liberation and zoospore germination of *Phytophthora* was studied.

7.2.1. Effect of pH on mycelial growth on GYP medium of *Phytophthora* species infecting black pepper

Phytophthora species varied in their mycelial growth at different pH. The pH 5 or 7 was optimum for most isolates. The pH 2.5 was not favourable for most of the isolates (**Fig. 10)**.

***P. capsici* (Isolate Nos: 1-51)**

The entire group I isolates grew at pH 2.5, 5, 7, and 9. Minimum growth was observed at pH 2.5 followed by pH 9 and maximum mycelial weight was given by pH 5. The isolates of group II had a different tendency; at pH 2.5 no mycelial growth was observed and minimum growth was observed at pH 9. Maximum growth was at pH 5 followed by pH 7. The isolates of group III also cannot grow at pH 2.5. The isolates of group IV had a different trend. They cannot grow at pH 2.5 and minimum growth was at pH 9. (**Table: 49)**.

Table: 49, Effect of pH on mycelial growth on GYP medium of *Phytophthora capsici* infecting

black pepper

Sl. No.	Isolate No.	Mean mycelial weight in mg.			
		pH 2.5	**pH 5**	**pH 7**	**pH 9**
1	96-8	0.13	0.26	0.23	0.21
2	97-50	0.12	0.28	0.25	0.16
3	97-51	0.14	0.26	0.26	0.15
4	97-52	0.13	0.27	0.25	0.17
5	98-7	0.14	0.28	0.26	0.13
6	98-135	0.11	0.28	0.27	0.14
7	98-157	0.15	0.26	0.23	0.21
8	98-177	0.15	0.26	0.20	0.17
9	99-139	0.09	0.27	0.23	0.11
10	98-24	0.00	0.25	0.22	0.14
11	98-76	0.00	0.34	0.22	0.11
12	98-131	0.00	0.20	0.19	0.11
13	98-140	0.00	0.33	0.28	0.15
14	99-158	0.00	0.25	0.19	0.11
15	99-106	0.00	0.28	0.21	0.13
16	98-49	0.00	0.27	0.28	0.16
17	98-81	0.00	0.23	0.26	0.15
18	98-144	0.00	0.25	0.26	0.17
19	98-167	0.00	0.24	0.26	0.14
20	98-172	0.00	0.22	0.23	0.17
21	98-198	0.00	0.24	0.24	0.23
22	99-103	0.00	0.18	0.19	0.02
23	99-104	0.00	0.16	0.20	0.05
24	99-125	0.00	0.23	0.24	0.04
25	99-144	0.00	0.24	0.26	0.17
26	99-166	0.00	0.24	0.29	0.15
27	99-167	0.00	0.31	0.32	0.08
28	97-45c	0.00	0.28	0.29	0.24
29	98-67	0.00	0.22	0.22	0.20
30	98-127	0.00	0.20	0.21	0.15
31	98-162	0.00	0.22	0.23	0.22
32	98-181	0.00	0.20	0.21	0.05
33	98-182	0.00	0.23	0.25	0.10
34	98-166	0.00	0.26	0.27	0.18
35	99-162	0.00	0.28	0.23	0.13
36	98-174	0.00	0.28	0.28	0.04
37	99-145	0.00	0.25	0.26	0.16

38	98-87	0.00	0.20	0.20	0.09
39	99-91	0.11	0.24	0.22	0.20
40	99-124	0.00	0.22	0.25	0.15
41	96-5	0.00	0.28	0.28	0.11
42	96-13	0.00	0.42	0.29	0.12
43	98-60	0.00	0.36	0.26	0.13
44	98-145	0.00	0.33	0.27	0.13
45	00-18	0.00	0.32	0.29	0.10
46	00-40	0.00	0.31	0.28	0.12
47	96-4	0.00	0.31	0.23	0.12
48	97-11a	0.00	0.32	0.25	0.12
49	96-11	0.00	0.41	0.31	0.13
50	97-55	0.00	0.41	0.32	0.11
51	98-17	0.00	0.44	0.33	0.11

Statistical Analysis of the effect of pH on mycelial growth on GYP medium of *Phytophthora capsici* infecting black pepper

pH and mycelial weight of *Phytophthora* isolates at different pH was analyzed statistically. There was considerable variation between different groups of *P.capsici* isolates. Minimum mycelial weight was observed in group III. Group III and II showed similar mycelial weight. Group I had maximum mycelial weight. **(Table: 50)**

Table: 50, ANOVA and DMRT of the effect of pH on mycelial growth on GYP medium of *Phytophthora capsici* infecting black pepper

pH and growth	Sum of Squares	df	Mean Square	F	Group	N	Mean value
Between Groups	.228	3	7.613	6.302	3.00	287	.1547
Within Groups	7.333	607	1.208		2.00	72	.1556
Total	7.561	610			4.00	144	.1876
					1.00	108	.2007

P. meadii **(Isolate Nos: 52-54):**

They cannot grow at pH 2.5 and minimum growth was at pH 9. The isolates 98-86, 98-192, 98-1 had maximum growth at pH 5 but the isolate 98-90 had maximum growth at pH 7. (**Table: 51)**.

Table: 51, Effect of pH on mycelial growth on GYP medium of *Phytophthora meadii* infecting black pepper

Sl. No.	**Isolate No.**	**Mean mycelial weight in mg.**			
		pH 2.5	**pH 5**	**pH 7**	**pH 9**
52	98-86	0.00	0.20	0.18	0.03
53	98-90	0.00	0.16	0.18	0.05
54	98-192	0.00	0.20	0.18	0.03

P. palmivora **(Isolate Nos: 55&56):**

The *P. palmivora* isolates cannot grow at pH 2.5 and minimum growth was at pH 9. The isolates had maximum growth at pH 5. (**Table: 52)**.

Table: 52, Effect of pH on mycelial growth on GYP medium of *Phytophthora palmivora* infecting black pepper

Sl. No.	**Isolate No.**	**Mean mycelial weight in mg.**			
		pH 2.5	**pH 5**	**pH 7**	**pH 9**
55	98-1	0.00	0.24	0.18	0.16
56	99-127	0.00	0.26	0.18	0.16

P. parasitica **(Isolate Nos: 57-60):**

The *P. parasitica* isolates cannot grow at pH 2.5 and their maximum growth was at pH 7 followed by pH 5. (**Table: 53)**.

Table: 53, Effect of pH on mycelial growth of *Phytophthora parasitica* infecting black pepper on GYP medium

Sl. No.	Isolate No.	Mean mycelial weight in mg.			
		pH 2.5	**pH 5**	**pH 7**	**pH 9**
57	99-186	0.00	0.33	0.47	0.24
58	99188	0.00	0.32	0.47	0.24
59	98-95	0.00	0.33	0.36	0.29
60	98-75	0.00	0.22	0.22	0.10

7.2.2. Effect of pH and sporulation of *Phytophthora* species infecting black pepper

The sporulation of the *Phytophthora* isolates was studied in different pH. The pH was adjusted in distilled water and the isolates grown in carrot agar medium were used. The selected pH was 2.5, 5, 7, and 9. All the 60 isolates were tested for their sporulation. All of them cannot sporulate at pH 2.5. Some of the isolates can grow at pH 2.5 but cannot sporulate. Sporulation was possible in pH 5, 7 and 9. Most of the isolates had maximum sporulation at pH 7 (**Fig.11).**

***P. capsici* (Isolate Nos: 1-51):**

In the case of group I isolates maximum sporulation was at pH 7 for the isolates 96-8, 98-135, 98-157 and 99-139. But the sporulation was high at pH 5 for the isolates

Fig: 10, Effect of pH on mycelial growth of *Phytophthora* species infecting black pepper

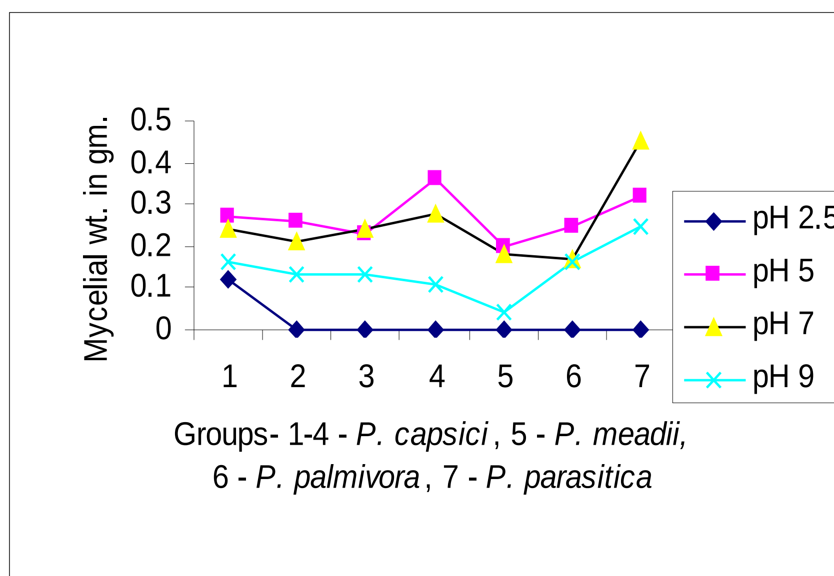
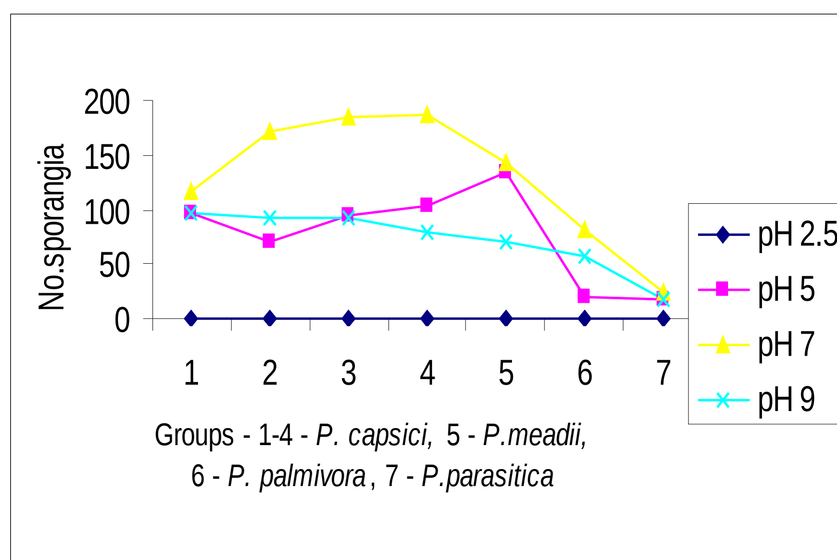


Fig: 11, Effect pH on sporulation of *Phytophthora* species infecting black pepper



97-50, 97-51, and 97-52 and for the isolate 98-7 maximum sporulation was at pH 9. In the case of the isolate 99-139 the sporulation was minimum in pH 5. The isolates of groups II, III and IV had maximum sporulation at pH 7. (**Table: 54)**.

Table: 54, Effect of pH and sporulation of *Phytophthora capsici* infecting black pepper

Sl.No	Isolate No.	Mean no. of sporangia produced / 3 microscopic fields			
		pH 2.5	pH 5	pH 7	pH 9
1	96-8	0	81.6	100.6	95.6
2	97-50	0	155	61.6	120
3	97-51	0	135	81.6	100
4	97-52	0	111	86	91.6
5	98-7	0	132.3	141.6	180.3
6	98-135	0	55	131.6	120
7	98-157	0	96.6	161	102
8	98-177	0	93.3	131.6	77.3
9	99-139	0	19	123.3	96.6
10	98-24	0	66.6	143.3	58.3
11	98-76	0	42.3	148.3	60
12	98-131	0	83.6	178.3	64
13	98-140	0	94.3	190	96
14	99-158	0	63.3	175	181.6
15	99-106	0	66.6	193.3	88.6
16	98-49	0	141.6	241.6	105
17	98-81	0	126.6	260.6	121.6
18	98-144	0	126.6	275	118.3
19	98-167	0	166.6	263.3	166.6
20	98-172	0	60	180	110
21	98-198	0	106	153.3	93.3
22	99-103	0	85.3	171.6	106.6
23	99-104	0	125	210	126.6
24	99-125	0	141.6	176.6	94.3
25	99-144	0	126	180	100
26	99-166	0	160	126	103.3
27	99-167	0	91	158.3	123.3
28	97-45c	0	100	185	103.3
29	98-67	0	106.6	126.6	80
30	98-127	0	228.3	298.3	98.3
31	98-162	0	81.6	275	157.6
32	98-181	0	201.6	330	118.3
33	98-182	0	21	71.6	23.6
34	98-166	0	83.3	190	71.6
35	99-162	0	61.36	155	51
36	98-174	0	116.6	201.6	124
37	99-145	0	95	221.6	150

38	98-87	0	93.3	134.3	108.3
39	99-91	0	45	152.6	34.3
40	99-124	0	48.3	154.3	44.3
41	96-5	0	140	178.3	102
42	96-13	0	131.6	201.6	85
43	98-60	0	140	161.6	64.3
44	98-145	0	100	159.3	105
45	00-18	0	110	190	94.3
46	00-40	0	160	258.3	94.3
47	96-4	0	103.3	255	98.3
48	97-11a	0	50	160	56.6
49	96-11	0	41.6	141.6	58.3
50	97-55	0	64.3	173.3	60
51	98-17	0	120	165	51.3

Statistical Analysis of the effect of pH and sporulation of *Phytophthora capsici* infecting black pepper

The pH and sporulation of *Phytophthora capsici* isolates at different pH was analyzed statistically. The isolates of different groups of *Phytophthora capsici* showed significant variation. The isolates of group II showed maximum sporulation. The isolates of group II had minimum sporulation. The group I and group IV were similar in sporulation. (**Table: 55)**.

Table: 55, ANOVA and DMRT of the effect of pH on sporulation of *Phytophthora capsici* infecting black pepper

pH and sporulation	Sum of Squares	df	Mean Square	F	Group	N	Mean value
Between Groups	49712.959	3	16570.986	2.884	3.00	12	61.7500a
Within Groups	3493336.784	608	5745.620		1.00	108	78.0556ab
Total	3543049.743	611			4.00	132	91.8636ab
					2.00	360	99.1722b

***P. meadii* (Isolate Nos: 52-54):**

Their sporulation was similar to *P. capsici*. No sporulation was at pH 2.5 and maximum was at pH 7. (**Table: 56)**.

Table: 56, Effect of pH and sporulation of *Phytophthora meadii* infecting black pepper

Sl.No:	**Isolate No.**	**Mean no. of sporangia produced / 3 microscopic fields**			
		pH 2.5	**pH 5**	**pH 7**	**pH 9**
52	98-86	0	121.6	136	41.3
53	98-90	0	105	116.6	100
54	98-192	0	173.3	173.3	71.6

***P. palmivora* (Isolate Nos: 55&56):**

Sporulation was less while comparing with *P. capsici*. Maximum sporulation was at pH 7. (**Table: 57)**.

Table: 57, Effect of pH and sporulation of *Phytophthora palmivora* infecting black pepper

Sl.No:	**Isolate No.**	**Mean no. of sporangia produced / 3 microscopic fields**			
		PH 2.5	**PH 5**	**PH 7**	**PH 9**
55	98-1	0	20	84.3	60.6
56	99-127	0	19.3	71.6	55.3

***P. parasitica* (Isolate No: 57-60):**

The two *P. parasitica* isolates, 99-186 and 99-188 showed maximum sporulation at pH 7. But 98-95 had maximum sporulation at pH 9. No sporangia were formed at pH 2.5. Minimum sporulation was at pH 9 for the isolates 99-186 and 99-188. (**Table: 58)**.

Table: 58, Effect of pH on sporulation of *Phytophthora parasitica* infecting black pepper

Sl.No:	Isolate No.	Mean no. of sporangia produced / 3 microscopic fields			
		pH 2.5	**pH 5**	**pH 7**	**pH 9**
57	99-186	0	13.3	21	8.6
58	99188	0	28.3	17.6	9
59	98-95	0	10	34.3	35.3
60	98-75	0	93.3	151.6	101.33

7.2.3. Effect of pH on indirect germination (zoospore liberation) of *Phytophthora* species infecting black pepper

No zoospore liberated at pH 2.5. Zoospores released at pH 5, 7 and 9. Maximum zoospore release was at 20 ^{0}C. (**Fig: 12).**

***P. capsici* (Isolate Nos: 1-51):**

The isolates of group I had maximum zoospore liberation at pH 7, but the isolate 96-8 had maximum zoospore liberation at pH 5. Zoospore liberation was minimum at pH 9. For the group II isolates the maximum zoospore liberation at pH 5. The group III isolates also had maximum zoospore liberation at pH 5. The group IV isolates also had a similar trend. The isolates of group IV had maximum zoospore liberation at pH 5 followed by pH 7 and minimum zoospore liberation was at pH 9. (**Table: 59)**.

Table: 59, Effect of pH on indirect germination (zoospore liberation) of *Phytophthora capsici* infecting black pepper

Sl.No:	Isolates No.	Mean no. of sporangia with indirect germination/ 3 microscopic fields			
		pH 2.5	**pH 5**	**pH 7**	**pH 9**
1	96-8	0	76.6	75	40
2	97-50	0	66	78.3	37.6
3	97-51	0	72.6	82.3	41.6
4	97-52	0	72.6	82.6	41.6
5	98-7	0	73.6	76	46.3
6	98-135	0	72.3	82.6	36.3
7	98-157	0	67.6	81	39
8	98-177	0	67.6	86	41.6
9	99-139	0	62.6	81.6	42.3
10	98-24	0	100	80	51.6
11	98-76	0	112.3	79.3	50
12	98-131	0	111.6	77.3	45.6
13	98-140	0	73.6	76	61.6
14	99-158	0	113.3	83.3	52.3
15	99-106	0	81.3	85	19.3
16	98-49	0	81	28.3	19.3
17	98-81	0	75	34	19.3
18	98-144	0	86.6	33.3	17.6
19	98-167	0	81.6	31.6	19.3
20	98-172	0	84.3	48.3	17.6
21	98-198	0	93.3	35.3	16.6
22	99-103	0	86	48	17.3
23	99-104	0	78	32.6	30
24	99-125	0	79	27.6	22.6
25	99-144	0	71.3	30	25
26	99-166	0	80	25.3	18
27	99-167	0	86.6	36	21
28	97-45c	0	82.6	36	24.3
29	98-67	0	88	35.3	21.3
30	98-127	0	78.6	38	23.6
31	98-162	0	98	34.3	23

32	98-181	0	88.3	37.6	22
33	98-182	0	71.6	30	18.3
34	98-166	0	96	27	19.3
35	99-162	0	93	25	20
36	98-174	0	97.3	14.3	20
37	99-145	0	71.6	35	14.3
38	98-87	0	40	26.6	17.6
39	99-91	0	102.6	84.3	61.6
40	99-124	0	106.3	68	33.3
41	96-5	0	98.3	80	51.6
42	96-13	0	115	87.6	48.3
43	98-60	0	92.6	80.6	50
44	98-145	0	108	85.3	45
45	00-18	0	96	91.6	47.3
46	00-40	0	123	87	43.3
47	96-4	0	114.3	86.6	38.6
48	97-11a	0	76.6	85	51.3
49	96-11	0	78.3	69.3	43.3
50	97-55	0	106.6	78.6	48.3
51	98-17	0	103.6	79.3	61.6

Statistical Analysis of the effect of pH on indirect (zoospore liberation) germination of *Phytophthora capsici* infecting black pepper

The pH and zoospore liberation of *Phytophthora capsici* isolates at different pH was analyzed statistically. There was significant variation between different groups. Minimum zoospores were released by group III. Maximum zoospores were released by group II. (**Table: 60)**.

Table: 60, ANOVA and DMRT of the effect of pH on indirect germination (zoospore liberation) of *Phytophthora capsici* infecting black pepper

pH and indirect germination	Sum of Squares	df	Mean Square	F	Group	N	Mean value
Between Groups	72170.819	3	24056.940	19.490	3.00	288	34.8611a
Within Groups	750459.861	608	1234.309		1.00	108	47.7222b

Total	822630.680	611			4.00	144	56.6250bc
					2.00	72	62.6667c

***P. meadii* (Isolate No: 52-54):**

In the case of *P. meadii*, the isolates 98-86 and 98-90 had maximum zoospore liberation at pH 7 followed by pH 5. But the isolate 98-192 showed maximum zoospore liberation at pH 5 followed by pH 7. (**Table: 61)**.

Table 61, Effect of pH on indirect germination (zoospore liberation) of *Phytophthora meadii* infecting black pepper

Sl.No:	**Isolates No.**	**Mean no. of sporangia with indirect germination/ 3 microscopic fields**			
		pH 2.5	**pH 5**	**pH 7**	**pH 9**
52	98-86	0	71.6	76.6	40
53	98-90	0	74	76	44
54	98-192	0	80	18	11

***P. palmivora* (Isolate Nos: 55&56):**

The *P. palmivora* isolates had maximum zoospore liberation at pH 5 followed by pH 7 and minimum at pH 9. (**Table: 62)**.

Table 62, Effect of pH on indirect germination (zoospore liberation) of *Phytophthora palmivora* infecting black pepper

Sl.No:	**Isolates No.**	**Mean no. of sporangia with indirect germination/ 3 microscopic fields**			
		PH 2.5	**PH 5**	**PH 7**	**PH 9**
55	98-1	0	81.6	65	33.3
56	99-127	0	71.6	67.6	32.6

***P. parasitica* (Isolate Nos: 57-60):**

P. parasitica isolates had maximum zoospore liberation at pH 5 followed by pH 7 and minimum at pH 9 and no zoospore liberation at pH 2.5. (**Table: 63).**

Table 63, Effect of pH on indirect germination (zoospore liberation) of *Phytophthora parasitica* infecting black pepper

Sl.No:	Isolate No.	Mean no. of sporangia with indirect germination/ 3 microscopic fields			
		PH 2.5	**PH 5**	**PH 7**	**PH 9**
57	99-186	0	27.6	18.3	13.3
58	99188	0	24.3	16.6	15
59	98-95	0	28.3	18	14.3
60	98-75	0	47.6	40	19.3

7.2.4. Effect of pH on zoospore germination of *Phytophthora* species infecting black pepper

Zoospore germination in different pH was studied in detail. For this study pH levels of 2.5, 5, 7 and 9 were selected. At pH 2.5 no germination occurred. But zoospore germinated at pH 5, 7 and 9 (**Fig : 13)**.

***P. capsici* (Isolate Nos: 1-51):**

For the group I isolates maximum germination was at pH 9 and minimum at pH 5. In the case of group II maximum germination occurs at pH 7, and at pH 5 and 9 almost same number of sporangia were germinated. The group III isolates showed variation in zoospore germination. (**Table: 64)**.

