DISTRIBUTION OF SPECIES OF PHYTOPHTHORA AFFECTING COCONUT AND PEPPER IN KERALA

By

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ABSTRACT OF A THESIS

submitted in partial fulfilment of the requirement

for the degree

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Kerala Agricultural University

DEPARTMENT OF PLANT PATHOLOGY

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VELLAYANI, THIRUVANANTHAPURAM

1996

To
My Teachers

DECLARATION

I hereby declare that this thesis entitled **"Distribution of species of *Phytophthora* affecting coconut and pepper in Kerala"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or society.

Vellayani
09.09.'96

VEENA. S.S.
(92-21-16)

CERTIFICATE

Certified that this thesis, entitled, "**Distribution of species of *Phytophthora* affecting coconut and pepper in Kerala**" is a record of research work done independently by **Veena. S.S.** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to her.

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CERTIFICATE

We the undersigned, members of the Advisory Committee of **Ms. Veena, S.S.**, a candidate for the degree of Doctor of Philosophy in Agriculture, with major in Plant Pathology, agree that the thesis entitled "**Distribution of species of *Phytophthora* affecting coconut and pepper in Kerala**" may be submitted by **Ms. Veena, S.S.** in partial fulfilment of the requirements for the degree.

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INTRODUCTION

INTRODUCTION

The variability present in the genus *Phytophthora* has been exhaustively reviewed about a decade ago by Erwin (1983). The earlier classification system for this genus was mainly designed for the identification of field isolates. Some species of *Phytophthora* were considered 'stable' while others which exhibited a high degree of variability were labelled 'unstable'. With a genetic system which runs parallel to higher organisms in terms of ploidy level and due to the existence of heterothallism, a high degree of variability is seen among different strains of *Phytophthora* making it extremely difficult to identify and group.

The genus *Phytophthora* is an infectious fungus found on almost all cultivated crops in Kerala owing to the high humidity condition prevalent during the monsoons. The fungus causes havoc to important cash crops including coconut, pepper, rubber, arecanut and cardamom. The exact identity of strains of *Phytophthora* infecting these crops is still not well understood. The present study envisages a better understanding of the host range, pathogenicity, species identification for better management of *Phytophthora* diseases in coconut and pepper.

Butler (1906) first reported bud rot of coconut from India. He (1925) identified the pathogen involved in bud rot as

P. palmivora. Recently bud rot was observed in a very destructive form in many parts of Kozhikode district and 80 to 90 per cent losses have been recorded in certain plantations.

The first authentic report of foot rot of pepper due to *Phytophthora* in India was in 1966 (Samraj and Jose, 1966) from Kerala, though the disease was noticed as early as in 1929 (Venkata Rao, 1929). Annual loss of 20 to 30 per cent of the pepper vines due to foot rot was recorded from some plantations of Kannur and Kozhikode districts and recently extensive losses to pepper due to this disease was recorded from Idukki and Wynad districts.

The presently accepted mode of classification of the genus *Phytophthora* revolves around the Waterhouse system, largely based on morphological characters and recognises about 50 species along with a few varieties and *Formae specialis* (Waterhouse, 1963). In the times to come a more natural system of classification may emerge based on molecular and genetic variations, isozyme patterns etc.

Controlling any soil borne plant pathogen in a crop is difficult, and *Phytophthora* diseases are no exception. Although numerous studies have reported antagonism among a great diversity of fungi, the extent of these interactions between *Phytophthora* and other groups under *in vitro* and in vivo conditions and then

possible importance in the reduction of disease remain largely unexplored. The main control measure adopted is prophylaxis with costly fungicidal umbrella. In a mixed cropping system, it is not clear whether it is necessary to adopt control measures to all the known host plants of *Phytophthora* when another crop is infected with this organism.

In the present study an attempt has been made to comprehend the distribution of species of *Phytophthora* affecting coconut and pepper in Kerala following the morphological parameters and host range studies. It also aimed at isolating viable antagonistic organisms against the pathogen so as to utilise them in future for biological control.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

One of the earliest plant pathogens identified about a century ago was *Phytophthora* infestans (Mont.) de Bary causing potato blight in Europe (1876). Since then about 70 species and their varieties of the genus *Phytophthora* have been reported on hundreds of host plants, with world wide distribution. There was several species of the fungus causing recurrent devastating damages to economic crop plants in many countries. Characteristically, the disease seems to appear overnight, before one could recognise its presence, the sweeping damage is already done.

P. infestans, *P. cactorum*, *P. citrophthora* and *P. cinnamomi* are prevalent in subtropical and temperate regions, while the various varieties and strains of *P. palmivora* are tropical in their habitat (Butler and Jones, 1949). Saksena (1981) reported that the genus *Phytophthora* has 21 species and varieties in India. According to Bilgrami *et al.* (1979), about eight species of *Phytophthora* are known to occur on plantation crops of Kerala.

Among the diseases of coconut, bud rot is the most common and perhaps the oldest. The earliest occurrence of bud rot was in Grand Cayman in 1834 (Tucker, 1926). Since then,

there had been several cases reported from various countries. *P. palmivora* (E.J. Butler) Butler was reported in Indonesia (Benett *et al.*, 1986), Philippines (Benigno, 1970), India (Lingaraj, 1969), Dominican Republic (Schieber, 1970) and Sarawak (Turner, 1964). *P. hevea* Thomps was reported in Ivory Coast (Quillec *et al.* 1984). *P. katsurae* Ko and Chang in Hawaii (Ooka and Uchida, 1984) and *P. parasitica* (Dastur) G.M. Waterh from Costa Rica (Rodriguez, 1982). Both *P. palmivora* and *P. katsurae* were reported on diseased coconut plants in Jamaica (Steer and Coates Beckford, 1990). *P. arecae* (L.C. Coleman) Pethybr. has also been reported causing epidemics of bud rot in India in the past (Mc Rae, 1924).

Butler in 1906 reported bud rot of coconut from India for the first time and the identity of the causal organism was accepted at International level in 1924 and in 1926 he proved that the pathogen involved in bud rot was *P. palmivora*. Radha and Joseph (1974) observed the disease to be generally sporadic in all the coconut growing states; however, exceptional cases where 30-40 affected palms in a single garden were also noticed. Recently bud rot was observed in a large proportion in many parts of Kozhikode district (Anonymous, 1992) and loss upto 90% have been recorded in certain plantations.

Phytophthora foot rot, or quick wilt, is by far the most important and devastating fungal disease on black pepper. (De Waard, 1980; Holliday, 1980 and Nambiar and Sarma, 1977).

Foot rot is the most destructive disease prevalent in all pepper growing tracts of India and takes a heavy toll of the crop (Sarma, *et al*. 1991).

The first authentic report of sudden collapse and death of the pepper vines came from Lampung (Indonesia) in 1885. Muller (1936) was the first to identify the causal agent of foot rot of pepper as *Phytophthora* sp. In India the disease was known as early as in 1902 when severe vine deaths were noticed in Wynad region of erstwhile Madras state (Menon, 1949). This was investigated by Barber (1902, 1903, 1905) and Butler (1906, 1918), but the investigations were inconclusive and etiology remained unresolved. Although isolation of *Phytophthora* was reported in Mysore area (Venkata Rao, 1929) it was Samraj and Jose (1966) who reported the '*Phytophthora* wilt' of black pepper in Kerala.

Controversy and confusion existed for decades regarding the identity and nomenclature of *Phytophthora* isolates pathogenic on black pepper. Prior to 1979, isolates from various parts of the world have been identified by different researchers as *P. capsici* in Indonesia, *P. colocasiae* in India and Malaysia, *Phytophthora* sp in Jamaica, *P. meadii* in Malaysia, *P. palmivora* in Brazil, India, Indonesia and Malaysia. *P. palmivora* var. *piperis* in India and Indonesia, *P. parasitica* in India or *P. parasitica* var. *piperina* in Bangladesh and India. By far, the

most frequently reported species from black pepper had been *P. palmivora*.

Since the 1960's the degree of confusion was somewhat lessened after the publications by Holliday and Mowat (1963), Turner (1969a) and Waterhouse (1974). Holliday and Mowat (1963), based on Waterhoues' identification at CMI, reported black pepper isolates in Sarawak, Malaysia, as atypical strain of *P. palmivora*. Waterhouse (1974) opined that the atypical strain differed considerably from the typical which is known to attack many tropical hosts. The 1976 Rothamsted Cocoa *Phytophthora* workshop (Brasier and Griffin, 1979; Griffin, 1977) contributed greatly to the clarification of the nomenclatural controvery in '*P. palmivora*' complex when the designation of '*P. palmivora*' MF_4 has been used by various workers on black pepper since 1979. However, some researchers (Kasim, 1978; Kunimoto *et al.*, 1976; Zentmyer *et al.* 1981) have used the nomenclature of *P. capsici* for isolates identifiable as '*P. palmivora* MF_4 because many morphological similarities exist between these two groups of *Phytophthora* isolates. Such a premature and casual nomenclatural change became problematical because of the existing species description of *P. capsici* (Leonian, 1922; Tucker, 1931; Waterhouse, 1963; Newhook *et al.*, 1978). Tsao and Alizadeh (1988) conducted detailed studies on large number of *Phytophthora* isolates from pepper collected from throughout the world and concluded that causal organism of foot rot of pepper is *P. capsici*.

The intensive work on etiology and symptomatology of bud rot disease of coconut in India was first carried out by Butler (1906). Subsequently, Butler (1910) Ashby (1927), Gadd (1927) Thomas *et al*. (1947), Menon and Pandalai (1958) and Radha and Joseph (1974) also studied various symptoms associated with the disease.

First visual symptom of bud rot disease is the paling and slight yellowing of the third or fourth leaf in coconut. Expression of similar symptoms of breakdown of the spindle is the next visible indication of the progress of the disease. The bud is rotten and the infection extends down to seventh or eighth leaf (Radha and Joseph, 1974). Lamberti *et al*. (1985) observed the symptoms of coconut in Philippines as premature senescence of the outer whorl of leaves, which hang down because the proximal portion of the petiole is broken, discolouration of the stem tissue and decay of the primary roots. Uchida and Aragaki (1992) concluded the symptoms as dark fruit rots associated with premature nut drop. Coconut trees with these symptoms gradually declined, initially by the death of the youngest leaf or spear leaf followed by other young leaves, and then finally the older leaves and eventually, only leafless trunks retained. Kurian (1992) also studied the symptomatology of bud rot of coconut in Karnataka and described as petiole rot, resulting in drooping of the leaves during summer months. Discoloured patches and water soaked lesions appeared on the petiole.

Three types of symptoms viz. leaf rot, collar rot and root rot are generally observed in a quick wilt infected plant. Muller (1936) observed greyish brown lesions upto 5 cm in diameter near the tip and margin of the lower leaves and noticed a few drops of yellowish fluid from the under side of the lesions. Samraj and Jose (1966) found small irregular black patches on the leaves, later enlarged in size and covered the bulk of the leaf area. Holliday and Mowat (1963) and Turner (1969a, 1969b) from Sarawak and Nambiar and Sarma (1976) from India observed fimbriate lesions during continuously humid conditions and concentrically zoned lesions under alternating wet and dry conditions.

A detailed symptomatological study of the collar rot type of infection was conducted by Muller (1936). He reported that diseased cortex rapidly turned from dark watery green to black. He also observed that the external symptoms were visible only after a complete decaying and disintegration of internal tissues. In his observations, the infection was usually noticed at a height of 30 cm from the base. Samraj and Jose (1966) observed that infection of vine was more common at a height of 25 cm above the ground level and it rarely occurred at any other region of the vine. The affected tissues became soft and decayed. The diseased leaves turned pale, flaccid and fell off.

In the case of root rot, the degree of damage depends upon the number of roots infected and the extent of rotting.

Holliday and Mowat (1957, 1963) reported that the infection started from fine roots of the plant. Once the cortex got infected, the disease spread to the main roots and then to the underground stem. When the stem became infected, visible symptoms appeared on the above ground parts of the plant, i.e., a halt in the growth of terminal shoots, wilting, rapid yellowing and shedding of the leaves and small shoots. Detailed symptomatology was also studied by Manomohan Das and Cheeran (1982); Anandaraj *et al*. (1991) and Sarma *et al*. (1991).

Identification of some *Phytophthora* species can be difficult due to lack of distinct morphological characters (Leonian, 1934; Brasier, 1971). In addition morphological traits may overlap between species and such characters can be highly variable and dependent on growing conditions. Problems may also arise because key morphological structures are difficult to produce with specific isolates. Among the most frequently used criteria . . . in the study of variability of different *Phytophthora* isolates are sporangial characters as size, shape, L/B ratio, diameter of exitpore, apical thickening, pedicel length etc. According to Al-Hedaithy and Tsao (1977), length of sporangium pedicel, or stalk appears to be fixed for a species under normal conditions, and is of high diagnostic value in identification of *Phytophthora* isolates.

Santhakumari (1987) isolated *Phytophthora* from bud rot affected coconut plants, the sporangia were spherical to

elongated, ellipsoid, papillate and caducous with a size of 30.66 um. Uchida and Aragaki (1992) observed that all isolates recovered from diseased coconut produced nondeciduous, papillate, ovoid sporangia in 49.0 um and with an average of 40+4 u, breadth ranged from 22.8 - 41.8 um. Blaha *et al*. (1994) working with *Phytophthora* isolates from coconut plantations in Indonesia and Ivory Coast observed loose sympodia of papillate, spherical, ovoid or obturbinate sporangia of coconut isolates with less than 10% deciduous sporangia. L/B ratio of the isolates ranged from 1.2:1 - 1.5:1. Mchau and Coffey (1994) in their study using 93 isolates of *P. palmivora* and 6 isolates of *P. arecae* revealed that all isolates of *P. palmivora* and 6 isolates of *P. arecae* revealed that all isolates in their study produced papillate, caducous sporangia borne either singly on simple sympodia or on irregular sporangiophores. There was a wide variability in sporangial shape and size within and between isolates. In general, sporangial morphology was predominantly ellipsoid or ovoid with a rounded base on occluded, short pedicel. The mean length of sporangia ranged from 31.0 - 56.4 um and mean breadth from 20.7 - 37.6 um. L/B ratios of sporangia ranged from 1.2 : 1 to 1.8:1. Pedicel lengths were less than 5.0 um and they were 1.8 - 2.7 um wide.

Since the first description of the disease and its causal fungus by Muller (1936) till 1970's, more than 10 species and variety names of *Phytophthora* have been reported in literature as responsible for causing diseases on pepper species.

Detailed morphological description of *Phytophthora* affecting pepper was made by Tsao and Tummakate (1977). According to them the sporangia of *Phytophthora* causing foot rot in Thailand were having long pedicellate sporangia often exceeding 100 um in length. Sporangial ontogeny was umbellate or fan shaped, this results in a cluster of long pedicelled, sporangia seemingly arising from a common point in a fan shaped or candelabrum. The isolates were usually narrowly ellipsoidal, obovoid, pyriform or even fusiform, there were occasional shorter, ovoid or broadly ellipsoid sporangia with a distinctly rounded base. The sporangia were of 48 x 22 um in size with a L/B ratio of 2.2.

Tsao *et al*. (1985) reported that the sporangium shape of Indonesian pepper isolates ranged from ovoid to obovoid and ellipsoid in most iolates. In addition, fusiform and pyriform sporangia with a characteristic tapered base were seen in some isolates, Of these isolates sporangia were papillate, caducous, producing detachable sporangia each with a long pedicel which ranged from 36 to 113 um. Sporangium arrangement was generally irregular but the umbellate or fan shaped arrangement was observed in some cultures. Santhakumari (1987) isolated *Phytophthora* isolates from foot rot affected black pepper, and found that the sporangia were ellipsoid to spherical, papillate, caducous with a size of 27-54 um.

Mammootty *et al*. (1991) conducted detailed studies on the morphological characters of six black pepper *Phytophthora* isolates collected from Kerala. The breadth of the hyphae ranged from 4-6 um while there was a wide variability in length of sporangiophore. Mean length of sporangiophore ranged from 108 um - 131.15 um with a range of 25 - 252.5 um. The mean pedicel length of these six isolates did not show much difference (3.5 - 3.8 um). According to Sarma *et al*. (1991), umbelloid ontogeny was noticed in all the isolates collected from Kerala and Karnataka. The sporangial shapes were ovoid to obovoid, pyriform with a tapering base or fusiform and were highly variable. The average L/B ratio of sporangia ranged from 1.7:1 - 2.8:1 and the average pedicel length ranged from 6.7 - 125 um.

Sastry and Hegde (1991) studied the morphological characters of pepper isolates from Karnataka and found that sporangia were elongated, caducous, with long pedicels (20-240 um) and L/B ratio of 1.7:1 - 3.0:1. Manohara and Sato (1992) investigated the morphology and physiology of 43 *Phytophthora* isolates from black pepper in Indonesia. Of the isolates, 42 had ellipsoid sporangia which were markedly papillate, tapered at the base and caducous with a long pedicel.

Mchau and Coffey (1995) conducted detailed morphological study of 113 isolates of *P. capsici* from different parts of the world and noted that the shapes of sporangia varied greatly between isolates and even within a single isolate.

Although, CAP2 and CAP3 isolates (identified by Oudeman and Coffey, 1991) produced a high percentage of sporangia which were ellipsoid, lanceolate and obovoid with umbellate arrangement. These characters were not restricted to these groups. The mean length and width of sporangia in CAPI ranged from 29.1 - 60.1 um and 21.5 - 38.9 um respectively, while, in CAP2 they ranged from 32.8 - 65.8 and 13.3 - 31.9 um. CAP3 sporangia had mean length of 32.9 - 48.4 and mean breadth of 17.4 - 29.0 um. The L/B ratio of sporangia ranged from 1.3:1 - 2.0:1, 1.4:1 - 2.1:1, and 1.4:1 - 2.0: 1 respectively among CAP1, CAP2 and CAP3 isolates. Mean pedicel length was another highly vairable character ranged from 34.7 - 123 um for CAP1, 59.1 - 130 um for CAP2 and 40.0 - 92.0 um for CAP3 isolates.

The morphology and ultrastructure of zoospores of *Phytophthora* had been studied by Bimpong and Hicknan (1975), Nogueira *et al*. (1977) and Hemmes (1983). Zoospores are basically ovoid in shape and posess a groove that runs longitudinally along the zoospores from the bluntly pointed anterior end to the rounded posterior end of the cell. The groove is shalow at each end and deepest in the centre where the two flagella, an anterior tinsel flagellum and posterior whiplash flagellum, are inserted at a common point. Zoospores swim, in water for variable periods of time, usually several hours at 25°C before encysting spontaneously.

Rosenbaum (1917) used six main criteria for the identification and seperation of fungal species of which, the presence or absence and the size of chlamydospores were the two important characters used. Later, Waterhouse (1963) also used this property as a criterion in the identification of the species. Chlamydospores of *Phytophthora* develop by spherical expansion of hyphal tips or by localized swelling of hyphal tubes to produce terminal or intercalary spores ranging from 8 - 15 um in diameter (Hemmes, 1983 and Hemmes and Lerma, 1986). According to Waterhouse (1983), the significance of Chlamydospore for classification in the genus as a whole is restricted since in some species chlamydospores are formed only by certain isolates. However, for separating some individual species such as *P. palmivora* MF_1 from *P. megakarya* or *P. meadii*, their presence or absence respectively is an useful indcator. Tsao *et al.* (1985) in their study with *P. capsici* from Indonesia failed to notice chlamydospores formation.

Uchida and Aragaki (1985) studied the importance of chlamydospore production in the identification of *P. capsici* and *P. palmivora*. They used 29 *P. capsici* isolates and five *P. palmovra* isolates from different crop plants. Twenty of 29 *P. capsici* isolates tested produced chlamydospores. Except for 3 isolates from egg plants, all the isolates producing chlamydospores were isolated from non solanaceous crops. All the isolates of *P. palmivora* produced chlamydospores. Alizadeh and

Tsao (1985) observed chlamydospore formation in *P. palmivora* MF_4 with a mean diameter of 27-29 um. Uchida and Aragaki (1985) and Sarma *et al*. (1991) also recorded production of chlamydospores by *P. capsici*.

Mchau and Coffey (1994) observed chlamydospore production by the majority of isolates of *P. palmivora* on V.8 juice agar with mean diameter of 30.2 ± 9.6 um. Blaha *et al*. (1994) also noted chlamydospore production by 32 out of 53 isolates of *P. palmivora*. Ristaino (1990) studied the variation among the isolates tested in his study produced chlamydospores in culture. However, some isolates of *P. capsici* from macadamia produced chlamydospores but they failed to induce infection on pepper. According to him, this character could be used to separate macadamia isolates of *P. capsici* from pepper and cucurbit isolates.

Mchau and Coffey (1995) studied the existence of CAP1, CAP2, CAP3 sub populations in *P. capsici* and they observed chlamydospores only in CAP2 and CAP3.

Sexuality is one of the complex area of *Phytophthora* biology. Both the terminology and concepts associated with the reproductive processes are ill defined. Heterothallism in *Phytophthora* was first demonstrated by Ashby (1922). Brasier (1983) gave the definition of sexuality to solve some of the confusion.

Homothallic species - Species that produce oospore promptly and abundantly in normal single isolate culture without special external stimuli.

Heterothallic species - Species in which single isolates exist either as an A1 or A2 types in culture results in prompt induction of oospore formation. Single isolates of either compatibility type may be included to self in response to certain external stimuli.

Secondarily homothallic isolates - Single isolates of heterothallic speices that are self compatible without external stimuli as a result of genetic rearrangement of the incompatibility system and that characteristically exhibit break down or regression to the A1 or A2 compatibility type.