Fig: 12, Effect pH on indirect germination (zoospore liberation) of *Phytophthora* species infecting black pepper

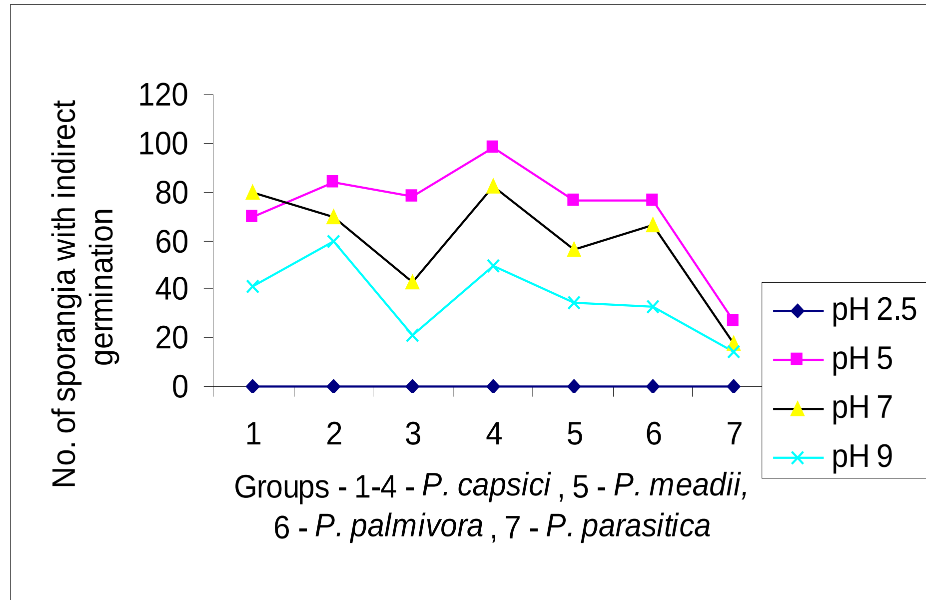


Fig: 13, Effect pH on zoospore germination of *Phytophthora* species infecting black pepper

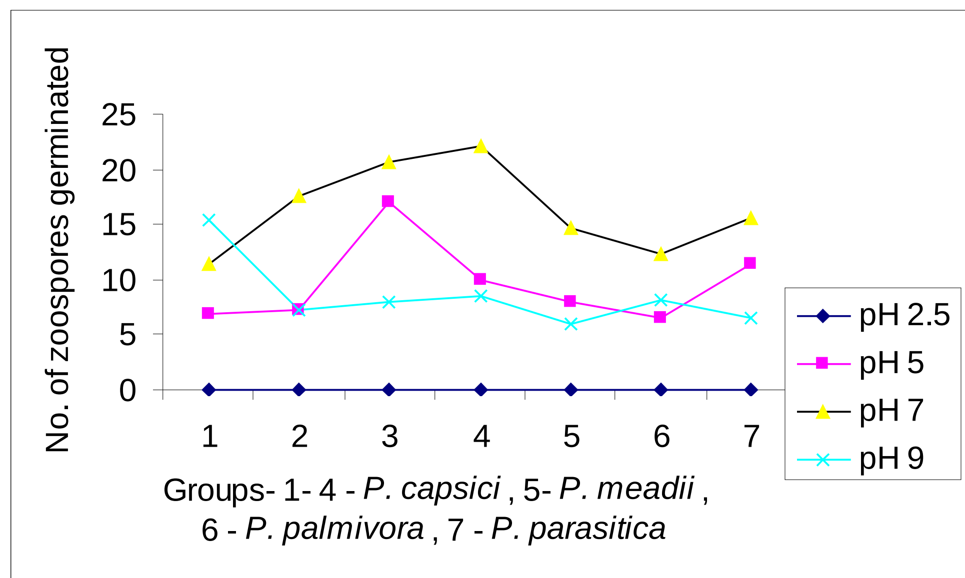


Table: 64. Effect of pH on zoospore germination of *Phytophthora capsici* infecting black pepper

Sl.No.	Isolate No.	Mean no. of zoospores germinated / 3 microscopic fields			
		PH 2.5	PH 5	PH 7	PH 9
1	96-8	0	6.3	12	14
2	97-50	0	6.6	12.6	14.6
3	97-51	0	8.3	12	20
4	97-52	0	5	9	15
5	98-7	0	7.3	11.3	13.6
6	98-135	0	8	11	13.6
7	98-157	0	9	11.6	16
8	98-177	0	6.6	11	17.3
9	99-139	0	5	12.3	14.3
10	98-24	0	6.6	18	7.6
11	98-76	0	7.3	16	6.6
12	98-131	0	7.6	17.6	6.3
13	98-140	0	7	19	8
14	99-158	0	7.3	19	8
15	99-106	0	7.6	15.6	6.6
16	98-49	0	17.3	15	6
17	98-81	0	17.3	14.6	6
18	98-144	0	16	14	6.6
19	98-167	0	22.3	19	6.6
20	98-172	0	17.6	22.6	7.3
21	98-198	0	21.6	20.6	7
22	99-103	0	19	23.3	6.6
23	99-104	0	19.3	19.6	6
24	99-125	0	21.3	18	6.6
25	99-144	0	22	20.6	5.3
26	99-166	0	21	20.3	5
27	99-167	0	20	23	7.6
28	97-45c	0	22	19.6	6
29	98-67	0	22	17.3	10.3

30	98-127	0	23.6	21	6.3
31	98-162	0	19.6	20	8
32	98-181	0	20	18.3	8.3
33	98-182	0	19	21	6
34	98-166	0	22.3	21.3	6.6
35	99-162	0	22.6	20.3	8
36	98-174	0	11.6	23	9
37	99-145	0	20.6	20.3	10.3
38	98-87	0	19.6	21	12.3
39	99-91	0	11	22	6.6
40	99-124	0	10.6	14.3	7
41	96-5	0	7	22	8
42	96-13	0	8.3	21.3	10.6
43	98-60	0	11	21	12
44	98-145	0	12	19.6	12
45	00-18	0	7.6	23.6	8.3
46	00-40	0	9.6	21.6	7.3
47	96-4	0	10	26	6.6
48	97-11a	0	11	20	6
49	96-11	0	12.3	20.3	7.3
50	97-55	0	10.6	23	7.3
51	98-17	0	10.6	21.6	7.3

Statistical Analysis on the effect of pH on zoospore germination of *Phytophthora capsici* infecting black pepper

pH and zoospore germination of *Phytophthora capsici* isolates at different pH levels was analyzed statistically. There was significant variation between the different groups of isolates. Group II had minimum germination and was similar to group I. Group III had maximum germination. (**Table: 65)**

Table: 65, ANOVA and DMRT of the effect of pH on zoospore germination of *Phytophthora capsici* infecting black pepper

pH and zoospore germination	Sum of Squares	df	Mean Square	F	Group	N	Mean value
Between Groups	1417.440	3	472.480	7.261	2.00	71	8.0563a

Within Groups	39562.617	608	65.070		1.00	108	8.4444a
Total	40980.057	611			4.00	144	9.9653ab
					3.00	289	11.8270b

***P. meadii* (Isolate Nos: 52-54):**

In *P. meadii* zoospore germination was not noticed at pH 2.5 and maximum sporulation was at pH 5. (Table**: 66**).

Table: 66. Effect of pH on zoospore germination of *Phytophthora meadii* infecting black pepper

Sl.No.	**Isolate No.**	**Mean no. of zoospores germinated / 3 microscopic fields**			
		PH 2.5	**PH 5**	**PH 7**	**PH 9**
52	98-86	0	8	12.6	6.3
53	98-90	0	8.3	15.3	6.6
54	98-192	0	7.6	16	3.6

***P. palmivora* (Isolate Nos: 55&56):**

In *P.palmivora* zoospore germination was not noticed at pH 2.5 and maximum sporulation was at pH 5. (**Table: 67)**.

Table: 67. Effect of pH on zoospore germination of *Phytophthora palmivora* infecting black pepper

Sl.No.	**Isolate No.**	**Mean no. of zoospores germinated / 3 microscopic fields**			
		PH 2.5	**PH 5**	**PH 7**	**PH 9**
55	98-1	0	5	11	10.3
56	99-127	0	7	13.6	8

***P. parasitica* (Isolate Nos: 57-60):**

In *P. parasitica* zoospore germination was not noticed at pH 2.5 and maximum sporulation was at pH 5. (**Table: 68)**.

Table: 68. Effect of pH on zoospore germination of *Phytophthora parasitica* infecting black pepper

Sl.No.	Isolate No.	Mean no. of zoospores germinated / 3 microscopic fields			
		PH 2.5	PH 5	PH 7	PH 9
57	99-186	0	12	14	7
58	99188	0	11.3	15	6
59	98-95	0	11	18	6.6
60	98-75	0	22	19.6	14.3

7.3. Effect of media on *Phytophthora* species infecting black pepper

The effect different solid media on the growth, sporulation, and zoospore liberation and zoospore germination of *Phytophthora* species was studied.

7.3.1. Effect of media on mycelial growth of *Phytophthora* species infecting black pepper

Growth of the 60 selected isolates was studied in four different media, carrot agar, potato dextrose agar, corn meal agar and papaya dextrose agar. Optimum growth was obtained at carrot agar. Most of the isolates had maximum growth in this media (**Fig. 14)**.

***P. capsici* (Isolate Nos: 1-51):**

The isolates of groups I and II had maximum radial growth in carrot agar followed by papaya dextrose agar and potato dextrose agar. In this group the isolates 98-131, 98-140, 99-106 and 98-76 had maximum growth in papaya dextrose agar instead of CA. Among the group III isolates most of them had maximum growth in CA but some isolates like 99-103 had maximum growth in papaya dextrose agar. The isolates differ in growth, the isolate 99-91 had maximum growth in CA and 99-124 had maximum growth in papaya dextrose agar. (**Table: 69)**.

Table: 69, **Effect of media on mycelial growth of *Phytophthora capsici* infecting black pepper**

Sl.No.	**Isolate No.**	**Radial growth in mm.**			
		Carrot agar	**Potato dextrose agar**	**Corn meal agar**	**Papaya dextrose agar**
1	96-8	31.6	28	26	30.3
2	97-50	32	27.6	26	29
3	97-51	30.6	28.3	25.3	28.6
4	97-52	30.3	26.3	27	30.6
5	98-7	29	22	21.3	22.3
6	98-135	29.6	25	24.6	20
7	98-157	35	23	27.3	28.6
8	98-177	32	22	25.3	29.3
9	99-139	30.3	23.6	23.3	25.3
10	98-24	24.3	18	23	24
11	98-76	25	20	24.3	26
12	98-131	25.3	23.6	26.6	27
13	98-140	25	20.3	24	27.6
14	99-158	22.3	20	27.3	21
15	99-106	23	23.6	25	29.3
16	98-49	39.3	19.6	24.3	28
17	98-81	34	25	26.3	27
18	98-144	45.3	20.6	24.6	29
19	98-167	39.3	25	25	32
20	98-172	33.3	25.6	22.6	32
21	98-198	33	28	25	31
22	99-103	34.3	27	30	36
23	99-104	40	25.6	23	30.3
24	99-125	36	20.6	25	31.6

25	99-144	36	25	24	24.3
26	99-166	40	25.3	24.6	26
27	99-167	39.3	25.3	25	26.3
28	97-45c	32.6	24.3	26.3	34
29	98-67	29.3	21	21.6	25
30	98-127	29	23	22.3	26
31	98-162	29.3	19	23	30
32	98-181	30.6	22	26	32.3
33	98-182	31.3	20	23	30
34	98-166	33.3	20	24.3	30
35	99-162	33	24	25	30.6
36	98-174	40	23.6	27	30.6
37	99-145	30.3	22.6	29.3	30.3
38	98-87	34.6	21	27	29.3
39	99-91	34.3	19.6	26	29.3
40	99-124	26	20.3	29.3	28
41	96-5	36	21.6	27	28.3
42	96-13	34.3	26	28	25.3
43	98-60	33.6	21.3	29.3	29.3
44	98-145	34	25	29	28
45	00-18	33.6	24.6	27.6	30.3
46	00-40	35.3	27.3	28.6	31.3
47	96-4	34.3	23.6	27.3	30.6
48	97-11a	34	22	29.3	28
49	96-11	30	24	28.6	29
50	97-55	35.6	23.6	30	30
51	98-17	33	19	27.6	28

Statistical Analysis of the effect of media on mycelial growth of *Phytophthora capsici* infecting black pepper

Growth of *Phytophthora capsici* isolates in different media was analyzed statistically. There was significant variation between the different groups of *P. capsici*. The group II had minimum growth and group III had maximum growth. (**Table: 70).**

Table: 70, ANOVA and DMRT of the effect of media on mycelial growth of *Phytophthora capsici* infecting black pepper

Media and mycelial growth	Sum of Squares	df	Mean Square	F	Group	N	Mean value
Between Groups	2224.638	3	741.546	3.172	2.00	72	23.9167a
Within Groups	142146.831	608	233.794		1.00	108	27.1481ab
Total	144371.469	611			4.00	144	28.4653b
					3.00	288	29.8542b

***P. meadii* (Isolate Nos: 52-54):**

These isolates showed maximum growth in Papaya dextrose agar and minimum in PDA. (**Table: 71)**.

Table: 71, **Effect of media on mycelial growth of *Phytophthora meadii* infecting black pepper**

Sl.No.	Isolate No.	Radial growth in mm.			
		Carrot agar	**Potato dextrose agar**	Corn meal agar	**Papaya dextrose agar**
52	98-86	25.6	21.3	27	29
53	98-90	29	23	25.6	29.3
54	98-192	36	26	28	28

***P. palmivora* (Isolate Nos: 55&56):**

Maximum growth was in CMA and minimum was in PDA. (**Table: 72)**.

Table: 72, **Effect of media on mycelial growth of *Phytophthora palmivora* infecting black pepper**

Sl.No.	Isolate No.	Radial growth in mm.

		Carrot agar	Potato dextrose agar	Corn meal agar	Papaya dextrose agar
55	98-1	24	23.6	31	26
56	99-127	30	17	28.3	26

P. parasitica **(Isolate Nos: 57-60):**

The *P.parasitica* isolates had maximum growth in CA followed by CMA. Minimum growth was in PDA. (**Table: 73)**.

Table: 73, **Effect of media on mycelial growth of *Phytophthora parasitica* infecting black pepper**

Sl.No.	**Isolate No.**	**Radial growth in mm.**			
		Carrot agar	**Potato dextrose agar**	**Corn meal agar**	**Papaya dextrose agar**
57	99-186	30	18	22	18.3
58	99188	30.6	21	28	20
59	98-95	33.6	21	23	16
60	98-75	21	21	28	28

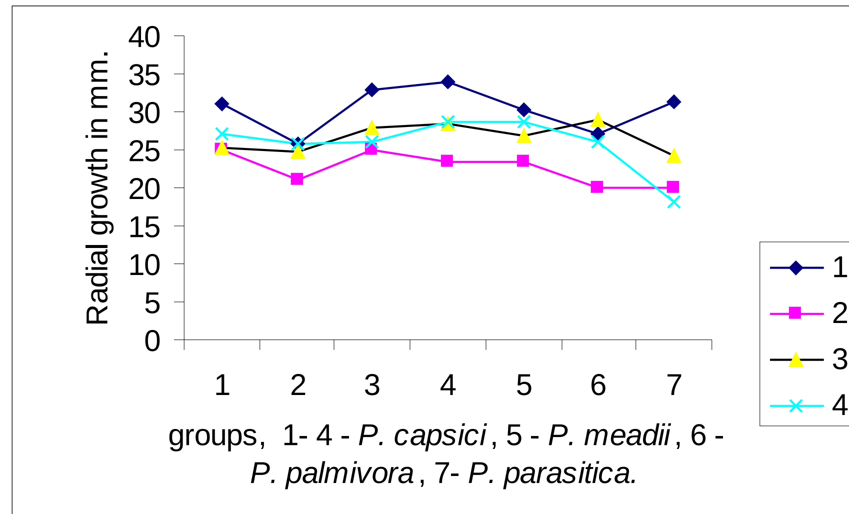
7.3.2. Effect of media on sporulation of *Phytophthora* species infecting black pepper

Sporulation of the isolates grown in carrot agar, potato dextrose agar, corn meal agar and papaya dextrose agar were tested. Maximum number of sporangia was produced in carrot agar followed by corn meal agar in most of the cases. Minimum sporulation was in potato dextrose agar or papaya dextrose agar. **(Fig: 15)**.

***P. capsici* (Isolate Nos: 1-51) :**

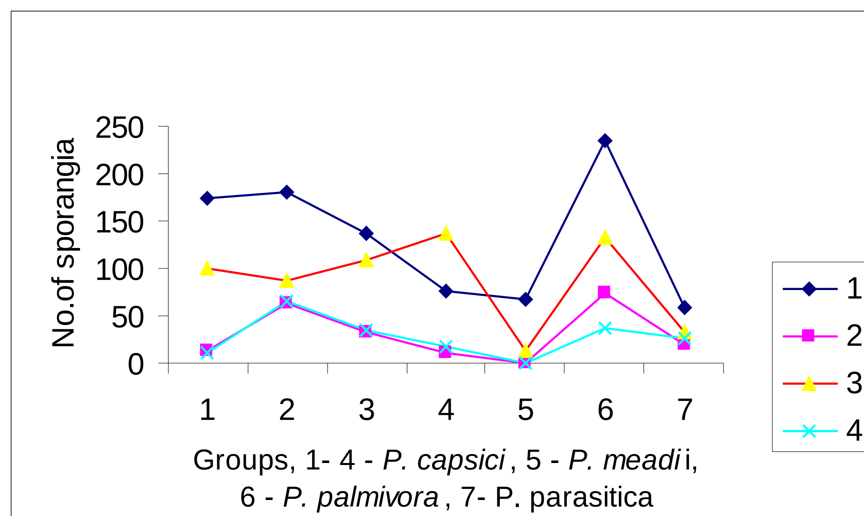
The isolates of groups I, II and III had maximum sporulation in carrot agar. In the case of group IV sporulation was very high in all the media tested while comparing with other groups. (**Table: 74).**

Fig: 14, Effect of media on mycelial growth of *Phytophthora* species infecting black pepper



1 - Carrot agar, 2 - Potato dextrose agar, 3 - Corn meal agar,
4 - Papaya dextrose agar.

Fig: 15, Effect of media on sporulation of *Phytophthora* species infecting black pepper



1 - Carrot agar, 2 - Potato dextrose agar, 3 - Corn meal agar,
4 - Papaya dextrose agar.

Table: 74, Effect of media on sporulation of *Phytophthora capsici* infecting black pepper

Sl.No.	Isolate No.	Mean no. of sporangia produced / 3 microscopic fields			
		Carrot agar	**Potato dextrose agar**	**Corn meal agar**	**Papaya dextrose agar**
1	96-8	168.3	12.3	105	12.3
2	97-50	196.6	12	101.3	13.3
3	97-51	163.3	14	98.3	14.3
4	97-52	205	12.6	94.6	13.6
5	98-7	148.3	13.3	105.3	10
6	98-135	125	16.6	115	8.6
7	98-157	153.3	14	71.6	10
8	98-177	205	15.3	75	9.3
9	99-139	206.6	14	93.3	8.3
10	98-24	168.3	66.6	71.6	69.3
11	98-76	153.3	50	81.6	51.3
12	98-131	196.6	51	85	61.6
13	98-140	183.3	63.3	85	63.3
14	99-158	203.3	81.6	122.6	76.6
15	99-106	183.6	61.6	71.6	67.6
16	98-49	131.6	75	125	36
17	98-81	160.6	68.3	121.6	69.3
18	98-144	153.3	61.6	131.6	67
19	98-167	133.3	58.3	121.6	68.6
20	98-172	129.6	56	146.6	50
21	98-198	158.3	76.3	140	49.3
22	99-103	105	45	106	63.3
23	99-104	125.3	31.6	109.3	33
24	99-125	138.6	40	108.3	38.3
25	99-144	118.3	52	108.6	34.3
26	99-166	118.6	50	96.6	30.6
27	99-167	119	28.6	125	30.6
28	97-45c	125	52.6	90	30

29	98-67	239	33.3	118.3	21
30	98-127	171.6	7	88.3	7
31	98-162	121	26	120	46.6
32	98-181	124.6	24.3	100	40.3
33	98-182	155	57.6	110	40
34	98-166	12.6.6	64	101.6	40
35	99-162	143.3	65	98.3	47.6
36	98-174	133.3	46.6	111	43.6
37	99-145	136.6	26.6	110.6	44.3
38	98-87	143.3	23.6	110.6	37.6
39	99-91	11.6	7	88.3	15
40	99-124	139.3	7.6	110	12.3
41	96-5	160	7.6	148.3	38.6
42	96-13	166.6	6.6	181.6	16.6
43	98-60	158.3	7	153.3	13.3
44	98-145	155	5	131.6	6.3
45	00-18	211	7	105	11.6
46	00-40	206.6	7.6	125	10.3
47	96-4	223.3	11.3	135	11
48	97-11a	223.3	8.6	128.3	13.3
49	96-11	173.3	10.3	101.6	12.6
50	97-55	186.6	30	146.6	60
51	98-17	168.3	10	113.3	7.6

Statistical Analysis of the effect of media on sporulation of *Phytophthora capsici* infecting black pepper

Sporulation of *Phytophthora capsici* isolates in different media was analyzed statistically. There was significant variation between the different groups of isolates. The isolates of group I had minimum sporulation. The isolates of group II had maximum sporulation. Groups II and IV were similar in sporulation. **(Table: 75)**

Table: 75, ANOVA and DMRT of the effect of media on sporulation of *Phytophthora capsici* infecting black pepper

Media on sporulation	Sum of Squares	df	Mean Square	F	Group	N	Mean value

Between Groups	25465.830	3	8488.610	2.184	1.00	108	74.6667a
Within Groups	2363150.502	608	3886.761		3.00	300	83.6633ab
Total	2388616.332	611			4.00	132	85.6212ab
					2.00	72	98.7778b

***P. meadii* (Isolate Nos: 52-54):**

The *P. meadii* isolates differ in their sporulation tendencies. They had maximum sporulation in carrot agar followed by papaya dextrose agar and potato dextrose agar. (**Table: 76).**

Table: 76, Effect of media on sporulation of *Phytophthora meadii* infecting black pepper

Sl.No.	Isolate No.	Mean no. of sporangia produced / 3 microscopic fields			
		Carrot agar	Potato dextrose agar	Corn meal agar	Papaya dextrose agar
52	98-86	58.3	5.3	15	7
53	98-90	65	7	17	6
54	98-192	78.3	7.6	8	8.3

***P. palmivora* (Isolate Nos: 55&56):**

The *P. palmivora* isolates had maximum sporulation in carrot agar and minimum in PDA. (**Table: 77).**

Table: 77, Effect of media on sporulation of *Phytophthora palmivora* infecting black pepper

Sl.No.	Isolate No.	Mean no. of sporangia produced / 3 microscopic fields			
		Carrot agar	Potato dextrose agar	Corn meal agar	Papaya dextrose agar
55	98-1	233.3	86.6	128.3	34.3

56	99-127	236.6	60	138.3	41.6

***P. parasitica* (Isolate Nos: 57-60):**

The *P. parasitica* isolates of had maximum sporulation in carrot agar followed by papaya dextrose agar and potato dextrose agar. (**Table: 78).**

Table: 78, Effect of media on sporulation of *Phytophthora parasitica* infecting black pepper

Sl.No.	**Isolate No.**	**Mean no. of sporangia produced / 3 microscopic fields**			
		Carrot agar	**Potato dextrose agar**	**Corn meal agar**	**Papaya dextrose agar**
57	99-186	27.6	18.3	7.6	26.6
58	99188	31.3	14	6.6	26
59	98-95	37.6	19.3	7.3	25
60	98-75	136.6	24.3	110	30.3

7.3.4 Effect of media on indirect germination (zoospore liberation) of sporangia of *Phytophthora* species infecting black pepper

All the 60 isolates were tested for zoospore liberation in the four media. In all the tested media zoospore liberation was possible but the number of zoospores liberated was different in different media. Maximum zoospore liberation was in carrot agar (**Fig**.**16).**

***P. capsici* (Isolate Nos: 1-51):**

Isolates of group I sporulated in all the media. Maximum zoospore liberation was in CA and minimum in CMA. Isolates of group II had zoospore liberation in all the media under study. Minimum zoospore liberation was in PDA. Group III also had similar trend and minimum zoospore liberation was in PDA and maximum in CA. Zoospore liberation was less in all the tested media. (**Table: 79).**

Table: 79, Effect of media on indirect germination (zoospore liberation) of sporangia of *Phytophthora capsici* infecting black pepper

Sl.no.	Isolate No.	Mean no. of sporangia with indirect germination/ 3 microscopic fields			
		Carrot agar	**Potato dextrose agar**	**Corn meal agar**	**Papaya dextrose agar**
1	96-8	121.6	56.6	43.3	54.3
2	97-50	125	57.6	42.6	50
3	97-51	140	56	46	50
4	97-52	140	48.3	39.3	46.6
5	98-7	131.6	57.6	52.6	56.6
6	98-135	140	52.6	48.3	53.3
7	98-157	121.6	53	48.3	42.6
8	98-177	120	57.6	43.3	47
9	99-139	85	45	50.3	45.3
10	98-24	70	28.3	57.6	46.3
11	98-76	71	28.3	48	51.6
12	98-131	60	30	40	52.6
13	98-140	51.6	18	42	52.6
14	99-158	65	35	39.6	58.3
15	99-106	83.3	34	50	58.6
16	98-49	75	41	53.6	56.6
17	98-81	71.6	40	41.6	67.6
18	98-144	91	35	43.3	56.6
19	98-167	78	36.6	38.3	56
20	98-172	78.6	41	47.6	62.3
21	98-198	87	48	47	57.6
22	99-103	91	42.6	465.6	61
23	99-104	98.3	35	45.6	55
24	99-125	92.3	38.3	47.6	46.6
25	99-144	82	40	48.3	40.6
26	99-166	91.6	39	37.6	55
27	99-167	91.3	45.3	36.3	64.3
28	97-45c	92	48.3	41.6	64.3
29	98-67	90	37.6	53	54.3
30	98-127	90.3	39	50	58
31	98-162	77.3	41.6	51	54

32	98-181	82.6	33.3	51	50
33	98-182	95	33.3	48.3	50
34	98-166	90	33.3	45	50
35	99-162	81.6	29.3	48.3	58.3
36	98-174	95	41.6	48.6	47.6
37	99-145	100	32	42.6	47.6
38	98-87	95	35	42	57.6
39	99-91	47.6	25	41	46.6
40	99-124	52.3	25.3	36.6	48.3
41	96-5	41	25	32.3	55
42	96-13	42	27.6	32.6	54.3
43	98-60	51.3	28.6	32	44.3
44	98-145	51.3	27.3	32.3	44.3
45	00-18	42.6	25.6	31	41.3
46	00-40	40.6	24.3	37.6	44.6
47	96-4	44	25	39.6	44.6
48	97-11a	41.6	25.3	38.3	37.6
49	96-11	47.6	25	44	36.6
50	97-55	47.6	24	40	43.3
51	98-17	51	28.6	40	45

Statistical Analysis of the effect of media on indirect germination (zoospore liberation) of sporangia of *Phytophthora capsici* infecting black pepper

The zoospore liberation of *P. capsici* in different media was analyzed statistically. The isolates of different groups showed significant variation in zoospore liberation in different media. Minimum zoospore liberation was observed in group IV and maximum zoospore liberation was in group I. (**Table: 80)**.

Table: 80, ANOVA and DMRT of the effect of media on indirect germination (zoospore liberation) of sporangia of *Phytophthora capsici* infecting black pepper

Media and indirect germination	Sum of Squares	df	Mean Square	F	Group	N	Mean value
Between Groups	63318.190	3	21106.063	12.400	4.00	132	38.1515a
Within Groups	1034913.68	608	1702.161		2.00	72	48.6806ab

	6						
Total	1098231.876	611			3.00	300	58.1967bc
					1.00	108	68.6111c

P. meadii **(Isolate Nos: 52-54):**

Maximum zoospore liberation occurred in CA and minimum in PDA. It was similar to *P. capsici*. (**Table: 81).**

Table: 81, **Effect of media on indirect germination (zoospore liberation) of sporangia of *Phytophthora meadii* infecting black pepper**

Sl.no.	**Isolate No.**	**Mean no. of sporangia with indirect germination/ 3 microscopic fields**			
		Carrot agar	**Potato dextrose agar**	**Corn meal agar**	**Papaya dextrose agar**
52	98-86	75	36	56.6	68.3
53	98-90	73.3	36	56.3	64.3
54	98-192	68.3	34	54	59.8

P. palmivora **(Isolate Nos: 55&56):**

The zoospore liberation was high in these isolates compared to *P. capsici*. Maximum zoospore liberation was in CA and minimum in PDA. (**Table: 82).**

Table: 82, **Effect of media on indirect germination (zoospore liberation) of sporangia of *Phytophthora palmivora* infecting black pepper**

Sl.no.	**Isolate No.**	**Mean no. of sporangia with indirect germination/ 3 microscopic fields**			
		Carrot agar	**Potato dextrose agar**	**Corn meal agar**	**Papaya dextrose agar**

55	98-1	125	60	66.6	83.3
56	99-127	110	62	65	83.3

***P. parasitica* (Isolate Nos: 57-60):**

Zoospore liberation was less in all the tested media while comparing with all other species. (**Table: 83).**

Table: 83, **Effect of media on indirect germination (zoospore liberation) of sporangia of *Phytophthora parasitica* infecting black pepper**

Sl.no.	**Isolate No.**	**Mean no. of sporangia with indirect germination/ 3 microscopic fields**			
		Carrot agar	**Potato dextrose agar**	**Corn meal agar**	**Papaya dextrose agar**
57	99-186	45	27.6	37	35
58	99188	38.3	24.6	36.6	34
59	98-95	37.6	25	31	32.6
60	98-75	61.6	42.3	45.6	52.6

7.3.4. Effect of media on zoospore germination of *Phytophthora* species infecting black pepper

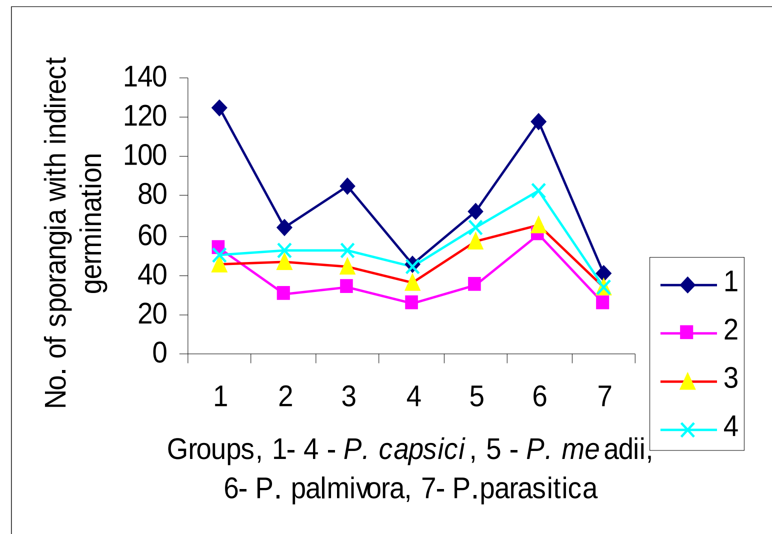
Zoospore germination of the 60 isolates was studied in different media. Maximum germination was in carrot agar for most of the isolates. (**Fig: 17).**

***P. capsici* (Isolate Nos: 1-51):**

The group I isolates had maximum zoospore germination in CA and minimum in PDA. Group II had similar rate of germination in all the media. All the isolates of group III had maximum germination in CA. But the isolate 98-67 of group III had maximum zoospore germination in papaya dextrose agar. Among the third group isolates 99-145,

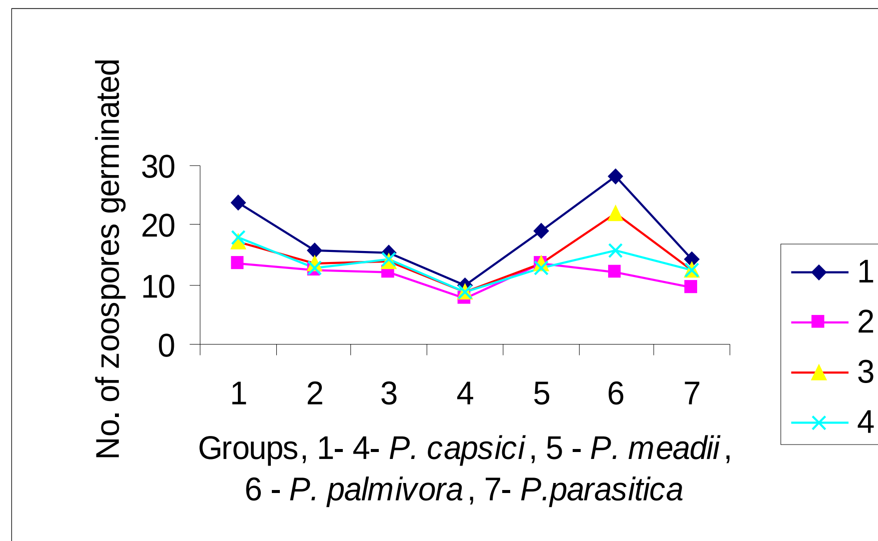
Fig: 16, Effect of media on indirect germination (zoospore liberation) of

***Phytophthora* species infecting black pepper**



1 - Carrot agar, 2 - Potato dextrose agar, 3 - Corn meal agar,
4 - Papaya dextrose agar

Fig: 17, Effect of media on zoospore germination of *Phytophthora* species infecting black pepper



1 - Carrot agar, 2 - Potato dextrose agar, 3 - Corn meal agar,
4 - Papaya dextrose agar

98-87 and 98-75 had maximum zoospore germination in carrot agar but the isolate 98-174 had maximum zoospore germination in papaya dextrose agar. The isolates of group IV had minimum germination in all the tested media. (**Table: 84).**

Table: 84, Effect of media on zoospore germination of *Phytophthora capsici* infecting black pepper

Sl. No.	Isolate No.	Mean no. of zoospores germinated / 3 microscopic fields			
		Carrot agar	**Potato dextrose agar**	**Corn meal agar**	**Papaya dextrose agar**
1	96-8	20	12.6	15	17
2	97-50	27.6	13	16.3	15
3	97-51	27.6	11.6	20	18
4	97-52	20	13.6	20	16
5	98-7	27	14.3	16.6	18.3
6	98-135	28	13.3	19.3	18.6
7	98-157	25	15.6	17	22
8	98-177	17.6	14	15.6	18
9	99-139	22.6	13.3	16.3	19.6
10	98-24	15.3	15.6	11	14.3
11	98-76	14.3	11.6	13.3	14
12	98-131	14	11	13.6	11.6
13	98-140	15	11	13	12
14	99-158	17	12	15.6	12
15	99-106	18	13.6	15	13.6
16	98-49	18	12	17	13.6
17	98-81	17.3	13.6	18	18.3
18	98-144	16.3	13	15.3	16.6
19	98-167	17.6	11.6	16.3	14.3
20	98-172	17	10.3	13.6	13.6
21	98-198	16.3	11.3	13	13.
22	99-103	13.3	13	12.3	13.3
23	99-104	16.6	11.6	15.3	16
24	99-125	16.6	9.6	15.3	15.6
25	99-144	15.3	9	12	13.6
26	99-166	18	12	12.6	13.3

27	99-167	19.3	14	15	16
28	97-45c	18.6	12.3	17	16.6
29	98-67	16.6	15.3	13.3	17
30	98-127	17	13.6	15	15
31	98-162	15	14.6	13.6	13.6
32	98-181	17	11.6	14	12.6
33	98-182	17	12.3	15	15.3
34	98-166	16	15.3	13.6	13.6
35	99-162	19	13	17	17
36	98-174	17	13	17	17.3
37	99-145	13.6	11	12.3	13.6
38	98-87	15.3	13	14.3	14
39	99-91	13.6	11	11.6	14
40	99-124	12	10	11	12.3
41	96-5	8.3	8	8.3	8.3
42	96-13	10.3	7.6	10.3	11
43	98-60	8	9	7.6	8
44	98-145	9	7.6	8	7.3
45	00-18	15	6.3	9.6	8.3
46	00-40	8	8.6	8	8.3
47	96-4	8.6	8.3	7.3	7.3
48	97-11a	11	8.3	10	10
49	96-11	10.3	7	9.6	9
50	97-55	10	6.3	7.6	9
51	98-17	11	9	10	10

Statistical Analysis of the effect of media on zoospore germination of *Phytophthora capsici* infecting black pepper

Zoospore germination of *P. capsici* in different media was analyzed statistically. There was significant variation between different groups of isolates. Minimum germination was observed in Group IV and maximum germination was showed by group I isolates. The groups II, III had similar trend in zoospore germination. **(Table: 85).**

Table: 85, ANOVA and DMRT of the effect of media on zoospore germination of *Phytophthora capsici* infecting black pepper

Media on	Sum of		Mean				Mean

zoospore germination	Squares	df	Square	F	Group	N	value
Between Groups	5488.884	3	1829.628	188.650	4.00	132	8.8485a
Within Groups	5896.703	608	9.699		2.00	72	13.6667b
Total	11385.587	611			3.00	300	14.4600b
					1.00	108	18.2315c

***P. meadii* isolates (Isolate no: 52-54):**

The *P. meadii* isolates had maximum germination in carrot agar and no germination occurs in potato dextrose agar and papaya dextrose agar. (**Table: 86).**

Table: 86, Effect of media on zoospore germination of *Phytophthora meadii* infecting black pepper

Sl. No.	Isolate No.	Mean no. of zoospores germinated / 3 microscopic fields			
		Carrot agar	Potato dextrose agar	Corn meal agar	Papaya dextrose agar
52	98-86	18	13	14	11
53	98-90	21.3	13.6	12.3	11.6
54	98-192	17.6	14.3	15	16

***P. palmivora* (Isolate Nos: 55&56):**

The *P. palmivora* isolates had maximum number of zoospores germinated in carrot agar. (**Table: 87).**

Table: 87, Effect of media on zoospore germination of *Phytophthora palmivora* infecting black pepper

Sl. No.	Isolate No.	Mean no. of zoospores germinated / 3 microscopic fields			
		Carrot agar	Potato dextrose agar	Corn meal agar	Papaya dextrose agar
55	98-1	29.3	12.6	21.6	10.6
56	99-127	27.3	11.6	22	20.6

***P. parasitica* (Isolate Nos: 57-60):**

The *P. parasitica* isolates also had maximum zoospore germination in carrot agar and minimum in PDA. (**Table: 88).**

Table: 88, Effect of media on zoospore germination of *Phytophthora parasitica* infecting black pepper

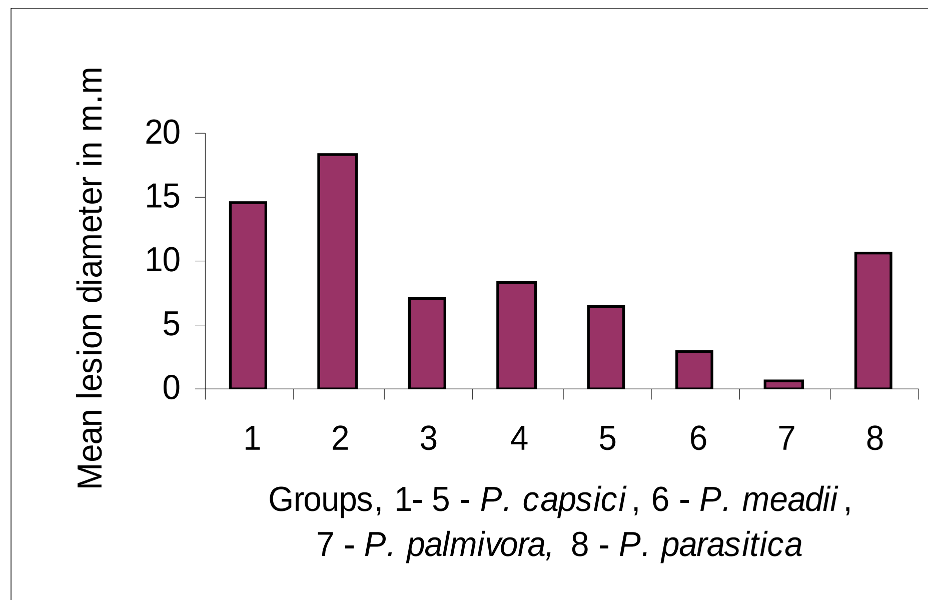
Sl. No.	Isolate No.	Mean no. of zoospores germinated / 3 microscopic fields			
		Carrot agar	Potato dextrose agar	Corn meal agar	Papaya dextrose agar
57	99-186	14.3	10	11.3	12
58	99-188	14	10	12	12
59	98-95	14	8.3	13.6	13.6
60	98-75	12.3	10.6	10.6	10.3

7. Pathogenicity / virulence of *Phytophthora* species infecting black pepper

7.1. Pathogenicity / virulence of *Phytophthora* species infecting black pepper on differentials of *Piper* species

The *P. capsici* isolates as well as other species like *P. meadii, P. palmivora* and *P. parasitica* isolates vary in their pathogenicity response. The *P. capsici* isolates consists of five races based on pathogenicity on differentials of *Piper* species and stem and root inoculation studies on Sreekara plants. (**Fig. 18 and Plate: 13 and 14)**.

Fig:18, Pathogenicity of *Phytophthora* species infecting black pepper on differentials of *Piper* species



1. *P. capsici* –Race - I (Isolates: 1-13)

They infected the entire *Piper* species tested except *P. arboreum*. The isolates were moderately virulent on Sreekara leaves. There were three isolates 98-76, 98-131, and 98-140 moderately virulent on *Piper betle* leaves and the rest ten isolates were less virulent on *P.betle*.

There were seven isolates 98-157, 98-177, 98-24, 98-76, 98-131, 98-140, and 99-106 moderately virulent with *P. longum* leaves and the rest six isolates were less virulent on *P. longum* leaves. All the isolates were moderately virulent on *P. chaba* leaves.

The five isolates 96-8, 97-51, 98-7, 99-139, and 99-158 were less virulent on *P. attenuatum* leaves where as the rest eight isolates were moderately virulent on *P. attenuatum* leaves. All the isolates were less virulent with *P. colubrinum*. The thirteen isolates were non virulent on *P. arboreum* leaves. The reaction of different isolates on *P.ornatum* was varied. The three isolates 98-7, 98-177, and 98-131, were highly virulent and the isolate 98-76 was less virulent on *P. ornatum* leaves. The rest nine isolates were moderately virulent on *P. ornatum* leaves.

The eight isolates 97-51, 98-157, 98-24, 98-76, 98-131, 98-140, 99-158 and 99-106 were moderately virulent on *P. hapnium* and the rest five isolates were less virulent isolates on *P .hapnium*. (**Table: 89).**

Table: 89, Pathogenicity/ virulence of *Phytophthora capsici* infecting black pepper on differential *Piper* species - Race - 1

Sl. no	Isolate no.	Mean lesion diameter after 72 hrs in mm								
		Piper nigrum	***Piper betle***	***Piper longum***	***Piper chaba***	***Piper attenuatum***	***Piper Colubrinum***	***Piper arborium***	***Piper ornatum***	***Piper hapnium***
1	96-8	21.8	9.8	11.3	17.8	10	11.3	0	15.3	9
2	97-50	22	10.8	10	22.3	19	10.6	0	15.3	8.3
3	97-51	21.3	12.3	12.5	27.6	10.3	10.3	0	21.8	17.8
4	98-7	20	9.8	9.1	21	9.6	7.3	0	25.3	10.5
5	98-157	22.16	11.16	21.16	24.3	25.5	8	0	19	19.6
6	98-177	20	11.16	21	26.8	24.5	10.6	0	34.3	11.6
7	99-139	18	9.5	10	15	6	6.8	0	17.5	9.8
8	98-24	22.3	13.5	20	20.3	18.8	6.6	0	21	20.3
9	98-76	22.16	21.83	22.83	16	20	6	0	11.16	17.5
10	98-131	20.3	16.3	21.16	20.3	22	9.16	0	27	21.3
11	98-140	20.5	20	18	20.5	22.6	8.83	0	21.16	20.5
12	99-158	23.3	13.6	11.3	19.8	12.8	9.5	0	16.4	20
13	99-106	22.3	11.6	20.16	19.5	20.1	6.6	0	15	23.5

2. *P.capsici* - Race 2 (Isolates: 14-38)

They infected the entire *Piper* species tested. They were highly virulent group of isolates. Except the isolates 97-52 and 98-135 all other isolates were highly virulent on Sreekara leaves. The

reaction of the isolates varied with *Piper betle* leaves. The isolate 98-81 was highly virulent. And the four isolates 98-181, 98-182, 97-52 and 98-135 were less virulent. The rest twenty isolates were moderately virulent on *Piper betle*.

There were five isolates 98-144, 98-198, 99-103, 99-167, and 97-45c highly virulent on *P. longum* leaves. But nine isolates 98-49, 98-81, 98-167, 98-172, 99-125, 99-144, 98-162, 98-87, and 97-52 were less virulent and the rest eleven isolates were moderately virulent with *P .longum* leaves.

When *P. chaba* leaves were tested the four isolates 98-49, 98-144, 98-181, and 99-145 were less virulent. And eleven isolates 98-81, 98-167, 98-172, 99-144, 98-127, 98-162, 98-182, 98-166, 99-162, 98-174, 97-52 were moderately virulent and the rest ten isolates were highly virulent with *P. chaba* leaves.

When *P. attenuatum* leaves were tested, the six isolates, 98-144, 98-167, 99-103, 99-144, 99-145, and 98-87, were less virulent isolates. The nine isolates, 98-49, 98-81, 98-172, 98-198, 99-104, 98-127, 98-162, 97-52 and 98-135 were moderately virulent with *P. attenuatum* leaves. But the rest ten isolates were highly virulent isolates with *P. attenuatum* leaves.

All the isolates were less virulent with *P. colubrinum* leaves. The isolates 98-81, 99-167, 98-67, 98-127, 98-162, and 98-181 were found to produce more than 10 mm lesion with *P .colubrinum* leaves. All of these isolates produce very small lesion with *P. arborium* leaves.

P. ornatum leaves show a different reaction with *Phytophthora* isolates. The six isolates, 98-144, 98-167, 98-172, 98-127, 99-145, and 98-87 were less virulent and the four isolates 98-49, 98-182, 98-166, 99-162 and 97-52 were highly virulent isolates with *P.ornatum* leaves. The rest fifteen isolates are moderately virulent with *P.ornatum* leaves.
All the isolates produce lesion with *P. hapnium* leaves. The three isolates 98-144, 98-182, 97-52 and 99-145, were less virulent and the ten isolates 98-167, 98-172, 99-103, 99-104, 97-45c, 98-127, 98-162, 98-181, 98-87, and 98-75 were highly virulent and the rest twelve isolates were moderately virulent with *P.hapnium* leaves. (**Table: 90)**

Table: 90, Pathogenicity/virulence of *Phytophthora capsici* infecting black pepper on differential *Piper* species - Race- 2

Sl. no	Isolate no.	Mean lesion diameter after 72 hrs in mm								
		Piper nigrum	***Piper betle***	***Piper longum***	***Piper chaba***	***Piper attenuatum***	***Piper Colubrinum***	***Piper arborium***	***Piper ornatum***	***Piper hapnium***
14	97-52	21.6	10	10.8	16.8	18.8	13.5	5	31.8	10.6
15	98-135	23	10	20	30.5	21	8.3	5	17.3	21.3
16	98-49	28.5	18.5	10.6	8.6	23	5.5	4.8	27.6	18.6
17	98-81	31.8	28.6	14.5	23.5	22	11.3	5.5	17.3	24
18	98-144	46	15.5	31.5	9.5	10.16	9.5	5.3	10.5	13.3
19	98-167	27	21.16	14.6	15.5	11	6.6	5.3	11.3	25.16
20	98-172	25.6	19.6	5	17.5	17.8	7	5.5	14.3	30.3
21	98-198	37.8	22.5	27.6	32.16	17.6	9	4.8	20.5	15.3
22	99-103	25.6	19.3	26.8	30.1	10.16	5.6	5.5	19.5	29
23	99-104	34	22.66	19	30.3	22.3	6	5.5	21.16	29.83
24	99-125	31.8	19.6	11.5	25	27.5	6.16	5.5	22.5	19.6
25	99-144	25.5	15.3	8.5	18.28	10.5	7	5.5	19	18.6

26	99-166	25.5	20.1	21.16	25.5	26.5	5.6	6	17.5	18.3
27	99-167	25.6	20.16	30.5	29	31.6	10.5	5.3	20.16	18.5
28	97-45c	27.3	21.3	35.6	25.6	27.5	8.1	4.8	22.5	28.5
29	98-67	35	20.5	20.3	26	29	10.16	5.5	18.8	21.8
30	98-127	25.6	20.8	23.3	23.16	20.6	14.16	5.16	13.3	38
31	98-162	26.8	16.6	6	20.5	22.6	12	5	16	28
32	98-181	34	12.6	22	11.6	25.3	10.16	5.3	15.6	27
33	98-182	31.6	10	21.3	16.8	26.5	5.6	6	28.16	11.6
34	98-166	40.3	19.3	22.6	21.5	28.83	9.5	5.6	27.5	19.6
35	99-162	26.6	23.6	23	20.3	26.8	10	5.16	26.5	23
36	98-174	30.3	19.5	19.8	17.16	27.16	8.5	8.3	20.5	23
37	99-145	26.8	17.3	23	12	10.3	7.3	6.6	9.1	10.6
38	98-87	30.83	21	13.6	29.8	10	5	5.5	8.16	30.6

3. *P. capsici* -Race – 3 (Isolates: 39-49)

They were not infecting *P. betle*, *P. longum*, *P. colubrinum* and *P. arboreum*. They were less virulent and they were not producing lesion with most of the tested species of *Piper*. All the eleven isolates were less virulent with Sreekara. There was no lesion development in the leaves of *P. betle* by eleven isolates. None of the isolates produce lesion with *P. longum*. The isolates were less virulent with *P. chaba*. Eleven isolates were less virulent with *P. attenuatum* leaves. They were not producing any lesion on *P. colubrinum*. None of the isolates produce lesion with *P. arboreum*. *P.*

ornatum leaves show a different reaction with the isolates. The three isolates 00-40, 97-11a, and 97-55, were moderately virulent. But the rest seven isolates were less virulent with *P. ornatum* leaves. In the case of *P. hapnium* leaves the seven isolates 96-13, 98-60, 00-40, 96-11, 97-55, and 98-17 were moderately virulent and the rest four isolates were less virulent. (**Table: 91).**

Table: 91, Pathogenicity / virulence of *Phytophthora capsici* infecting black pepper on differential *Piper* species (Race- 3)

Sl. no	**Isolate no.**	**Mean lesion diameter after 72 hrs in mm**								
		Piper nigrum	***Piper betle***	***Piper longum***	***Piper chaba***	***Piper attenuatum***	***Piper Colubrinum***	***Piper arborium***	***Piper ornatum***	***Piper hapnium***
39	96-5	13.6	0	0	7.8	9.6	0	0	10.3	10.3
40	96-13	12.5	0	0	11.6	9.5	0	0	11.5	18.8
41	98-60	13	0	0	9.3	10.6	0	0	12	15.5
42	98-145	9	0	0	8.3	9.8	0	0	12.56	12.6
43	00-18	9.8	0	0	8.3	9.16	0	0	15.5	11.8
44	00-40	12	0	0	10	9.6	0	0	12.8	17.16
45	96-4	11.3	0	0	10.3	8.1	0	0	13.8	10.5

46	97-11a	13	0	0	9.16	8.83	0	0	16.1	10
47	96-11	10	0	0	11.3	8.16	0	0	8.83	19.8
48	97-55	14	0	0	9.6	14	0	0	17.3	20
49	98-17	8.6	0	0	10.3	8.16	0	0	10.3	20.1

4. *P. capsici* - Race 4 (Isolates: 50)

The isolate was not infecting *P. ongum* and *P. arboreum*. It was less virulent on Sreekara, *P. betle*, *P. chaba* and *P. colubrinum*. It was moderately virulent on *P. attenuatum* and *P. hapnium*. It was highly virulent on *P. hapnium*. (**Table: 92).**

Table: 92, Pathogenicity /virulence of *Phytophthora capsici* infecting black pepper on differential *Piper* species (Race- 4)

Sl. no	Isolate no.	Mean lesion diameter after 72 hrs in mm								
		Piper nigrum	***Piper betle***	***Piper longum***	***Piper chaba***	***Piper attenuatum***	***Piper Colubrinum***	***Piper arborium***	***Piper ornatum***	***Piper hapnium***
50	99-91	14	5	0	14.8	19	5	0	26.3	18.3

5. *P. capsici* - Race - 5 (Isolate No: 51)

The isolate was not infecting *P. longum*, *P. colubrinum* and *P. arboreum*. It was less

virulent on Sreekara, *P. betle*, *P. chaba*, *P. ornatum* and *P. hapnium*. It was moderately virulent on *P. attenuatum*. (**Table: 93).**

Table: 93, Pathogenicity /virulence of *Phytophthora capsici* infecting black pepper differentials of *Piper* species (Race- 5)

Sl. no	**Isolate no.**	**Mean lesion diameter after 72 hrs in mm**								
		Piper nigrum	***Piper betle***	***Piper longum***	***Piper chaba***	***Piper attenuatum***	***Piper Colubrinum***	***Piper arborium***	***Piper ornatum***	***Piper hapnium***
51	99-124	13	5	0	13.8	15.6	0	0	13.5	9.8

Statistical Analysis of Pathogenicity /virulence of *Phytophthora capsici* infecting black pepper on differentials of *Piper* species

Pathogenicity of different *Piper* species on *Phytophthora capsici* was analyzed statistically. There was significant variation between different groups. The isolates of Race 5 had minimum lesion development. Race 2 produced maximum pathogenicity. The races 3, 4 and 5 were similar in pathogenicity. (**Table: 94)**.