Secondary homothallic species (Yet to be confirmed in *Phytophthora*) - Species exhibiting true homothallism but derived from a permanent rearrangement of the heterothallic incompatibility system.

The heterothallic behaviour of different *Phytophthora* species was the topic of the study by several workers. Smoot *et al*. (1958) and Gallindo and Gallegly (1960) observed the existence of two mating types in *P. infestans*. Gallindo and Gallegly (1960) showed that hybridization occurred between strains of opposite mating types, one strain producing antheridium and the other strain producing oogonium. They showed

that the mated strains were bisexual. In a particular mating, the A1 strain sometimes produced antheridium, and A2, the oogonium and sometimes the reverse. Relative sexuality was also demonstrated in *P. palmivora* by Hugenin (1973). Sansome *et al.* (1979) also demonstrated both crossing and selfing in pairs of *P. palmivora*. Ko (1978) observed that a strain of one mating type can induce one of the opposite mating type to produce oospores by placing the two different strains on opposite side of a polycarbonate membrane. He postulated that a hormone x 1 produced by strains of A1 induces reproduction in A2 strains and a hormone x 2 produced by A2 strain induce sexual reproduction in A1 strain. Ko (1980) further postulated 16 types of chemical regulation of sex among *Phytophthora* species based on the various possible combinations of production and non production, and response to and non response to, the x 1 and x 2 sex hormones. There are many reports of oospore formation in single cultures of species generally considered heterothallic (Brasier, 1972; Shephered, 1978 and Tsao *et al.* 1980).

Brasier (1972) paired A1 and A2 isolates of *P. palmivora* on opposite sides of cellulose acetate membrane. Numerous oospores were produced on other side of membrane. Although, such oospore production is generally less regular and less abundant than when the same strain is paired with one of the opposite mating types. Tsao *et al.* (1986) reported formation of oospores in single culture of certain strains of both mating

types in *P. palmivora* MF_4. Oospores were produced in aged cultures of six month old or older subcultures taken from part of the culture containing oospores themselves produced oospores. However, subcultures from the margin of the colony did not produce oospores. This according to them may be due to the fact that substances required for oospore production was produced only slowly but can accumulate to a concentration sufficient to initiate rapid oospore production in subcultures.

Sastry and Hegde (1987) reported that isolates of *P. palmivora* from *Piper nigrum* in Karnataka comprised both A^1 and A^2 mating types in the ratio of 6:4. According to Blaha *et al.* (1994), *Phytophthora* isolates from coconut were heterothallic and produced oogonia with amphigynous antheridia, when mated with an opposite mating type. Manohara and Sato (1992) studied physiology of 43 isolates from black pepper in Indonesia and found that all isolates were heterothallic and sexual reproduction occurred when paired with the mating isolate. They observed 2 mating type viz. A_1 and A_2 from the 43 isolates.

Klisiewicz (1970) reported the best development of oospores in *Phytophthora sp* was between 15-24°C. While, Chung and Kang (1990) found the optimum temperature range for oospore formation in paired cultures of A_1 and A_2 of *P. capsici* to be 20-24°C.

The work of Tsao *et al.* (1985) with isolates collected from black pepper in Indonesia showed a variation in length of

antheridia from 10-14 u, with 13-14 u and that of oogonial diameter 28-31 u, width 13-14 u and oospore diameter 23-27 um. The isolates of *Phytophthora* (Suspected to be *P. katsurae*) isolated by Uchida and Aragaki (1992) from coconut from Hawaii had a oogonia of diameter of 22.1 - 30.5 um. The oospores measured 20.5 + 1.5 um. Santhakumari (1987) noted that the oogonia of *P. capsici* were amphigynous with a size of 28-52.5um. According to Mchau and Coffey (1995), mean oogonial and oospore diameter of *P. capsici* varied greatly among isolates within subgroups as well as from a single isolate. The average diameter of the oospores ranged from 22-36.6 um. Certain compatible pairings produced abundant oospores, while others produced only very sparse.

Phytophthora encompasses species with a wide variety of nutrition demands, ranging from the very simple to the highly complex (Hohl, 1975). It represents an intermediate form between the preferentially saporphytic genera and the obligate parasites of perenosporales. The first attempt to grow *Phytophthora* in artificial media may be credited to de Bary who cultivated and observed *P. infestans* (de Bary, 1876) and *P. palmivora* (de Bary, 1881) on various plant fragments. Brefeld (1881) was probably the first one to grow *P. infestans* under strictly saprophytic condition (Brefeld, 1883).

Effect of different types of media on the growth characters of *Phytophthora sp* were studied by various workers

(Fries, 1938; Robbins, 1938). Manomohandas (1982) carried out a study to find out the effect of media on growth of *P. palmivora* and concluded that oat meal agar, potato dextrose agar and carrot agar permitted a luxurious growth of the fungus. Santhakumari (1987) observed max growth of *Phytophthora* on soy bean meal agar, sugarcane juice agar, wheat meal agar and carrot agar. According to Agarwal *et al.* (1990) best media for growth of *Phytophthora* were oat meal agar and chick pea agar.

Light enhances (Brasier, 1969; Rocha and Machadu, 1973), inhibits (Cohen *et al.* 1975), or has no influence on (Aragaki and Hine, 1963; Harnish, 1965) or growth of *Phytophthora* sp. Some species sporulates better in the dark. Investigations using monochromatic radiation have indicated that asexual sporulation is significantly enhanced by wave length in the near UV and low region of the spectrum (Ribeiro *et al.*, 1976; Englander and Roth, 1980). Light also has a striking effect on the morphology of sporangia. Hendrix (1967) reported that sporangia of *P. palmivora* and *P. capsici* formed in the light differed considerably in size and shape from those produced in the dark. The L/B ratio of sporangia from cultures grown in dark were found to be significantly lower than those reported from those species in taxonomic keys. Also, the shape of sporangia in dark grown cultures varied to the point that, *P. palmivora* could be confused with other closely related species (Hendrix, 1967). According to Hyun *et al.* (1981), *P. capsici* failed to produce

sporangia in the dark, while under continuous cool white fluorescent light, abundant sporangia were produced. Similar results were reported by Sagir *et al*. (1982) and Alizadeh and Tsao (1985). Studies of Narula and Mehrotra (1984) on *P. colocasiae* have shown that darkness favoured vegetative growth of the fungus, while for sporangial production, continuous light was necessary.

Although the optimal pH for sporangium formation varies with the species and among isolates of the same species, sporangial production in *Phytophthora* was noticed within a range of 4 to 9 (Divinagracia, 1969; Zentmyer and Marshall, 1959). Narula and Mehrotra (1984) observed that *P. colocasiae* grew between pH 5.0 and 9.0 with maximum growth and sporangial production at pH 7.0 and 8.0 respectively.

Radha and Joseph (1974) conducted the artificial inoculation study using one to two year old seedlings of coconut, cut bits of coconut petioles and tender leaves. In their study, the seedlings failed to produce the symptoms under natural condition, and they concluded that during post inoculation period, relative humidity was not sufficient for the infection. However, they got successful infection with controlled condition.

Uchida and Aragaki (1992) studied *Phytophthora* fruit and heart rots of coconut in Hawaii. In their study coconut fruits were inoculated by spraying entire detached clusters of

large green or small immature fruits with zoospore suspension or by placing discs covered with mycelium from a 3 day old colony grown on 2%. VJA close to the stem end of detached fruits and fruit rot symptoms on large green coconuts appeared 3.5 days following artificial inoculation. Apart from fruits, young coconut seedlings approximately 1.5 m tall were also used for stem and foliar inoculation. They recorded heart rot symptoms on stem inoculated plants. While successful infection on mature leaves, petioles and exposed shoots were not common.

Turner (1971) reported that *P. palmivora* from pepper was very specific in its host range. He further showed that *Piper* sp tested from S.E. Asia were highly susceptible, while those from American Tropics were resistant or slightly to moderately susceptible. Manomohan Das (1982) conducted cross inoculation study with one year old pepper cuttings and tender leaves of coconut. He found that pepper plants produced water soaked lesions within 2 days of inoculation and water soaked lesions appeared on the coconut leaves, and these lesions turned dark brown and started spreading to adjacent tissues within 2 days. Sarma *et al*. (1991) practiced root and stem inoculation on pepper plants using 3 month old rooted cuttings. Mammooty *et al*. (1991) conducted inoculation studies on leaves, stems and roots of pepper using zoospore suspension prepared from one week old cultures of the fungus. On leaves, symptoms appeared within 24 hrs after inoculation. Lesions coalesced, and covered large area

of the lamina. Immature leaves took infection more easily than the matured ones. Infection on stem and branches was noticed within 3-5 days after completely wihin 14-20 days. Root succumbed to infection within 24-48 hrs after inoculation and got killed within 15-20 days.

P. palmivora has been recorded to infect 138 species belonging to different families of angiosperms (Chee, 1969). Radha and Joseph (1974) reported that *Phytophthora* isolates from coconut could infect one year old arecanut, mango, *Atrocarpus hirsuta*, *Atrocarpus integrifolia*, *Hibiscus*, *Bougainvillea* sp and rubber and infection was established on all hosts other than *Hibiscus* within 48 hrs after inoculation and the leaves withered and dropped within a week time. Campelo and Luz (1981) identified *P. palmivora* from 3% of cocoa plants showing brown rot. The same organism was isolated from blighted seedlings of castor bean (Uchida and Aragaki, 1988) and fruit rot disease of pomegranate (Mero and Bangar, 1989). Manomohan Das and Cheeran (1986) noted that *Phytophthora* isolates from pepper, arecanut, rubber, cocoa, coconut and cardamom could cross inoculate each other.

Sarma and Nambiar (1982) found that *P. palmivora* isolates from pepper in Kerala infected roots of *Piper beetle*, *Piper longum*, *P. attenuatum*, cocoa pods, tender leaves of rubber, castor and caused mild rotting of capsules of cardamom

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Phytophthora isolates from cocoa, cardamom, betel vine, palmyrah, oil palm, arecanut and ficus showed differential reactions on leaves and root system of black pepper. Bandara *et al.* (1985) revealed that isolates from pepper were host specific whereas the cocoa isolates were pathogenic on pepper, papaw and cardamom. Sastry and Hegde (1987) found *Phytophthora* isolates from arecanut, black pepper, cocoa and cardamom were cross inoculable.

According to Ristaino (1990) *P. capsici* apart from black pepper also infect tomato, egg plant, cucumber, water melon, pumpkin, squash, cocoa and macadamia and isolates of *P. capsici* from cucurbits are pathogenic on both pepper and cucurbits. Mammootty *et al.* (1991) working with six isolates of Phytophthora from six different hosts ie. pepper, arecanut, coconut, rubber, cocoa and cardamom observed that these isolates are cross inoculable and the symptoms they produced on the plants were identical. Cross inoculable nature of *P. capsici* was also reported by Hartman (1993). He found that *P. capsici* could infect cabbage, cucumber, pepper potato, tobacco and tomato plants.

The effect of plant extracts on *Phytophthora* was studied by few workers. Whitefield *et al.* (1982) found that the steam-volatile extract of *Acasia pulchella* restricted mycelial growth, suppressed sporangial production and germination of *P. cinnamomi* in culture. Deacon and Mitchell (1985), in their

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lab experiments using roots of oats and the grass, *Arrhenatherum elatius* caused attraction and subsequent lysis of zoospores of *P. cinnamomi*. Awuah (1994) reported that the crude steam distillate from *Ocimum gratissimum* sprayed on to infection courts on detached cocoa pods moments after inoculation with *P. palmivora*, completely inhibited the pathogen and black pod lesion development on 75% of the infection courts. However, fungitoxicity of the extract on pods was lost within 3 hours of application.

Although, most fungi are insensitive to bacterial antibiotics, members of perenosporales (including Pythiacae) behave like prokaryotic organisms in that they are sensitive to antibacterial antibiotics (Tsao, 1983), especially to those with anti gram -ve or broad spectra. The antibiotics especially at high concentrations, inhibit the growth and or spore germination of many, though, not at all, *Phytophthora* sp (Tsao, 1983). Some notable examples of antibacterial antibiotics known to inhibit Pythaceous fungi are Chloramphenicol (Carnes, 1976), Chlorotetracycline (Eckert and Tsao, 1962), Kanamycin (El-Goorani and Tarabeith, 1973), Neomycin (Ersek, 1975), Novobiocin (Butler and Hine, 1958), Oxytetracycline (Eckert and Tsao, 1962), Polymixin (Banihashemi and Mitchell, 1976), Streptomycin (Eckert and Tsao, 1962) and tetracycline (Eckert and Tsao, 1962). Streptomycin is extremely inhibitory to mycelial growth of *P. citrophthora* and *P. heveae*, but only partially inhibitory to

that of *P. capsici*, *P. cinnamomi* and *P. parasitica* (Eckert and Tsao, 1962; El-Goorani and Tarabeith, 1973). Chloramphenicol inhibited zoospore germination (Carnes, 1976). Leary *et al.* (1981) studied variability in growth of 35 isolates of *P. cinnamomi* from eight countries and 18 different hosts on several antibiotics. They observed that the A1 isolates were significantly more resistant to Cyclaheximide than A_2 isolates and that the effect of streptomycin was influenced by the nutrient status of the medium used.

Several new chemicals with novel features for the control of diseases caused by *Phytophthora* have been launched into the market during the recent years. Among the systemic fungicides used for the control of *Phytophthora*, the most widely used is acylalanine. (Benson, 1979; Englander *et al.* 1980 and Ramachandran *et al.* 1982). Under *in vitro* conditions metalaxyl inhibited mycelial growth, chlamydospore and sporangium formation of *P. cinnamomi* and *P. nicotianae* (Margot, 1982). The concentration required for inhibition of sporangium formation was 25 times lower, and those for inhibition of chlamydospore formation was 100 times lower than those required for inhibition of mycelial growth.

Ramachandran *et al.* (1982) tested sensitivity of two systemic fungicides, Ridomil (metalaxyl) and Aliette (Aluminium tris phosphonate) to *P. palmivora* by poison food technique.

Ridomil at 100 and 200 ppm caused 80% and 86% inhibition respectively, in the radial growth of the fungus and complete inhibition was noticed at 300 ppm. Aliette caused 22% and 50% inhibition of the fungus at 500 and 1000 ppm respectively and there was no growth at 1500 ppm. Tey and Wood (1983) tested eight fungicides *in vitro* against *P. palmivora* pathogenic to cocoa, and found metalaxyl greatly decreased mycelial growth at < 1 ppm. According to Ramachandran (1990) besides soil application, granular formulations of metalaxyl was also equally effective.

Various species of *Phytophthora* differ in their response to metalaxyl (Fuller and Gisi, 1985; Farih *et al.*, 1981 and Karkenaar and Sijpestejn, 1981). Wide variability is reported even among the isolates (Hunger *et al.*, 1982: and Shew, 1984) and races (Bruck *et al.* 1980) within a single species. Ramachandran *et al.* (1988) found *Phytophthora* isolates from black pepper, arecanut, cardamom and cocoa showed wide variabilities in their sensitivity to metalaxyl compared to those of rubber and nutmeg. Black pepper and cocoa isolates were comparatively less sensitive to metalxyl.

Mchau and Coffey (1995) studied resposne of 113 isolates of *P. capsici* to metalaxyl and observed considerable variation in growth response. Some isolates were completely inhibited at 0.5 um/ml. The CAPI sub group contained more

isolates which showed greater insensitivity to metalaxyl than CAP2 and CAP3.

Controlling any soil borne plant pathogen in a crop is difficult and *Phytophthora* diseases are no exception. Situation to exist in nature when susceptible vegetation in the presence of the pathogen is not diseased under climatic conditions that are normally suitable for disease development. These situations provide working models of successful biological and ecological control (Shea and Patricia, 1983). Although numerous studies have reported antagonism among a great diversity of fungi the extent of these interactions between *Phytophthora* and other groups under *in vitro* and in vivo conditions and their possible importance in the mechanism of fungal propagule reduction remain largely unexplored (Malajaźuk 1983). Novel approaches using biological agents currently known to have efficacy against *Phytophthora* may offer better possibilities for long term control than using only chemicals (Ribeiro and Linderman, 1991). Despite the various obstacles some progress is being made towards identifying potentially active anti *Phytophthora* microbial agents.

Trichoderma spp are becoming well known for their capacities to control soil borne pathogens, including *Phytophthora*. *Trichoderma* have attracted special interest because of their ability to induce development of sex organs in

some normally sterile isolates of *Phytophthora* spp (Brasier, 1971, Pratt *et al*., 1972; Reeves and Jackson 1972). Malajcuk (1983) grew *Phytophthora* and *Trichoderma* on agar and *Trichoderma* eventually overgrew the *Phytophthora* culture and parasitized the hyphae. The first successful application of *Trichoderma* to control *P. cactoorum* on apple was by Valdebenito Sanhueza (1987) *Trichoderma* reduced mortality from natural or artificial inoculation to zero and no infection have occurred. Al-Heeti (1989) supported the antagonistic property of *T. harzianum* against *P. cryptogen* and *P. megasperma* F. sp *glycinea*. Finlay and Mc Cracken (1991) showed antagonistic property of *Trichoderma* spp towards *P. cinnamomi*. Phukan and Baruah (1991) tested *Trichoderma* spp against *P. infestans* and came out with successful result. Similar results were also obtained by Lederer *et al*. (1992) in *P. cactorum* and *T. viride* against *P. infestans* (Arrora 1993).

Cristinzio (1987) found antagonistic property of *Trichoderma* spp against *P. capsici*. Working with foot rot disease of pepper, Anandaraj and Sarma (1991) observed that among several isolates of antagonistic fungi tried against *P. capsici*, *Trichoderma* spp and *Gliocladium virens* were the best under *in vitro* and in field conditon.

Dutta (1984) recorded *Penicillium* spp were most frequently isolated from healthy rather than from *P. cinnamomi* infected soil (Maas and Kotze, 1989). *Aspergillus fumigatus*,

A. niger and *Penicillium islandicum* could inhibit the growth of *Phytophthora arecae* (Bopaih *et al.*, 1991). Phukan and Baruah (1991) also reported the antagonistic property of *Penicillium* spp against *P. infestans*. Arora (1993) identified *Penicillium viridicatum* and *Penicillium aurantiogriseum* as an antagonist of *P. infestans*. Fang and Tsao (1995) evaluated *Penicillium fumiculosum* in the green house for its ability to suppress *Phytophthora* root rots of rhododendron and sweet orange and they could obtain successful result. Inhibitory effect of *Aspergillus candidus* against *P. cinnamomi* was reported by Duvenhage and Katze (1993).

In vitro, the filtrate of *Cladobotryum amazonense* inhibited mycelial growth of *P. capsici* and *P. citrophthora* (Bastos and Figueiredo, 1982).

Cristinzio (1987) noted the antagonistic property of *Chaetomium* spp against *P. capsici* while Arora (1993) found that culture preparation of *Chaetomium brasiliense*, *Myrothecium varucaria* have antagonistic properties against *P. infestans*.

Pythium nunn is a mycoparasite; first found in soils of Colorado (Lifshitz, 1984b). This fungus is antagonistic to several root disease fungi including two *Phytophthora* spp. It's antagonistic activity against host fungi included coiling around, penetration and lysis of host hyphae or reproductive structures (Lifshitz, 1984a). Fang and Tsao (1995) studied the antagonistic

property of *Pythium nunn* against *Phytophthora cinnamomi*, *P. citraphthora* and *P. parasitica* and they observed pronounced mycoparasitism by *P. nunn* on hyphae, sporangia, chlamyolospores and sexual organs of these three *Phytophthora* spp. Eventhough Lifschitz *et al*. (1984) described a slow reaction between *Pythium nunn* and *Phytophthora* sp, Fang and Tsao (1995) observed the reaction between these two fungi as quick and observed root rot suppression of azalea and sweet orange at a concentration of 1000 propagules/gram.

Epicoccum purpurascens has been reported to be a marked inhibitor of *P. cactorum in vitro* (Gilmore 1986) and *P. cinnamomi* (Finlay and Ward, 1987). The fungal interaction between *P. cinnamomi* and *E. purpurascens* in culture resulted in extreme antagonism through antibiosis, parasitism involving destruction of *Phytophthora* hyphae after contact, and the production of a wide range of antifungal compounds.

Gees and Coffey (1989) identified a strain of *Myrothecium roridum*, that was active in suppressing *P. cinnamomi* on highly susceptible seedling, of *Persea indica* in green house trials using natural or artificial inoculum. This fungal biocontrol agent exhibited good rhizosphere competence on *Persea indica*. According to Finlay and Mc Cracken (1991), *Myrothecium verucaria* is capable of antagonising *P. cinnamomi* in opposition culture by antibiosis and mycoparasitism.

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Mycorrhizal fungi have been studied for the potential to reduce root infection caused by *Phytophthora* spp. But, confusion and disagreement exist on whether or not mycorrhizae reduce *Phytophthora* sp. Several studies (Dehene, 1982 and Sehenck, 1989) suggest that mycorrizhal fungi do not reduce infections by *Phytophthora* sp. But other studies on different hosts suggest the contrary (Bartschi, 1981).

Many soil bacteria have been found to exert inhibitory effects on species of *Phytophthora* but few reports exist on specific bacteria that inhibit the pathogens in woody plant species. Utkhede (1987) reported good control of *P. cactorum* infections on apple tree by application of bacterial antagonist *Enterobacter aerogenes* strain B88. The mode of action of these antagonists was suggested to be production of antibiotics. When seedlings of *Capsicum annum* were raised in pots containing seed bed soil formulated with *Pseudomonas ceparia* and transplanted into field with the pot soil, initial incidence of *P. capsici* was delayed for 30-79 days (Lee *et al.*, 1990). Stirling and Hayward (1992) isolated 164 bacteria from the rhizosphere of avocado, out of these, three fluorescent pseudomonads and *Serratia* showed antagonistic activity against *P. cinnamomi*. Sarathchandra *et al.* (1993) reported the efficiency of *Pseudomonas* to inhibit the growth of *P. nicotianae* var *nicotianae*.