Table: 94, ANOVA and DMRT of Pathogenicity / virulence of *Phytophthora capsici* infecting black pepper on differentials of *Piper* species

Pathogenicity	**Sum of Squares**	**df**	**Mean Square**	**F**	**Group**	**N**	**Mean value**
Between							

Groups	58832.142	4	14708.036	213.748	5.00	54	6.4074a
Within Groups	189159.149	2749	68.810		3.00	594	7.0471a
Total	247991.292	2753			4.00	54	8.3333a
					1.00	702	14.5897b
					2.00	1350	18.3726c

2) *P. meadii* isolates (Isolates: 52-54)

They were less virulent with most of the *Piper* species studied. There were three isolates, 98-86, 98-90, and 98-192. They were less virulent with Sreekara and no lesion was produced with *P. betle*, *P. longum*, *P. attenuatum*, *P. colubrinum*, and *P. arboreum*. But with *P. chaba* leaves the isolate 98-192 produced a small lesion. The isolates were less virulent with *P. ornatum* and *P. hapnium* leaves. (**Table: 95).**

Table: 95, Pathogenicity / virulence of *Phytophthora meadii* infecting black pepper on differentials of *Piper* species

Sl. no	Isolate no.	Mean lesion diameter after 72 hrs in mm

		Piper nigrum	*Piper betle*	*Piper longum*	*Piper chaba*	*Piper attenuatum*	*Piper Colubrinum*	*Piper arborium*	*Piper ornatum*	*Piper hapnium*
52	98-86	3.6	0	0	0	0	0	0	5.3	8
53	98-90	5.5	0	0	0	0	0	0	10.5	10
54	98-192	10.6	0	0	4.16	0	0	0	10.16	10.3

3) *P. palmivora* (Isolate No: 55&56)

These isolate, 98-1 from Thamarassery, Calicut was a soil isolate and 99-127 was from KAU, Trichur was a leaf isolate, were very less pathogenic with the *Piper* species. They were not producing lesion with any of the species of *Piper* tested. Very small lesion was produced with *P. chaba* alone. (**Table: 96).**

Table: 96, Pathogenicity /virulence of *Phytophthora palmivora* infecting black pepper on differentials of *Piper* species

Sl. no	Isolate no.	Mean lesion diameter after 72 hrs in mm								
		Piper nigrum	*Piper betle*	*Piper longum*	*Piper chaba*	*Piper attenuatum*	*Piper Colubrinum*	*Piper arborium*	*Piper ornatum*	*Piper hapnium*
55	98-1	0	0	0	5.3	0	0	0	0	0
56	99-127	0	0	0	5.8	0	0	0	0	0

4) *P. parasitica* isolate (Isolate No: 57-60)

They produced lesion with most of the species tested. The isolates were 99-186 collected from Belur, Karnataka was leaf isolate, 99-188 collected from Andhrapradesh also was a leaf isolate and 98-95 collected from Adivarum, Kerala was a stem isolate. The isolates 99-186 and 99-188 were moderately virulent and 98-95 was less virulent with Sreekara leaves. They were producing no lesion with *P. betle* and *P. longum* leaves. All of them were less virulent with *P. chaba*. The isolates 99-186 and 99-199 were moderately virulent and 98-95 was less virulent with *P.attenuatum* leaves. They were less virulent with *P.colubrinum* and non virulent with *P. arboreum* and *P. hapnium* leaves. Two of them 99-186 and 99-188 were highly virulent with *P. ornatum* leaves and 98-95 was less virulent with *P. ornatum* leaves. (**Table: 97).**

Table: 97, Pathogenicity / virulence of *Phytophthora parasitica* infecting black pepper on differentials of *Piper* species

Sl. no	Isolate no.	Mean lesion diameter after 72 hrs in mm								
		Piper nigrum	*Piper betle*	*Piper longum*	*Piper chaba*	*Piper attenuatum*	*Piper Colubrinum*	*Piper arborium*	*Piper ornatum*	*Piper hapnium*
57	99-186	17.5	0	0	7.6	18.16	7.8	0	32.5	0
58	99-188	19.16	0	0	7.16	18.8	5.6	0	34.16	0
59	98-95	8.6	0	0	5.8	10	6	0	9.6	0
60	98-75	26.3	22.6	10.66	28.5	29.16	5.16	5.16	19.6	27.5

It was clear from the study that the pathogenicity of *P. capsici*, *P. meadii*, *P. palmivora* and

P. *parasitica* vary very much. The *P*. *capsici* isolates were grouped into five races based on pathogenicity. Comparison of Races (?) of *Phytophthora capsici* infecting black pepper is given in **Table 98.**

Table: 98, Comparison of Races (?) of *Phytophthora capsici* infecting black pepper

Races	**Pathogenicity**	**Morphological group**	**Morphological characters**	**Physiological characters**	**Source of the isolates**
Race I Isolate No: 1-13	Infected 8 *Piper* species Not infected *P. arboreum*	Morphological Group I (7 isolates) and Group II (6 isolates)	**Colony:** Chrysathemum like and white cottony **Caducity %** –51-75. **Pedicel length Range -** 39-150 µm. **Sporangia:** Ovoid to obovoid and fusiform **LB ratio**: 1.4-2.3. **Chlamydospores:** produced. **Mating type**: A1	**Effect of temperature** All the isolates of race I except two grew and sporulate at 35 ^{0}C. No zoospore liberation at 35 ^{0}C. No zoospore germination at 10 ^{0}C and 35 ^{0}C. **Effect of pH** The seven isolates of race I grew at all the pH tested. But the 6 isolates of morphological group II cannot grow at pH 2.5. No sporulation. zoospore liberation and zoospore germination occurs at pH 2.5	Leaf – 7, Stem -2, Root - 3, Soil -1.

				Effect of media All the isolates of Race I had maximum growth and sporulation in CA. Zoospore liberation was maximum in CA and minimum in CMA. Zoospore germination was maximum in CA and minimum in PDA.	
Race II Isolate No: 14-38	Infected all the nine *Piper* species tested	Morphological Group I (2 isolates) and Group III (23 isolates)	The first two isolates had **Colony**: Chrysanthemum like **Caducity**: 51-75 %. **Pedicel length range**: 39-94 µm. **Sporangia**: Ovoid **LB ratio**: 1.4-1.6. **Chlamydospores**: produced.	**Effect of temperature** All the isolates grew well at 35 ^{0}C. No zoospore release at 35 ^{0}C. **Effect of pH** No growth, sporulation, zoospore liberation and zoospore germination occurs at pH 2.5. **Effect of media** Maximum growth, sporulation,	Leaf -7 , Stem -1 , Spike - 1, Berry-1, Soil- 6, Root -4.

			Mating type: A1 The rest 23 isolates had **Colony morphology:** Chrysanthemum like**,** stellate, and floral. **Caducity**: 26- 50 %. **Pedicel length range**: 71 -192 µm. **Sporangia**: Ovoid, obovoid, obpyriform, or with distorted shapes. **LB ratio**: 2-3.5. **Chlamydospores:** were not produced in 18 and produced in 5 isolates. **Mating type**: A1	zoospore liberation and zoospore germination was in CA for most of the isolates. Minimum sporulation was in PDA, zoospore liberation in CMA and zoospore germination in PDA.	
Race III Isolate No:39- 49	Infected *P.nigrum* (Sreekara), *P. chaba*, *P.*	Morphological Group IV	**Colony**: Uniform cotton wool like aerial mycelium. **Sporangia**: Papillate	**Effect of temperature** They cannot tolerate 35 ^{0}C. They cannot sporulate at 10 ^{0}C. No zoospore liberation and zoospore	leaf - 7, root - 2, soil - 2.

	attenuatum *P. ornatum* *P. hapnium*		ovoid to obovoid and fusiform LB ratio: 1.6-2.9. **Caducity**: 76-100. **Pedicel length range**: 70-167 µm **Chlamydospores**: not produced. **Mating type**: A2 – 3 isolates Neuter- 8 isolates.	germination occurs at 10 ^{0} C and 35^{0}C. **Effect of pH** No growth, sporulation, zoospore liberation and zoospore germination occurs at pH 2.5 **Effect of media** Maximum growth was in CA. Minimum sporulation was in PDA. Zoospore liberation was maximum in CA and minimum in PDA.	
Race IV Isolate No:50	Infected *P.nigrum* (Sreekara) *P. betle* *P. chaba* *P. attenuatum*	Morphological Group III (11isolates).	**Caducity**: 26 %. **Pedicel length**: 121 µm. **Sporangia:** Ovoid, obovoid, obpyriform, or with distorted shapes. **LB ratio**: 2.	**Effect of temperature** Growth was minimum at 35 ^{0}C. At 10 ^{0}C and 35 ^{0}C no sporulation, zoospore liberation and zoospore germination.	Leaf- 1

	P. colubrinum *P. ornatum* and *P. hapnium*		**Chlamydospores**: Produced. **Mating type**: A1	**Effect of pH** The isolate grew at all the pH tested. No sporulation, zoospore liberation and germination occurs at pH 2.5. **Effect of media** The isolate had maximum growth and sporulation in CA and minimum in PDA.	
Race V Isolate No:51			**Caducity**: 26 %. **Pedicel length**: 136 µm. **Sporangia**: Ovoid, obovoid, obpyriform, or with distorted shapes. **LB ratio**: 2. **Chlamydospores**: not produced. Mating type was A1	**Effect of temperature** Growth was minimum at 35 ^{0}C. At 10 ^{0}C and 35 ^{0}C no sporulation, zoospore liberation and zoospore germination.	Stem- 1

				Effect of pH The isolate grew at all the pH except pH 2.5. No sporulation, zoospore liberation and germination occurs at pH 2.5. **Effect of media** It had maximum growth in CMA and minimum in PDA. Maximum sporulation was in CA.	

7.2. Pathogenicity/ virulence of *Phytophthora capsici* infecting black pepper by stem and root inoculation on Sreekara variety of black pepper plants

Representatives of each race *P. capsici* were tested and the racial picture of *P. capsici* was further confirmed (**Fig. 19, 20 and** Plate **15).**

***P. capsici* (Race 1):**

The isolates of Race 1 were moderately virulent and produced 50-55% stem infection and 45-55% root infection. The pathogenicity on stem, roots and leaves were comparable. (**Table: 99).**

Table: 99, Pathogenicity / virulence of *Phytophthora capsici* infecting black pepper by stem and root inoculation on Sreekara variety of black pepper plants (*P. capsici*- Race 1)

Sl no.	Isolate no.	Mean percent root infection after 14 days	Mean percent stem infection after 4 days
1	97-50	55	55
2	99-139	45	50
3	98-24	50	55
4	99-158	45	50

***P. capsici* (Race 2):**

The isolates of Race 2 were highly virulent and produced 45-65 % stem infection and 40-80 % root infection. The pathogenicity on stem, roots and leaves were comparable. (**Table: 100).**

Fig: 19, Pathogenicity of *Phytophthora* species infecting black pepper by stem inoculation on Sreekara variety of black pepper plants

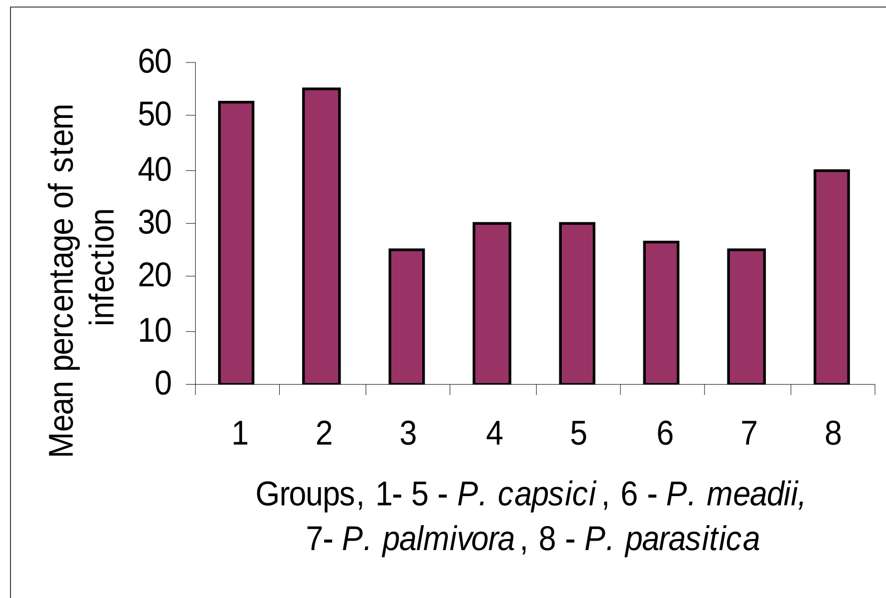


Fig: 20, Pathogenicity of *Phytophthora* species infecting black pepper by root inoculation on Sreekara variety of black pepper plants

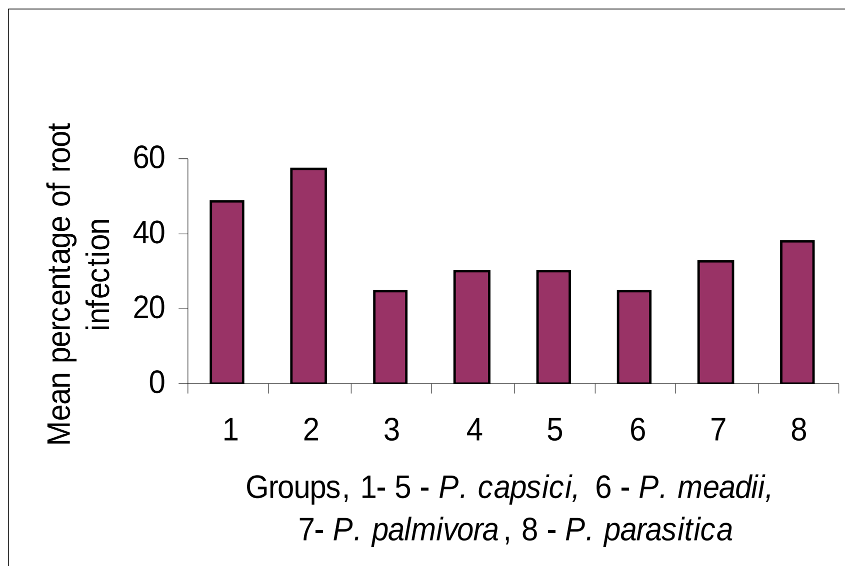


Table: 100, Pathogenicity/ virulence of *Phytophthora capsici* infecting black pepper by stem and root inoculation on Sreekara variety of black pepper plants-

***P. capsici* - Race - 2:**

Sl no.	Isolate no.	Mean percent root infection after 14 days	Mean percent stem infection after 4 days
5	99-103	75	65
6	99-167	80	65
7	99-166	70	65
8	98-67	45	45
9	98-127	45	45
10	98-166	45	45
11	98-87	40	55

***P. capsici* -Race 3:**

The isolates of Race 3 were less virulent and produced 25 % stem infection and 25 % root infection. The pathogenicity on stem, roots and leaves were comparable. Pathogenicity of (**Table: 101).**

Table: 101, Pathogenicity / virulence of *Phytophthora capsici* infecting black pepper by stem and root inoculation on Sreekara variety of plants - *P. capsici* -Race 3:

Sl no.	Isolate no.	Mean percent root infection after 14 days	Mean percent stem infection after 4 days
14	97-55	25	25
15	98-17	25	25
16	98-60	25	25
17	00-40	25	25

Pathogenicity/ virulence of *Phytophthora capsici* infecting black pepper by stem and root inoculation on Sreekara variety of black pepper plants- *P. capsici* -Race 4:

The isolates of Race 4 were less virulent and produced 30 % stem infection and 25 % root infection. The pathogenicity on stem, roots and leaves were comparable. (**Table**: **102)**.

Table: 102, Pathogenicity/ virulence of *Phytophthora capsici* infecting black pepper by stem and root inoculation on Sreekara variety of black pepper plants -*P. capsici* -Race 4:

Sl no.	Isolate no.	Mean percent root infection after 14 days	Mean percent stem infection after 4 days
12	99-91	25	30

***P. capsici* -Race 5:**

The isolates of Race 5 were less virulent and produced 30% stem infection and 30% root infection. The pathogenicity on stem, roots and leaves were comparable. (**Table: 103)**.

Table: 103, Pathogenicity / virulence of *Phytophthora capsici* infecting black pepper by stem and root inoculation on Sreekara variety of black pepper plants- *P. capsici* -Race 5:

Sl no.	Isolate no.	Mean percent root infection after 14 days	Mean percent stem infection after 4 days
13	99-124	30	30

Statistical Analysis of Pathogenicity / virulence of *Phytophthora capsici* infecting black pepper by stem inoculation on Sreekara variety of black pepper plants

Stem pathogenicity of *Phytophthora capsici* isolates on Sreekara plants was analyzed statistically. There was significant variation between different groups. Minimum pathogenicity was found in group 3. The Races 3, 4 and 5 are weakly pathogenic and statistically similar. The Races 1 and 2 were highly pathogenic and similar. (**Table: 104)**.

Table: 104, ANOVA and DMRT of Pathogenicity / virulence of *Phytophthora capsici* infecting black pepper by stem inoculation on Sreekara plants

Pathogenicity on stem	Sum of Squares	df	Mean Square	F	Group	N	Mean value
Between Groups	14845.588	4	3711.397	27.945	3.00	20	25.0000a

Within Groups	10625.000	80	132.813		4.00	5	30.0000a
Total	25470.588	84			5.00	5	30.0000a
					1.00	20	52.5000b
					2.00	35	55.0000b

Statistical Analysis of Pathogenicity / virulence of *Phytophthora capsici* infecting black pepper by root inoculation on Sreekara plants

Root pathogenicity of *Phytophthora capsici* isolates on Sreekara plants was analyzed statistically. There was significant variation between different groups of *Phytophthora capsici* isolates. Minimum lesion was produced in race 3. Maximum lesion was produced in Race 2. Races 1 and 2 were highly virulent compared to races 3, 4 and 5 which were similar in pathogenicity. (**Table: 105).**

Table: 105, ANOVA and DMRT of Pathogenicity / virulence of *Phytophthora capsici* infecting black pepper by root inoculation on Sreekara plants

Pathogenicity on roots	Sum of Squares	df	Mean Square	F	Group	N	Mean value
Between Groups	15662.553	4	3915.638	19.507	3.00	20	25.0000a
Within Groups	16058.036	80	200.725		4.00	5	30.0000a
Total	31720.588	84			5.00	5	30.0000a
					1.00	20	48.7500b
					2.00	35	57.1429b

2) *P. meadii* isolates

The *Phytophthora meadii* isolates were less virulent and produced 25 % stem infection and 25 % root infection. The pathogenicity on stem, roots and leaves were comparable. (**Table: 106).**

Table: 106, Pathogenicity/ virulence of *Phytophthora meadii* infecting black pepper by stem and root inoculation on Sreekara plants

Sl no.	Isolate no.	Mean percent root infection after 14 days.	Mean percent stem infection after 4 days.
18	98-192	25	25
19	98-86	25	25
20	98-90	25	25

3) *P. palmivora*

The *Phytophthora palmivora* isolates were less virulent and produced 25 % stem infection and 25 % root infection. The pathogenicity on stem, roots were comparable. But they were not producing lesion on pepper leaves. (**Table**: **107)**.

Table: 107, Pathogenicity/ virulence of *Phytophthora palmivora* infecting black pepper by stem and root inoculation on Sreekara plants

Sl no.	Isolate no.	Mean percent root infection after 14 days	Mean percent stem infection after 4 days.
21	98-1	25	25
22	99-127	25	25

4) *P. parasitica* isolate

The *Phytophthora parasitica* isolates were moderately virulent and produced 40% stem infection and 40 % root infection. The pathogenicity on stem, roots and leaves were comparable. (**Table**: **108)**.

Table: 108, Pathogenicity / virulence of *Phytophthora parasitica* infecting black pepper by stem and root inoculation on Sreekara plants

Sl no.	Isolate	Mean percent root infection	Mean percent stem infection

	no.	after 14 days	after 4 days
23	99-186	40	40
24	99-188	40	40
25	98-75	40	40

It was clear from the study that the pathogenicity of *P. capsici*, *P. meadii*, *P. palmivora* and *P. parasitica* vary very much. The *P. capsici* isolates were grouped into five races based on pathogenicity.

8. Cross infectivity of *Phytophthora* species on different plantation crops

For cross infectivity studies *Phytophthora* from different crops were used. They were 1) rubber isolates (*P. meadii*), 2) cocoa isolates (*P. palmivora*), 3) cardamom isolates (*P. meadii*), 4) areca nut isolates (*P. meadii)*, 5) black pepper isolates (*P. capsici*), 6) betelvine isolates (*P. parasitica* and *P. capsici*), 7) coconut isolates (*P. palmivora*). From each crop six isolates were selected and they were tested on rubber leaf petiole, cocoa pods, areca nut, black pepper leaves, betel vine leaves, and coconut.

The rubber isolates produced lesion on areca nut, cocoa and rubber. Maximum lesion diameter was obtained with rubber and minimum was with areca nut. They were moderately virulent with cocoa and areca nut and highly virulent with rubber.

The cocoa isolates produce lesion on areca nut, cocoa, pepper and rubber and no lesion development was in betel vine and coconut. They were highly virulent on rubber and cocoa and were less virulent on areca nut. The isolates 98-130, and 99-6 were less virulent on pepper and the rest four isolates were moderately virulent on pepper.

Cardamom isolates were pathogenic to areca nut, rubber, pepper and cocoa and non pathogenic to betel vine and coconut. They were less virulent on areca nut and moderately virulent on rubber and less virulent on pepper. They were highly virulent on cocoa.

Areca nut isolates were pathogenic on areca nut and rubber and non virulent on pepper, betel vine, cocoa and coconut. They were highly virulent on rubber. Black pepper isolates were pathogenic on areca nut, rubber, pepper, betel vine, cocoa and coconut. They were moderately virulent on areca nut and highly virulent on robber and pepper. Five isolates were moderately virulent on betel vine but the isolate 97-50 was less virulent on betel vine. Five isolates were less virulent on cocoa but 98-127 was moderately virulent on cocoa. Four isolates were moderately virulent on coconut but 99-103 and 98-127 were less virulent on coconut.

Betel vine isolates were pathogenic to all the tested crops except cocoa. They were less virulent on areca nut. Four isolates were less virulent on rubber but the isolates 98-203 and 98-127 were moderately virulent on pepper. Three isolates were moderately virulent on betel vine but the isolates 98-203, 98-154, and 98-197 were highly virulent on betel vine. The isolate 98-99 was highly virulent on coconut and the rest five isolates were moderately virulent on coconut.

The coconut isolates were pathogenic to areca nut, rubber, cocoa and coconut. They were not pathogenic to pepper and betel vine. They were less virulent to areca nut, pepper and cocoa. But the isolate 96-20 was moderately virulent on cocoa. All the isolates were highly virulent on coconut. (**Table: 109)**. Host range and pathogenicity of *Phytophthora* isolates on black pepper are given in (**Table: 110)**.

Table: 109, Cross infectivity of *Phytophthora* species on different plantation crops

Group	**Sl.No**	**Isolate no**	**Mean lesion diameter in the host after 72 hrs**					
			Arecanut	Rubber	Black pepper	Betel vine	Cocoa	Coconut
I Rubber	1	99-151	16	33	0	0	18	0
	2	98-38	19	35	0	0	20	0
	3	99-175	15	36	0	0	22	0
	4	98-25	18	42	0	0	20	0

	5	98-26	19	31	0	0	19	0
	6	98-31	18	31	0	0	18	0
II Cocoa	7	98-11	8	37	19	0	35	0
	8	98-130	13	30	14	0	35	0
	9	99-6	11	32	11	0	44	0
	10	97-3	11	30	18	0	33	0
	11	99-108	12	29	18	0	31	0
	12	99-4	12	27	22	0	30	0
III Cardamom	13	99-169	9	19	12	0	46	0
	14	99-168	5	15	14	0	33	0
	15	99-180	6	18	12	0	30	0
	16	99-164	9	18	12	0	31	0
	17	99- 183a	6	18	12	0	31	0
	18	99-181	6	18	12	0	34	0
IV Arecanut	19	97-10	27	37	0	0	0	0
	20	98-32	35	36	0	0	0	0
	21	99-85	30	47	0	0	0	0
	22	99-185	34	40	0	0	0	0
	23	99-90	31	27	0	0	0	0
	24	99-89	32	36	0	0	0	0
V Black pepper	25	99-167	19	28	27	22	14	18
	26	98-81	22	28	33	23	13	15
	27	98-24	18	26	24	13	14	20
	28	99-103	18	31	26	22	14	14
	29	98-127	22	36	27	22	15	14
	30	97-50	22	27	23	9	12	16
VI Betel vine	31	98-203	7	21	15	37	0	18
	32	99-124	7	11	16	22	0	18
	33	98-121	12	9	16	20	0	22
	34	98-99	7	13	14	22	0	25

	35	98-154	7	9	20	27	0	24
	36	98-197	6	16	25	36	0	19
VII Coconut	37	98-160	6	8	0	0	13	33
	38	97-46	7	8	0	0	10	37
	39	99-99	7	8	0	0	10	37
	40	96-22	6	5	0	0	13	34
	41	96-20	7	7	0	0	22	30
	42	96-21	8	6	0	0	12	30

From the study it was clear that *Phytophthora* isolates from rubber, arecanut, and coconut were not pathogenic to black pepper

Table: 110, Host range of *P.capsici* isolates

Sl. No:	Host range of *P.capsici* isolates
1	*P.capsici* isolates infected all the tested hosts arecanut, rubber, black pepper, betle vine, cardamom, cocoa and coconut.