Treatment with *Bacillus* Str-AC-1 was highly effective against *P. capsici* on *Capsicum*, both under controlled growing

condition and in the field (Kim *et al.*, 1989). Pen and Raymundo (1994) identified the antagonistic property of *Bacillus subtilis* against *P. nicotianae* var. *parasitica* when used as seed treatments.

Of 4087 actinomycetes isolated from soil by Lee *et al.* (1990), *Streptomyces purvullus* showed activity against *P. capsici* and *P. parasitica*. Finlay and Mc Cracken (1991) isolated three *Streptomyces* spp which were inhibitory to all of the *P. cinnamomi* isolates tested. Chung and Ser (1992) identified antagonistic property of 2 isolates of *Streptomyces* viz. *S. bikinensis* and *S. echinoruber* against *P. nicotianae*. Pena and Raymundo (1994) applied *Streptomyces* sp as seed treatments and inhibited growth of the *Casuarina* damping off fungus *P. nicotianae* var. *parasitica* in the lab.

The variability is a common phenomenon within and among species of *Phytophthora*. In order to say a particular isolate varies, the fungus in question has to be compared with a typical fungus or a 'type species'. Leonian (1934) questioned the wisdom of this restriction. 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". According to

Hansen (1991) variation is the inevitable product of our observational and experimental techniques and the consequence of imperfect taxonomy. Variation is also the real and natural product of growth and reproduction in all organisms.

The variability is expressed in different ways. Erwin (1983) reviewed variability within and among species of *Phytophthora*. He studied the variability under four category.

Morphological variability
Physiological variability
Pathogenic variability
Resistance to fungicides

Drechsler (1931) recorded variation in *P. megasperma* and noted the variation in oogonia and oospores and commented that sporangia are too variable for profitable to statistical metric treatment.

Leonian (1934) opined that a given species of *Phytophthora* is not an absolute entity, but a fluctuating group of organisms more or less loosely bound in a flexible orbit and hence it is unwise to regard any species as firmly fixed. He studied morphological variability in *P. parasitica* var. *rhei* and found high degree of variability in the sporangial size of 3 variants. Brasier and Griffin (1979) conducted a study to find out the morphological difference seen among the various isolates

of *P. megakarya* and observed big degree of variability in shapes of sporangia, chlamydospore and oogonia. Hamm and Hansens (1982) reported that isolates of *P. megasperma* varied in morphological properties, even among the zoospore progeny of a single parent.

Alizadeh and Tsao (1985) noted much variation in chlamydospore production among the 25 isolates of *P. capsici*. Sarma and Tsao (1988) observed a wide range of variation in morphological characters of *P. capsici*. Their results clearly indicated that even among one species, great variations existed depending upon the climate and other conditions. Uchida and Aragaki (1992) also found lot of variability among chlamydospore production, pedicel length, L/B ratio and sporangial ontogeny in *P. palmivori* isolates from Hawaii.

Temperature and nutrition are two important factors that affect size of various structures and mycelial growth. Ribeiro *et al*. (1975) working with *P. cinnamomi* observed that the oospores from root extract were larger than those from the carrot agar, corn meal agar and V-8 juice agar. Sarma and Tsao (1988) studied the growth characters of ten black pepper isolates of *P. capsici* on carrot agar and V-8 juice agar and found that the isolates grew well in carrot agar, and the same isolate exhibited difference in the growth characters under different nutritional conditions.

Variation in the production of melanin pigment among several single oospore cultures of *P. capsici* was recorded by Timmer *et al.* (1970) in a synthetic medium containing tyrosine. Pigment was produced only by some species. Shepherd (1976) also found that *P. cinnamomi* produced little or no pigment on a medium containing tyrosine, but that pigment production of A1 compatibility types of *P. parasitica* was double that of the A2 isolates.

Shephered and Pratt (1973) reported that an ecotype of *P. drechsleri* from northern Australia had an upper temperature limit of 36-37.5°C, whereas for the southern ecotype, it was 33.36°C. Sarma and Tsao (1991) conducted an experiment to study the effect of temperature on different isolates of *P. capsici* and found that the isolates grew better at 28°C, the isolates had a linear growth of 8.9 - 14.0 mm/day compared to 0.7 - 5.5 mm/day at 35°C.

Variation in pathogenicity among isolates within a species has long been recognized. Recognition of this variation is of paramount importance to plant pathologists and to plant breeders. Loss of virulence with continued culturing is not an uncommon phenomenon (Caten, 1971. Erwin, 1966 and Jeffrey *et al.*, 1962).

Giddings and Berg (1919) and Berg (1926) showed that isolates of *P. infestans* from the potato produced only small

necrotic spots on tomato leaves compared to the blighting of the entire tomato leaf by the tomato isolates. Races of *P. infestans* were first noted by Schick (1932) in some seven year plants after the introduction of resistant hybrid potato cultivars. Physiological races are common in *P. megasperma*. Hildebrand (1959) noted different levels of virulence among the isolates. Polach and Webster (1972) showed variation in virulence of *P. capsici* strains. Faris (1986) tested virulence in six isolates of *P. megasperma* f sp. *medicaginis* and found that there were 2 levels of virulence among the isolates. Dodan and Shyam (1995) used 26 isolates of buck eye rot pathogen collected from different tomato growing areas of Himachal Pradesh (*P. nicotianae* var. *parasitica*) to study variability and found that there was significant difference in linear growth and aggressiveness of various isolates of the fungus.

Metalaxyl was the first of several highly selective and effective fungicides toxic to *Phytophthora* spp to be introduced (Urech *et al*. 1977). Although these fungicides have been highly successful for control of foliar pathogen within the oomycetes, resistance to acylalanine fungicides within the population of *P. infestans* has been found in potato fields in Netherlands (Davidse *et al*., 1981). Bower and Coffey (1985) recovered phosphorus acid and tolerant isolate of *P. capsici* which could withstand a concentration of 900 ppm while sensitive isolates failed to grow even at 150 ppm. Mchau and Coffey (1995) also

found considerable variation in growth response to metalaxyl by different isolates of *P. capsici*.

Leary *et al.* (1982) compared the growth rates of 35 isolates of *P. cinnamomi* from eight countries and 18 different hosts at 25°C on media containing antibiotics. They observed significant differences among isolates in presence of every single antibiotic.

With the development of new technologies, now it is possible to study the variability at the genetic level. These include protein electrophoresis (Kaosiri and Zentmyer, 1980), Isozyme patterns (Clare and Zentmyer, 1966), DNA and RNA polymorphism (Forster *et al.*, 1988), RFLP, RAPDS, Karyotype analysis and chromosome electrophoresis (Sansome, 1975) Immunological probes etc.

Mchau and Coffey (1994) have resolved 99 isolates of *P. palmivora* and *P.arecae* based on starch gel electrophoresis for isozyme analysis combined with morphological studies of 17 electrophoretic types (ETs). The morphological characters of all the 17 ETs were confluent. The isolates of *P. arecae* clustered with *P. palmivora* in ET7 and ET8 the most common ETs found in *P. palmivora*. Despite the reports regarding morphological differences between the two, Mchau and Coffey (1994) have demonstrated that *P. palmovra* and *P. arecae* are conspecific.

Isozyme and morphological analysis conducted by Mohau and Coffey (1994) revealed highest level of genetic variability amongst *P. palmivora* isolates from coconut and durian in Indonesia, Malaysia and Thailand.

Griffin (1977) has divided *P. palmivora* isolates from Cocoa into 4 morphological forms MF1-4, of which MF-1 and MF-2 were found to be synonymous with *P. palmivora* (Brasier and Griffin 1979) and MF-3 as a seperate species *P. megakarya* based on Karyotype analysis. Based on an isozyme analysis of twelve papillate species of *Phytophthora*, Oudemans and Coffey (1990) have demonstrated that the species of *P. palmivora*, *P. megakarya* and *P. capsici* are only distantly related and that the difference between the species are so high.

Blaha *et al.* (1994) have studied the isozyme pattern of 36 *Phytophthora* isolates from coconut in Indonesia and 15 isolates of *Phytophthora* from coconut in Ivory Coast. The coconut isolates from Indonesia confirmed to the *P. palmivora* group while the isolates causing premature nutfall in Ivory coast were found to have a distinct isozyme pattern and were assigned to *P. katsurae*.

When 84 isolates of *P. capsici* were subjected to isozyme analysis. 19 Electrophoretic types were resolved

(Oudemans and Coffey, 1990). Subsequent cluster analysis revealed the distinct clusters, CAP1, CAP2, CAP3 and the isolates earlier described as 'MF_4' were distributed in all subgroups. Based on the isozyme diversity, *P. capsici* is one of the most genetically complex species of these. CAP1, is the group containing maximum diversity in geographical distribution and isozyme banding. Recently in 1995 Mchau and Coffey analysed the morphology. Physiology and isozyme data for 113 isolates of *P. capsici*, the isolates belonging to the earlier grouping by Oudemans and Coffey (1991) fell into two distinct sub groups ie. Cap A and Cap B. Cap A is characterized by rounded sporangia and irregular sporangial ontogeny while Cap B has ellipsoid lanceolate sporangia, umbellate sporangial ontogeny and chlamydospore formation. These two subgroups have been recorded on *Piper nigrum* from India.

MATERIALS AND METHODS

MATERIALS AND METHODS

Investigations on the 'Distribution of species of *Phytophthora* affecting coconut and pepper in Kerala' were carried out at the Department of Plant Pathology, College of Agriculture, Vellayani, Thiruvananthapuram and at Indian Institute of Spices Research Marikunnu, Kozhikode. Diseased specimens of bud rot of coconut and foot rot of pepper were collected from seven districts in Kerala representing five different cropping systems viz. pure plantations of coconut; pure plantations of pepper; mixed plantations of coconut and pepper, mixed plantations of coconut pepper and arecanut and mixed plantations of coconut, pepper and cocoa.

The locations and cropping patterns of plantations were as follows.

Cropping pattern	Area	District
Pure coconut garden	Badakara	Kozhikode
	Alapuzha	Alapuzha
	Pilicode	Kasaragod
	Vellanikkara	Thrissur
	Chowa	Kannur
Pure pepper garden	Ambalavayal	Wynad
	Kozhikode	Kozhikode
	Manjappara	Wynad
	Pulpally	Wynad

Coconut and pepper	Ambalavayal	Wynad
	Chowa	Kannur
	Pilicode	Kasargodu
	Vellayani	Thiruvananthapurm
	Marunthonkara	Kozhikode
	Malapuram	Kozhikode
Coconut, pepper and arecanut	Thamarsseri	Kozhikode
Coconut, pepper and cocoa	Kozhikode	Kozhikode
	Malapuram	Kozhikode

Diseased specimens from coconut and pepper were collected during rainy (July - September) and Summer (December - April) seasons of 1992 to 1995.

Isolation and purification

Phytophthora cultures used for the present study were isolated from bud rot affected portions of coconut palms and leaves, vines and roots of foot rot affected pepper. *Phytophthora* cultures were also isolated from the soils collected from the diseased areas. The fungi were brought into pure culture by standard isolation procedures and by baiting technique (Ribeiro, 1978).

To find out the best medium suitable for growth studies, the following seven media were tried. For this study only two *Phytophthora* isolates were used (coconut C_2 and pepper P_{30}).

1. Potato Dextrose Agar
2. Oat meal agar
3. Carrot agar (200 gm/lit)
4. Coconut leaf extract agar (200 gm/lit)
5. Pepper leaf extract agar (200 gm/lit)
6. Coconut water agar (200 ml/lit)
7. Czapek's Dox agar

Based on the performance of *Phytophthora* isolates on the above media, oat meal agar was used for isolation and carrot agar for maintaining the pure cultures. Stock cultures of all the isolates were maintained on carrot agar medium and renewed at 60 days interval.

Mass multiplication

Phytophthora isolates from coconut and pepper were mass multiplied on carrot bits. For this, carrot bits were sterilized in conical flasks and inoculated with *Phytophthora* aseptically and incubated at room temperature. Within 5-7 days, white cottony growth of the fungus was noticed on the carrot bits. After confirming the identity of the fungus, they were used for further investivations.

Morphological studies

Colony morphology

To study the colony morphology, all the isolates were inoculated centrally in petri dishes containing the required

medium and incubated in dark at room temperature for three days. Colony morphology was examined against a black background. For all cultural studies 9 cm petri dishes containing 15 ml media were used.

Growth rate on agar medium

Petri dishes of 9 cm containing 15 ml carrot agar medium were inoculated in the centre with a 1 cm disc of the fungus taken from the leading edge of a 3-5 day old cultures on carrot agar and incubated in the dark at 25°C. Two measurements of colony diameter were recorded at right angles at 24 hr intervals till the growth reached the periphery of the petri dish. Three replicates were kept for each trial. The growth rate per day was calculated by dividing the diameter of the isolate by number of days.

Sporangia production and morphology

In order to find out the time required for sporangial production, all the [illegible] isolates were grown on carrot agar and kept under continuous light and incubated at room temperature. Samples were taken after every 24 hr for a period of 144 hrs.

Small bits (1 cm diameter) were cut from the grown culture and placed on a glass slide and 1-2 drops of distilled water added and the agar melted over a flame. The fungal mat was squashed using an inoculation needle and finally a cover slip was

placed over the fungal mat and was examined under high power of a microscope (430X). Sporangial dimensions and mycelial width were measured from these preparations using ocular micrometer and drawings were made using a prism type camera lucida attached to microscope.

Pedicel length of the sporangia was measured from slide culture preparations. For this 10 ml plain agar medium was melted and poured into sterile petri dish to form a thin layer. After the medium soldified, one cm agar squares were cut out using sterile dissecting needle and a flamed glass rod. Using a sterile forceps, a flamed microscope slide was placed under the cover of a petri dish lid, the inside of which was also flamed. The agar block was transferred rapidly to the centre of the cooled slide. A small bit of the mycelium was placed at the centre of each edge of agar block. Using sterile forceps, the cover slip was placed centrally upon the upper surface of the agar square and the slide was then transferred to a moist chamber. The earliest suitable time before excessive sporangia production was chosen to terminate growth. The coverslip was lifted vertically from the agar block without twisting and was placed on a sterile microscope slide with a drop of lactophenol on it. The agar blcok was removed from the slide and a drop of lactophenol was placed on the slide. Then a clear cover slip was gently lowered over the above slide. These slides were observed under microscope and the pedicel length was measured by ocular micrometer.

Zoospore production

Sporangia were induced to release zoospores by modifying the technique of Ribeiro, 1978. Discs of 1.5 cm size were cut out from the edges of 2-3 day old cultures of the fungus grown on carrot agar medium and placed in the centre of a petri dish with fresh carrot agar medium and incubated for 4-5 days under laboratory conditions. From this, discs were cut and put in sterilized petri dish into which sterilized water was added, so that a thin film of water remained over the culture disc. Petri dish with the culture discs were exposed to continuous fluorescent light for 72 hrs. After this, water in the petri dish was decanted and fresh water was filled in it. Petri dish was then kept in refrigerator for 10-15 minutes, and taken out and examined for zoospore release.

Chlamydospore production

Submerged culture method developed by Kadooka and Ko (1973) was modified to produce chlamydospores of *Phytophthora* isolates. Five day old mycelial mat of *Phytophthora* grown in 10 per cent carrot juice broth at 24°C was removed and washed in sterile distilled water. This mycelial mat was then submerged in conical flasks containing sterile distilled water for 4-8 weeks at 16°C in the dark. Cultures were then examined under low power (100 x) of microscope for chlamydospore formation.

Compatibility types

Compatibility types of *Phytophthora* isolates from pepper and coconut were evaluated by pairing them with standard A1 and A2 strains of *Phytophthora* obtained from the Department of Plant pathology, I.I.S.R., Marikunnu, Kozhikode. Carrot agar enriched with 3 ppm B - sitosterol was used for growing the isolates used for the study. The procedure involved cutting mycelial discs of one cm diameter from advancing margins of an actively growing colony of each of the 62 cultures of unknown mating type. A disc of the unknown culture was placed at the centre of the plate and discs of the known A1 and A2 cultures were placed three cm away from each disc. Following inoculation the petri dishes were wrapped with black paper and incubated at 20-24°C. The contents of each inverted petri dishes were examined udner a microscope at 100 X magnification from 13-25 day after inoculation. The dimensions of oogonia, antheridia and oospores were measured from randomly chosen sexual structures under 430 X magnification. Microphotographs of these structures were taken. Based on the number of oogonia observed per field the isolates were grouped as 'abundant' (where there were > 20 oogonia per field), 'moderate' (between 1-5 oogonia per field) and 'sparse' (1-5 oogonia per fields).

Effect of light on growth of *Phytophthora*

Out of the 16 coconut and 46 pepper isolates, only five *Phytophthora* isolates representing.

1. Isolate from bud rot affected coconut from a pure coconut garden - C_2.

2. Isolate from bud rot affected coconut from a mixed garden of coconut and pepper - C_3

3. Isolate from foot rot affected pepper from a pure pepper garden - P_{42}

4. Isolate from foot rot affected pepper from a mixed garden of pepper and coconut - P_{20}

5. Isolate from soil from a foot rot affected pure pepper garden - P_{42}

were used to study the effect of light under three different conditions on sporulation.

1. Continuous dark:- Inoculated petri dishes were wrapped with black paper and kept in darkness.

2. Alternating light and dark - cultures were incubated under laboratory conditions.

3. Continuous light. The cultures were kept under continuous fluorescent light.

All the plates were incubated at room temperature. Observations on the effect of light on linear growth and sporulation were recorded after every 24 hrs for a period of 144 hrs (6 days).

Effect of pH on growth of *Phytophthora*

The effect of pH of the medium on the growth and sporangial production of five isolates (P_{20}, P_{30}, P_{42}, C_2 and C_3) was studied using carrot agar as the basal medium. The basal medium had a pH of 6.7. The pH was brought down to five and six by adding 0.1 NHCI and to pH 7.8 and 9 by using 0.1 N NaOH. The pH was tested and adjusted after sterilizing the medium using a pH meter. After adjusting the pH, the media was poured in to a nine cm petri dish and inoculated with one cm disc of actively growing culture of *Phytophthora*. The plates were incubated at room temperature and linear growth was recorded for a period of seven days.

Pathogenicity tests

Coconut

For artificial inoculation studies, coconut leaves, nuts and one to two year old seedlings were used.

Leaves: Unwounded spindle leaves of coconut were used for artificial inoculation. Four to five day old actively growing culture grown on carrot agar was used for inoculation studies. The leaves were washed free of dust with clean sterilized water and culture bits were placed on the leaves. The inoculated leaves were then incubated at moisture saturation under, laboratory conditions till the symptom development. Uninoculated leaves served as the control.

Nuts Tender coconut with intact perianth and stalk were used for artificial inoculation. Culture bits were placed on the unwounded perianth and the inoculated nuts were incubated at moisture saturation in humidity chambers and kept in the laboratory for 15 days. Nuts with moist cotton bits on the perianth served as the control.

Seedlings: One to two year old coconut seedlings grown in cement troughs were used for the inoculation studies. Three types of inocula viz. three to five day old mycelial mat of the fungi grown on carrot agar media, sterilized carrot bits inoculated with the fungi and zoospore suspension, were used for artificial inoculation. The inoculum was placed/sprayed at the leaf axil of the spindle leaf. Immediately after inoculation, all the leaves of the seedlings were tied together for 48 hrs to retain the humidity at the leaf axil. The plants were kept under observation for a period of two months.

Pepper Three month old rooted pepper cuttings (variety - Panniyur I) grown in polythene bags were used for artificial inoculation studies. Three to four day old culture bits of the different isolates grown on carrot agar medium were used for artificial inoculation on leaves and stem of the rooted cuttings.

Leaves Third leaf from the top was used for artificial inoculation. The leaves were cleaned free of dust particles using sterile distilled water and the inoculum (cultural bits)

was placed on the upper and lower sides. The leaves were then covered with polythene bags for 24 hrs during dry season. Leaves sprayed with water served as the control.

Stem For stem inoculation, culture bits of the fungus were placed at the collar region of the plants and was covered by moist cotton wool. During dry season, the whole plant was covered by a polythene cage for 24-48 hrs, while during rainy season, the plants were not covered. The plants were kept for observation for a period of 15 days.

Cross inoculation studies

All the isolates of *Phytophthora* from pepper were used for artificial inoculation on coconut and the coconut isolates were used to inoculate the pepper plants by following the methods described under pathogenicity test.

Host range

Eight crop plants, nine ornamental plants and 12 weeds commonly found growing in coconut and pepper gardens were artificially inoculated with *Phytophthora* isolates of coconut and pepper to find out the host range of these fungi. Inoculation was done on the plants/twigs kept in Knop's solution.

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The composition of Knop's solution

Calcium nitrate	-	0.8 g
Potasium nitrate	-	0.2 g
Potassium dihydrogen phosphate	-	0.2 g
Magnesium sulphate	-	0.2 g
Ferrous sulphate	-	trace
Distilled water	-	1000 ml.

The leaves of the plants were inoculated following the method described under pathogenicity tests. The plants used for the study were

Cultivated plants

Cucumber	-	*Cucumis sativus*
Jack	-	*Artocarpus integrifolia*
Colocasia	-	*Colocasia esculenta*
Tapioca	-	*Manihot esculentus*
Papaya	-	*Carica papaya*
Cocoa	-	*Theobroma cacao*
Rubber	-	*Hevea brasiliensis*
Thippali	-	*Piper longum*

Ornamental plants

Bougain villea	-	*Bougainvillea glabra*
Hibiscus	-	*Hibiscus rosasinensis*
Alocasia	-	*Alocasia* sp

Aglaonema	-	*Aglaonema commutatum*
Acalypha	-	*Acalypha wilkesiana*
Croton	-	*Codium variegatum*
Clitoria	-	*Clitoria ternata*
Dieffenbachia	-	*Dieffenbachia bowmannii*
Myrabilus	-	*Myrabilus jalappa*

Weed plants

Eupatorium	-	*Eupatorium odoratum*
Morinda	-	*Morinda tinctoria*
Antagonum	-	*Antagonum leptopus*
Synendrella	-	*Synendrella nodiflora*
Cycla	-	*Cycla pectata*
Tridax	-	*Tridax procumbens*
Castor (wild)	-	*Ricinus communis*
Lantana	-	*Lantana camara*
Gopuramthangi	-	*Andrographis echiodes*
Demon's comb	-	*Abutilon indicum*
Knoxia	-	*Spermocoea stricta*
Rangoon creeper	-	*Quisgalis indica*

Effect of botanicals on growth and symptom development

Growth

Effect of sterilized leaf extract of bougainvillea, Ocimum and neem on the growth of five isolates of *Phytophthora* (C_2, C_3, P_{20}, P_{30} and P_{42}) was tested using carrot agar as the

basal medium. For preparing crude extract, 25 g of the leaves of bougainvillea, ocimum and neem were crushed using a mortar and pestle by adding small quantities of water. After properly macerating, the contents were transferred to one litre conical flask and the volume made upto 250 ml. This was sterilized at 1.02 kg/m^2 for 15 minutes. Twenty ml of this sterilized leaf extract was added to 100 ml of sterilized molten carrot agar. The growth and sporangial production in it was studied using the methodology described by Zentmyer (1955) for poison food technique.

Disease development

Effect of botanicals on disease development was tested on three month old rooted pepper cuttings. The crude and sterilized leaf extract of bougainvillea, Ocimum and neem was sprayed directly on the pepper leaves using an atomizer and it was artificially inoculated with an isolate of *Phytophthora* from pure pepper garden (P_{30}) immediately, three days and seven days after spraying. Leaves sprayed with water served as the control. The size of the lesion on the inoculated plants was measured for a period of five days.

Effect of antibiotics on growth and sporulation

The effect of five different antibiotics on the growth of two isolates of *Phytophthora* (viz., coconut (C_2) and pepper (P_2) was studied at different concentrations as shown below.

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Antibiotic	Concentration in ppm
(Streptomycin sulphate) Streptomycin	50, 800
(Ampicillin trihydrate) Ampicillin	50, 800
(Erythromycin estolate) Erythromycin	50, 800
Cephalaxin	50, 800
(Nalidix acid) Negadix	50, 800

Carrot agar was used as the basal medium. The methodology followed was similar to the poison food technique described by Zentmyer (1955). Based on the initial observations, a detailed study was conducted using eight different concentrations of streptomycin (50, 200, 400, 600, 800, 1000 and 2000 ppm) and all the 62 isolates. Linear growth and sporulation of the isolates under the different concentrations were recorded at 48 hrs interval.

***In vitro* evaluation of fungicides**

The comparative efficacy of four fungicides against one coconut (C_2) and pepper (P_2) isolates of *Phytophthora* were tested under laboratory conditions at different concentrations as shown below.

Name of fungicide	Concentration in ppm
Copper oxy chloride	500, 1000 and 2000
Ridomil (Methyl DL-N-2,6-dimethyl phenyl)-N-(2 methoxyacetyl) alaninate	500, 1000 and 2000

Bavistin (Methyl-3-benzimidazole carbamate)	500, 1000 and 2000
Bordeaux Mixture	0.25, 0.5 and 1%

The effect of different fungicides on the inhibition of radial growth of *Phytophthora* isolates on carrot agar medium was tested by the poison food technique described by Zentmyer (1955). A detailed study was conducted using three concentrations of Ridomil (50, 100 and 150 ppm) and all the 62 isolates. Linear growth and sporulation of the isolates under the different concentrations were recorded at 48 hrs interval.

Antagonism

Antagonistic effects of phylloplane and soil microorganisms against *Phytophthora* isolates from coconut and pepper were studied.

Isolation of microorganisms

With a view to locate antagonistic organisms against *Phytophthora*, microorganisms found associated with healthy and diseased coconut and pepper plants were isolated. Microorganisms from soils collected from pepper and coconut gardens of different parts of Kerala were also isolated for this study.

Phylloplane micorflora from 25 coconut and two pepper varieties were isolated.

Coconut varieties

1. Keraganga	2. Andaman giant	3. TxD
4. Komadan	5. SS	6. W.C.T.
7. Spikeless	8. Cochin China	9. Philippines
10. Lacadive ordinary	11. Tembli	12. Fiji
13. Lacadive micro	14. Yellow dwarf	15. Sanramon
16. Ganga bondam	17. Jaint	18. Chawaghat dwarf
19. Kappadam	20. Java	21. Ceylon
22. Andaman ordinary	23. Malayan dwarf	
24. DxT	25. SSa	

Pepper varieties

1. Panniyur - 1
2. Karimunda

Leaf washings and dilution plate technique (Walksman, 1922) was used to study the qualitative aspect of microflora on the leaf surface and soil. Leaf samples were collected from coconut and pepper plants and brought to the laboratory in fresh polythene bags. Each sample was cut into small bits using sterile scissors and approximately 10 gms of the sample and was transferred aseptically into 250 ml flask containing 100 ml of sterilized water and shaken for 20 minutes in a mechanical shaker to detach the propagules from the surface. Samples of microflora were obtained by plating 0.5 ml of leaf washings in 15 ml of the respective agar medium in sterile petri dishes for each group of

microorganisms. The media used wer rose bengal streptomycin agar for fungi, nutrient agar for bacteria and Coon's glycerol arginate agar for actinomycetes. The petri dishes were incubated for 72 hrs in case of bacteria and fungi and 10-14 days in the case of actinomycetes. The phylloplane microorganisms were separated and maintained in respective agar media for further studies. Fungal cultures were maintained on Potato dextrose agar and actinomycetes and bacteria on nutrient agar medium. The identification of the fungi were done referring to relevant literature.

Fungi, bacteria and actinomycetes isolated from leaves and soil were paired seperately on carrot agar media to study colony interactions with *Phytophthora* isolates from coconut and pepper. Methods outlined by Skidmore and Dickinson (1976) were followed for studying interactions of *Phytophthora* with phylloplane and soil fungi. Agar block (1 cm diameter) containing four day old growth of mycelia of both *Phytophthora* and the fungi were placed three cm apart on carrot agar in petridish and incubated at room temperature for 10 days. Three replicates were maintained for each treatment.

The method for testing *in vitro* antagonism of bacteria against *Phytophthora* was adapted from similar studies by Utkhede and Rahe (1983). The test organisms were either singly streaked at a spacing of three cm from *Phytophthora* or streaked on either

side of the centralised *Phytophthora* placed upon carrot agar in nine cm petridishes. The paired cultures were examined after incubation at room temperature for 48-96 hr and the nature of the reactions were noticed.

The actinomycetes isolated from the phylloplane and soil were tested for their antagonism using the cross streak assay method followed by Ahmed and Ahmed (1963). The actinomycetes was streaked at a spacing of three cm from the test fungus inoculum placed on carrot agar in petridishes and incubated at room temperature for 48 to 96 hrs. Observations on colony interactions were then recorded.

Twenty eight isolates of fungi which were found effective in inhibiting growth of *Phytophthora* under *in vitro* conditions were used for evaluating their efficiency in inhibiting foot rot diseases of pepper in potted plants. One cm agar discs of seven day old culture of antagonistic orgnisms grown on carrot agar was mixed thoroughly with 20 ml of sterilized distilled water and was filtered through a muslin cloth. This spore suspension was sprayed on the leaves of three month old rooted pepper cuttings using an atomizer. The leaves were then inoculated with *Phytophthora* isolate (P_{20}) as described under pathogenicity tests, immediately, three days and seven days after spraying with the mycoparasites. Plants sprayed with water served as the control and the lesion size was measured for a period of seven days.

Statistical analysis

Cluster analysis was performed to group/cluster the 62 isolates based on morphological characters such as sporangial length, breadth, L/B ratio and pedicel length using Mahalanobis D^2 statistic.

The distance between two isolates is given by

$$D^2 = \sum_{i=1}^{P} (y_i(k) - y_i(1))^2$$ with p characters where $y_i(k) - y_i(1)$ is the difference in the mean values for the character 'i' with respect to k^{th} and 1^{th} isolates. As such with n isolates, nC_2 distances were worked out. The isolates were grouped based on their D^2 values using Tocher method (Rao, 1952).

RESULTS

RESULTS

Symptomatology

Coconut

On a full grown coconut tree initial visible symptoms of bud rot were paling of the leaves in the inner whorl and discolouration of the heart leaf. The basal tissues of the leaf rot quickly and spear leaf collapse. The collapsed spear leaf could be easily separated from the crown. In advanced stages of disease the tender part of the crown including the bud completely rot into a slimy mass and emitted foul smell while older leaves with bunches remain healthy.

The but rot affected palms in different areas showed similar type of symptom. Symptoms of bud rot were artificially induced on 1-2 year old coconut seedlings grown in cement troughs by inoculating with different *Phytophthora* isolates of coconut collected from different parts of Kerala (Plate 1). All the isolates produced withering and discolouration of the central leaves within a period of 1-3 weeks. These leaves could be easily pulled out, and base of the leaves were rotten emitting foul smell. Even after death of the spindle leaf, the seedlings remained without showing additional symptoms for 2-3 months. Then it dried up.

Pepper

The fungus infects all parts of the plant. Infection on the leaves starts as water soaked lesions and readily expands into large dark brown spots with a fimbriate margin (Plate 2). Tender leaves were the most susceptible. Foliar infection led to varying degrees of defoliation.

Collar and root infections were fatal and the infected vine succumbed within 10-20 days. Collar and root infections were not observed until foliar yellowing was noticed. Infection started as wet slimy dark patch on the collar and rotting occured as the disease progressed. The symptom expression was gradual. The earliest symptoms were yellowing of the leaves, followed by defoliation. Infection occured on the fine feeder roots and later spread to the main roots and collar.

No variation in the symptoms of foot rot affected pepper was observed in different parts of Kerala. Forty six isolates of pepper obtained from various regions were inoculated on 3 month old rooted pepper cuttings and the symptomatology studied. On leaves the first visible symptom was pale coloured water soaked lesions. Initial lesions appeared within a period of 24-48 hrs after inoculation. This water soaked area turned from light to dark brown within 24-72 hrs. Lesions expanded and coalesced rapidly and covered large area of the lamina. Immature leaves took infection more easily than the matured ones. When the

Plate 1.

Coconut seedlings - artificially inoculated with *Phytophthora palmivora* isolate C_1

Plate 2.

Rooted pepper cuttings showing foot rot symptom


C1

pathogen was inoculated separately on both the sides of mature and immature leaves. More lesions appeared on the leaves inoculated on the lower surfaces. Five to ten days after the appearance of first symptom, defoliation took place.

Infection on stem took place within 2-5 days after inoculation and the stem portion above the infected area completely wilted within 1-2 weeks. Initial symptom on the stem was the appearance of a water soaked region which turned to dark brown colour within 2 to 3 days. Flaccidity of younger leaves followed by yellowing of younger leaves and flaccidity of matured leaves were the other symptoms observed. This was followed by leaf shedding.

Root and fruit symptoms were not observed as the study was conducted using 3 month old cuttings.

Collection and purification of *Phytophthora* spp

Locations

Diseased specimens of but rot of coconut and foot rot of pepper were collected from seven districts of Kerala comprising five different cropping patterns during 1992-95 as mentioned in the materials and methods.

The pathogens causing foot rot of pepper and bud rot of coconut were isolated from naturally infected plants. The fungi

were isolated in oat meal agar using the standard techniques. The isolates were maintained in carrot agar slants by periodic subculturing. One set of isolates grown in carrot agar was kept under refrigerated condition while another set was kept under room temperature. To avoid the loss of virulence of the cultures they were occasionally passed through the corresponding host plants and reisolated. Koch's postulates were proved using all the 62 isolates from coconut and pepper.

Place of collection of the isolates and cropping pattern practiced in the area are given in Table 1.

Sixteen *Phytophthora* isolates were purified from bud rot infected coconut palms from six districts viz. Alapuzha, Thrissur, Kozhikode, Kannur, Kasaragod and Wynad. Out of these 16 isolates, seven were from bud rot infected coconut palms from pure coconut gardens while nine were from mixed gardens of coconut and pepper where coconut was used as a standard for trailing the pepper vines. The *Phytophthora* isolates from Alapuzha, Kozhikode and Thrissur were from bud rot infected plants seen in pure coconut gardens while the isolates from Wynad were from Coconut-pepper mixed gardens. Kannur and Kasargod isolates of *Phytophthora* were obtained both from pure coconut and coconut-pepper mixed gardens. All the isolates were collected during the rainy seasons (July-September).

Table 1 Cropping pattern and place of collection of *Phytophthora* isolates

Isolate number	Place of collection		Cropping pattern
	District	Place	
C_1	Alapuzha	Alapuzha	Coconut
C_2	Kozhikode	Badagara	Coconut
C_3	Wynad	Ambalavayal	Coconut, pepper
C_4	Wynad	Ambalavayal	Coconut, pepper
C_5	Wynad	Ambalavayal	Coconut, pepper
C_6	Thrissur	Vellanikkara	Coconut
C_7	Kannur	Chowa	Coconut + pepper
C_8	Kannur	Chowa	Coconut
C_9	Kannur	Chowa	Coconut
C_{10}	Kannur	Chowa	Coconut
C_{11}	Kannur	Chowa	Coconut
C_{14}	Kannur	Chowa	Coconut+pepper
C_{12}	Kasaragod	Pilicode	Coconut
C_{13}	Kasaragod	Pilicode	Coconut
C_{15}	Kasaragod	Pilicode	Coconut+Pepper
C_{16}	Kasaragod	Pilicode	Coconut+Pepper
P_1	Kozhikode	Kozhikode	Coconut,Pepper, Cocoa
P_4	Kozhikode	Kozhikode	Pepper
P_5	Kozhikode	Kozhikode	Pepper
P_6	Kozhikode	Kozhikode	Pepper
P_7	Kozhikode	Kozhikode	Pepper

Isolate number	Place of collection		Cropping pattern
	District	Place	
P_8	Kozhikode	Kozhikode	Pepper
P_2	Kozhikode	Badagara	Coconut, pepper
P_9	Kozhikode	Thamarasseri	Coconut, Arecanut, pepper
P_{10}	Kozhikode	Thamarasseri	Coconut, Arecanut, pepper
P_{11}	Kozhikode	Thamarasseri	Coconut, Arecanut, pepper
P_{18}	Kozhikode	Thamarasseri	Coconut, Arecanut, pepper
P_{19}	Kozhikode	Thamarasseri	Coconut, Arecanut, pepper
P_{12}	Kozhikode	Marunthonkara	Coconut, pepper
P_{13}	Kozhikode	Marunthonkara	Coconut, pepper
P_{20}	Kozhikode	Marunthonkara	Coconut, pepper
P_{14}	Wynad	Ambalavayal	Pepper
$P1_5$	Wynad	Ambalavayal	Pepper
P_{16}	Wynad	Ambalavayal	Pepper
P_{17}	Wynad	Ambalavayal	Pepper
$P2_1$	Wynad	Ambalavayal	Pepper
$P2_2$	Wynad	Ambalavayal	Pepper
P_{23}	Wynad	Manjappara	Pepper
P_{24}	Wynad	Manjappara	Pepper
P_{25}	Wynad	Manjappara	Pepper
P_{26}	Wynad	Manjappara	Pepper
P_{27}	Wynad	Manjappara	Pepper

Isolate number	Place of collection		Cropping pattern
	District	Place	
P_{28}	Wynad	Manjappara	Pepper
P_{29}	Wynad	Manjappara	Pepper
P_{30}	Wynad	Manjappara	Pepper
P_{31}	Wynad	Manjappara	Pepper
P_{32}	Wynad	Manjappara	Pepper
P_{33}	Wynad	Manjappara	Pepper
P_{34}	Wynad	Manjappara	Pepper
P_{35}	Wynad	Manjappara	Pepper
P_{36}	Wynad	Manjappara	Pepper
P_{37}	Wynad	Manjappara	Pepper
P_{38}	Wynad	Manjappara	Pepper
P_{3}	Thiruvananthapuram	Vellayani	Coconut+Pepper
P_{46}	Thiruvananthapuram	Vellayani	Coconut+Pepper
P_{39}	Kozhikode	Malappuram	Coconut, Pepper
P_{40}	Kozhikode	Malappuram	Coconut, Pepper
P_{41}	Kozhikode	Malappuram	Coconut,Pepper, cocoa
P_{42}	Kozhikode	Malappuram	Coconut, Pepper
P_{43}	Kozhikode	Malappuram	Coconut, Pepper
P_{44}	Wyanad	Pulpally	Pepper
P_{45}	Wyanad	Pulpally	Pepper

Fourty six *Phytophthora* isolates were purified from foot rot affected pepper plants from three districts viz. Kozhikode, Thiruvananthapuram and Wynad. Out of the 46 isolates, 29 were from foot rot affected pepper from pure pepper gardens, 10 from mixed gardens of coconut and pepper, where coconut was used as standard for trailing the pepper vines, 5 from coconut, pepper and arecanut mixed gardens and 2 from coconut, pepper and cocoa mixed gardens. The *Phytophthora* isolates from Wynad were from pure pepper gardens and those from Thiruvananthapuram were from mixed gardens of coconut and pepper while the isolates obtained from Kozhikode were from four cropping patterns viz. pure pepper gardens, mixed gardens of coconut and pepper; coconut, pepper and cocoa and coconut, pepper and arecanut. All the isolates were collected from March-December.

Colony morphology

The colony morphology of all the 62 *Phytophthora* isolates of coconut and pepper were studied by growing them on carrot agar medium.

Coconut

The 16 coconut isolates showed six distinct colony pattern (Plate 3). They were as follows.

Type 1 Mycelium dull white slightly cottony with sharply defined leading edges. C_2, C_5, C_6.

Type II The growth almost similar to Type I, but the aerial mycelium was sparse being more or less absent from the peripheral areas of the colony. The edges of the colony well defined. C_1

Type III Mycelial growth dense with well defined margin, slow growth rate. C_3, C_4

Type IV No distinct colony pattern, aerial mycelium was profuse giving the culture a cottony appearance. C_7, C_{10}, C_{11}, C_{12}, C_{13} and C_{14}

Typve V Moderately dense colonies displaying either stellate or rosette appearance. C_{15} and C_{16}

Type VI The colony characters similar to Type IV but with less dense radiating mycelial growth. C_8 and C_9

Six (C_7, C_{10}, C_{11}, C_{12}, C_{13} and C_{14}) out of the 16 cultures belonged to type IV. All these isolates were collected from Kannur and Kasaragod districts from pure coconut plantation or a coconut garden with pepper as an intercrop. Isolate from Alapuzha (C_1 belonged to a separate type (Type I). The isolates C_{15} and C_{16} which showed stellate growth pattern (Type V) were collected from Pilicode region with a coconut + pepper cropping pattern.

There were variations in colony morphology within cropping patterns and regions of collection. To investigate the

stability of these colony characteristics, the experiment was repeated and in every case the colony morphology was found to be similar to that of the parent culture.

Pepper

The colony morphology of the pepper isolates was highly variable and exhibited seven different types of colony characteristics (Plate 4). They were grouped as follows.

Type I Mycelium dull white dense without a uniform surface

P_{10}

Type II Similar to the type I, with uniform surface and well defined margin.

P_2, P_8, P_{17}, P_{20}, P_{21}, P_{22}, P_{24}, P_{25} and P_{38}

Type III Mycelium dull white with sparse aerial mycelium and slight concentric rings

P_1, P_7, P_{16}, P_{26}, P_{27}, P_{31}, P_{32}, P_{33}, P_{34} and P_{37}

Type IV Mycelium white with sparse and coarse aerial mycelium.