Statistical analysis of cross infectivity of *Phytophthora* species on different plantation crops

Cross pathogenicity of *Phytophthora* isolates on different crops was analyzed statistically. There was significant variation between different groups of isolates. The coconut isolates had minimum pathogenicity compared to others. Maximum amount of lesion was produced with black pepper isolates. The coconut, arecanut, cardamom and rubber isolates were similar in pathogenicity. The betel vine and cocoa isolates were also similar in pathogenicity based on the analysis. **(Table: 111)**.

Table: 111, ANOVA and DMRT of Cross infectivity of *Phytophthora* species on different plantation crops

Cross infectivity	Sum of Squares	df	**Mean Square**	**F**	**Group**	**N**	**Mean value**
Between	23650.680	6	3941.780	24.5	7.00	215	9.8000a

Groups							
Within Groups	247726.957	1540	160.862		4.00	216	11.3935a
Total	271377.637	1546			3.00	252	11.6230a
					1.00	216	12.0880a
					6.00	216	14.5787 b
					2.00	216	15.5278 b
					5.00	216	22.5046c

9. Characterization of *Phytophthora capsici* isolated from other hosts

9.1. Morphology of *Phytophthora capsici* isolated from other hosts

The *P. capsici* isolates obtained from other hosts were studied for their morphology and pathogenicity. The isolates were from betel vine, cocoa, *Capsicum*, *P. chaba* and *Bauhinia purpurea*. Five isolates from betle vine were studied and they showed umbellate ontogeny and long sporangial pedicels. The isolates 98-120, 98-200 and 98-122 showed white cottony colony morphology but the isolates 99-158 and 98-197 had stellate colony pattern.

All the cocoa isolates were studied morphologically and two isolates were *P. capsici* and the rest of the isolates were *P. palmivora*. The *P. capsici* isolates were with white cottony (98-64) and stellate colony morphology (00-7) and umbellate ontogeny. Two isolates were with long sporangial pedicels. They were identified as *P. capsici* .All others were with small pedicels (3.8-4.5) and sympodial ontogeny. They were *P. palmivora*. *P. capsici* isolates from cocoa infected cocoa pods as well as pepper leaves.

The isolate 98-47 from *Capsicum* had white cottony mycelium and umbellate ontogeny and long sporangial pedicels. The LB ratio was 1.5. Both of the *P.capsici* isolates from *P. chaba* had white cottony mycelium and umbellate ontogeny and long sporangial pedicels. LB ratio was >2. The P. *capsici* isolated from *Bauhinia purpurea* had white cottony mycelium and umbellate ontogeny

and long sporangial pedicels. LB ratio was > 2. (**Table: 112).**

Table: 112, Morphology of *P. capsici* isolated from other hosts

Sl no	**Isolate no**	**Host**	**Ontogeny**	**Sporangial length µm**	**Sporangial breadth µm**	**L/B**	**Pedicel length µm**
1	98-200	P.betle	Umbellate	45±3	25.9±1.5	2	89
2	98-122	*P.betle*	Umbellate	40.5±3	18.7±1.4	2.1	117.3
3	98-197	*P.betle*	Umbellate	39.8±1.8	22.2±2	1.75	52.8
4	98-120	*P.betle*	Umbellate	42.7±2.7	21.4±1.5	2.12	101.9
5	98-158	*P.betle*	Umbellate	55.4±3.1	27.2±1.6	2.33	102
6	98-64	*Cocoa*	Umbellate	38±2.3	21±1.1	1.8	85
7	00-7	*Cocoa*	Umbellate	40±2.2	20±1.2	1.9	96
8	98-47	*Capsicum*	Umbellate	42±1	20±1	1.5	101
9	98-33	*P.chaba*	Umbellate	40.4±2.2	21±1.2	1.9	104
10	99-163	*P.chaba*	Umbellate	38±2	20±1	1.8	98
11	97-5	*Bauhinia purpurea*	Umbellate	40.5±2.3	21.8±1.3	1.9	102

9.2. Pathogenicity of *P. capsici* isolated from other hosts

Isolates from different hosts like betel vine, *P. chaba*, *Capsicum*, *Cacao*, and *Bauhinia purpurea* was used in the study. It was found that all these isolates were pathogenic on black pepper roots. One *capsicum* and cocoa isolates were found to be less pathogenic than all other isolates.

It was found that all these isolates were pathogenic on black pepper leaves. Pathogenicity of *P. capsici* isolated from cocoa was less than all other isolates (14.5 mm). Two betel vine isolates were less virulent. (**Table: 113)**.

Table: 113, Pathogenicity of *P. capsici* isolates on black pepper roots and leaves

Sl no	*P. capsici* isolates	Source of isolate	Reaction on black pepper	
			Percentage of root infection	Lesion diameter on pepper leaves 72 hr.
1	98-200	Betel vine	60	27.5
2	98-122	Betel vine	70	32.5
3	98-197	Betel vine	60	35
4	98-120	Betel vine	60	27.5
5	98-158	Betel vine	80	42.5
6	00-7	Cocoa	70	30.3
7	98-64	Cocoa	50	14.5
8	98-47	*Capsicum*	50	25.6
9	99-163	*P.chaba*	60	28
10	98-33	*P chaba*	60	27
11	97-5	*Bauhinia purpurea*	60	25
12	99-103	Black pepper	90	40

7. Polyacrylamide gel electrophoresis of mycelial proteins of *Phytophthora* species infecting black pepper

Variability of *Phytophthora* isolates from black pepper was studied by electrophoresis of total proteins by SDS –PAGE. The gels were visually observed and there was considerable variation between different *Phytophthora* species and among *P.capsici*. UPGMA cluster analysis of the isolates were done using NTSYS programme. Based on the banding patterns the isolates can be grouped into four major clusters, A, B, C and D. (**Plate: 16** and Fig**. 21)**.

Cluster A – (*P. capsici)*

The upper major cluster is cluster A had similarity of 79.5 % and it consisted of *P. capsici* isolates, and it had two sub clusters, ie. s.1 and s.2.

s.1, The upper sub cluster had 100 % similarity and consisted of 6 *P. capsici* isolates. The isolate 99-103 showed chrysanthemum like pattern, 98-67 showed stellate pattern. The isolates 99-91 and 99-124 showed floral pattern and LB ratio was more than 2. The above described four isolates were *P. capsici* isolates of morphological group III and they consisted of Race II, IV and V. The rest two isolates 98-60 and 97-55 had uniform cotton wool like aerial mycelium and caducous with long pedicels. They were *Phytophthora capsici* of morphological group IV and Race III.

s.2, The lower sub cluster consists of three *P.capsici* isolates 98-24, 99-139, and 97-50. The first isolate 98-24 was with white cottony mycelium and LB ratio 2. But the isolates 99-139 and 97-50 were with chrysanthemum like colony pattern. LB ratio was 1.5. They were caducous with long pedicels and their LB ratio was 1.5. They were isolates of morphological group I and II and Race 1.

Cluster B – (*P. meadii)*

This cluster consisted of three *P. meadii* isolates 98-86, 98-192, and 98-90. They were 100 % similar and caducous with intermediate pedicels. The mean pedicel length was (12.6 µm). Among these isolates 98-86 and 98-90 had sporangia of sympodial ontogeny but the isolate 98-192 had irregular ontogeny. The isolate 98-86 and 98-192 had LB ratio of 1.45, whereas 98-90 had an LB ratio of 1.6.

Cluster C- (*P. palmivora)*

This cluster consisted of two *P.palmivora* isolates 98-1 and 99-127. They were 100 % similar and had white cottony mycelium with faded pattern. The LB ratio was 1.6 and they were caducous with small pedicels (5µm).

Cluster D - (*P. parasitica)*

This cluster consisted of *P. parasitica* isolates 98-95, 99-188 and 98-75, they were 100 % similar. They had white fluffy aerial mycelium LB ratio was 1.2 and they were non-caducous isolates.

8. Isozyme characterization of *Phytophthora capsici* infecting black pepper

The *P. capsici* isolates infecting black pepper, which clustered together, based on isozyme data, were grouped into three major groups, and in the lowest portion of the dendrogram the isolate 98-87 was seen. There was a correlation between clustering of the isolates and two important morphological characters, like caducity, colony morphology and the L / B ratio of the sporangia. In addition, there was also a correlation between the virulence and the grouping of the isolates. (**Fig: 22).** Isozyme profiles of *Phytophthora* species with Superoxide dismutase, Catalase, Malic enzyme, Malate dehydrogenase, Diaphorase, and Isocitrate dehydrogenase are given in (**Plates: 17 – 22)**,

Cluster A

This cluster had similarity of 84 % and it consisted of two sub clusters, ie. s1 and s2.
s1. The upper cluster had a similarity 87 % and it consisted of eleven *P. capsici* isolates of morphological group II and Race 2 and highly virulent. They were 99-144, 99-166, 98-144, 99-125,

98-198, 98-81, 99-103, 98-49, 99-104, 98-167, and 99-167 which had long caducous sporangia and a high LB ratio with values 2.2 - 3. The isolates had chrysanthemum like colony morphology, which were fast growers.

s.2. The second sub cluster had similarity of 85 % and it consisted of group of six *P.capsici* isolates of morphological group II and Race 1 namely, 98-172, 98-131,99-158, 98-24, 98-140, and 99-106, which was distinctly different from the other isolates of the cluster showed white cottony type of colony morphology and slow growth. Their LB ratio was 1.8-2.3. These isolates were moderately virulent. But the isolate 98-172 was different from this group and was similar to the first sub cluster.

Cluster B

This cluster had similarity of 83 % and it consisted of three sub clusters, ie. b.1 and b.2.

b.1. The first sub cluster had similarity of 95 % and it consisted of eight *P. capsici* isolates of morphological group IV and Race 3 namely, 96-13, 97-11, 96-4, 00-40, 96-5, 00-18, 98-60, 98-145 which were fast growers and LB ratio was 1.6-2.9. They were with uniform cotton wool like aerial mycelium and highly caducous sporangia. They were neuter isolates, which did not produce oospores. They were less virulent isolates, in contrast to the highly virulent and moderately virulent isolates observed in cluster A.

b.2 The second sub cluster had similarity of 89 % and it consisted of five isolates namely 99-91, 99-124, 98-76, 98-174, and 99-145, they had floral colony pattern, LB ratio 2-3. They were highly virulent isolates. The isolate 99-91 was *P. capsici* of morphological group III and Race 4**,** the isolate 99-124 of morphological group III and Race 5. The isolate 98-76 was *P. capsici* isolate of morphological group II and Race 1**,** and the isolates 98-174 and 99-145 were *P. capsici* of morphological group III and Race 2**.**

b.3 The third sub cluster had similarity of 89 % and consisted of nine *P. capsici* isolates of morphological group I and Race 1 namely, 98-157, 98-7, 97-50, 97-51, 97-52, 98-177, 99-139, 98-135, and 96-8. This cluster consisted of similar isolates, which were typical, and different from the rest of the *P. capsici* isolates, they had chrysanthemum like pattern. In addition, these isolates had a characteristic LB ratio, which was around 1.5. These were only moderately virulent. These isolates

were grouped as Cap A isolates described by Coffey and Oudemans (1991).

Cluster C

This cluster had similarity of 85 % and it consisted of two sub clusters.

c.1. This sub cluster had similarity of 89 % and contain eight *P. capsici* isolates of morphological group III and Race 2, namely, 98-181, 98-127, 99-162, 98-182, 97-45C, 98-162, 98-67, and 98-166. These isolates showed stellate colony pattern and LB ratio was high (2.3-3.5). They were highly virulent isolates.

c.2. The sub cluster had similarity of 94.5 % and contain three *P. capsici* isolates of morphological group IV and Race 3, namely 97-55, 96-11, and 98-17. They were *P. capsici* isolates with A2 mating type. Their LB ratio was around 2 (1.6-2). They were less virulent.

d. There was single *P. capsici* isolate (98-87) of morphological group II and Race 2. This isolate showed floral pattern, irregular ontogeny and high LB ratio (3) and it was highly virulent.

Isozyme analysis of *Phytophthora* species other than *P. capsici*

It was classified based on morphology and isozyme variability, these isolates were observed to be generally less virulent on black pepper. The isolates clustered into four major clusters. (**Fig: 23).**

Cluster A (*P. parasitica*):

The *Phytophthora* isolates of cluster A had 89 % similarity, which were identified as *P. parasitica*, viz, 99-186, 98-95, 99-188, with sympodial ontogeny and L/B ratio of 1.2-1.3, formed a separate cluster. These isolates were less virulent.

Cluster B (*P. meadii*):

The isolates, 98-86, 98-192, and 98.90 were identified as *P. meadii* and had similarity of 89 %. Among these isolates 98-86 and 98-90 had sporangia of sympodial ontogeny but the isolate 98-192 had irregular ontogeny. The isolate 98-86 and 98-192 had L/B ratio of 1.45, whereas 98-90 had an L/B ratio of 1.6. These isolates were less virulent.

Cluster C (*P. parasitica*):

A single isolate 98-75 was included in this cluster was a *P. parasitica* isolate. It had floral colony pattern. The LB ratio was 1.2. All the other *P. parasitica* isolates were less virulent but 98-75 was a highly virulent isolate and produced lesion on all the tested *Piper* species.

Custer D (*P. palmivora*):

The cluster D consisted of *Phytophthora palmivora* isolates with a similarity of 89 % and contains isolates 99-127 and 98-1 with an L/B ratio of 1.6. They were less virulent.

12. Molecular characterization of *Phytophthora* species infecting black pepper using Internal Transcribed Spacer regions of rDNA gene repeates

Phytophthora isolates from black pepper tissue and soil collected from different localities in India was studied morphologically. Some of the morphologically varied isolates were selected and examined at molecular level using the ITS 4 and ITS 6 primers to amplify the internal transcribed spacer regions of rDNA gene repeat. Polymerase chain reaction was done for the selected isolates. The amplified product was divided into three aliquots. The first 5µl was used to check the presence of amplified product of 862 bp by agarose gel electrophoresis of 1.4%. All the isolates formed a single band at the same position so they were *Phytophthora*. Restriction digestion was done with the last two aliquots. First was with Msp1 and the second with Taq 1. After restriction digestion the product was electrophoresed in 2.5% agarose gel at 40v for 4 hours. The size of the restriction fragments were estimated by comparison with 100bp molecular size ladder loaded at both ends of

the gel. The gel was documented with gel documentation unit. They were analyzed by UPGMA cluster analysis by the computer programme NTsys. The isolates were grouped based on morphology and its patterns. There were three major clusters, A, B, C and D. **(Plate 23 and Fig: 24**.)

Cluster A ***(P. capsici)***

The first major, Custer A, had similarity of 71.5 % consisted of *P.capsici* isolates, it forms two sub clusters s.1and s.2.

s.1: This sub cluster consisted of *P. capsici* isolates which were 100 % similar and consisted of 97-50, 99-139, 00-40, 98-60, 97-55. The first two isolates 97-50 and 99-139 were with chrysanthemum like colony pattern, LB ratio around 1.5. They were *P.capsici* isolates of morphological group I and Race I. The isolates 00-40 and 98-60 were with uniform cotton wool like aerial mycelium and LB ratio less than 2. The last isolate 97-55 had the same colony pattern but it had A2 mating type and LB ratio was less than two. They were *P.capsici* isolates of morphological group IV and Race III. It was clear that these isolates were caducous with long pedicels and had LB ratio less than two. These isolates were moderately or less virulent.

s.2: This sub cluster consisted of *P. capsici* isolates which were 100 % similar. All the isolate were caducous with long pedicels. The isolates 98-67, 98-166, 98-87, 99-103, 99-91, 99-124, 98-24, 99-158 were included in this group. Among them 98-67 and 98-166 had stellate colony pattern. The isolate 99-103 had chrysanthemum like colony pattern. The isolates 99-91 and 99-124 had floral colony pattern. All the isolates had LB ratio more than two. They were *P. capsici* isolates of morphological group III and Race II, IV and V. They were highly virulent isolates. The isolates 98-24, 99-158 had white cottony colony and LB ratio around 2. They were moderately virulent isolates. They were *P.capsici* isolates of morphological group II and Race 1.

Cluster B *(P. meadii)*:

The second major cluster consisted of two *P.meadii* isolates 98-86 and 98-192 and they were

100 % similar. Their LB ratio was 1.45 and less virulent.

Cluster C (*P. parasitica*):

The third major cluster consists of 98-1 a *P. palmivora* isolate and had 86 % similarity. It showed white cottony mycelium with faded pattern. The LB ratio was 1.6 and a less virulent isolate.

Cluster D (*P. palmivora):*

The last major cluster contains 99-186 a *P. parasitica* isolate and had 86 % similarity, which showed white fluffy aerial mycelium LB ratio was 1.2 and was a less virulent isolate

Table, 114: Morphological and molecular characterization of *Phytophthora capsici* pathogenic to black pepper

Group name	No of clusters	Morphological groups	Races
Morphological Group	4 morphological groups (MO)	MO 1 MO 2 MO 3 MO 4	Race 1 Race 1 Race 2, 4 and 5 Race 3
Polyacrylamide gel electrophoresis	2 clusters.	1. MO 1 and 2 2. MO 3 and 4.	Race 1 Race 2, 3, 4 and 5.
Isozyme analysis	3 clusters and a single isolate	1. MO 3 and 4 2. MO 1, 3 and 4 3. MO 3 and 2	Race 2 and 3 Race 1, 2, and 3 Race 1, 2, 4, 5 and a single isolate.
ITS analysis of rDNA	2 clusters	1. MO 1 and 4 2. MO 2 and 3	Race 1 and 3 Race 3, 2, 4 and 5

Direct correlation between morphological and molecular characterization is not obtained. The racial picture of P. *capsici* is not directly correlated with the morphological or molecular data. There were four morphological groups and five races in *P. capsici*. Based on isozyme analysis there were three main clusters and a single isolate. Based on the ITS analysis *P. capsici* isolates were broadly classified into two molecular sub groups. Morphological and molecular characterization of *Phytophthora capsici* pathogenic to black pepper is given in (**Table: 114)**.

DISCUSSION

DISCUSSION

In India black pepper apart from being a monocrop, is predominantly an important component of mixed crop in high density multi species cropping system where more than one *Phytophthora* species is involved in the disease of certain crop species of the cropping system. Hence it is of paramount importance to know whether more than one *Phytophthora* species is involved in foot rot / leaf rot of black pepper. If more than one species is involved which is the most dominant species involved need to be clarified. For any future breeding programmes in black pepper the variability of *Phytophthora* species involved and the races if any need to be established. The present investigations were undertaken to fill these gaps in our understanding. Detailed studies on biology of the pathogen were undertaken specially on the adaptability of the pathogen to variable temperatures and pH levels. These results were examined to see whether any correlation exists between the pathogenic groups (Races?) of *P. capsici* and their molecular parameters of *P. capsici* causing foot rot of black pepper.

Foot rot disease of black pepper is a serious disease and is the major production constraint in all pepper growing countries (Sarma and Premkumar, 1991). The present study aims to find out the involvement of *Phytophthora* species in the disease development, and their variability. Detailed studies on *Phytophthora* species were conducted on morphological physiological, pathological and molecular level. The identity of the black pepper *Phytophthora* has been a long controversy. Muller (1936) identified the causal agent in Indonesia as *P. palmivora* var. *piperina*, but this varietal name was not accepted (Stamps et al.1990). Much of the older literature attributes this disease to *P. palmivora* (Holliday and Mowat, 1963, Turner, 1973). Tsao et al. (1985 b) considered the isolates of black pepper are totally different from *P. palmivora* sensu stricto but accommodated into '*P. palmivora*' MF4. It was later redescribed as *P. capsici* sensu lato (Tsao and Alizadeh, 1988; Tsao, 1991; Mchau and Coffey, 1995).

Identity of *Phytophthora capsici* infecting black pepper

Out of the 173 isolates of *Phytophthora* infecting black pepper 60 were selected based on the morphology and colony characters, these isolates were thoroughly examined for their identity based on the *Phytophthora* key (Stamps et al. 1990). Of the sixty *Phytophthora* isolates studied 51 belong to *P. capsici* (85 %), 3 belongs to *P. meadii* (5%), 2 belongs to *P. palmivora* (3.3 %) and 4 belongs to *P. parasitica* (6.6 %). *P. capsici* is the most predominant (85 %) and distribution of *P. meadii*, *P. palmivora* and *P. parasitica* are meagree but important. Three other species viz., *P. meadii* 5 % (3 out of 60), *P. palmivora* 3.3 % (2 out of 60) and *P. parasitica* 6.6 % (4 out of 60) have been identified as pathogenic to black pepper. Four isolates from Thailand examined in 1981 / 82 were distinctly different from *P. capsici*. “These isolates were identified as *P. parasitica* Dast. (= *P. nicotianae* Breda de Haan)” (Tsao and Kueprakone, unpublished data Tsao, 1991) and more than one species of *Phytophthora* was thus reported on black pepper (Tsao, 1991). This is in contrast to the earlier reports of *Phytophthora capsici* infecting black pepper, three new *Phytophthora* species have been recorded on black pepper for the first time in India in the present study. From the studies carried out at university of California on black pepper *Phytophthora* revealed that not a single isolate belonged to *P. palmivora* (Tsao, 1991 was reported pathogenic to black pepper.. However in the present case *P. palmivora* has been recorded for the first time. In the case of cocoa in India, more than one *Phytophthora* species viz, *P. palmivora*, *P. capsici*, *P. citrophthora* and *P. megakarya* were reported (Chowdappa and Chandramohan, 1997).

Morphology of *Phytophthora* species infecting black pepper

Based on the morphology, *P. capsici* isolates have been categorized in to 4 groups which are discussed further.

The geographical distribution of the isolates varied for different groups of isolates. Among the group I isolates seven were from Karnataka and two from Kerala. But in the case of group II, four isolates were from Kerala and only two from Karnataka. Twenty five isolates of group III and 11 isolates of group IV were from Kerala, Tamil Nadu and Karnataka. This indicated the distribution of all the four morphological groups in Kerala and Karnataka predominantly.

The source of the isolates of different groups varied. Group I isolates were from root (4), stem (1) and leaf (4). Group II consisted of isolates from leaf (4), stem (1) and soil (1). Group III consisted of isolates from leaf (12), stem (2), berry (1), spike (1), root (3), and soil (6). Group IV consisted of isolates from soil (2), leaf (7) and root (2). This indicated that the group III isolates were from almost all parts of the plant. There was no specific pattern of distribution of the isolates based on the geographical region and source of isolation.

Colony morphology and growth rate on carrot agar of *Phytophthora* species

The isolates varied in their colony morphology. They showed chrysanthemum like pattern, stellate pattern, white cottony, floral pattern, uniform cotton wool like aerial mycelium and white fluffy were observed in the present study. The *P. capsici* were separated into four groups based on morphology. The isolates of group I showed chrysanthemum like colony and aerial mycelium was sparse, group II showed white cottony, group III showed chrysanthemum like, stellate, and floral patterns and group IV showed uniform cotton wool like aerial mycelium (**Plate: 2**). Three of the *P. meadii* isolates showed white cottony with faded pattern and one isolate showed stellate pattern. The *P. palmivora* isolates showed white cottony faded pattern. The *P. parasitica* isolates showed white fluffy without any specific pattern. Such colony patterns were described earlier by Waterhouse et al. (1983) as chrysanthemum type with sectors narrowly petaloid, stellate type with colony radiating like a star, in some without any pattern. Colonies of some isolates are "densely fluffy" or "scanty fluffy" but some colonies were appressed (Erwin 1965; Pratt and Mitchell, 1973; Stack and Miller, 1985; Erwin and Li, 1986). Variability in colony pattern, viz, white cottony and petaloid patterns of black pepper *Phytophthora* isolates was reported earlier (Sarma et al. 1982). The colony patterns like stellate pattern, white cottony, floral pattern, and uniform cotton wool like aerial mycelium and chrysanthemum like patters were described by Brasier and Griffin (1979) on cocoa isolates of *P. palmivora*. Petalloid colony pattern and fairly dense aerial mycelium was reported in isolates from cocoa (Brasier and Griffin, 1979).

The revised description of *P. capsici* (Tsao and Alizadeh, 1988) includes colony morphology on carrot agar as highly variable, often radiating with striation or with petalloid or floral pattern, or with sparse or uniformly dense cotton wool like aerial mycelium, or entire colony or more dense in the central portion of the colony, with advancing margin lobed, sharply defined or diffused.

Sastry and Hegde (1989) reported black pepper *Phytophthora* isolates produced petalloid pattern of growth, where as the arecanut and small cardamom isolates produced abundant fluffy aerial mycelium.

Santhakumari (1987) noted white to dirty white petalloid mycelial growth in cocoa (*P. palmivora*), coconut (*P. palmivora*), and pepper (‘*P. palmivora*’ MF4) in carrot agar medium. The cardamom (*P. nicotianae* var. *nicotianae*) isolates showed pure white with appressed coralloid pattern. Arecanut (*P. meadii*) isolate showed pure white fluffy mycelial growth with smooth margin.