P_3, P_5, P_{11}, P_{12}, P_{13}, P_{14}, P_{28}, P_{29}, P_{30}, P_{34} and P_{36}

Type V Mycelium white, sparse, aerial mycelial colony display either stellate or rosette appearance

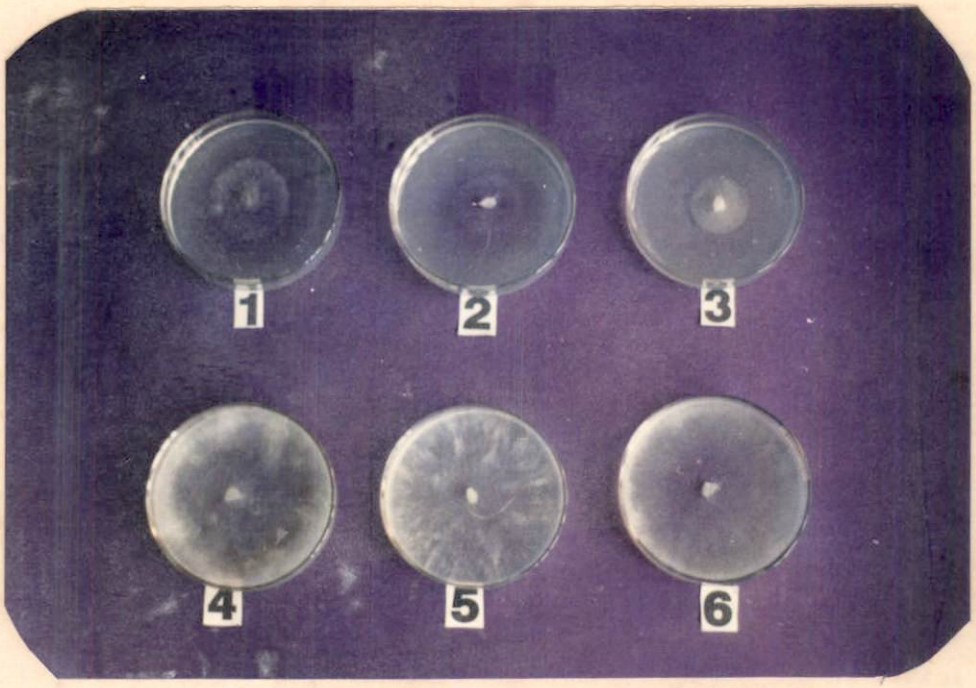
P_{39}, P_{40}, P_{41} and P_{45}

Plate 3.

Growth patterns of *P. palmivora* on carrot agar media

Plate 4.

Growth patterns of *P. capsici* on carrot agar media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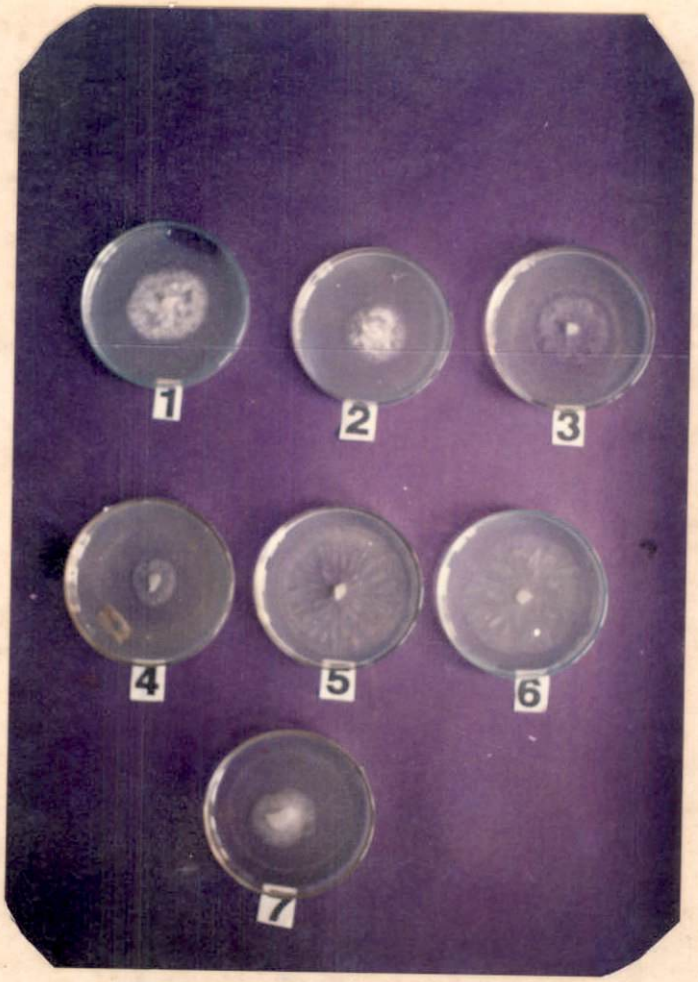
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Type VI Mycelial characters similar to type V, instead of stellate or rosette appearance, mycelium radiating nature from the centre.

P_9 P_{42}, P_{43}, P_{44} and P_{46}

Type VII Mycelium white, fast growing with a well defined margin

P_4, P_6, P_{15}, P_{18}, P_{19} and P_{23}

Except type VII, all others were dull white in colour. Types V and VI, had practically no aerial mycelium. Excluding P_9 (Thamarsseri isolate with coconut pepper and arecanut mixed cropping pattern) all other isolates in these groups (Types V and VI) were soil isolates from Kozhikode, Wynad and Thiruvananthapuram districts. The isolate from Thamarasseri (P_{10}) which showed a distinct colony morphology was isolated from diseased pepper, coconut, pepper and arecanut mixed farm. The colony characters of the various isolates were stable and the characters did not change even after repeated culturing on carrot agar medium. Isolates with some morphological characteristics were observed from different cropping patterns and regions.

Growth rate on carrot agar medium

Two distinct growth patterns were noticed among the 16 coconut isolates. The isolates from Kannur and Kasaragod (C_7 to C_{16}) were very fast growing and they filled a 9 cm petridish

within a period of 48 hrs (Table 2). Most of these isolates grow to a diameter of more than 7 cm within 24 hrs of inoculation. The isolate C_{13}, which was collected from a pure coconut garden from Pilicode had the maximum growth rate of 8.5 cm within 24 hrs, while, the isolate C_5 (coconut and pepper mixed garden) from Ambalavayal could cover only 1.7 cm during this period. The growth rate of the fast growing isolates ranged from 5 to 8.5 cm during the first 24 hrs compared to 1.7 to 2.6 cm for other slow growing isolates.

Pepper isolates could be grouped into three classes based on the number of days required to completely fill a nine cm petridish. Fast growing (required less than five days), medium (five to seven days) and slow growers (eight to nine). All the isolates collected from foot rot infected soil from Kozhikode, Wynad and Thiruvananthapuram districts (P_{39}, P_{40}, P_{41}, P_{42}, P_{43}, P_{44}, P_{45} and P_{46}) were fast growers. These isolates completely covered a 9 cm petridish within 72 hrs (Table 3). The growth rate of these isolates during the first 24 and 48 hrs ranged from 2.2 to 3.8 and 5.3 to 7.4 cm respectively. The soil isolates were fast growers irrespective of the place of collection and the cropping pattern. Among the isolates obtained from infected leaves, the isolate P_3 from Vellayani (coconut-pepper) had the maximum growth rate of 2.4 cm per 24 hrs while the minimum growth rate of 0.7 cm per 24 hrs was noticed with P_{19} (Thamarasseri, pepper, coconut and arecanut isolates (except soil isolates of

Table 2 Growth rate of *Phytophthora* isolated from coconut on carrot agar

Isolate Number	Growth (cm)		Days required to cover 9 cm petri dish	Growth rate
	after 24 hrs	after 48 hrs		
C_1	1.9	3.2	6	1.6
C_2	2.2	3.7	5	1.8
C_3	2.2	3.8	5	1.9
C_4	1.8	3.5	6	1.7
C_5	1.7	2.5	6	1.25
C_6	2.6	3.9	5	1.95
C_7	8.0	9.0	2	4.5
C_8	8.0	9.0	2	4.5
C_9	6.6	9.0	2	4.5
C_{10}	7.2	9.0	2	4.5
C_{11}	7.0	9.0	2	4.5
C_{12}	7.5	9.0	2	4.5
C_{13}	8.5	9.0	2	4.5
C_{14}	8.0	9.0	2	4.5
C_{15}	5.0	9.0	2	4.5
C_{16}	7.2	9.0	2	4.5

Table 3 Growth rate of *Phytophthora* isolated from pepper on carrort agar

Isolate Number	Growth in cm after			Days required to cover 9 cm petri dish	Growth rate
	24 hrs	48 hrs	72 hrs		
P_1	2.7	4.2	5.1	7	1.7
P_2	2.6	4.3	6.0	6	2.0
P_3	2.8	5.0	7.2	4	2.4
P_4	2.0	4.3	5.8	5	1.9
P_5	2.5	4.0	4.6	7	1.5
P_6	1.8	4.3	5.8	5	1.9
P_7	1.7	3.2	5.1	6	1.9
P_8	1.0	1.7	2.9	8	0.96
P_9	3.1	5.0	6.0	5	2.0
P_{10}	2.4	4.1	5.4	6	1.8
P_{11}	1.8	2.6	4.0	7	1.3
P_{12}	1.6	2.4	3.6	7	1.2
P_{13}	1.7	2.3	3.4	7	1.1
P_{14}	1.6	2.7	3.3	7	1.1
P_{15}	1.7	3.2	5.0	6	1.6
P_{16}	1.0	1.8	2.3	9	0.76
P_{17}	1.0	1.3	2.4	9	0.8
P_{18}	1.3	2.5	3.4	7	1.1
P_{19}	1.0	1.6	2.1	9	0.7
P_{20}	2.0	3.5	4.5	6	1.5
P_{21}	1.7	2.9	4.2	7	1.4
P_{22}	1.0	2.0	2.9	8	0.96

Isolate Number	Growth in cm after			Days required to cover 9 cm petri dish	Growth rate
	24 hrs	48 hrs	72 hrs		
P_{23}	2.1	3.4	4.2	7	1.4
P_{24}	1.8	3.2	4.6	7	1.5
P_{25}	1.2	2.4	3.6	7	1.2
P_{26}	1.0	2.4	3.0	8	1.0
P_{27}	1.5	2.8	4.0	8	1.3
P_{28}	1.9	3.4	4.4	7	1.4
P_{29}	1.5	3.0	3.9	7	1.3
P_{30}	1.2	2.5	3.7	6	1.2
P_{31}	1.7	2.8	4.0	8	1.3
P_{32}	1.5	3.0	4.2	7	1.4
P_{33}	1.1	2.6	3.4	8	1.1
P_{34}	1.7	2.9	4.3	7	1.4
P_{35}	1.8	2.0	4.0	7	1.3
P_{36}	1.3	2.9	3.9	7	1.3
P_{37}	1.3	2.4	3.3	7	1.1
P_{38}	1.4	2.4	3.6	7	1.2
P_{39}	3.7	7.0	9.0	3	3.0
P_{40}	2.2	6.4	9.0	3	3.0
P_{41}	3.8	7.2	9.0	3	3.0
P_{42}	3.6	7.4	9.0	3	3.0
P_{43}	3.8	6.8	9.0	3	3.0
P_{44}	3.7	6.5	9.0	3	3.0
P_{45}	3.4	6.0	9.0	3	3.0
P_{46}	2.0	5.3	9.0	3	3.0

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pepper and Kannur and Kasaragod isolates of coconut) had a growth rate between one and two cm per day (38 isolates). Growth rate was maximum during 24-48 hrs compared to 0-24 or subsequent periods.

Sporangial characters

Coconut

All the *Phytophthora* isolates, except those collected from bud rot infected coconut palms from Kannur and Kasargod (C_7 - C_{16}) districts, produced abundant sporangia on carrot agar. Wide variability in sporangial shape within and between isolates were noticed (Table 4). Sporangia were borne either singly on simple sympodia, or on irregular sporangiophores (Plate 5). Sporangial shapes ranged from lemon to spherical. In addition, fusiform and pyriform sporangia were also noticed. In all these types both papillate and non papillate sporangia were observed (Fig. 1). The Alapuzha isolate (C_1) collected from pure coconut garden produced almost equal number of lemon (54.2%) and spherical (48.8%) sporangia. The isolate C_6 from pure coconut garden of Thrissur had only 20% sperical and 30% lemon shaped sporangia. The remaining 50% of the sporangia did not have any well defined shape. All the three different shapes of sporangia were seen in isolates C_2 to C_5; with 36 to 57.1 per cent lemon, 28.6 to 48.8 per cent spherical and 10 to 20 per cent other types of sporangia. All the Kannur and Kasaragod isolates (C_7 to C_{16})

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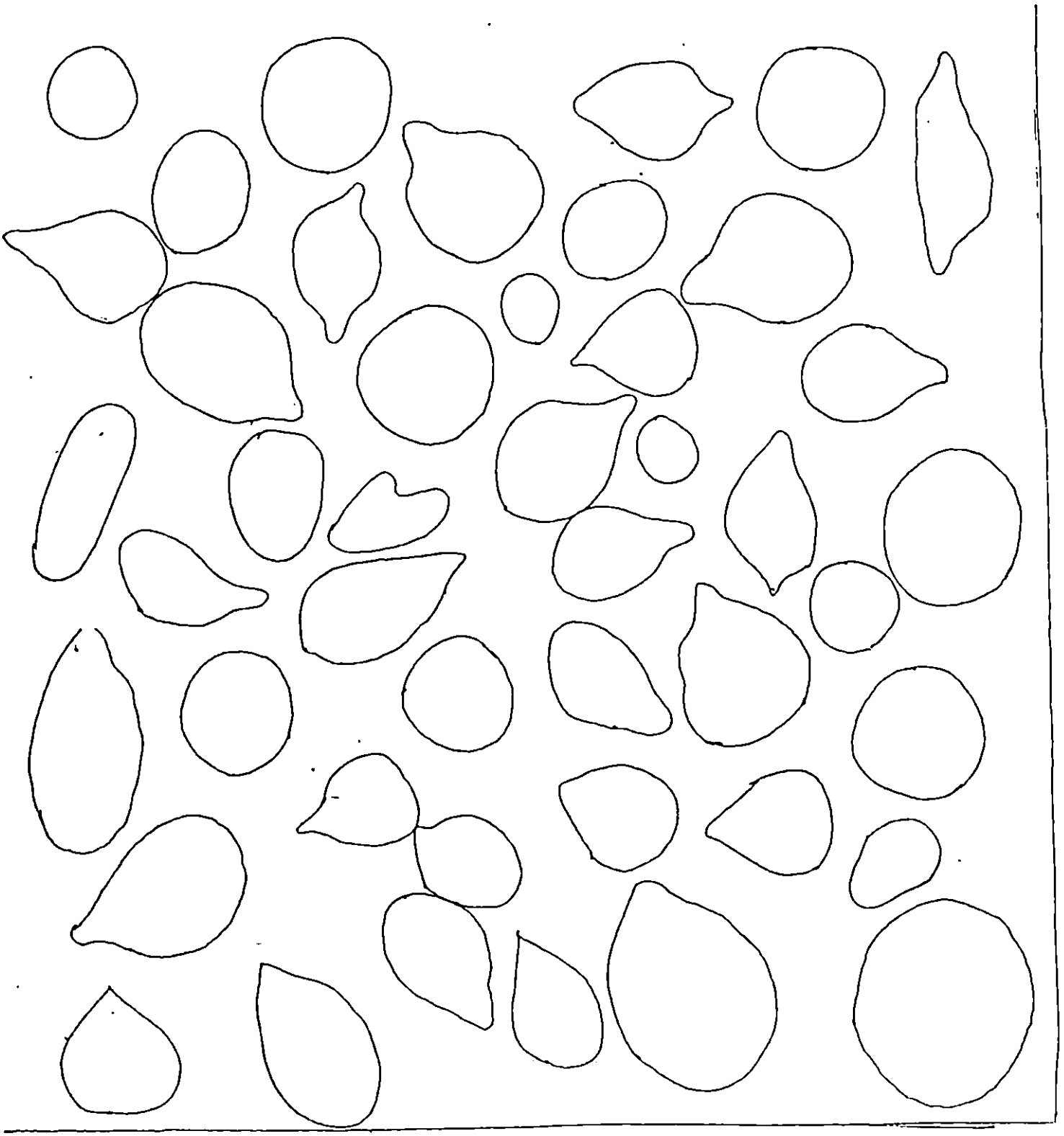
Table 4 Percentage distribution of shapes of sporangia of *Phytophthora* isolates from coconut and pepper

Isolate number	Lemon shaped	Spherical	Other
C_1	54.2	48.8	0
C_2	50.0	40.0	10
C_3	36.3	48.5	15.2
C_4	57.1	28.6	14.3
C_5	36.0	44.0	20.0
C_6	30.0	20.0	50.0
C_7	71.0	29.0	-
C_8	80.0	20.0	-
C_9	71.0	29.0	-
C_{10}	30.8	69.2	-
C_{11}	67.0	33.0	-
C_{12}	71.0	29.0	-
C_{13}	28.6	71.4	-
C_{14}	71.4	28.6	-
C_{15}	44.4	55.6	-
C_{16}	25.0	75.0	-
P_1	46.2	30.8	23.0
P_2	71.4	7.1	21.5
P_3	47.8	43.5	8.7
P_4	87.5	12.5	-
P_5	58.3	4.2	37.5
P_6	45.0	5.0	50.0

Isolate number	Lemon shaped	Spherical	Other
P_7	57.1	14.3	28.6
P_8	64.3	14.2	21.4
P_9	33.3	22.2	44.4
P_{10}	63.3	33.3	3.3
P_{11}	24.1	17.2	38.7
P_{12}	36.6	13.3	50.0
P_{13}	34.2	16.8	49.0
P_{14}	21.4	7.1	71.4
P_{15}	33.3	-	66.7
P_{16}	35.7	7.1	57.2
P_{17}	33.3	7.1	59.6
P_{18}	40.0	20.0	40.0
P_{19}	33.3	22.2	44.4
P_{20}	24.0	-	76.0
P_{21}	33.3	-	66.7
P_{22}	33.3	4.8	61.9
P_{23}	33.3	-	66.7
P_{24}	17.6	8.8	73.5
P_{25}	11.8	-	88.2
P_{26}	33.3	-	66.7
P_{27}	13.3	6.6	80.0
P_{28}	33.3	-	66.7
P_{29}	20.0	-	80.0
P_{30}	28.6	14.2	57.2

Isolate number	Lemon shaped	Spherical	Other
P_{31}	19.2	3.8	76.9
P_{32}	36..9	-	63.1
P_{33}	45.2	3.0	51.7
P_{34}	18.4	-	81.6
P_{35}	22.2	5.5	72.2
P_{36}	26.7	6.7	66.7
P_{37}	22.2	-	77.8
P_{38}	65.0	-	75.0
P_{39}	-	91.6	8.4
P_{40}	50.0	40.0	10.0
P_{41}	47.8	47.8	4.4
P_{42}	47.6	52.4	-
P_{43}	41.2	52.9	5.9
P_{44}	43.5	56.5	-
P_{45}	31.7	60.8	7.5
P_{46}	22.2	72.2	5.5

Fig. 1. Variability in sporangial shape - coconut isolates



failed to produce sporangia under normal growing conditions. Sporangia were produced only when exposed to cold treatment. All the induced sporangia of these coconut isolates were either lemon shaped or spherical.

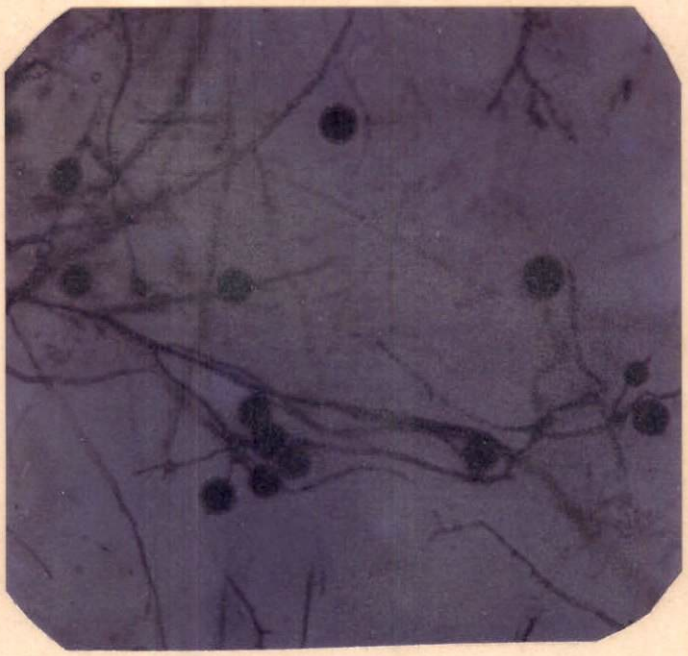
Pepper

Sporangial arrangement of *Phytophthora* isolates from pepper was generally umbellate (Plate 6). However, this umbellate arrangement of sporangia was rare (P_{33}, P_{11} and P_{18}) or less frequent (P_{13}, P_{24} and P_{18}) in some isolates. Sporangium shapes varied greatly within and between isolates (Fig. 2).

Isolates P_4, P_{42} and P_{44} produced only lemon and spherical shaped sporangia. Ten isolates viz. P_{15}, P_{20}, P_{23}, P_{25}, P_{28}, P_{29}, P_{32}, P_{34}, P_{37} and P_{38} did not produce any spherical shape sporangia. All these, except P_{20}, were isolated from pure pepper gardens located in Wynad districts while P_{20} was isolated from a pepper-coconut garden in Kozhikode district. The *Phytophthora* isolated from a diseased pepper in Malapuram with a cropping pattern of coconut - pepper did not produce any lemon shaped sporangia. In this isolate, 91.6% of the sporangia were spherical. All the pepper isolates collected from soil (P_{39}-P_{46}) had a high percentage of (>40%) spherical and a low percentage of irregular (Nil to 17.5%) sporangia. In these soil isolates, except for P_{44} and P_{45} (which were collected from a pure pepper garden) all the other isolates were from a pepper intercropped with coconut or coconut and cocoa gardens.

Plate 5. Sporangia of *P. palmivora* isolate

Plate 6. Sporangia of *P. capcici* isolate



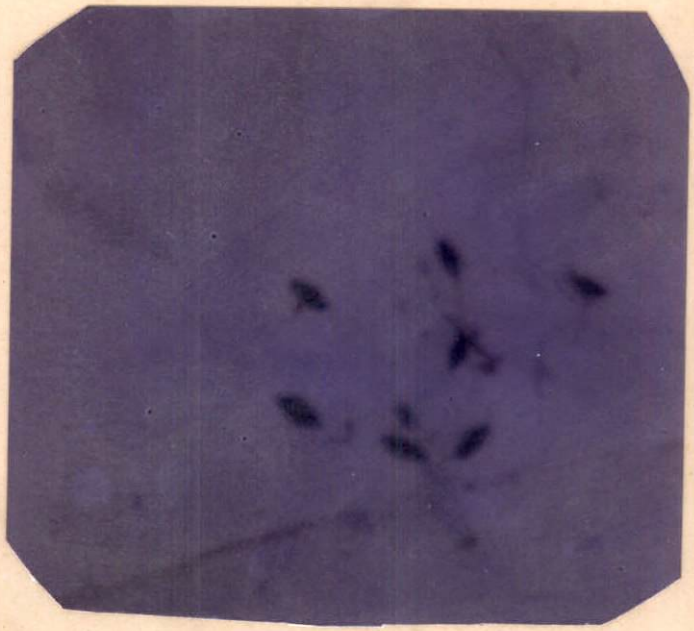
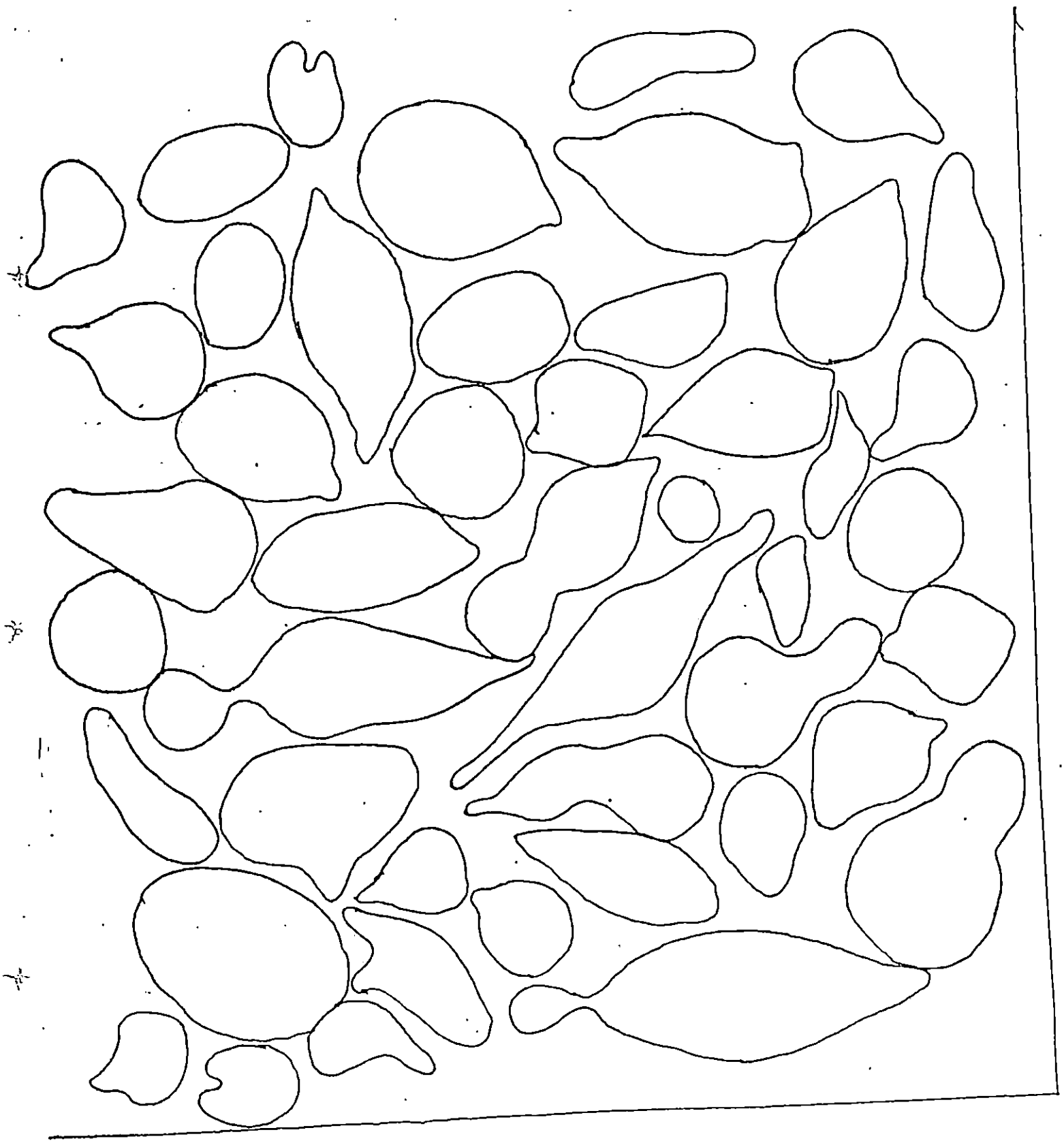


Fig. 2. Variability in sporangial shape - pepper isolates



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Sporangial size

Coconut

There was a wide variability in sporangial size within and among the isolates (Table 5).

Length

Based on the average length of sporangia produced on carrot agar medium, *Phytophthora* isolates from coconut can be grouped into two viz. those with sporangial length of 20 to 30 um and those >30 um. Average length of sporangia of Kasaragod, Kannur and Alapuzha isolates did not show much variation and the length ranged from 22.2 to 29.5 um. The cropping pattern followed in this area was either pure plantations of coconut or a coconut-pepper mixed cultivation. The sporangial length of the remaining isolates (except C_4) were almost two times that of the first group and it ranged from 49.9 to 54.8 um while C_4 had a length of 30.7 um. Maximum variation in the sporangial length (29.1 to 83.2) was noticed in C_3 isolate from coconut-pepper gardens in Ambalavayal, while the minimum was recorded in C_8 where the difference between maximum and minimum was only 2.6 um (22.3 - 24.9 um).

The variation in sporangial breadth of coconut isolates C_1 to C_6 was almost of the same pattern as that observed in sporangial length. The average minimum breadth (14.52) as with sporangial length was noticed in C_1 isolate. Based on

sporangial breadth, the six isolates could be grouped into three viz. those with a breadth of 30-40 um (C_2, C_3 and C_5) those with 20-30 um (C_4, C_6) and those below 20 um (C_1).

L/B ratio of coconut isolates ranged from 1.12 (C_{14}) to 1.8 (C_6). Majority of the isolates had an L/B ratio of 1.5 (Table 5).

The characters of the sporangia of *Phytophthora* from Kannur and Kasaragod were studied after artificially inducing them giving cold treatment. The sporangial characters of these isolates (C_9-C_{16}) were almost uniform and different from that of other coconut isolates. The average length of these isolates ranged from 22.2 to 29.5 um and breadth 19.4 to 29.1 um. Most of the sporangia of these isolates were round with an L/B ratio 1.0 to 1.3 compared to 1.12 to 1.8 for other coconut isolates (Table 5).

Pepper

Sporangial length of pepper isolates ranged from 12.5 to 104 um (Table 6) However, 29 out of the total of 46 isolates had sporangial length between 35-50 um. All the isolates obtained from Thamarasseri soils during summer season measured less than 35 um. Maximum average sporangial length (64.5 um) was observed for P_{18} isolate collected from Thamarasseri with a cropping pattern of pepper, coconut and arecanut, while the

Table 5 Sporangial characteristics of isolates of *Phytophthora* from coconut

Isolate number	Length (um)	Breadth (um)	L/B ratio
C_1	22.04 ± 1.67 (20.8 - 25)	14.5 ± 1.29 (12.5 - 16.6)	1.52 ± 0.09 (1.43 - 1.66)
C_2	54.80 ± 13.2 (37.4 - 79.04)	34.8 ± 6.8 (24.9 - 41.6)	1.58 ± 0.30 (1.0 - 2.1)
C_3	49.9 ± 20.16 (29.1 - 83.2)	36.6 ± 6.8 (25.0 - 41.6)	1.34 ± 0.38 (1.1 - 2.0)
C_4	30.7 ± 6.20 (25 -41.6)	27.4 ± 6.2 (20.8 - 37.4)	1.12 ± 0.07 (1.0 - 1.2)
C_5	55.7 ± 15.9 (37.4 - 74.8)	35.76 ± 11.19 (25-54)	1.57 ± 0.22 (1.28-1.77)
C_6	52.8 ± 11.7 (37.4 - 74.8)	28.7 ± 3.60 (25-33.3)	1.80 ± 0.29 (1.24-2.24)
C_7	21.5 ± 4.47 (22.3 - 33.2)	27.5 ± 4.40 (20.8 - 33.2)	1.0 (1.0 ± 0)
C_8	24.1 ± 1.80 (22.3 - 24.9)	20.8 ± 2.90 (20.8 - 24.9)	1.17 0.09 (1.0 - 1.25)
C_9	27.04 ± 2.20 (24.9 - 29.1)	26.3 ± 3.3 (20.8 - 29.1)	1.03 ± 0.80 (1.0 - 1.2)
C_{10}	25.6 ±5.50 (20.8 - 29.1)	19.4 ± 3.40 (16.6 - 24.9)	1.30 ± 0.35 (1.0 - 2.0)
C_{11}	22.2 ± 2.10 (20.8 - 24.9)	21.7 ± 2.80 (16.6 - 24.9)	1.02 ± 0.08 (1.0 - 1.25)
C_{12}	29.5 ± 4.50 (20.8 - 33.3)	29.1 ± 4.30 (20.8 - 33.3)	1.01 ± 0.04 (1 - 1.4)
C_{13}	27.4 ± 2.20 (24.9 - 29.1)	26.6 ± 3.70 (20.8 - 29.1)	1.04 ± 0.09 (1.0 - 1.21)
C_{14}	27.5 ± 2:12 (24.9 - 29.1)	26.3 ± 2.1 (24.9 - 29.1)	1.05 ± 0.07 (1 - 1.16)
C_{15}	23.2 ± 3.28 (20.8 - 29.1)	22.6 ± 3.27 (20.8 - 29.1)	1.02 ± 0.07 (1 - 1.2)
C_{16}	24.50 ± 4.40 (20.8 - 33.3)	22.6 ± 3.0 (20.8 - 29.1)	1.07 ± 0.09 (1.0 - 1.2)

Table 6 Sporangial characteristics of isolates of *Phytophthora* from pepper

Isolate number	Sporangial Length (um)	Sporangial Breadth (um)	L/B ratio
P_1	37.4 ± 15.8 (20.8 - 62.4)	24.9 ± 5.1 (20.8 - 33.3)	1.51 ± 0.61 (1.0 - 2.49)
P_2	29.1 ± 6.6 (20.8 - 37.4)	17.8 ± 2.7 (14.5 - 20.8)	1.6 ± 0.42 (1.0 - 2.0)
P_3	48.2 ± 16.1 (29.1 - 87.36)	36.6 ± 7.2 (25 - 41.6)	1.29 ± 0.8 (1.11 - 1.75)
P_4	34.9 ± 4.7 (29.12 -41.6)	24.1 ± 3.48 (20.8 - 29.1)	1.37 ± 0.23 (1.16 - 1.66)
P_5	35.7 ± 4.7 (29.12 - 41.6)	24.1 ± 3.4 (20.8 - 29.1)	1.48 ± 0.07 (1.4 - 1.6)
P_6	40.8 ± 3.49 (37.4 - 45.7)	27.4 ± 5.5 20.8 - 33.3)	1.51 ± 0.22 (1.24 - 1.79)
P_7	39.5 ± 8.5 (25 - 54)	24.9 ± 4.8 (16.6 - 33.3)	1.64 ± 0.59 (1.2 - 3.25)
P_8	39.5 ± 9.6 (16.6 - 45.7)	30.3 ± 6.2 (16.6 - 37.4)	1.29 ± 0.15 (1.0 - 1.57)
P_9	49.9 ± 27.5 (25- 104)	29.5 ± 6.3 (20.8 - 41.6)	1.62 ± 0.63 (1.0 - 3.12)
P_{10}	61.5 ± 27.0 (20.8 - 104)	32.0 ± 5.54 (20.8 - 37.4)	1.90 ± 0.76 (1.0 - 2.9)
P_{11}	54 ± 22.9 (20.8 - 91.5)	33.6 ± 10.0 (20.8 - 58.2)	1.59 ± 0.52 (1.0 - 2.56)
P_{12}	44.5 ± 10.5 (29.1 - 66.5)	32.8 ± 5.69 (25 - 41.6)	1.37 ± 0.34 (1.0 - 2.28)
P_{13}	38.2 ± 6.16 (29.12 - 45.7)	30.8 ± 6.3 (20.8 - 37.4)	1.26 ± 0.15 (1.0 - 1.4)
P_{14}	43.2 ± 7.0 (33.3 - 54)	30.3 ± 9.6 (16.6 - 41.6)	1.53 ± 0.44 (1.0 - 2.50)
P_{15}	39.5 ± 10.0 (25 - 49.9)	24.9 ± 6.79 16.6 - 37.4)	1.62 ± 0.39 (1.2 - 2.5)

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Isolate number	Sporangial Length (um)	Sporangial Breadth (um)	L/B ratio
P_{16}	42.4 ± 12.9 (29.1 - 62.4)	30.8 ± 3.7 (25 - 33.3)	1.37 ± 0.34 (1.0 - 1.87)
P_{17}	64.5 ± 18.5 (41.6 - 87.36)	33.6 ± 6.3 (25 - 41.6)	1.96 ± 0.65 (1.25 - 3.0)
P_{18}	67.8 ± 13.17 (49.92 - 91.5)	34.5 ± 8.08 (25 - 49.9)	2.03 ± 0.5 (1.49 - 3.16)
P_{19}	56.98 ± 21.4 (33.3 - 91.5)	29.5 ± 6.0 (25 - 41.6)	2.02 ± 0.93 (1.1 - 3.6)
P_{20}	43.3 ± 7.6 (29.1 - 54)	30.7 ± 6.15 (25 - 41.6)	1.44 ± 0.34 (1.1 - 1.99)
P_{21}	41.6 ± 9.6 (25 - 58.24)	29.5 ± 3.6 (25 - 33.3)	1.40 ± 0.27 (1.0 - 1.83)
P_{22}	45.8 ± 5.06 (41.6 - 54)	30.7 ± 4.48 (25 - 37.4)	1.5 ± 0.28 (1.12 - 1.85)
P_{23}	42.7 ± 5.2 (37.4 - 49.9)	30.3 ± 5.2 (25 - 37.4)	1.40 ± 0.17 (1.22 - 1.7)
P_{24}	47.4 ± 2.29 (45.76 - 49.92)	32.4 ± 3.48 (29.12 - 37.4)	1.49 ± 0.15 (1.32 - 1.7)
P_{25}	43.3 ± 11.4 (29.12 - 70.7)	26.2 ± 3.42 (20.9 - 33.3)	1.65 ± 0.44 (1.33 - 2.8)
P_{26}	47.5 ± 13.3 (25 - 62.4)	29.1 ± 5.8 (20.8 - 37.4)	1.69 ± 0.67 (1.0 - 3.0)
P_{27}	43.7 ± 8.6 (33.3 - 49.9)	26.9 ± 2.9 (25 - 29.12)	1.62 ± 0.36 (1.28 - 2.49)
P_{28}	44.9 ± 3.28 (41.6 - 49.9)	25.4 ± 9.16 (25 - 33.3)	1.60 ± 0.18 (1.37 - 2.0)
P_{29}	45.76 ± 6.24 (37.4 - 58.24)	28.6 ± 4.35 (25 - 37.4)	1.62 ± 0.35 (1.24 - 2.32)
P_{30}	53.2 ± 13.5 (33.3 - 70.7)	29.1 ± 3.9 (20.8 - 33.3)	1.81 ± 0.31 (1.14 - 2.14)

Isolate number	Sporangial Length (um)	Sporangial Breadth (um)	L/B ratio
P_{31}	51.48 ± 21.4 (20.8 - 91.52)	30.16 ± 6.94 (20.8 - 41.6)	1.74 ± 0.84 (1.0 - 3.6)
P_{32}	64 ± 16.6 (37.4 - 91.5)	34.5 ± 8.78 (25 - 49.92)	1.87 ± 0.3 (1.4 - 2.32)
P_{33}	52 ± 4.49) (45.76 - 58.2)	29.9 ± 3.82 (25 - 37.4)	1.76 ± 0.29 (1.3 - 2.16)
P_{34}	44.0 ± 4.8 (37.4 - 54)	33.3 ±4.3 (25- 37.4)	1.32 ± 0.16 (1.11 - 1.6)
P_{35}	44.9 ± 8.2 (37.4 - 54)	30.36 ± 6.2 (20.8 - 37.4)	1.52 ± 0.41 (1.12 - 2.49)
P_{36}	55.7 ± 19.3 (20.8 - 83.2)	32.8 ± 5.7 (20.8 - 41.6)	1.67 ± 0.48 (1.0 - 2.49)
P_{37}	49.5 ± 5.3 (41.6 - 58.2)	35.12 ± 3.0 (29.12 - 33.3)	1.42 ± 0.3 (1.22 - 1.62)
P_{38}	40.3 ± 3.42 (33.3 - 45.7)	31.6 ± 2.9 (29.1 - 37.4)	1.28 ± 0.12 (1.1 - 1.42)
P_{39}	26.6 ± 8.5 (20.8 - 49.9)	21.6 ± 4.3 (16.6 - 29.1)	1.23 ± 0.25 (1.0 - 1.71)
P_{40}	20.8 ± 5.5) (12.5 - 29.1)	20.3 ± 5.5 (12.5 - 29.1)	1.02 ± 0.08 (1.0 - 1.25)
P_{41}	30.0 ± 3.8 (25-33.3)	25.7 ± 2.6 (25 - 29.1)	1.16 ± 0.11 (1.0 - 1.33)
P_{42}	30.8 ± 3.5 (25- 37.4)	24.1 ± 3.2 (20.8 - 29.1)	1.32 ± 0.2 (1.0 - 1.6)
P_{43}	24.9 ± 3.3 (20.8 - 29.12)	22.8 ± 3.5 (16.6 - 29.12)	1.10 ± 0.16 (1.0 - 1.5)
P_{44}	54.9 ± 12.6 (45.76 - 66.56)	32.8 ± 4.9 (25 - 41.6)	1.7 ± 0.51 (1.0 - 2.66)
P_{45}	39.1 ± 4.0 (33.3 - 45.76)	24.9 ± 3.9 (20.8 - 29.12)	1.59 ± 0.23 (1.28 - 2.0)
P_{46}	35.36 ± 7.8 (25 - 45.76)	32.8 ± 8.6 (25 - 41.6)	1.09 ± 0.15 (1.0 - 1.5)

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minimum length of 20.8 um was recorded for the isolate from Malapuram (P_{40}) with a cropping pattern of pepper and coconut.

As compared to sporangial length, breadth is less variable. The average sporangial breadth varied from 17.8 (P_2) to 36.6 um (P_3) with a range of 12.48 to 58.24 um. Out of 46 isolates, 34 isolates had a sporangial breadth between 25-35 um. Only Eight isolates, from Thamarasseri, had a width of less than 25 um.

The L/B ratio of the isolates ranged from 1.0 (indicating round nature of sporangia (P_{40}) to 3.6 (P_{19} and 31) indicating highly elliptical nature. The L/B ratio of 27 out of 46 pepper isolates was more than 1.5 indicating oval to elliptical nature of sporangia.

In general, pepper isolates were longer than coconut isolates. The coconut isolates, which have got maximum sporangial length (C_5) was obtained from mixed gardens of Ambalavayal. Only 4 out of 46 pepper isolates had a sporangial length of less than 30 um, while only 3 isolates of coconut had a sporangial length more than 30 um. Compared to sporangial length much variation was not observed in sporangial breadth between coconut and pepper isolates. The maximum breadth exhibited by both the isolates were equal. The L/B ratio of pepper isolates was higher compared to coconut isolates. Only 3 coconut isolates had L/B ratio more than 1.4 while, L/B ratio of 32 pepper isolates were more than 1.4.

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Pedicel length and caducity

Coconut

Pedicel length and caducity of only six (C_1-C_6) out of the 16 coconut isolates were measured as sporangial production was not noticed on carrot agar medium for Kannur and Kasaragod isolates of coconut. Even when induced, sporangial production was very sparse and enough numbers were not obtained for taking observations (Table 7). Pedical length of all the coconut isolates were less than 30 um. The maximum caducity of 23.6 was recorded with Alapuzha isolates (C_1) from a pure coconut garden. The maximum pedicel length of 25.7 um was noted in the isolate C_2 from Badagara. The isolates C_3, C_4 and C_5, collected from the same region with same cropping pattern had a pedicel length of 5-10 um (C_1, C_4 and C_5). The caducity of the isolates ranged from 11.1 (C_4) to 23.6 (C_1). There was no relationship between the pedicel length and caducity.