The growth rate varied among the different groups of *P. capsici* the isolates. The variability depends on the colony pattern. The *P. capsici* (Group I) isolates showed chrysanthemum like pattern and 30-37 mm radial growth per 72 hours. The *P. capsici* isolates of (Group II) showed white cottony colony were very slow in growth and the radial growth of 22-30 mm per 72 hours. The *P. capsici* isolates of (Group III) had chrysanthemum like, floral and stellate patterns and the radial growth was 26-46 mm per 72 hours. The *P. capsici* (group IV) isolates showed uniform cotton wool like mycelium grew fast with radial growth of 32-38 mm per 72 hours. The *P. meadii* isolates showed white cottony with faded pattern and the radial growth was 26-37 mm per 72 hours. *P. palmivora* isolates showed white cottony with faded pattern and the radial growth was 24-30 mm per 72 hours. *P. parasitica* isolates showed white fluffy aerial mycelium and the radial growth was 30-35 mm per 72 hours. Shepherd and Pratt (1974) studied *P. cinnamomi* from Australia and reported the variability in growth rate. Zentmyer et al. (1976) showed the variation in colony diameters of *P. cinnamomi*. Variability in growth rate was reported for *P. palmivora* from cocoa (Brasier and Griffin, 1979) and *P. meadii* and *P. palmivora* isolates of rubber (Dantanarayana et al. 1984). Suseela Bhai (1998) reported similar variability in growth rate of *P. meadii* isolates from small

cardamom.

The colony characters of the four groups of *P. capsici* isolates showed variation. Out of the four groups of *P. capsici* isolates group II were slow in growth rate. The *P. capsici* isolates could be differentiated into four morphological groups based on their colony characters and growth rate as mentioned above. The three species of *Phytophthora* viz, *P. meadii*, *P. palmivora*, *P. parasitica* varied in their colony characters and growth rate.

Sporangial ontogeny of *Phytophthora* species

Ontogeny of sporangiophore is one of the criterion looked for, in *Phytophthora*. It is generally sympodial or umbellate. Among the 51 *P. capsici* 50 isolates showed umbellate ontogeny and one isolate (98-87) showed non umbellate and non sympodial (Irregular) ontogeny.

P. capsici showed both umbellate and sympodial ontogeny of sporangium formation. Tsao and Alizadeh (1988) in the revised description of *P. capsici* Leonian emend A. Alizadeh and P.H. Tsao 1922, described that umbellate ontogeny was the result of little or no elongation of sporogenous hyphae. Umbellate ontogeny was described for rubber isolates from Thailand and black pepper isolates from Indonesia and India (Tsao and Alizadeh, 1988). Black pepper isolates from India were identified as '*P. palmivora*' MF4 (Sarma et al. 1982; Tsao et al. 1985 b). Detailed investigation carried at California University, Riverside confirmed the identity of 10 isolates from different geographical regions of Kerala and Karnataka as "*P. palmivora*" MF4 (Tsao et al.1985 b, Tsao, 1991).

Two of the *P. meadii*, and all the *P. palmivora* and *P. parasitica* isolates had sympodial ontogeny. Sympodial ontogeny was common to most of the reported *Phytophthora* species. Sympodial ontogeny was reported for *P. meadii* of rubber (Dantanarayana et al. 1984). In the revised key of *Phytophthora* (Stamps et al. 1990), *P. palmivora* and many other species were considered sympodial. One isolates each from *P. capsici* and *P. meadii* had irregular ontogeny. Irregular branching was reported in *P. heveae* isolates (Erwin and Ribeiro, 1996).

The black pepper isolates with umbellate ontogeny varied in sporangial shapes. They were ovoid, pyriform with tapering base or fusiform. Double septate sporangia were noticed in some isolates (Sarma et al. 1988).

High variability of sporangial shapes, of *P. capsici* isolates from black pepper in India, Indonesia and Malaysia has been reported earlier (Tsao, 1991). In the present study the *P. capsici* isolates varied in their morphology of sporangia. The *P. capsici* isolates were grouped into four morphological groups based on colony characters, sporangial characters like size, shape, LB ratio, caducity, pedicel length, chlamydospore production and mating types (**Plate: 3 - 5**). *P. capsici* isolates of group I had ovoid sporangia. The size and shape were almost stable. L/B ratio was around 1.4 – 1.6 and papillate. They were caducous (51-75 %) with long pedicels (31-94 µm). Aragaki and Uchida (2001) reported that *Phytophthora capsici* isolates with broad sporangia, sporangial length to breadth ratio less than 1.8, predominantly round sporangial bases were reported as *P. tropicalis* sp. nov. by these characters. This group of isolates could be assigned to *P. tropicalis* based on their characters. The *P. capsici* isolates of group II had white cottony slow growing mycelium. They had ovoid to obovoid and fusiform and papillate sporangia. Their LB ratio was (1.8-2.3). The group II isolates had sporangia with tapering bases, septation etc. Sporangia papillate mostly round based and occasionally with two or three papillae were seen. They were caducous (51-75 %) with long pedicels (65-150 µm). The *P. capsici* isolates of group III had elongated sporangia papillate or nonpapillate and shape was varied from fusiform, obovoid, obpyiform and distorted shapes. LB ratio was (2 – 3.5). They were caducous (26-50 %) with long pedicels (38.7-192 µm). The isolate 98-87 of group III was different from other isolates and showed distorted balloon like sporangia with very long pedicels (192 µm). The *P. capsici* isolates of group IV were similar to the second group isolates and had ovoid to obovoid and fusiform papillate sporangia. Their LB ratio was around (1.6 - 2.1). They were caducous (76-100 %) with long pedicels (70.2-167 µm). (**Plate: 6 - 8**)

The caducity of sporangia was described by Waterhouse (1974 c). Not all the species of the shed sporangia but only those which might be termed ‘aerial’ and normally live in and sporulate on

leaves, twigs, stems and fruits. The sporangium of the species also has a prominent papilla with deep apical thickening. In contrast species inhabiting roots or attaching the collar do not shed their sporangia, which either have shallow papilla or are non papillate and have inconspicuous apical thickening. In the present study isolates from root and leaves did not showed any such pattern The importance of caducity and their relation to disease spread needs indepth investigation.

The *P. meadii* isolates showed sympodial or irregular ontogeny and ovoid to ellipsoid sporangia. The LB ratio was 1.45 to 1.6, caducous, sporangia were papillate. The descriptions were comparable to the *P. meadii* isolates described by Dantanarayana et al. (1984) in rubber and Suseela Bhai (1998) in small cardamom.

The *P. palmivora* isolates had sympodial ontogeny of sporangiophore, white cottony mycelium with faded pattern, sporangia globose to ovoid, LB ratio around 1.6 and caducous. The isolates contain prominent papilla present with short occluded pedicels. They were similar to the *Phytophthora palmivora* isolates described by Brasier and Griffin (1979) on cocoa.

The *P. parasitica* isolates showed sympodial ontogeny of sporangiophore, white fluffy aerial mycelium, globose sporangia, prominent papilla, hyphal swellings and internal proliferations present. They were non-caducous. It was reported by various workers that *P. parasitica* Dast. (or *P. nicotianae* var. *parasitica* (Dast) Waterhouse) possesses either caducous (Tucker, 1931; Waterhouse, 1963, 1974 a and b) or non-caducous sporangia (Frezzi, 1950, Kunimoto et al 1976). Results of the study by Al- hedaithy and Tsao (1979a) showed that very few sporangia of *P. parasitica* were detachable and each detached sporangium carried either no pedicel or a piece of hyphae of irregular length. *P. parasitica* on black pepper was recorded from Thailand (Tsao, 1991).

Chlamydospore morphology of *Phytophthora* species

Variability in chlamydospore production in *Phytophthora* isolates from black pepper was a distinct feature. Some produced only few chlamydospores some never produced chlamydospores at cultural conditions, but some isolates produced abundant chlamydospores. The study of Alizadeh and Tsao (1985 b) showed that some '*P. palmivora*' MF4 isolates produced chlamydospores and some did not produce chlamydospores. Absence of chlamydospore was noted by Leonian as well as by others in isolates obtained from pepper (Tucker, 1931). *Phytophthora palmivora* isolates produced abundant chlamydospores (Kadooka and Ko, 1973).

All the *P. capsici* isolates of group I and group IV produced chlamydospores. Seven isolates of group III produced chlamydospores. None of the group II isolates produced chlamydospores (**Plate: 10**). Two of the *P. meadii* of and all *P. palmivora* and *P. parasitica* produced chlamydospores. The size of the chlamydospores varied with isolates. After chlamydospore

induction by incorporating β-sitosterol in the medium only some isolates produced chlamydospores. The chlamydospores produced after induction was small. Since morphology of chlamydospores did not vary significantly among species, its significance in identification is limited. *P. palmivora* produced chlamydospores abundantly but only some isolates of *P. megakarya* or *P. meadii* showed chlamydospores formation. In this case presence or absence may be a useful presumptive indicator (Waterhouse et al. 1983).

Suseela Bhai (1988) studied chlamydospore production in *P. meadii* from small cardamom in detail. Chee (1973) reported the formation of chlamydospores in shallow water at 15 ^{0}C and none in submerged waters. Kunimoto et al. (1976) had observed chlamydospores in *P. nicotianae* var. *parasitica* in water when washed and mycelial mats were kept in sterile distilled water for four weeks at 16 ^{0}C. Chlamydospore production was not seen in fresh water cultures. '*P. palmivora*' MF 4 cultures of more than ten days old produced chlamydospores in carrot agar and the production varied among the isolates (Sarma et al. 1988).

Mating type and oospore production of *Phytophthora* species

Among the fifty one *P. capsici* forty A1, three A2, and eight neuter isolates were present. The occurrence of isolates with A2 mating type among *P. capsici* was low. Majority of black pepper *P. capsici* isolates belong to A1 mating type and only 4.86 % isolates belong to A2 mating type. Among the *P. meadii* one was with A1 mating type and two were neuter isolates. The *P. parasitica* isolates were of three A1 and one neuter. Two of the *P. palmivora* isolates were A2. In the present study majority of *P. capsici* isolates were A1 mating type (**Plate: 11**).

Santhakumari (1987) reported that cholesterol, cholesterol acetate, and β-sitosterol favoured oospore formation. Ten *Phytophthora* isolates collected from Kerala and Karnataka were found to be of A1 mating type (Sarma et al. 1991). Sastry and Hegde (1987a) had reported the presence of A1 and A2 mating type in *P. palmivora* in the ratio of 6 : 4 and A1 mating type in *P. meadii* infecting arecanut. Presence both A1 and A2 mating type had been

reported from Karnataka in black pepper *Phytophthora* isolates (Sastry, 1982).

All the oospore producing A1 and A2 isolates produced amphigynous antheridia. The position of the antheridium whether amphigynous or paragynous was one of the diagnostic character used for identification of *Phytophthora* species. Usually the morphology of oogonia and oospores does not differ enough among most species to be of a diagnostic value. Sexual mechanism within the isolates of '*P. palmivora*' group (*P. palmivora*, *P. arecae* and *P. meadii*) was studied in detail (Brasier, 1972).

The oogonial size varies within different morphological form; this character has little diagnostic value for separating L and S form of MF4 types (Brasier and Griffin, 1979). Variation in oogonial size was not considered as a taxonomic criterion for characterization of the species (Kaosiri et al. 1980).

In the present study the first three morphological groups showed A1 mating type and morphological group IV consisted of three A2 and eight neuter (sterile) isolates. Thus the presence of A1, A2 and neuter isolates among *P. capsici* has been recorded

Variability in the time of oogonia formation was also a notable feature among black pepper isolates. (**Plate: 11**). There were isolates which formed oogonia within 10 to 14 days and most of them reached maturity at 21 days. Formation of oogonia in *Phytophthora* isolates of plantation crops in Ribeiro's medium within five days was already reported by Santhakumari (1987). Most of the rubber isolates referable to *P. meadii* are of the A1 compatibility type and a few fell into the A2 compatibility type (Dantanarayana et al. 1984). Sastry and Hegde (1987a) studied the mating behaviour of *P. meadii* and A1 and A2 mating type of *P. palmivora*.

Biology of *Phytophthora* species infecting black pepper

Effect of temperature on *Phytophthora* species infecting black pepper

Temperature, nutrition and pH are the important factors that affect the growth and other phases of development. The mycelial growth, sporulation, zoospore liberation and zoospore germination of *Phytophthora* species infecting black pepper vary with temperature. Since Tucker (1931) all the keys have used growth at minimum and optimum temperatures as useful criteria for classification at species level (Waterhouse et al. 1983).

Studies on temperature relation to isolates of *Phytophthora* of cocoa, arecanut, coconut and black pepper showed temperature optima of 25-30 ^{0}C which include species such as *P. palmivora*, *P. meadii*, and *P. nicotianae* var. *nicotianae* (Sastry and Hegde, 1987). Ribeiro (1978) also observed an optimum range of 28-30 ^{0}C for these species (*P. palmivora*, *P. arecae*, *P. meadii*, and *P. nicotianae* var. *nicotianae*) and using this as a criterion, he opined them as closely related species. For all the isolates studied 10 ^{0}C and 35 ^{0}C was unfavourable for mycelial growth, sporulation, and zoospore liberation and zoospore germination.

Optimum temperature was 28 ^{0}C for almost all the isolates. The *P. capsici* isolates of group I could tolerate high temperature of 35 ^{0}C. Most of the *P. capsici* isolates of group II also grew at 35 ^{0}C. The isolate 98 - 87 grew well at 35^{0}C. The group IV and most of the group III isolates did not tolerate 35 ^{0}C and 10 ^{0}C and they did not grow well at this temperature. The *P. meadii* and *P. palmivora* isolates did not grow well at 10 ^{0}C and 35 ^{0}C. The *P. parasitica* isolates behave differently and they could tolerate 10 ^{0}C and 35 ^{0}C. The present study indicated that the *P. capsici* isolates of the four groups and *P. meadii*, *P. parasitica* and *P. palmivora* isolates infecting black pepper varied in their response to temperature.

Roncadori (1965) found that six species of *Phytophthora* grew best at 20 ^{0}C while nineteen species and one var. had optimum temperature between 25-30 ^{0}C. *P. parasitica* var. *nicotianae* fell in the second group of Waterhouse (1963) . Westesteijn (1973) also recorded optimum temperature

range of 25-30 ^{0}C for growth of *P. nicotianae* var. *nicotianae*. Sarma et al. (1988) reported the maximum growth of black pepper *Phytophthora* isolates between 25 - 30 ^{0}C. Zentmyer et al. (1976) reported cardinal temperature for growth of *P. cinnamomi* varied from 5-16 ^{0}C, minimum, 20 - 32 ^{0}C optimum and 30 - 36 ^{0}C maximum. These ranges were considerably lower than those given in Waterhouse key (1963), which were 5 ^{0}C minimum, 24-28 ^{0}C optimum and 32-34 ^{0}C maximum. Stamps (1985) reported the minimum temperature for growth 10 ^{0}C, optimum 28 ^{0}C, and maximum 35 ^{0}C for *P. capsici* isolates. The L and S isolates from cocoa vary in their temperature requirements. The optimum temperature 24 - 26 ^{0}C for L type and it is lower than that for the S and for MF4 the temperature was (28 - 30 ^{0}C). Temperature relation especially the upper limit for growth was used in *Phytophthora* taxonomy (Waterhouse, 1963).

Sharma and Sharma (1982) studied the variation of *P. nicotianae* var. *parasitica* from brinjal fruit, *Capsicum* leaf, and tomato root and tomato fruit. Difference in growth rate at nine temperatures ranging from 12 - 37^{0}C was studied. Growth difference at different temperatures were less marked and were non significant. at 21 ^{0}C and 36 ^{0}C. At 12 ^{0}C, 15 ^{0}C and 30 ^{0}C the isolates of brinjal fruit (P1), *Capsicum* leaf (P2), tomato root (P3) behaved as one group and tomato fruit (P4) was significantly different from all other isolates.

Sastry and Hegde (1989) reported the isolates of *Phytophthora* from black pepper produced maximum growth at 30 ^{0}C. The temperature range of 25 ^{0}C to 30 ^{0}C was reported optimum for all isolates of *Phytophthora* in general.

Sarma (2000) reported variability of *Phytophthora* isolates over a wide range of temperatures. In that maximum growth and sporulation was reported at 28 ^{0}C followed by 20 ^{0}C. At 10 ^{0}C and 35 ^{0}C growth and sporulation was minimum or nil.

Mchau and Coffey (1995) reported that isolates from CAP 1 with the exception of three isolates grew well at 35 ^{0}C. Four isolates of CAP 2 was poor at 35 ^{0}C, but the remaining isolates grew well. Growth of CAP 3 isolates was poor or absent at 35 ^{0}C. Optimum temperature for growth among all the isolates varied from 24-33 ^{0}C.

Sporulation of the 60 *Phytophthora* isolates from black pepper varied with different temperatures. Maximum sporulation was at 20 ^{0}C and followed by 28 ^{0}C and 35 ^{0}C. Temperature of 10 ^{0}C was not favourable for sporulation. The *P. capsici* isolates of group I and group II sporulated at all the four temperatures. Minimum sporulation was at 35 ^{0}C. Among the group III isolates one did not sporulate at 10 ^{0}C and another at 35 ^{0}C. The group IV isolates also had similar trends, but one did not sporulate at 35 ^{0}C. None of the group IV isolates sporulated at 10 ^{0}C and their sporulation was very less at 35 ^{0}C. Two of the *P. meadii* isolates did not sporulate at 35 ^{0}C. The *P. palmivora* did not sporulate at 10^{0}C and the *P. parasitica* sporulated at all the tested temperatures.

Zoospore liberation (indirect germination of sporangia) was maximum at 20 ^{0}C and followed by 28 ^{0}C. At 35 ^{0}C zoospore liberation did not occur. Minimum zoospore release was noticed at 10 ^{0}C. Zoospore liberation was not seen at 35 ^{0}C and was minimum at 10 ^{0}C for *P. capsici* isolates of group I and group II. The *P. capsici* isolates of group IV did not liberate zoospores at 10 ^{0}C and 35 ^{0}C. The *P. meadii* and *P. palmivora* isolates did not liberate zoospores at 10 ^{0}C and 35 ^{0}C. The *P. parasitica* isolates also did not liberate zoospores at 35 ^{0}C.

All the 60 *Phytophthora* isolates infecting black pepper were studied for zoospore germination. Even though zoospore liberation was noticed at 10^{0}C and 35^{0}C by certain isolates but they did not germinate at these temperatures. Different groups of isolates varied in their zoospore

germination. Among the *P. capsici*, group III had maximum germination and which was similar to group I. Minimum germination occurred with *P. capsici* of group IV. Group II also was slow in germination.

Maximum temperature for germination and germ tube growth was 30 ^{0}C and minimum were 10 ^{0}C and 45 ^{0}C for *P. drechsleri* f.sp. *cajani* (Singh and Chauhan, 1988).

Effect of pH on *Phytophthora* species infecting black pepper

Maximum mycelial growth was observed at pH 5 and pH 7. Many of the isolates did not grow at pH 2.5 and 9. The mycelial growth of the isolates, at pH 2.5 and 9 was minimum. The growth behaviour of the isolates at different pH ranges did not vary very much. The *P.capsici* isolates of group I had maximum growth at pH 5 followed by 7 and less at pH 2.5 and 9. The isolates of group II did not grow at pH 2.5 and was minimum at pH 9. The isolates of group III did not grow at pH 2.5 and showed minimum growth at pH 9 and was maximum at pH 7. The group IV isolates did not grow at pH 2.5 and maximum at pH 5. Most of the *P. meadii* isolates and *P. palmivora* isolates did not grow at pH 2.5 and showed minimum growth at pH 9. The *P. parasitica* isolates grew only at pH 5 and pH 7.

The sporulation of the sixty isolates varied very much. They did not sporulate at pH 2.5. Most of the isolates had maximum sporulation at pH7. In the case of group I isolates maximum sporulation was at pH 5 or 7. The second, third, and fourth, groups of isolates had maximum sporulation at pH 7 and no sporulation at pH 2.5. Their sporulation at pH 5 and 9 were almost equal. Similar trend was noticed with P. *meadii*, *P. palmivora*, *P. parasitica* isolates. Suseela Bhai (1998) reported maximum sporulation at pH 4 and 7 for *P. meadii* isolates of small cardamom isolates. Kennedy and Erwin (1961) reported that sporangia might form within a wide range of pH from 4 - 9. Ribeiro (1983) reported that the optimum pH for sporangium formation varied within a species and among isolates of same species.

Zoospore liberation of the isolates in different pH was studied. Zoospore liberation did not occur at pH 2.5 where as sporulation and zoospore release was noticed at pH 5, 7 and 9. The zoospore germination of the isolates varied. Germination did not occur at pH 2.5. The *P. capsici* isolates of first group showed maximum zoospore germination at pH 9 and followed by pH 7 and minimum at pH 5. Kasim and Prayitno (1979) found that the optimum pH range of *P. capsici* as 6 - 7 under continuous light. Kueh and Kew (1982) found that the optimum pH for *P. palmivora* ranged between 6.5 -7. Nair (1979) found that soil pH 6 to 7 were favourable for the survival of the pathogen. Sato (1994) reported that pH 8 was optimum for the indirect germination of *P. infestans*. In the present study at higher pH (5 - 9) was favourable to indirect germination of sporangia of black pepper isolates.

Effect of media on *Phytophthora* species

The growth of the isolates in different natural media was studied. Optimum growth was observed in carrot agar. Sporulation, zoospore liberation and zoospore germination was also optimum in this media. The isolates of group I had maximum radial growth in carrot agar followed by papaya dextrose agar and minimum growth in potato dextrose agar. The isolates of group II were also similar to the second group but some isolates had maximum growth in papaya dextrose agar. Among the group III isolates most of them had maximum growth in carrot agar but some had maximum growth in papaya dextrose agar. Some of the group IV isolates had maximum growth in carrot agar and others had maximum growth in papaya dextrose agar. Among the *P. meadii* isolates two had maximum growth in papaya dextrose agar and one grew well in carrot agar. The *P. palmivora* isolates grew well in carrot agar and then in corn meal agar. The *P. parasitica* isolates had maximum growth in carrot agar followed by corn meal agar.

Sastry and Hegde (1989) reported maximum growth of *Phytophthora* species on carrot agar followed by corn meal agar. Minimum growth was obtained on lima bean agar.

Sporulation of the isolates in different media was studied in detail. Maximum number of sporangia were produced in carrot agar and followed by corn meal in most of the cases. Minimum sporulation was in potato dextrose agar or papaya dextrose agar for most of the isolates. *P. meadii*, *P. palmivora* and *P. parasitica* isolates had maximum sporulation in carrot agar. The *P. parasitica* isolates had minimum sporulation in corn meal agar.

All the isolates vary in zoospore liberation in different media. In the first group isolates maximum zoospore liberation occurred in carrot agar and not much variation was noticed in the rest of the three media. In the second group isolates, maximum zoospore liberation was in carrot agar and minimum was in potato dextrose agar. The third group isolates also had maximum zoospore liberation in carrot agar and minimum and almost equal in potato dextrose agar and papaya dextrose agar. Some of the group IV isolates had maximum zoospore liberation in carrot agar and others in papaya dextrose agar. The *P. meadii* isolates were different from others in their zoospore liberation. They had maximum zoospore liberation in carrot agar and no zoospore liberation in potato dextrose agar and papaya dextrose agar. The *P. palmivora* isolates had maximum zoospore liberation in carrot agar followed by papaya dextrose agar. The *P. parasitica* isolates had maximum germination in carrot agar and almost similar zoospore liberation in papaya dextrose agar and corn meal agar. Zoospore liberation was very less in potato dextrose agar.

The zoospore germination of the sixty isolates in different media was studied in detail. Maximum germination was in carrot agar for most of the isolates. The *P. capsici* isolates of the first, second, and third groups had maximum germination in carrot agar. The group IV isolates had maximum germination in carrot agar and minimum germination in potato dextrose agar. The *P. meadii* isolates had maximum germination in carrot agar and no germination occurs in potato dextrose agar and papaya dextrose agar. The *P. palmivora* isolates and *P. parasitica* isolates had maximum germination in carrot agar. Growth, sporulation, chlamydospore formation, and sexual reproduction in relation to survival, perennation and their ecological significance are the areas of future research.

Jain et al. (1980) recorded the best growth of the fungus *P. nicotianae* var. *nicotianae* on natural media. Tucker in 1931, Hendrix and Apple in 1964 and Tiwari in 1973, also found natural media to be better for growth of *Phytophthora* species, *P. parasitica* var. *nicotianae* and *P. parasitica* var. *piperina* respectively. Sarma (2000) reported the variability of the isolates with different solid media. Radial growth of *P. capsici* from black pepper was studied in different solid media and comparisons were made. Maximum growth was found in carrot agar followed by papaya dextrose agar, corn meal agar and least growth was in potato dextrose agar. Sarma et al. (1988) reported that the black pepper *P. capsici* isolates grew better in carrot agar compared to V8 juice agar. Santhakumari in 1987 did a comparative study of 12 natural media. She tested natural media like soybean meal agar, sugar cane juice agar, wheat meal agar, potato dextrose agar, barley meal agar, and carrot agar and found that maximum growth were observed in soybean meal agar, sugar cane juice agar followed by carrot agar.

Pathogenicity of *Phytophthora* species - A racial picture

Pathogenic variability of the isolates on nine *Piper* species was studied. Based on pathogenicity the *P. capsici* isolates were grouped into five races (**Table: 98). The isolates of Race 1 infected all *Piper* species except *P. arboreum*.** They were moderately virulent on Sreekara leaves. **The isolates of Race 2 infected all the tested *Piper* species.** The isolates of Race 2 were highly virulent on Sreekara leaves.