Pepper

Pedicel length varied greatly within and between the isolates (Table). The average pedicel length varied from 24.9 (P_{21}) to 74 um (P_9) with the range of 16.6 (P_{14}, P_{21}, P_{22} and P_{37}) to 166.4 (P_{40}). The isolates showing shorter pedicels were from pure pepper gardens of Ambalavayal. Except P_{41} (Thamarasseri, coconut, pepper, cocoa) all other soil isolates

Table 7 Pedicel length and degree of caducity of *Phytophthora* isolates from coconut and pepper

Isolate number	Pedicel length (um)	Caducity (%)	Isolate number	Pedicel length (um)	Caducity (%)
P_1	51.15 ± 16.8 (29.1 - 83.2)	40	P_{16}	35.8 ± 7.1 (25 - 45.7)	40
P_2	38.2 ± 6.13 (29.1 - 45.7)	40	P_{17}	58.6 ± 24 (25 - 104)	41.1
P_3	37.8 ± 10.4 (25 - 58.2)	26.6	P_{18}	50.3 ± 19.6 (20.8 - 83.2)	37.5
P_4	67.9 ± 30.8 (37.4 - 104)	50	P_{19}	59.02 ± 12.0 (41.6 - 79.04)	36.3
P_5	43.2 ± 12.8 (29.1 - 66.5)	48.5	P_{20}	48.6 ± 6.2 (37.4 - 58.2)	25
P_6	69.0 ± 15.0 (37.4 - 87.36)	55.5	P_{21}	24.9 ± 7.3 (16.6 - 33.3)	44.4
P_7	46.5 ± 6.4 (37.4 - 64.4)	40	P_{22}	24.5 ± 7.45 (16.6 - 37.4)	17.6
P_8	65.1 ± 8.9 (54.0 - 79.0)	54.2	P_{23}	42.4 ± 13.2 (25 - 37.4)	53.8
P_9	74.0 ± 14.4 (49.9 - 99.8)	68.4	P_{24}	50.7 ± 10.5 (41.6 - 74.8)	45.4
P_{10}	63.6 ± 14.0 (41.6 - 79.04)	50	P_{25}	58.2 ± 19.8 (29.12 - 87.36)	50
P_{11}	45.3 ± 16.6 (29.12 - 83.2)	50	P_{26}	35.4 ± 12.6 (33.3 - 45.76)	20
P_{12}	39.4 ± 15.0 (25 - 54)	26.6	P_{27}	70.3 ± 15.9 (49.9 - 10.4)	36
P_{13}	51.9 ± 13.0 (37.4 - 83.2)	52.8	P_{28}	38.6 ± 15.0 (16.6 - 66.5)	46.4
P_{14}	31.1 ± 10.2 (16.6 - 49.9)	16.6	P_{29}	42 ± 8.19) (37.4 - 49.9)	41.1
P_{15}	47.4 ± 10.2 (33.2 - 58.2)	18.7	P_{30}	34.6 ± 5.5 (25 - 45.7)	36.8

Isolate number	Pedicel length (um)	Caducity (%)	Isolate number	Pedicel length (um)	Caducity (%)
P_{31}	46.1 ± 21.6 (25 - 87.3)	52	P_{42}	68.64 ± 26.06 (29.12 - 120.6)	45.4
P_{32}	61.5 ± 8.2 (45.7 - 70.7)	44.4	P_{43}	68.6 ± 12.0 (45.76 - 87.36)	60
P_{33}	48.3 ± 5.2 (41.6 - 48.24)	36.3	P_{44}	66.9 ± 16.8 (37.4 - 91.5)	46.1
P_{34}	37.4 ± 11.6 (20.8 - 58.2)	50	P_{45}	70.72 ± 16.64 (45.7 - 99.8)	51.5
P_{35}	41.6 ± 10.7 (25 - 48.2)	46.6	P_{46}	67.3 ± 16.5 (37.4 - 91.5)	61.5
P_{36}	39.9 ± 5.9 (33.3 - 49.9)	52.3	C_1	6.6 ± 2.9 (4.16 - 12.5)	23.6
P_{37}	34.7 ± 12.8 (16.6 - 58.2)	40.7	C_2	25.7 ± 8.05 (12.5 - 37.4)	21.8
P_{38}	32.0 ± 6.79 (25 - 45.7)	28.5	C_3	21.6 ± 5.4 (12.5 - 29.12)	18.5
P_{39}	68.6 ± 33.5 (25 - 120.61)	48.7	C_4	9.15 ± 3.6 (4.16 - 16.6)	11.1
P_{40}	73.2 ± 46.7 (24.9 - 166.44)	50	C_5	5.4 ± 2.0 (4.1 - 8.3)	13.6
P_{41}	40.8 ± 10.5 (25 - 54)	46	C_6	23.3 ± 4.49 (16.6 - 29.1)	16.2

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had more than 65 um pedicel length. The pedicel length of pepper isolates were more than two times that of coconut isolates. In general the pepper isolates were more caducous than the coconut isolates. The caducity in pepper isolates ranged from 16.6 (P_{14}) to 68.4 (P_9). Caducity of majority of the pepper isolates (19 out of 46) ranged from 40-50%.

Zoospore production

Zoospore production from sporangia was induced by cold treatment and the number and size of zoospores were measured. The number of zoospores released from sporangia ranged from 6-15 and the encysted zoospores measured 4.16 to 8.32 um. Variation in the number and size of zoospores were not observed among the different isolates.

Chlamydospore production

All the coconut isolates and five isolates of pepper from Manjappara near Ambalavayal in Wynad district produced chlamydospores. All the pepper isolates which produced chlamydospores (P_{26}, P_{27}, P_{32}, P_{33}, P_{35}) were from pure pepper gardens. The chlamydospores were produced singly in terminal or intercalary position on vegetative hyphae.

Cluster analysis

The 62 isolates of *Phytophthora* (16 coconut isolates and 46 pepper isolates) collected from different areas were

grouped based on Mahalanobis D^2 analysis with respect to the morphological characters viz. sporangial length, breadth, L/B ratio and pedicel length. Based on the analysis, the isolates could be clustered into 8 groups (Table 8) and two isolates (C_1 and C_5) remained independently.

Out of the 16 coconut isolates, 11 were grouped in cluster I. All the Kannur and Kasaragod isolates of coconut and one isolate of Ambalavayal belonged to this cluster. Cluster II, III and IV include only pepper isolates. Out of 46 pepper isolates, 21 isolates coming under cluster II and the isolates were spread over the regions and different cropping patterns.

Cluster V is the unique group whic include both coconut and pepper isolates.

The mean sporangial length ranged from 26.2 um - 61.6 um represented by cluster I and cluster IV respectively (Table 9). The sporangial breadth ranged from 21.8 um (cluster VI) - 33.2 um (cluster VIII) and L/B ratio varied from 1.07 (cluster I - 1.91 (cluster IV). The mean pedicel length ranged from 11.1 um (cluster I) - 68.7 um (cluster III).

The isolates of cluster I were characterised by the least sporangial length, L/B ratio and pedicel length; Sporangial breadth was also not high (Fig. 3). The isolates of cluster II ranked 5th in respect of sporangial length, L/B ratio and

Table 8 Clustering of *Phytophthora* isolates

Cluster number	Isolate number	Number of isolates under the cluster
Cluster I	C_4, C_7, C_8, C_9, C_{10}, C_{11} C_{12}, C_{13}, C_{14}, C_{15} and C_{16}	11 isolates
Cluster II	P_1, P_3 P_5, P_7, P_{12} P_{13} P_{14}. P_{15} P_{16} P_{20}, P_{23}, P_{24}, P_{26}, P_{28}, P_{29}, P_{31}, P_{33}, P_{34} P_{35} P_{37}, and P_{38}	21 isolates
Cluster III	P_4, P_6, P_8, P_{39}, P_{40}, P_{42}, P_{43}, P_{45} and P_{46}	9 isolates
Cluster IV	P_{17}, P_{32}, P_{10}, P_{18} P_{19} and P_{44}	6 isolates
Cluster V	P_{30}, P_{22}, P_{21} C_2, C_3 and C_6	6 isolates
Cluster VI	P_2 and P_{41}	2 isolates
Cluster VII	P_9, P_{25} and P_{27}	3 isolates
Cluster VIII	P_{11} and P_{36}	2 isolates

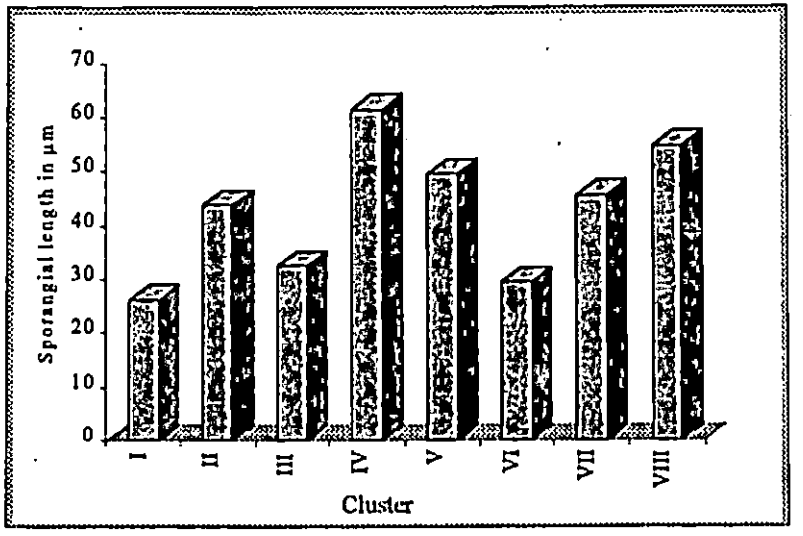
C_1 and C_5 could not be clustered with any isolates

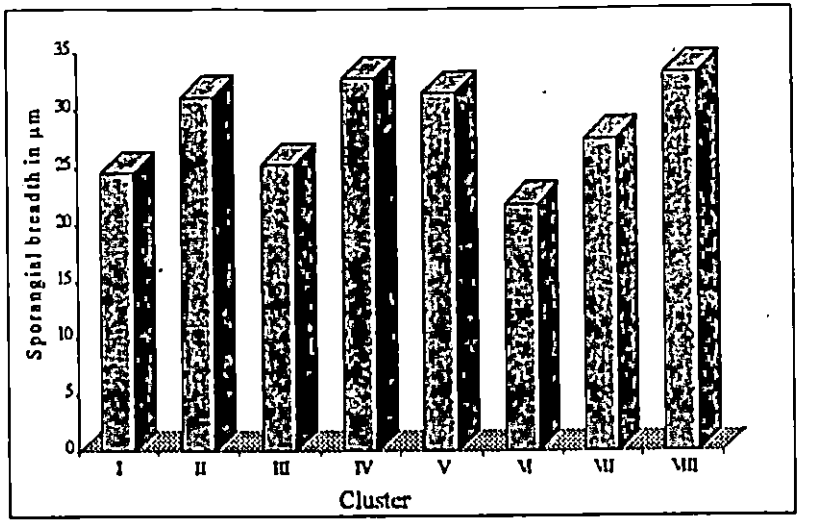
Table 9 Cluster characters of *Phytophthora* isolates

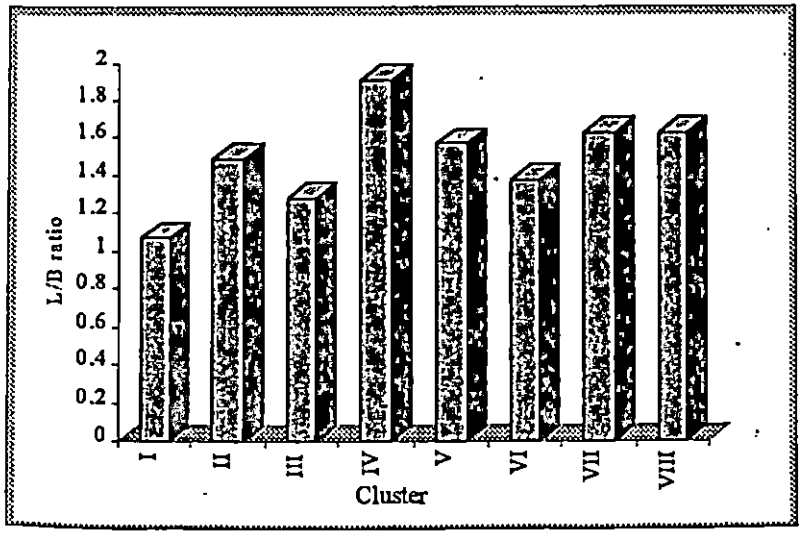
Characters / Cluster number	Sporangial length (um)	Sporangial breadth (um)	L/B ratio	Pedicel length (um)
I	26.2 (8)	24.5 (7)	1.07 (8)	11.1 (8)
II	43.9 (5)	31.2 (4)	1.49 (5)	42.0 (5)
III	32.5 (6)	25.3 (6)	1.28 (7)	68.7 (1)
IV	61.6 (1)	32.8 (2)	1.91 (1)	59.9 (3)
V	49.6 (3)	31.5 (3)	1.57 (4)	25.7 (7)
VI	29.5 (7)	21.8 (8)	1.38 (6)	39.5 (6)
VII	45.6 (4)	27.5 (5)	1.63 (2)	67.5 (2)
VIII	54.8 (2)	33.2 (1)	1.63 (2)	42.6 (4)

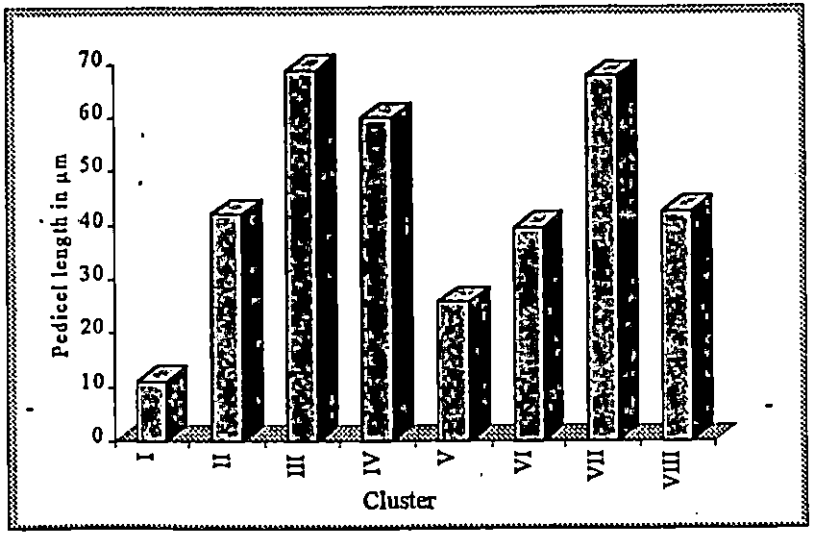
Figures in paranthesis indicate the rank

Fig.3 Divergence in *Phytophthora* isolates









pedicel length, but ranked 4th with respect to sporangial breadth. Eventhough, sporangial length and breadth assumed 6th rank and L/B ratio ranked 7th in case of cluster III isolates, they had the maximum pedicel length. High sporangial length and L/B ratio was recorded by cluster IV isolates, while stood 2nd and 3rd with respect to sporangial breadth and pedicel length. The Vth cluster recorded 3rd in sporangial length and breadth and 4th in L/B ratio, but the pedicel length is less (7th in rank). The VIth cluster was characterized by L/B ratio and pedicel length in 6th position, sporangial length in 7th position and least in sporangial breadth.

In case of VIIth cluster, the isolates were ranked 2nd in L/B ratio and pedicel length, 4th in sporangial length and 5th in sporangial breadth. The VIIIth cluster had the maximum sporangial breadth, 2nd in sporangial length and L/B ratio but 4th in pedicel length.

Compatibility types

Phytophthora isolates of coconut and pepper (except Kannur and Kasaragod isolates of coconut) failed to produce sexual structures in carrot agar medium. In order to induce sexual structures, the isolates were grown on carrot agar medium enriched with B sitosterol and mated with A_1 or A_2 mating types obtained from IISR, Calicut. In presence of the opposite mating type all the isolates produced oospores (Plate 7). However,

there was variations in the abundance, dimensions and days required for the production of sexual structures.

All the coconut isolates except C_7 - C_{16} produced oospores when paired with A_1 and the pepper isolates produced oospores when mated with A_2 mating types. The measurements of sex organs of one isolate of coconut and 25 isolates of pepper were not recorded due to low frequency of formation of oospores.

Nine isolates of pepper (all the soil isolates, P_{39}-P_{46}, and an isolate from Manjappara, pure pepper garden, P_{27}, produced abundant number of oospores within 12 to 15 days (Plate 8). Twelve pepper isolates (P_1 to P_9, P_{15}, P_{16} and P_{18}) and five coconut isolates produced moderate number of sexual structures, while the remaining isolates produced only very few sexual structures (Table 10).

The antheridia of homothallic *Phytophthora* (Kannur and Kasaragod isolates C_7-C_{16}) are in general bigger than the other coconut and pepper isolates (Table 11). Among the heterothallic isolates, antheridia of *Phytophthora* of coconut with a length of 9.36 - 17. 69 um and breadth of 7.28 to 17.68 um were bigger than the pepper isolates. The length and breadth of pepper isolates ranged from 6.2 - 16.64 and 5.9 - 14.56 um respectively. The oogonia and oospore of heterothallic isolates of both coconut and pepper were bigger than the homothallic isolates of coconut. As with antheridia, the oogonia and oospore of heterothallic coconut

Table 10 Comparative abundance of sexual strucutres formed by Phytophthora isolates

Cultures used	Presence	Cultures used	Presence
P_1	++	P_{27}	+++
P_2	++	P_{28}	+
P_3	++	P_{29}	+
P_4	++	P_{30}	+
P_5	++	P_{31}	+
P_6	++	P_{32}	+
P_7	++	P_{33}	+
P_8	++	P_{34}	+
P_9	++	P_{35}	+
P_{10}	+	P_{36}	+
P_{11}	+	P_{37}	+
P_{12}	+	P_{38}	+
P_{13}	+	P_{39}	+++
P_{14}	+	P_{40}	+++
P_{15}	++	P_{41}	+++
P_{16}	++	P_{42}	+++
P_{17}	+	P_{43}	+++
P_{18}	++	P_{44}	+++
P_{19}	+	P_{45}	+++
P_{20}	+	P_{46}	+++
P_{21}	+	C_1	+
P_{22}	+	C_2	++
P_{23}	+	C_3	++
P_{24}	+	C_4	++
P_{25}	+	C_5	++
P_{26}	+	C_6	++

\+ - Rare 1-5/10 fields
++ - Moderate 1-5
+++ - Abundant > 20

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Table 11 Sex organ dimensions of *Phytophthora* isolates from coconut and pepper

	Antheridia		Oogonia	Oospore
	(Vertical)	(Hortizontal)	(diameter)	(diameter)
P_1	10.4 ± 2.08 (8.32-12.48)	8.32 ± 0	35.6 ± 4.2 (29.12-41.6)	31 ± 3.6 (29.12-37.4)
P_2	8.32 ± 4.16 (4.16-12.48)	8.32 ± 0 (8.32)	35.36 ± 9.2 (20.8-49.92)	30.8 ± 8.6 (20.8-45.7)
P_3	8.32 ± 0	6.76 ± 1.72 (4.16-8.32)	35.7 ±7.7 (29.12-49.92)	30.2 ± 6.8 (29.12-45.7)
P_4	15.25 ± 3.92 (12.48-20.8)	11.09 ± 3.9 (8.32-16.64)	27.4 ± 4.6 (20.8-32.28)	23.2 ± 5.0 (20.8-33.2)
P_5	16.64 ± 0 (16.64)	14.56 ± 2.0 (12.48-16.64)	46.9 ± 3.6 (45.76-54.0)	41.8 ± 4.3 (41.6-49.9)
P_6	14.56 ± 2.0 (12.48-16.64)	12.48 ± 0 (12.48)	39.9 ± 4.9 (33.28-45.76)	35.4 ± 4.3 (33.28-45.7)
P_7	12.48 ± 5.8 (8.32-20.8)	12.48 ± 5.8 (4.16-16.64)	36.4 ± 7.4 (24.96-45.76)	31.2 ± 6.3 (24.9-41.6)
P_8	9.36 ± 1.8 (8.32-12.48)	7.28 ± 1.8 (4.16-12.48)	33.28 ± 5.8 (24.96-45.76)	28.2 ± 6.0 (24.9-41.6)
P_9	6.24 ± 2.0 (4.16-8.32)	7.28 ± 3.4 (4.16-12.48)	34.5 ± 3.73 (28.12-41.6)	29.8 ± 4.5 (24.9-37.4)
P_{15}	6.9 ± 1.96 (4.16-8.32)	6.9 ± 3.92 (4.16-12.48)	45.76 ± 5.8 (41.6-54)	41.2 ± 6.3 (37.4-49.9)
P_{16}	8.32 ± 0 (8.32)	10.4 ± 2.08 (8.32-12.48)	38.0 ± 5.18 (29.12-41.6)	33.71 ± 4.8 (29.1-37.4)
P_{18}	12.48 ± 6.5 (4.16-20.8)	12.48 ± 6.5 (4.16-20.8)	38.48 ± 7.9 (24.96-45.76)	33.7 ± 6.4 (24.9-41.6)
P_{27}	8.32 ± 2.6 (4.16-12.48)	6.6 ± 1.5 (4.16-8.32)	26.6 ± 11.3 (24.96-37.4)	22.3 ± 10.2 (20.8-33.2)
P_{39}	8.32 ± 1.68 (4.16-12.48)	7.6 ± 3.7 (4.16-12.48)	29.82 ± 3.6 (29.12-37.4)	24.7 ± 3.4 (20.8-33.2)