The isolates of Race 3 did not infect *P. longum*, *P. colubrinum*, and *P. arboreum*. The isolates of Race 4 did not infect *P. longum*, and *P. arboreum.* The isolates of Race 5 did not infect *P. longum*, *P. colubrinum* and *P. arboreum.*

The *P. meadii* isolates did not infect *P. betle*, *P. longum*, *P. attenuatum*, *P. colubrinum*,

and *P. arboreum*. Very small lesion was produced with *P. chaba* and less virulent on *P. ornatum* and *P. hapnium*. **The *P. palmivora* did not infect any of the tested *Piper* species except *P. chaba*.** But they were pathogenic to the roots and stem of Sreekara plants. **The *P. parasitica* isolates did not infect *P. arboreum*, *P. betle*, *P. longum* and *P. hapnium*.**

Variation in pathogenicity among the isolates had long been recognized (Caten, 1971). The isolates with variation in virulence could be assigned to pathogenic races or physiologic races. Hildebrand (1959) reported physiological races in *P. megasperma* (1959). Faris (1985) tested virulence of six isolates of *P. megasperma* f. sp. *medicaginis* and found that there were two levels of virulence among the isolates. Liang et al. (2003) reported the different degrees of black shank resistance in different *P. nicotianae* var. *parasitica* strains. Daayf and Platt (2003) reported two genotypes of *P. infestans*. Zhang et al. (2003) reported different levels of resistance to tobacco black shank. Kapsa (2002) conducted comparative studies and found that stem isolates were more pathogenic than leaf isolates. But no such a correlation was obtained in the present study. Aldwinckle et al. (1975) observed differential interaction between apple cultivars and *P. cactorum* isolates. The fifteen isolates were placed into four groups according to their interaction. The studies of Singh and Dubey (2005) are comparable to the present study as they classified *P. dreschsleri* f. sp. *cajani* into four variable races based on pathogenicity tests. Based on the pathogenic studies in the present investigation five races have been identified in *P. capsici*. Races of *Phytophthora infestans* were isolated and identified by Malcolmson (1979). The presence of different races of *P. infestans* was studied in detail based on the differentials is comparable to the present study and work of Phukan and Baruah (1991) is also a similar one.

The pathogenicity of the selected isolates on stem and root was tested. Different isolates show varied reaction on the stem and roots of Sreekara cuttings. The *P. capsici* isolates of Race 1 had maximum stem infection and root infection. The *P. capsici* isolates of Race 2 was moderately virulent on root and stem. The *P. capsici* isolates of Race 4 and 5 were less virulent on root and stem. The *P. meadii* isolates and *P. palmivora* isolates were less virulent on stem and root of pepper cuttings. The *P. parasitica* isolates were moderately virulent on stem and roots of Sreekara.

Turner (1973) did root inoculation and found *P. palmivora* varied in their reaction to pepper cultivars. The most resistant variety was Indian cultivar Balancotta. There was no apparent relationship between pathogenicity of the isolates and their geological origin. Yang et al. (1989) found that 108 isolates of *P. capsici* obtained from diseased *Capsicum annum* were grouped into four types based on morphology. However they were pathogenically different.

Cross infectivity of *Phytophthora* species

P.capsici isolates infected all the tested hosts arecanut, rubber, black pepper, betel vine, small cardamom, cocoa and coconut. The rubber (*P. meadii*), arecanut (*P. meadii*), and coconut (*P. palmivora*) isolates did not infect black pepper. But cocoa (*P. palmivora*) small cardamom (*P. meadii*), black pepper (*P. capsici*) and betel vine (*P. parasitica* and *P. capsici*) isolates infected black pepper. In the present study *P. capsici* showed wide host range (**Table: 109**).

The development of new strains as result of interspecific hybridization under field conditions was reported earlier (Sastry, 1982; Santhakumari, 1987). The cross infectivity results of *Phytophthora* isolates from rubber, pepper, arecanut, coconut, cardamom, and cocoa are variable. '*P. palmivora'* MF4 from pepper infected roots of *P. betle*, *P. longum*, *P. attenuatum*, cocoa pods, rubber leaves and caster (Sarma and Nambiar, 1982). Based on the cross inoculation studies, arecanut, rubber, pepper, cardamom, and coconut might serve as the hosts for black pepper infection.

Cross inoculation studies conducted by Mammootty et al. (1988) with six *Phytophthora* isolates from six hosts showed the symptoms produced on the same hosts were more or less identical. Studies with *Phytophthora* from small cardamom, coconut and rubber revealed that they could cross infect the hosts. The results indicated that *P. palmivora* or its strain caused capsule rot of small cardamom (Radha and Joseph, 1974). Muniz et al. (2003) investigated the pathogenicity of

three isolates of *P. nicotianae* on tomato in laboratory and found pathogenic to all tested hosts. Azevedo and Silva (1986) inoculated detached fruits of *Capsicum annum*, tomato, cucumber and melon and squash cultivars with an isolate of *P. capsici* and found that all fruits are susceptible. Steekelenburg (1988) showed, *P. capsici* caused root rot and crown rot in *Capsicum annum* plants in Netherlands (1977). It was pathogenic to *Capsicum*, tomato and occasionally egg plant.

Sastry and Hegde (1987 a) studied *P. meadii* from arecanut, cocoa and small cardamom; '*P. palmivora*' MF4 from *P. nigrum* and *P. nicotianae* var. *nicotianae* from citrus were obtained from plantations in Karnataka. They showed cross infectivity among themselves. The cross inoculation studies have special importance in the present study because the presence of four *Phytophthora* species in black pepper. "From the more practical point of view, it is of particular interest to note that the crossing of two different species, if occurring in nature, may produce a wide range of progeny, greatly variable in morphology, physiology and pathogenic aggressiveness. This might well contribute to the evolution of new *Phytophthora* population and their pathogenic adaptation" (Boccas, 1980).

Study of *Phytophthora capsici* infecting black pepper isolates from other hosts

P.capsici isolates obtained from other hosts like, betel vine, cocoa, *capsicum*, *P.chaba* and *Bauhinia purpurea* were studied. Among the five *P. capsici* isolates from betel vine, three showed white cottony colony morphology and two showed stellate colony pattern. Their ontogeny was umbellate. Their LB ratio was in the range of 1.7-2.3 and they had long pedicels. They were similar to the *P. capsici* isolates obtained from black pepper. Among the *Phytophthora* isolates cocoa two were *P. capsici*. The colony morphology of one was white cottony, and the other was stellate pattern. Their LB ratio was 1.8 and 1.9 respectively. *P. capsici* isolates were reported on cocoa in Kerala by Chowdappa et al. (1993).

The *Phytophthora capsici* isolates from *Capsicum* showed white cottony mycelium, umbellate ontogeny and LB ratio 1.5 and with long pedicels. Two of the *P. capsici* isolates from *P. chaba* showed white cottony mycelium, umbellate ontogeny and long pedicels, and LB ratio less than 2. The *P. capsici* isolates from *Bauhinia purpurea* showed white cottony mycelium, umbellate

ontogeny and long pedicels and LB ratio less than 2. Based on the revised description of Tsao and Alizadeh (1988) the isolates were identified as *P. capsici* Leonian emend A. Alizadeh and P. H. Tsao.

All these *Phytophthora capsici* isolates were found to be pathogenic to black pepper. The studies done by Resnik et al. (1980) were similar to the present study. They cross-inoculated *P. palmivora* isolates from cocoa, rubber and black pepper by inserting mycelial discs in to the stem barks of these hosts. The petioles of rubber seedlings were similarly inoculated. All produced lesion on cocoa seedlings. Morphological form 1 and 4 were most virulent. Black pepper and rubber seedlings produced smaller lesions. All of them were pathogenic to rubber. The most virulent was *P. palmivora* from rubber and the least virulent isolates were *P. palmivora* (MF1) from cocoa. All caused leaf shedding when inoculated on petioles. Thankamma (1983) found that *P. nicotianae* var. *nicotianae* from pomegranate, *P. nigrum* and *Hibiscus rosasinensis* and *P. meadii* from brinjal and *Artocarpus hirsuta* were pathogenic to rubber. The isolate of *P. heveae* from mango and guava did not infect rubber. Steekelenburg (1980) showed, *P. capsici* caused root and crown rot of *Capsicum annum* and was pathogenic to *Capsicum*, tomato and eggplant.

Polyacrylamide gel electrophoresis of mycelial proteins of *Phytophthora* species

The *Phytophthora* isolates can be grouped into different types based on protein electrophoretic patterns. The UPGMA cluster analysis of the 17 selected isolates were separated into two main groups, cluster A consists of *P. capsici* isolates and cluster B, C and D consists of other species. The cluster A consists of two sub clusters and they were different based on their morphological characters like colony pattern, LB ratio, sporangial characters etc. The cluster B consisted of *P. meadii*, and cluster C consisted of *P. palmivora* and cluster D consisted of *P. parasitica*. Kaosiri and Zentmyer reported in 1980 that each morphological form produced consistent major bands distinctly different from those of other morphological forms. Isolates with similar protein patterns often come from widely separated geographical origins and occasionally from widely differing hosts (Hansen et al. 1986). Chowdappa and Chandramohan (1995) used

PAGE in distinguishing species of *Phytophthora* on cocoa using protein-banding patterns alone. Hamm and Hansen (1983) distinguished *P. pseudosugae* as a new species of *Phytophthora* from *P. cactorum*. It has been used along with other criteria for identification of *P. citricola* from avocado by Zentmyer et al (1974), *P. palmivora* from milkweed by Feitenberger et al.(1984), *P. drechsleri* from cucumber by Ho et al. (1984). Two distinct groups of *P. megasperma* were distinguished based on protein patterns (Farris et al. 1986; Hansen and Hamm, 1986; Irwin and Dale, 1982). The studies on electrophoretic protein patterns of three species of *Phytophthora* associated with black pod disease of cocoa revealed that the isolates of the same species were readily distinguished both quantitatively and qualitatively and by visual similarity (Chowdappa and Chandramohan, 1995).

Isozyme analysis of *Phytophthora* species infecting black pepper

Isozymes were defined as variant forms of an enzyme, using polymorphic forms of enzymes variability. Isozymes are ideal tools in molecular identification. By isozyme analysis the different species of *Phytophthora* can be separated. There was report of such separation.

All the sixty isolates were characterized by isozymes to understand the genetic variability existing among the isolates obtained from black pepper. Six isoenzymes were used in the study. The *P. capsici* isolates were analysed separately and they clustered into three main clusters and a single isolate in the bottom of the similarity tree. Based on morphology, LB ratio, and virulence the isolates were grouped into three major clusters, A, B and C. The upper major cluster consists of two sub clusters. The first sub cluster consists of isolates with chrysanthemum like colony morphology, LB ratio more than two. They were *P. capsici* (Race 2) and highly virulent. The second sub cluster consists of white cottony isolates, LB ratio varied, and *P. capsici* (Race 1) with moderate virulence. Cluster B consists of three sub clusters, the first sub cluster form the white cottony sterile isolates, and they do not form oospores. They were P. *capsici* (Race 3) and less virulent isolates in contrast to the highly virulent and moderately virulent isolates of cluster. The second sub cluster consists of isolates with floral colony morphology, LB ratio more than two. They consisted of Race 1, 2, 4 and 5 and highly virulent. The third sub cluster consists of isolates with chrysanthemum like colony

pattern, moderately virulent and LB ratio around 1.5. They were *P. capsici* of Race 1. The third cluster consisted first sub cluster of eight isolates with stellate colony pattern and LB ratio more than 2 and highly virulent. They were *P. capsici* (Race 2). The second sub cluster contains three A2 isolates from *P. capsici*. Their LB ratio was around 2 and was less virulent. They were *P. capsici* of (Race 3). The last portion of the similarity tree consisted of single isolate 98- 87. It had irregular ontogeny, LB ratio 3, highly virulent and *P. capsici* of (Race 2)

The *Phytophthora* species infecting black pepper other than *P. capsici* is clustered into four. They were cluster A consisted of *P. parasitica*, cluster B consisted of *P. meadii*, cluster C consisted of *P. parasitica*, and cluster D consisted of *P. palmivora*. Here the P. *parasitica* isolate 98-75 formed a separate cluster, this isolate was morphologically similar to *P. parasitica* but it was a highly virulent isolate and infected the entire *Piper* species tested. But all other *P. parasitica* isolates were less virulent compared to this isolate.

Isolates of *P. capsici*, including those previously identified as '*P. palmivora*' MF4 separated into three groups CAP 1, CAP 2 and CAP 3. Those in CAP 1 were widely distributed geographical location on a range of hosts. CAP 2 was primarily on black pepper and other hosts. The black pepper from India and Indonesia contain CAP 1 and CAP 2. CAP 3 contains isolates from cocoa (Oudemans and Coffey, 1991a). Mchau and Coffey, (1995) extended the study of *P. capsici* and found that isolates clustered into subgroups Cap A and Cap B**.** The isolates described by *P. tropicalis* fell within both CAP 2 and CAP 3. The Cap A and Cap B contained isolates from widely distributed hosts and geographical origin. The Cap A isolates had round sporangia and exhibited irregular sporangial ontogeny. Cap B isolates produced ellipsoid lanceolate sporangia and 50 % had umbellate ontogeny. Nygaard et al. (1989) grouped *P. megasperma* into three formae specialis based on isozyme variability.

Morphologically closely related species such as *P. parasitica*, I or *P. cambivora* and *P. cinnamomi* were clearly differentiated as distinct genetic entities. Recent studies revealed that *P. capsici*, *P. citrophthora* and *P. megakarya* are complex species (Forster et al. 1990: Oudemans,

1990) clustering into subgroups, which may represent distinct biological species that could reflect recent speciation events.

There are reports of identification of *Phytophthora* species especially *P. nicotianae* var. *parasitica*, *P. capsici* and *P. melonis* through disc gel electrophoresis (Kaw et al.1982). Bielenin et al. (1988) did a similar line of work for studying isozyme variability of a large number of isolates of *Phytophthora* from different hosts around the world. They identified four distinct groups in *P. drechsleri* and six groups in *P. cryptogea*, which can becomparable to the present study. These results were in agreement with the studies conducted by Oudeman and Coffey (1991a). Similarly isozyme variability among the isolates of *P. cinnamomi* from Australia and Papua New Guinea were reported (Old et al. 1984).

In the present study that the isolates formed three major clusters and each cluster consisted of morphologically similar sub clusters. The isolates of b1 sub cluster formed a highly similar cluster. The present study is similar to the study of Mchau and Coffey (1995). It has been observed in this study that the majority of *P. capsici* isolates postulated as belonging to Cap B type described by Mchau and Coffey (1995), grouped together but were subdivided into two clusters based on their colony morphology, virulence and the relative difference in their L/B ratios. Morphologically, they were characterized by ellipsoid-lanceolate sporangia produced on umbellate sporangiophore with higher L/B ratios of values equal to or more than 2.0. In contrast, the Cap A isolates produced more or less rounded sporangia, with L/B ratios of around 1.5. A gradation in the virulence was seen in the grouping of the isolates based on isozyme variability. The highly virulent isolates were found in the upper portion of the similarity tree, while the virulence of the isolates grouped towards the lower portion decreased gradually. Thus, the *P. capsici* isolates in cluster A were highly virulent, while those in Cluster B showed different degrees of virulence.

Molecular characterization using Internal Transcribed spacer regions of rDNA gene repeates

The internal transcribed spacer regions (ITS1 and ITS 2) of the ribosomal gene repeat from the *Phytophthora* species were amplified using the polymerase chain reaction and sequenced. Sequences from *P. cambivora, P. cinnamomi, P. citricola, P. cryptogea, P. drechsleri, P. fragariae* var. *frageriae, P. frageriae* var. *rubi, P. megasperma* var. *megasperma* and *P. nicotianae* were compared with Published sequences and Phylogenetic trees were produced. The resultant grouping of species generally agreed with grouping established using classical morphological criteria, primarily sporangial morphology (Cook and Duncan, 1997). Ristaino et al. (1998) amplified *Phytophthora* by ITS primers, ITS 5 and ITS 6. Restriction digests with Msp1 separated *P. capsici* from *P. citricola* and separated *P. cactorum* from *P. infestans* and *P. mirabilis*.

The study has shown that RFLP analysis of ITS regions was used to distinguish between the different species of *Phytophthora* involved in foot rot disease of black pepper. The internal transcribed spacer regions (ITS4 and ITS 6) of the ribosomal gene repeat from the *Phytophthora* species were amplified using the polymerase chain reaction and sequenced. The ITS analysis indicated the presence of sub groups within *P. capsici.* They were considered as separate species since their morphological and ITS pattern variations are substantial for the division of the species into two biological species itself. Several earlier reports also suggest the same thing. These include the separation of *P. capsici* into two or three forms based on isozyme criteria. (Oudeman and Coffey, 1991 a, b; Mchau and Coffey, 1994); a high degree of mitochondrial DNA diversity (Forster et al. 1989), and the detection of two groups based on ITS sequence analysis (Forster et al. 2000).

The first major cluster A of *P. capsici* forms two sub clusters, s1 and s2. They were morphologically and pathogenically also different and they can be considered as two molecular sub groups. The upper sub cluster consists of Race 1 and 3. The lower sub cluster consists of Race 1, 2, 4 and 5. The sub cluster a1 can be considered as Cap A and a2 as Cap B. The three different species *P. meadii*, *P. palmivora* and *P. parasitica* were clustered separately. The existence of two molecular sub groups among *P. capsici* was substantiated by the works of Chowdappa et al. (2003 a and b). They can be considered as *P. capsici* and *P. tropicalis* sp.nov. (Aragaki and Uchida 2001)

Chowdappa et al (2003 a) studied *Phytophthora capsici* isolates from cocoa, black pepper, betel vine and bell pepper collected from different localities in India were examined at the molecular level using the ITS 1 and ITS 2 primers. Majority of isolates from black pepper and all isolates from cocoa, betel vine and bell pepper displayed restriction digestion patterns similar to Cap A patterns while few isolates on black pepper had Alu 1 and Msp digests of ITS similar to Cap B patterns.

Stamps *et al.* (1990) in his revised tabular key to the species of *Phytophthora* set out characters for 67 species of *Phytophthora* and varieties including 19 new ones described since 1978. Erwin (1983) reviewed variability within and among species of *Phytophthora.* Erwin and Ribeiro (1996) reviewed the morphology of *Phytophthora*. *P. capsici* have been described (Leonian, 1922) as having generally ovoid sporangia with extremely variable sizes and brown coloured zoospores, lacking chlamydospores being pathogenic to pepper plants (*Capsicum annum* L). Later, many isolates of *Phytophthora* from other hosts, which exhibited similar morphological features, have been included under the species of *P. capsici*. *Phytophthora* isolates from tropical crops such as black pepper and cocoa, previously described as *P. palmivora* MF4 and some of which form chlamydospores, were designated as an amended description of this species (Tsao and Alizadeh, 1988). In contrast a new species, *P. tropicalis* (previously identified as *P. capsici*) for isolates of tropical origin on morphological grounds has been proposed (Uchida and Aragaki, 1989; Aragaki and Uchida, 2001). Later three isozyme subgroups were reported in *P.capsici*, CAP 1, CAP 2 and CAP 3. The isolates described as *P. tropicalis* fell in both CAP 2 and CAP 3. Later studies showed that there were two sub groups namely Cap A and Cap B described by Mchau and Coffey, 1994 and Aragaki and Uchida, 2001).

The amplification with ITS 1 and ITS 4 yielded a PCR product of 860 bp for *P. capsici*, 900 bp for *P. arecae*and 920 bp for *P. nicotianae*. The ITS - restriction fragment length polymorphism patterns of *P. arecae*, *P. capsici*, *P. meadii*and *P. nicotianae*significantly varied. The isolates of the same species, however, showed identical banding patterns. The results were almost similar irrespective of the enzyme used (Chowdappa et al. 2003 b). The four main species of *Phytophthora*

in black pod disease of cocoa can be readily distinguished based on RFLP - ITS analysis (Appiah et al. 2004)

In the present study the molecular data is not correlating with the pathogenic groups or races in *P. capsici*. Gupta and Rajeev (1999) reviewed that in some studies there is lack of polymorphism in micro propagated plants screened through molecular markers. So it is clear that the morphological or pathologic variation may not be reflected in the molecular studies like RFLP, RAPD PCR or isozymes.

There may be instances were the polymorphism cannot be detected because the molecular methods used are inadequate to detect the morphological or pathogenic variation. Seven land races of little millet formed six distinct groups with high inter-group distances. But molecular analysis of diversity reported that the land races were all genetically uniform and any observed diversity may be due to environmental variation. The contradictory result only emphasized the fact that the lack of polymorphism need not imply lack of genetic divergence (Arunachalam et al. 2005).

In our work the pathogenicity groups were not reflected in the molecular studies. So the gene for pathogenicity may be different and the methods used were inadequate to reflect the genetic variation between the races. The work of Arunachalam et al. (2005) is a similar where diversity obtained in morphological studies is not reflected in the molecular studies.

As discussed, earlier there is no correlation between the morphological groups of *P. capsici* with racial picture that emerged in the present study. Nor there is any correlation of the molecular data of the fungus with racial picture. This would mean the necessity of reexamining this, with large population of *P. capsici* using more differentials including more land races of black pepper to reconfirm findings or other wise. It is possible that there is an independent evolution of races with out any bearing on morphology & molecular aspects of the pathogen. The study on the other has brought out new information that another three new *Phytophthora* species infecting black pepper exist in the region, that calls for a

regular monitoring of the *Phytopthoras* during regular disease surveillance .The racial picture of the pathogen needs a relook with large population of *P. capsici*. This is important in planning future breeding programmes focusing on development of resistance in black pepper for *P. capsici*, the most serious pathogen of black pepper all over the pepper growing countries

SUMMARY AND CONCLUSIONS

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Phytophthora foot rot continued to be a major production constraint in black pepper causing serious crop losses not only in India but also in other countries where black pepper is grown commercially. This most devastating disease of black pepper caused severe crop loss in India especially in southern states of India, Kerala, Karnataka, Tamil Nadu, and Andhrapradesh and also in North East Region of India. Earlier studies established *Phytophthora capsici* as the causal agent. The identity of causal agent was a controversy for long, ultimately resolving its identity as *Phytophthora capsici* Leonian (1922) emend. A. Alizadeh and P.H. Tsao. However its biology and variability were not studied in detail and documented. The present investigation was undertaken with a very large population of *Phytophthora* isolates from black pepper to study if more than one *Phytophthora* species is involved in foot rot of black pepper. Even in *P. capsici* there was no documented information about the pathogenic variability / virulence and racial picture of if any. These two lacunae in our understanding have become the basis of present investigation. The results and conclusions obtained in the present investigations have been outlined below.

1. **The present investigation has brought out clearly that *P. capsici* is the predominant pathogen (85% isolates) of black pepper. In addition *P. parasitica* (6.6% isolates), *P. meadii* (5% isolates) and *P. palmivora* (3.3% isolates) were also identified as pathogens of black pepper pointing to the possible threat in future by these three species of *Phytophthora*, in addition to *P. capsici*.** *Phytophthora* species were isolated adopting selective medium from various infected plant parts like leaf, stem, collar, spike, and root and rhizosphere soil of the affected plants. 60 isolates were used for characterization of which 32 were from leaf, 7 from stem, 9 from root, one each from collar and berry and 10 from soil. They were obtained from black pepper plantations from Kerala, Karnataka a Tamil Nadu and Andhra pradesh. The percentage of other species was meagree while considering *P. capsici* which is predominant.

2. **Based on the morphology and biology, *P. capsici* isolates of black pepper were grouped**

into four.

Considerable variation was noticed in the colony morphology of these isolates. Some isolates were slow growing and others were fast growing. The *P. capsici* isolates of morphological group I showed chrysanthemum like colony and aerial mycelium was sparse. The isolates of morphological group II showed white cottony colony and slow growth. The isolates of morphological group III showed chrysanthemum like pattern, stellate pattern and floral pattern. The isolates of morphological group IV showed uniform cotton wool like aerial mycelium and fast growth. **The isolates of morphological group II with white cottony colony were slow growing and the isolates of morphological group III with chrysanthemum like pattern and morphological group IV with uniform cotton wool like aerial mycelium were fast growing.**

The *P. meadii* isolates had white cottony with faded pattern and floral pattern. The *P. palmivora* isolates had white cottony with faded pattern. The *P.parasitica* isolates had white fluffy aerial mycelium.

2.1. All of them showed predominantly umbellate ontogeny of sporangiophore with caducous sporangia of long pedicels, size, with LB ratio of 1.4-3.5.

Considerable variation was noticed in the morphology of sporangia their ontogeny, caducity, pedicel length and shapes of sporangia. The *P. capsici* isolates had two types of sporangial ontogeny. 50 isolates had umbellate ontogeny and one was irregular. Sporangial shape varied in different isolates.

The *P. capsici* isolates of group I had umbellate ontogeny and ovoid sporangia. LB ratio was around 1.5. The sporangia were prominently papillate, and caducity (51-75 %) and pedicel length range was (31-94 µm).

The *P. capsici* isolates of group II had umbellate ontogeny and LB ratio was 1.8-2.3. Sporangia were ovoid, obovoid and fusiform in shape and prominent papilla present. Their

caducity was similar to the group I isolates (51-75 %) and pedicel length range was (65-127 µm).

The *P. capsici* isolates of group III were with varied shapes of sporangia like ovoid, obovoid, obpyriform and distorted shapes. The sporangia were papillate or semipapillate with LB ratio of 2 – 3.5. Central vacuole was present in some sporangia. Their caducity was less 26-50 % and pedicel length range was 38-192 µm. The isolate 98-87 of group III was different from other isolates. It had irregular ontogeny and LB ratio was 3, with very long pedicels and pedicel length of 192 µm, distorted balloon like sporangia and caducity was 26 %.

The *P. capsici* isolates of group IV showed umbellate ontogeny and papillate ovoid sporangia with LB ratio of 1.6 - 2.1. Caducity was maximum in this group of isolates (76-100 %) and pedicel length was 70-167 µm.

The *P. meadii* isolates were with white cottony with faded pattern sympodial or irregular ontogeny papillate sporangia and shape was ovoid and LB ratio less than 1.6. Their caducity was 51-75 %. But their pedicel length was (10-14 µm).

The *P. palmivora* isolates were with sympodial ontogeny. Sporangia were globose to ovoid and prominently papillate and LB ratio around 2. They were highly caducous (76-100 %) and pedicels were small, < 5 µm. The *P. parasitica* isolates were non caducous, with sympodial ontogeny and sporangia were globose and LB ratio > 1.5.

2.2. Chlamydospores were absent in the morphological group II. Most of the *P. capsici* isolates were producing chlamydospores. The entire group I, and group IV and seven isolates of the group III produced chlamydospores. Two of the *P. meadii* isolates (98-86 and 98-90) produced chlamydospores and 98-192 did not produce chlamydospores. All *P. palmivora* and

P. parasitica isolates also produced chlamydospores. The chlamydospore diameter was also variable.

2.3. Studies on mating type of *P. capsici* showed that A1 (88.4%) as the most predominant followed by A2 (1.73%) and (4.62%) were sterile isolates. Out of the 60 isolates 51 belong to *P. capsici*, 3 belong to *P. meadii*, 2 to *P. palmivora* and 4 to *P. parasitica*. . Among the three *P. meadii* isolates two were sterile and one A1. Two of the *P. palmivora* isolates were A2. Among the four *P. parasitica* three isolates were A1 and one sterile.

2.4. Temperature and pH ranges were highly variable indicating the plasticity of *P. capsici* for their adaptability to variable ranges of edaphic factors which points to the survival capability of the pathogen.

The sixty isolates showed variation in different temperature. 10 ^{0}C and 35 ^{0}C were unfavorable for growth but 20 ^{0}C and 28 ^{0}C were favourable for growth. Maximum growth occurs at 28 ^{0}C and 72hrs interval. Different groups of isolates varied in their response to different temperatures.

Sporulation of the sixty isolates was studied and maximum sporulation occurs at 20 ^{0}C and just followed by 28 ^{0}C. Minimum sporulation was at 35 ^{0}C. Temperature and zoospore liberation of the isolates were studied at different temperature. Zoospore liberation was maximum 20 ^{0}C followed by 28 ^{0}C. 10 ^{0}C and 35 ^{0}C were not favourable for zoospore liberation. All the sixty isolates were studied for zoospore germination. 20 ^{0}C was favourable for zoospore germination and maximum zoospore germination was at this temperature and followed by 28 ^{0}C. The temperature 10 ^{0}C and 35 ^{0}C were not favourable for zoospore germination.

All the 60 isolates were studied for their variability with various pH. PH 5 or 7 were optimum for mycelial growth for most of the isolates. Minimum growth or no growth was at pH 2.5 and pH 9. All the sixty isolates were studied for their sporulation in different pH. Maximum sporulation was noticed at pH 7 and followed by pH5 in most of the isolates. At pH 2.5 no sporulation was noticed. pH and zoospore liberation of the isolates were studied. At pH 2.5 there was no zoospore liberation. Zoospore liberation was at pH 5, pH 7 and pH 9. Minimum zoospore liberation was at pH 9. Zoospore germination of the isolates at various temperatures was studied for sixty isolates. Zoospore germination was noticed at pH 5, pH 7 and pH 9. But no germination was noticed at pH 2.5.

Growth of sixty *Phytophthora* isolates was studied in various media like carrot agar, potato dextrose agar, cornmeal agar and papaya dextrose agar. Maximum growth was obtained in carrot agar for most of the isolates. Minimum radial growth occurs in potato dextrose agar medium. Sporulation of the all isolates was studied in these media and maximum sporulation was obtained in carrot agar and just followed by corn meal agar. The minimum sporulation was in potato dextrose agar or papaya dextrose agar. Zoospore liberation was tested for all the isolates. Maximum zoospore liberation was in carrot agar for most of the isolates. Zoospore liberation was minimum in potato dextrose agar for most of the isolates. Zoospore germination of the all isolates was studied and maximum germination was in carrot agar for most of the isolates. Minimum germination was in potato dextrose agar for most of the isolates. The group III isolates had maximum zoospore germination in papaya dextrose agar. The group IV isolates had minimum zoospore germination in all the tested media.

3. *P. capsici* isolates was found pathogenic to arecanut, rubber, black pepper, betel vine, small cardamom, cocoa and coconut. Thus the cross infectivity studies proved the wide host range of *P. capsici*.

4. *Phytophthora* species from other plantation crops viz, rubber, arecanut & coconut

isolates were not pathogenic to black pepper. Cocoa, cardamom, and betel vine isolates infected black pepper.

5. *Bauhinia purpurea* and *Piper chaba* were recorded for the first time as the new hosts of *P. capsici* and can serve as the alternate hosts for *P. capsici,* adding to the possible inoculum source for black pepper infection. The *P. capsici* isolates obtained from other hosts were studied for their morphology and pathogenicity. The isolates were from betel vine, cocoa, *Capsicum*, *P. chaba* and *Bauhinia*. The isolates from other hosts showed variability in morphology and pathogenicity. Among the betel vine isolates three had white cottony mycelium and two had stellate colony morphology. All of them had umbellate ontogeny and long pedicels. Two of the *P. capsici* isolates from cocoa had stellate or white cottony mycelium and umbellate ontogeny and long sporangial pedicels. The *Capsicum*, *P. chaba* and *Bauhinia* isolates had white cottony mycelium and umbellate ontogeny and long pedicels.

6. *In addition, P. capsici* isolates from *Cacao*, and *Capsicum*, and betel vine were found pathogenic to black pepper pointing to the possibilities of these becoming as alternate hosts of *P. capsici* thus adding up to be the potential sources of inoculum for black pepper infection. The *P. capsici* from *Capsicum* and cocoa were found to be less virulent on black pepper leaves than all other isolates. Virulence of *P. capsici* (98-64) from cocoa was found to be less compared to all other isolates (14.5 mm). So also the two betel vine isolates were less virulent.

7. The variable reaction of *P. capsici* isolates on 9 *Piper* species as differentials including *P. nigrum* clearly indicated the existence of 5 possible races of *P. capsici* in black pepper based on the study with a population of 51 *P. capsici* isolates. Based on the pathogenicity the isolates can be categorized as follows.

Race 1: Isolates with white cottony and chrysanthemum like pattern. They had LB ratio >2. They were moderately virulent on Sreekara leaves and infecting all the tested hosts except *P.*

arboreum.

Race 2: Isolates had chrysanthemum like, floral and stellate pattern and highly virulent on Sreekara leaves. They infected all the tested host leaves and produced comparatively large lesions on the tested hosts.

Race 3: They had uniform cotton wool like aerial mycelium. They did not infect *P. arboreum*, *P. colubrinum*, *P. betle* and *P. longum*. They were less virulent on Sreekara leaves.

Race 4: Isolate had floral pattern and did not infect *P. longum* and *P. arboreum.*

Race 5: Isolate had floral pattern and did not infect *P. longum*, *P. colubrinum* and *P. arboreum.*

The *P. capsici* isolates of Race 1 were moderately virulent on pepper stem and root. The *P. capsici* isolates of Race 2 were highly virulent on pepper stem and root. The *P. capsici* isolates of Race 3 were pathogenically weak on pepper stem and root. The *P. capsici* isolates of Race 4 and 5 were less virulent on pepper stem and root.

The *P. parasitica* isolates were moderately virulent and *P. meadii* and *P. palmivora* were less virulent on pepper stem and roots. The *P. palmivora* isolates, which did not produce lesion on Sreekara leaves, were pathogenic on pepper root and stem.

8. Besides this, some of the *Phytophthora* isolates from other plantation crops viz. *P. meadii* from rubber, cardamom and cocoa, *P. palmivora* isolates form cocoa and coconut known to be predominantly A2, point to the possibilities of mixing up of *Phytophthora* spp under high density multi species cropping systems there by leading to possibility of development of new races/ biotypes pathogenic to a broad spectrum of hosts.

9. Based on polyacrylamide gel electrophoresis the *P. capsici* cluster into two sub

clusters. Variability of *Phytophthora* isolates was studied by electrophoresis of total proteins by SDS PAGE. Seventeen isolates were studied by protein electrophoresis. The isolates clustered into four major groups. The first cluster consisted of *P. capsici* isolates and it had two sub clusters. The first sub cluster consisted of isolates of morphological group III and IV and Race 2, Race 3, Race 4 and Race 5. The second sub cluster consisted of isolates of morphological group I and II and Race 1. The lower major clusters consisted of *P. meadii*, *P. palmivora* and *P. parasitica*.

10. Based on the isozyme analysis 51 *P. capsici* isolates fell into three major clusters and an isolate was separated out and clustered alone in the lowest portion of the similarity tree. The cluster A consists of two sub clusters The first sub cluster consisted of *P. capsici* isolates of morphological group III and race 2. The second sub cluster consisted of *P. capsici* isolates of morphological group II and race 1. The cluster B consisted of two sub clusters b1, b2 and b3. The sub cluster b1 consisted of *P. capsici* isolates of morphological group IV and race 3. The sub cluster b2 consisted of isolates of group III and Race 2, 4 and 5. The cluster C consisted of two sub clusters c1 and c2. The sub cluster c1 consists of *P. capsici* isolates of morphological group III and race 2. The sub cluster c2 consists of *P. capsici* isolates of morphological group IV and race III. The last isolate was seen in the lower most portion of the similarity tree was a *P. capsici* isolates of morphological group III and race 2.

11. Based on the DNA polymorphism, ITS analysis *P. capsici* fall in to two molecular groups corresponding to Cap A and Cap B. The upper sub cluster consisted of isolates of morphological groups I and IV form Cap A group of ITS analysis and lower sub cluster consisted of morphological groups II and III form the Cap B group of ITS analysis. The first sub cluster contains Race 1, Race 3, Race 4 and Race 5 and the second sub cluster consists of Race 1 and Race 2. The second, third and fourth major clusters contain respectively *P. meadii*, *P. palmivora* and *P. parasitica.*

12. The above (9, 10, and 11) findings indicate that there is no direct correlation

between the racial picture and the molecular data available in the present investigation. However the molecular data has some correlation with morphological groups. The morphological groups II and III form the Cap B group of ITS analysis and groups I and IV form Cap A group of ITS analysis.

Conclusion

For the first time four *Phytophthora* species viz. *P. capsici*, *P. meadii*, *P. parasitica* and *P. palmivora* have been found pathogenic to black pepper of which *P. capsici* is the most serious pathogen. *P. capsici* showed remarkable variability in morphology, physiology and virulence. *P. chaba* and *Bauhinia purpurea* were recorded for the first time as new hosts of *P. capsici*..

The study also points out the possible threat arising out of emergence of new biotypes with a broad spectrum of host range because of the existence of A1 and A2 mating type of *Phytophthora* species involved in high density multi species cropping system. in the region. Besides the fact that *P. capsici* isolates of Cocoa, betel vine & chillies were found pathogenic , did indicate the likely inoculum build up that would be detrimental if these crops are grown in the vicinity of black pepper plantations.

The role of chlamydospores in survival of the fungus though known but their absence in some of the *P. capsici* isolates and its relation to the pathogenic fitness needs in depth study to relate it to the disease spread. Similarly the role of pedicel length of sporangia, their caducity a their role in disease spread is important from epidemiological angle and needs investigation

Pathogenic variability based on the pathogenic reaction of 51 *P. capsici* isolates on 9 *Piper* spp as differentials clearly brought out for the first time the existence of a racial picture of *P. capsici* into 5 races. However no correlation existed on the racial picture with morphological and molecular grouping. (**Table: 114**) This indicated the possible evolution of independent races in *P. capsici.* This would mean the confirmation or otherwise of the existence of races is called for involving

more number of land races of black pepper as differentials, in addition to the existing *Piper* spp and also large population of *P. capsici*. These points to the need and relevance for a careful planning of future breeding programmes of black pepper to develop host resistance to *P. capsici* utilizing races of *P. capsici* in screening programmes, with an ultimate aim of developing black pepper varieties with durable resistance. The present study opened up new opportunities and avenues both for disease management & breeding programmes in black pepper

The present investigations calls for the extension of the present studies with larger population of *P. capsici* isolates o from black pepper, and also for inclusion of more number of land races of black pepper in the differential hosts, to confirm the present racial picture and also how the racial picture correlates with molecular data if any.

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APPENDIX

APPENDIX

Preparation of culture media

1.Carrot agar medium (Kaosiri et al. 1978, 1980; Brasier and Griffin 1979)

Carrot	200gm
Agar	20gm
Water	1litre

Preparation

Sliced, fresh carrots (200 g) are comminuted in a blender in 500 ml distilled water for 40 s at high speed and filtered through four layers of cheese cloth. The juice is then squeezed from the residue. Make up the juice to 1 litre. Add 20 gm agar and boil to dissolve and dispense into flasks or tubes and sterilize.

2.Corn meal agar

Corn meal agar	17gm
Water	1litre

(Hi media laboratories, Pvt. Limited, Bombay- 400086, India.

Preparation:

Suspend 17 gms in 1 liter distilled water. Boil to dissolve completely and dispense into flasks or tubes. Sterilize by autoclaving.

3.Potato dextrose agar

Agar	20 gm
Potato	200gm
Dextrose	17gm
Distilled water	1litre

Preparation:

Sliced potatoes were peeled and boiled in distilled water for half an hour. The extract was filtered through muslin cloth. After making up to 1 liter, 20 g dextrose is added and boiled, dispensed in flasks and tubes and sterilized.

4.Papaya dextrose agar medium

Papaya	200gm
Dextrose	20gm
Agar	20gm

Preparation:

Boiled papaya pieces were filtered through a double layer of muslin cloth. The extract was made upto 1 litre after adding 20gm dextrose and 20 gm agar and boiled, dispensed into flasks and sterilized.

5. Clarified carrot agar medium

Carrot agar medium is made and it is boiled again to remove the froth; when the sediments are removed the carrot agar become colourless and then add 30µg/litre of β sitosterol dissolved in

dimethyl sulphoxide (DMSO) and heated to dissolve. The medium should be thoroughly stirred and dispensed into 250 ml flasks.

Inorganic salt solution for sporangia production

6. Petri' s solution (Petri, 1917; Tucker 1931; Hildebrand 1959)

The solution contains (g/litre) Ca $(NO_3)_2$(0.4) (0.0024 M), $MgSO_4$ (0.15) (0.001M), KH_2PO_4 (0.15) (0.001M), KCl (0.06) ($8x\ 10^{-4}$ M), and distilled water to 1 litre. From a suitable organic medium mycelial discs are transferred (e.g; V8 juice agar) to 10 ml of solution per petri plate at 12-20 ^{0}C. The salt solution is replaced with distilled water after 3-4 days.

7. Tryptophan supplemented Carrot agar

Clarified carrot agar is supplemented with sitosterol (30), tryptophan (20), $CaCl_2 2H_2O$ (100), and thiamine, and incubated in dark at 20^{0}C (Chee et al 1976).

8. Lactophenol

Phenol crystals melted	20 ml
Lactic acid	20ml
Glycerol	40 ml
Water	20 ml

9. Cotton blue

Cotton blue	1g
Water	20ml

Reagents for Polyacrylamide Gel Electrophoresis

Extraction buffer

Sodium phosphate buffer PH 7

0.2 m solution of monobasic sodium phosphate (NaH2 PO4.2H2O) – 39 ml

0.2 m solution of dibasic sodium phosphate (Na2HPO4.2H2O)- 61 ml

Add both solutions and made upto 200ml.

Preparation of stock solutions

1) Acrylamide stock solution

Acrylamide ------ 30g

Bis acrylamide ----- 0.8g

Add 30g acrylamide to 50ml sterile distilled water and dissolve by stirring. Then add 0.8g Bis acrylamide and dissolve it completely. Make up to 100 ml.

2) 5 M Tris cl, pH 8.8

Tris(FW 121.1). ---- 1.5 M 18.17g

Dissolve in 50ml sterile distill water and adjust the pH to 8.8 with Con. HCl .Make up to 100ml.

3) 0.5M Trs Cl, pH 6.8

Tris (FW 121.1) ----6.05g

Dissolve in 50 ml sterile distilled water and adjust the pH to 6.8 by adding 4N HCl. Make up to 100 ml.

4) 10% SDS solution

SDS (FW 288.4) ----0.35M, g.

Add this to 25ml water dissolves by gentle stirring and make up to 50 ml.

5) 10% APS

Ammonium per sulphate(FW 228.2) ---- 0.44mM, 0.1g

Dissolve in 1ml sterile distilled water.

Fresh APS crackles when water is added. Prepare just prior to use.

6) 2X sample treatment buffer

(0.125M Tris Cl, 4% SDS, 20% glycerol 2% 2- Mercaptoethanol, pH 6.8, 10 ml.)

0.5M Tris Cl, pH 6.8----- 2.5 ml.

10% SDS, 0.35 M solution -----0.4 ml

Glycerol (FW 92.09) ------------- 2 ml

2- Mercaptoethanol (FW 78.13) --------------0.31g

Bromophenol blue (FW 691.9) ---------------0.2mg

Sterile distilled water is added to make up to 10 ml.

Divide into 1 ml aliquots and store at –40 to –8 ^{0}C.

7) Electrophoresis buffer

Tris FW 121.1) -------- 0.756g

Glycine (FW 75.07) --------- 3.6g

SDS (FW 288.4) ----------0.25g

Add sterile distilled water to make up to 250 ml.

Reagents for PAGE (two gels)

Separating gel	(12%)	Stacking gel(4%)
Acrylamide stock	6 ml	1340µl
1.5M tris Cl, pH 8.8	3.75ml	----
0.5M Tris Cl, pH 6.8	-----	2.5ml
10% SDS	150 µl	100 µl
Distilled water	5.05 ml	6ml
10% APS	100 µl	50 µl
Temed	10 µl	5 µl
Total volume	15 ml	10 ml.

Staining solution

Coomassie brilliant blue R-250-- 0.2g

Methanol -------- 40 ml

Distilled water --------50 ml

Glacial acetic acid --------10 ml

Total -- -------100ml

Destaining solution

Methanol -------- 40 ml

Distilled water -------- 50 ml

Glacial acetic acid ----------10 ml

Total -----------100ml.

Reagents for Isozyme analysis

The reagents for electrophoresis

Resolving gel 10%

	Reagents	**Volume (ml)**
2.	Resolving gel buffer stock (1.5M Tris-Cl, pH8.8)	3.8
3.	Distilled water	6.05
4.	10%APS	0.15
5.	TEMED	20µl
Total		

Stacking gel –5%

	Reagents	**Volume (ml)**
1.	Acrylamide: Bisacrylamide (30:0.8)	1.0
2.	Stacking gel buffer stock (1M Tris-Cl, pH6.8)	0.75
3.	Distilled water	4.19
5.	10%APS	0.06
6.	TEMED	20µl
Total		

1) Superoxide dismutase (SOD)

Stain solution: 75ml

Na_2EDTA - 5.625mg

Riboflavin - 3.0mg

NBT - 7.5mg

1M Tris-Cl, pH8.0 - 3.75ml

Volume was made up with distilled water.

Staining procedure

1. Covered gel with stain solution (75ml/gel)
2. Incubated gel at 37^{0}c for 20 min. in dark.
3. Placed gel under light.
4. Clear achromatic bands against blue background.

2) Diaphorase (DIA)

Stain solution : 75ml

NADH	-	37.5mg
MTT	-	9mg
DCPIP	-	1.5mg
Tris-Cl, pH 8.0	-	7.5ml

Volume was made up with distilled water.

Staining procedure

Gels were immersed in the stain solution and incubated in dark at room temperature for 10 – 15 minutes, until clear bands appeared.

3) Malate dehydrogenase (MDH)

Stain solution : 75ml

Malic acid - 2011.35mg (eq.to 2.0M) dissolved in a small qty. of water, pH adjusted to pH 8.0

NAD	-	33.3mg
NBT	-	16.65mg
PMS	-	3.33mg
1.0M Tris-Cl, pH8.0	-	7.5ml

Volume was made up with distilled water

Staining procedure

The gels were incubated in the stain solution for 15 minutes in dark at room temperature till the bands appeared. The reaction was stopped by immersing the gels in 7% acetic acid

4) Malic enzyme (ME)

Malic acid	-	2011.35mg, dissolved in a small qty. of water, pH adjusted to 8.0
1.0M $MgCl_2$	-	1.5ml
NADP	-	15mg
PMS	-	1.5mg
NBT	-	15mg
1M Tris-Cl, pH8.0	-	7.5ml

Volume made up with distilled water.

Staining procedure

The gels were incubated in the stain solution in dark at room temperature for 15minutes, until clear bands appeared.

5) Isocitrate dehydrogenase

Stain solution : 75ml

Isocitric acid	-	41.6mg
$MgCl_2$	-	166.6mg
NADP	-	33.3mg
NBT	-	8.3mg
PMS	-	1.7mg
1.0M Tris-Cl, pH8.0	-	7.5ml

Volume was made up with distilled water

Reagents for ITS analysis

1.GYP Medium (**Glucose yeast peptone**) broth as modified by Hall *et al*. (1969)

L.Asparagine	20gm
$FeSO_4 7H2o$	20mg
$CaCl2.2H_2o$	10mg
$MgSO4.7H_2O$	0.1gm
KH_2PO_4	0.47gm
$K_2H_2PO_4$	0.26gm
Thiamine hydrochloride	10mg
$ZnSO_4.7H_20$	1 µg/ml
$CuSO_4.5H_2O$	0.02µ/ ml
$Mncl_2$	2mg

pH adjusted to 6

2. PVPH incorporated Carrot Agar (1L)

Carrot	200gm
Agar	20gm

Volume made upto 1000ml using sterile distilled water. For every 100 ml of carrot agar 10ml of PVPH was added.

3.Extraction buffer

Tris base	100mM
EDTA	20mM
Sodium Chloride	1.4M
SDS	10%
Sterile Distilled Water	

Preparation of Commonly Used Stock Solutions Stock Solutions for Agarose Gel Electrophoresis

1.TAE Buffer (50X)

Method of Preparation

Weigh 242g Tris base and dissolve in 600ml of water. Add 57.1 ml glacial acetic acid and 100ml of 0.5M EDTA (pH 8.0). Stir well. Make up to 1 liter with water. Dispense in aliquots and sterilize by autoclaving.

TAE buffer is used as 1X.

2.Ethidium Bromide (10mg/ml)

Method of Preparation

Add 1g of Ethidium bromide to 100ml of water. Stir on a magnetic stirrer for several hours to ensure that the dye has dissolved. Wrap the container in aluminium foil and store at room temperature.

EtBr is a powerful mutagen. Gloves should be worn when working with the solutions containing this dye.

3.Gel loading buffer 6X

0.25% Bromophenol blue
0.25% Xylene cyanol FF
15% Ficoll (Type 400; Pharmacia biotech)
Store at room temperature.

4.1 M Tris.Cl (pH 8.0)

Method of Preparation

Dissolve 121.1g of Tris base in 800ml of water. Adjust the pH to 8.0 by adding 42ml concentrated HCl. Allow the solution to cool to room temperature before making final adjustments to the pH. Adjust the volume to 1 liter with water. Sterilize by autoclaving. If the 1M solution has a yellow colour, discard it and obtain better quality Tris.

5.0.5 M EDTA (pH 8.0)

Add 186.1g of $Na_2EDTA.2H_2O$ to 800 ml of water. Stir vigorously on a magnetic stirrer.

Adjust the pH to 8.0 with NaOH (approx. 20g of NaOH pellets). Dispense into aliquots and sterilize by autoclaving.

The disodium salt of EDTA will not go into solution until the pH of the solution is adjusted to approx. 8.0 by the addition of NaOH.

4.5 M NaCl

Dissolve 292.2g of NaCl in 800ml of H_2O and adjust the volume to 1 liter with H_2O. Dispense into aliquots and sterilize by autoclaving.

5.Sucrose

Dissolve 34.23g of sucrose in 80ml of water. Make up the volume to 100ml. Filter sterilize.

6.20% SDS

Dissolve 408.1g of $CH_3COONa.3H_2O$ in 800ml of H_2O. Adjust the pH to 5.2 with glacial acetic acid. Adjust the volume to 1liter with H_2O. Dispense into aliquots and sterilize by autoclaving. Always use fresh. Wear a mask when weighing SDS and wipe down the weighing area and balance after use. There is no need to sterilize 10% SDS.

7.3M Sodium acetate (pH 5.2)

Dissolve 408.1g of $CH_3COONa.3H_2O$ in 800ml of H_2O. Adjust the pH to 5.2 with glacial acetic acid. Adjust the volume to 1liter with H_2O. Dispense into aliquots and sterilize by autoclaving.

8.Equilibrated phenol

Remove the redistilled phenol containing 0.1% hydroxyquinoline from the freezer. Allow it to warm to room temperature, and then melt it at 68_0C. To the melted phenol, add an equal volume of 0.5M Tris.Cl (pH 8.0). Stir the mixture on a magnetic stirrer for 15 minutes, and then turn off the stirrer. When the two phases have separated, remove the aqueous phase as much as possible. Repeat the above process by adding equal volume of 0.5M Tris.Cl (pH 8.0) until the pH of the phenolic phase is >7.8. After the phenol is equilibrated and the final aqueous phase has been removed, add 0.1 volume of 0.1M Tris.Cl (pH 8.0) containing 0.2% β-mercaptoethanol. Wear gloves, protective clothing and safety glasses when handling phenol. All manipulations should be carried out in a chemical hood. The phenol may be stored in this form for period's upto 1 month.

9.Phenol: Chloroform (1:1)

Mix equal amounts of phenol and chloroform. Equilibrate the mixture by extracting several times with 0.1M Tris.Cl (pH 7.6). Store the equilibrated mixture under an equal volume of 0.01M Tris.Cl (pH 7.6) at 4^0C in dark glass bottle.

10.Chloroform: Isoamyl alcohol

Mix 96ml of chloroform with 4ml of isoamyl alcohol. Store in a dark colour bottle