	Antheridia		Oogonia	Oospore
	(Vertical)	(Hortizontal)	(diameter)	(diameter)
P_{40}	9.8 ± 2.5 (4.16-12.48)	5.9 ± 2.0 (4.16-8.32)	31.4 ± 3.89 (24.96-37.4)	26.2 ± 3.4 (24.9-33.2)
P_{41}	7.68 ± 2.6 (4.16-12.48)	9.2 ± 2.8 (4.16-12.48)	34.12 ± 4.0 (24.96-41.6)	29.2 ± 4.6 (24.96-41.6)
P_{42}	9.0 ± 2.8 (4.16-12.48)	10.7 ± 2.65 (8.32-12.48)	35.7 ± 3.5 (29.12-41.6)	31.0 ± 3.6 (29.1-37.4)
P_{43}	8.7 ± 2.7 (4.16-12.48)	8.7 ± 2.1 (4.16-12.48)	33.2 ± 4.3 (24.96-41.6)	28.4 ± 3.4 (24.9-37.4)
P_{44}	8.32 ± 3.16 (4.16-12.48)	8.6 ± 3.2 (4.16-12.48)	31.4 ± 3.98 (24.96-37.4)	26.8 ± 4.2 (24.9-37.4)
P_{45}	9.0 ± 2.8 (4.16-12.48)	9.36 ±2.3 (6.24-12.48)	31.8 ± 4.3 (22.96-41.6)	26.2 ± 4.8 (24.9-37.4)
P_{46}	8.32 ± 2.4 (4.16-12.48)	7.6 ± 1.5 (4.16-8.32)	29.4 ± 4.4 (20.8-37.4)	24.3 ± 5.0 (20.8-33.2)
C_2	13.8 ± 5.18 (8.32-20.8)	12.48 ± 5.8 (8.32-20.8)	42.9 ± 5.1 (33.28-49.92)	37.1 ± 5.8 (33.2-45.7)
C_3	12.48 ± 3.39 (8.32-16.64)	11.09 ± 1.96 (8.32-12.48)	46.2 ± 1.96 (33.28-41.6)	35.3 ± 3.0 (33.28-37.4)
C_4	9.36 ± 8 (8.32-12.48)	7.28 ± 1.8 (4.16-8.32)	39.52 ± 4.6 (32.28-45.76)	34.8 ± 5.2 (32.28-41.6)
C_5	13.0 ± 4.6 (8.32-20.8)	13.0 ± 5.18 (4.16-16.64)	38.6 ± 6.1 (24.96-49.2)	33.7 ± 7.0 (29.96-45.7)
C_6	17.69 ± 3.4 (12.48-20.8)	17.68 ± 3.4 (12.48-20.8)	46.8 ± 9.47 (37.4-62.4)	42.6 ± 10.2 (33.2-58.2)
C_7	18.02 ± 2.42 (16.64-20.8)	11.1 ± 2.4 (8.32-12.48)	22.5 ± 2.3 (20.8-24.96)	18.1 ± 2.6 (16.6-20.8)
C_8	24.26 ± 2.8 (20.8-29.12)	21.4 ± 1.55 (20.8-24.9)	27.7 ± 4.5 (20.8-33.28)	23.4 ± 5.3 (20.8-29.12)
C_9	23.32 ± 2.3 (20.8-24.96)	19.1 ± 3.7 (16.64-24.9)	27.4 ± 2.2 (24.9-29.12)	22.8 ± 2.2 (20.8-24.9)

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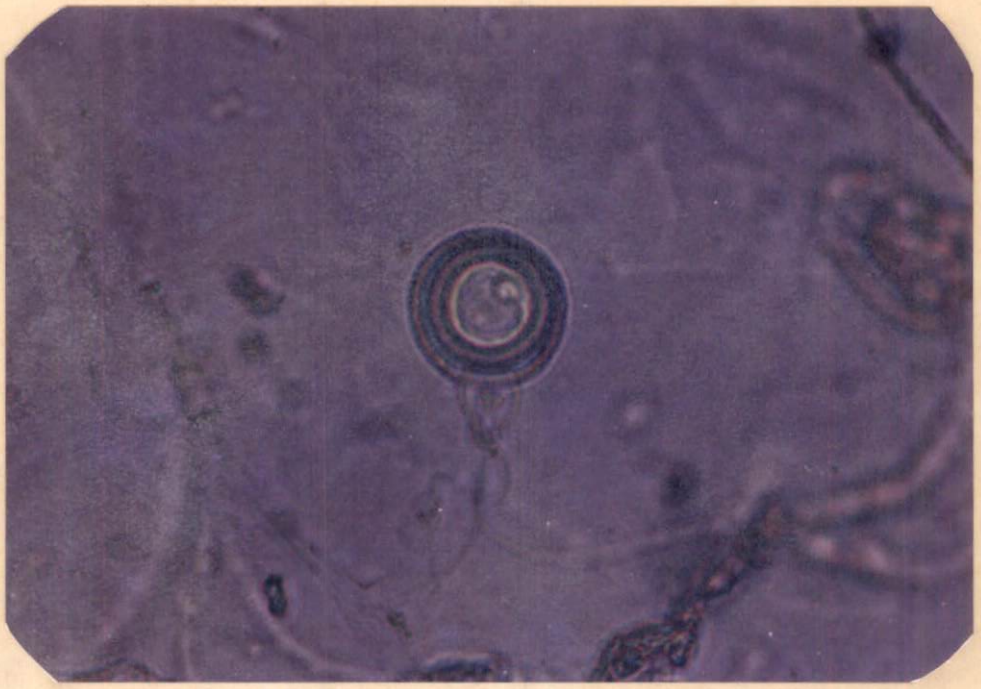
	Antheridia		Oogonia	Oospore
	(Vertical)	(Hortizontal)	(diameter)	(diameter)
C_{10}	24.12 ± 3.1 (20.8-29.9)	21.6 ± 3.1 (16.64-24.9)	29.9 ± 3.1 (24.96-33.3)	25.2 ± 3.7 (24.9-33.3)
C_{11}	14.1 ± 2.2 (12.48-16.64)	11.6 ± 1.8 (8.32-12.48)	18.3 ± 2.3 (16.68-20.8)	14.4 ± 1.6 (12.48-16.6)
C_{12}	20.8 ± 2.96 (16.64-24.96)	16.6 ± 2.19 (12.48-20.8)	24.1 ± 3.48 (20.8-29.9)	20.8 ± 4.2 (16.6-24.9)
C_{13}	22.9 ± 2.4 (20.8-24.96)	20.8 ± 3.4 (16.64-24.96)	26.6 ± 4.7 (20.8-33.28)	21.6 ± 4.3 (20.8-29.9)
C_{14}	18.3 ± 3.7 (12.48-20.8)	16.6 ± 2.93 (12.48-20.8)	27.4 ±2.25 (24.96-29.9)	22.1 ± 3.2 (20.8-29.9)
C_{15}	14.1 ± 2.24 (12.48-16.04)	12.5 ± 0 (12.5)	21.6 ± 5 (16.64-24.96)	16.9 ± 3.2 (12.48-20.8)
C_{16}	12.48 ± 0 (12.48)	9.15 ± 1.86 (8.32-12.48)	15 ± 2.24 (12.48-16.64)	13 ± 1.8 (12.48-16.04)

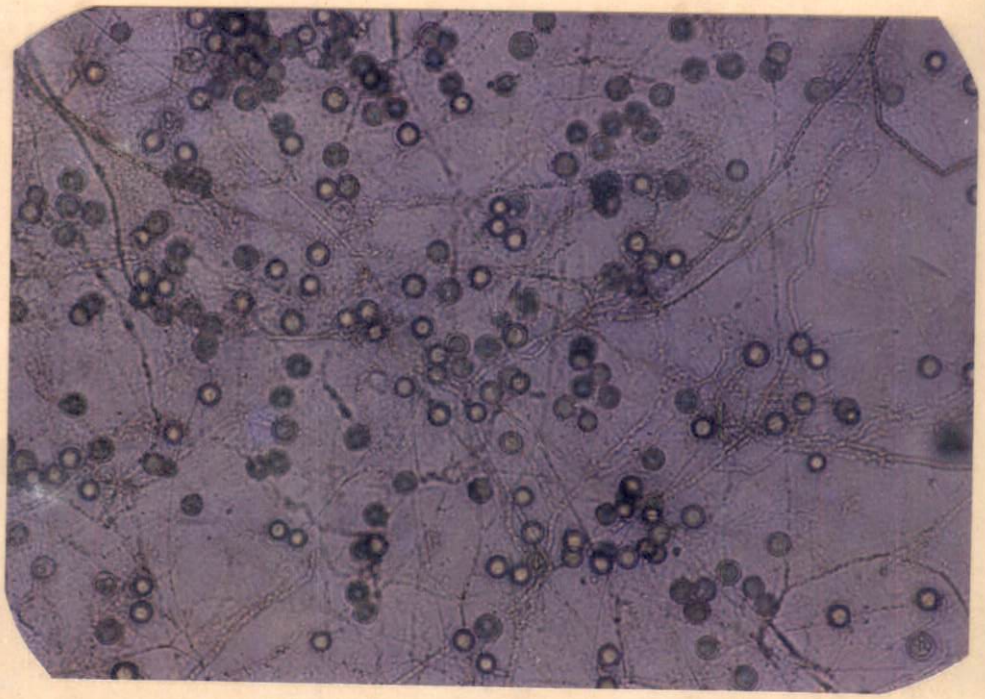
Plate 7.

Oogonia of *Phytophthora* with amphigynous antheridia

Plate 8.

Soil isolates of *P. capsici* producing abundant oospores





isolates with a diameter range of 38.6 - 46.8 and 33.7 - 42.6 um were bigger than the pepper isolates with a diameter of 26.6 - 46.9 and 22.3 - 41.8 um.

Effect of different media on growth of coconut and pepper isolates

The radial growth

The growth of coconut (C_2) and pepper isolate (P_{30}) was compared at room temperature on seven different media. Both the isolates failed to grow on Czapek Dox, pepper leaf extract and coconut leaf extract media (Table 12).

The maximum growth of C_2 was noticed when it was grown on oats media, while, the growth of the isolates on carrot agar and PDA were significantly better than on coconut water agar (Fig. 4). For P_{30} isolate also, maximum growth was noticed when grown on oat meal agar. However, for this isolate, growth on carrot agar and coconut water were significantly better than that of PDA.

Effect of different media on sporangial characters

Two coconut isolates (C_2, C_3) and three pepper isolates (P_{20}, P_{30}, P_{42}) were grown on 4 different media viz. coconut water agar, carrot agar, PDA and oats and its effect on the sporangial characters was studied using five day old cultures.

Table 12 Effect of different media on radial growth of *Phytophthora* isolates

Media used	Growth after 2 days		Growth after 4 days		Growth after 6 days	
	Coconut C_2	Pepper P_{30}	Coconut C_2	Pepper P_{30}	Coconut C_2	Pepper P_{30}
Coconut water	3.6	4.0	5.0	5.0	7.0	7.2
Carrot agar	3.5	4.0	5.5	5.0	6.9	7.0
Potato Dextrose agar	3.0	3.5	5.3	4.0	7.5	5.1
Oats	5.6	4.5	7.6	5.5	9.0	7.5
Czapak Dox	0	0	0	0	0	0
Host extract (Pepper leaf)	0	0	0	0	0	0
Host extract (Coconut leaf)	0	0	0	0	0	0

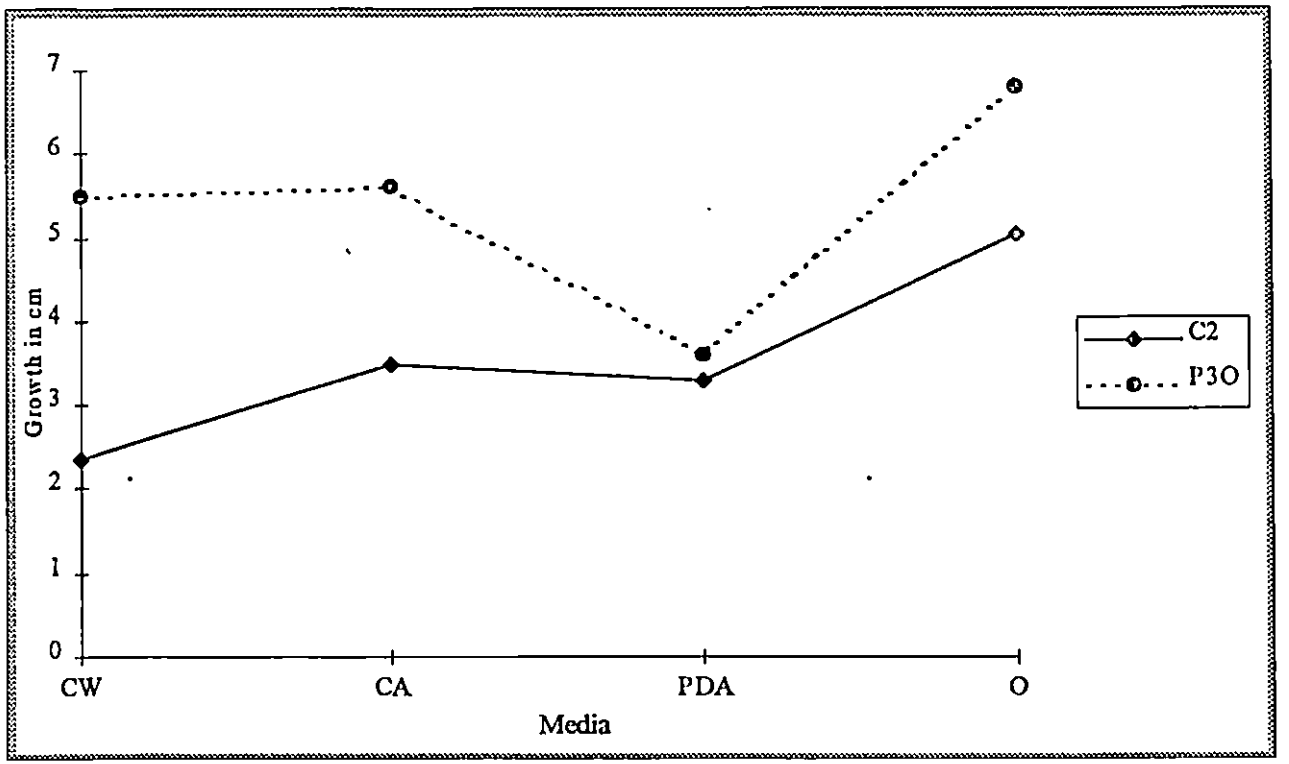
CD

Isolate (A) - 0.042 AB - 0.085

Media (B) - 0.060 AC - 0.070

Period (C) - 0.051 BC - 0.100

Fig-4 Effect of media on growth of selected *Phytophthora* isolates



CW - Coconut Water
CA - Carrot Agar
PDA - Potato Dextrose Agar
O - Oats

The sporangial length of the isolates varied with the host and the medium (Table 13). In general maximum sporangial length was noticed in the isolate P_{30}, C_2 and P_{20} and they did not differ significantly from one another. While, least sporangial length was noticed by P_{42} which was on par with C_3 (Fig. 5). All the isolates produced longer sporangia when grown on coconut water and shortest on oats except by the isolate C_3 where the shortest was noticed when grown on PDA.

Maximum sporangial breadth was noticed when the isolates were grown in coconut water while, smallest size sporangia were generally seen on PDA (Fig. 6). The sporangial breadth of P_{20} and P_{30} did not differ significantly when grown on different media. Among the pepper isolates P_{42} has the narrowest sporangia.

Pedicel length

The pedicel length of 2 coconut isolates (C_2 and C_3 and 3 pepper isolates (P_{20}, P_{30} and P_{42}) were compared by growing them on four different media namely coconut water agar, carrot agar, oatmeal agar and PDA. In general, the pedicel length of coconut isolates were less influenced by the media compared to that of pepper isolates (Table 14). The maximum variation of pedicel length of coconut isolate was only 9.1 um (16.6 - 25.7 um) while it was 57.4 um in pepper isolates. When pedicel length of all the isolates were compared, it was higher on those grown

Table 13 Effect of different media on sporangial length and breadth of Phytophthora isolates

Sporangial length

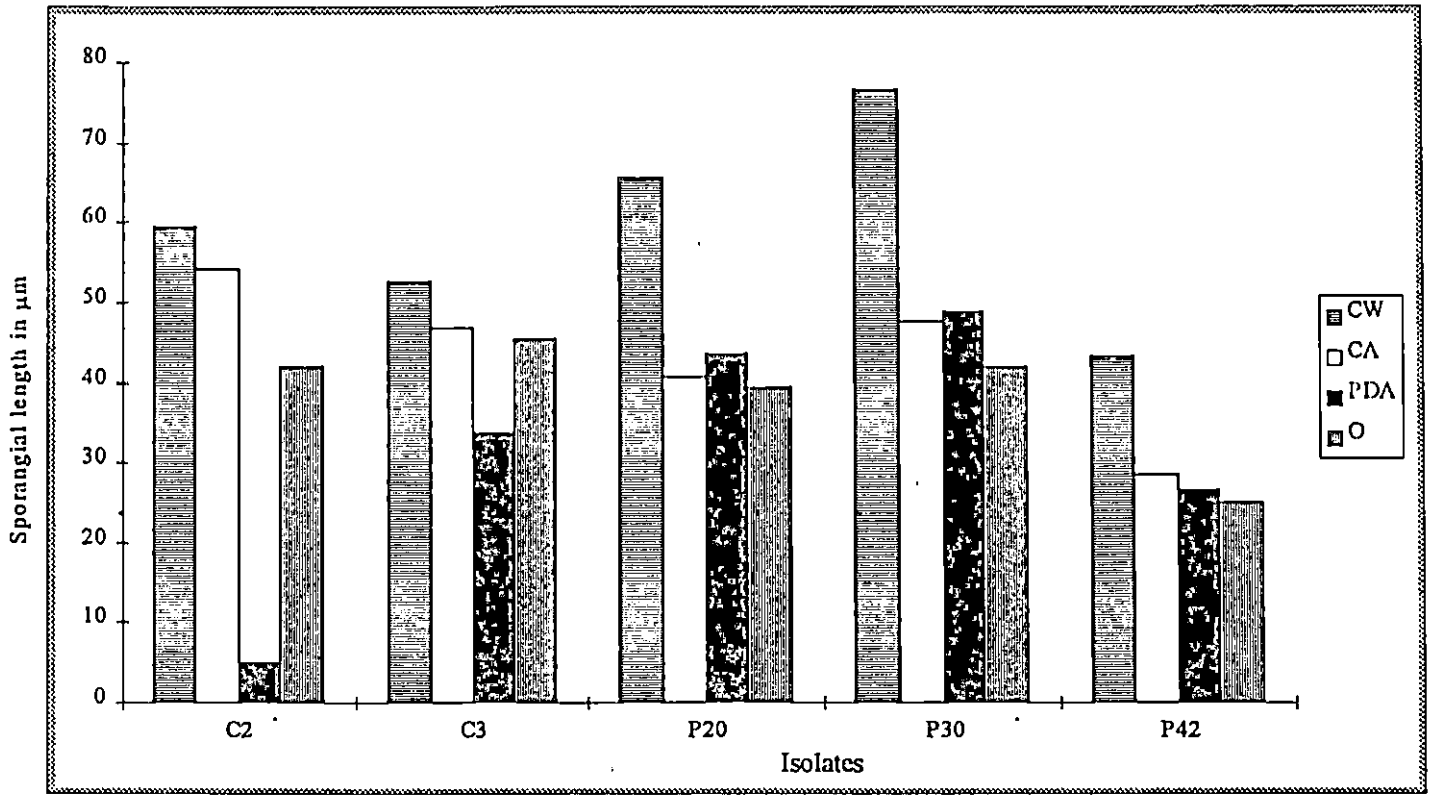
Cultures used	Coconut water	Carrot agar	PDA	Oats
C_2	60.3 ± 14.9 (37.4-79.04)	54.8 ± 13.2 (37.4-74.8)	51.1 ± 13.2 (41.6-93.12)	42.4 ± 6.6 (33.28-49.92)
C_3	52.4 ± 20.0 (33.28-79.04)	47.5 ± 9.3 (32.28-66.56)	33.8 ± 3.2 (29.12-37.4)	45.76 ± 9.1 (37.4-62.4)
P_{20}	67.39 ± 23.7 (45.76-112.3)	42.4 ± 19.5 (20.8-74.8)	43.68 ± 5.2 (37.4-54.0)	39.9 ± 10.0 (29.12-58.24)
P_{30}	79.04 ± 21.12 (33.28-10.4)	49 ± 19.0 (24.96-74.8)	47.84 ± 4.6 (41.6-54)	42.29 ± 6.5 (33.28-54)
P_{42}	35.06 ± 11.52 (16.64-58.24)	29.12 ± 8.32 (16.64-41.6)	26.8 ± 6.1 (24.96-33.28)	24.96 ± 3.7 (20.8-29.5)

Sproangial breadth

Cultures used	Coconut water	Carrot agar	PDA	Oats
C_2	39.52 ± 5.2 (29.12-45.76)	34.84 ± 6.8 (24.9-41.6)	43.38± 2.0 (41.6-45.76)	40.7 ± 6.1 (33.28-49.92)
C_3	51.5 ± 20.8 (33.28-79.04)	40.4 ± 5.7 (33.28-45.76)	33.3 ± 3.5 (29.12-37.4)	41.6 ± 3.7 (37.40-45.76)
P_{20}	33.97 ± 5.4 (20.8-37.4)	28.28 ± 3.1 (24.96-33.28)	25.6 ± 1.5 (24.96-29.12)	31.6 ± 4.24 (24.96-37.4)
P_{30}	35.7 ± 3.3 (33.28-41.6)	26.3 ± 3.10 (24.96-29.12)	26 ± 3.4 (20.8-29.12)	28.4 ± 2.8 (24.96-33.28)
P_{42}	25.6 ± 5.5 (16.64-33.28)	23.2 ± 4.2 (16.64-33.28)	20.8 ± 7.7 (12.48-33.28)	24.12 ± 3.1 (20.8-29.12)

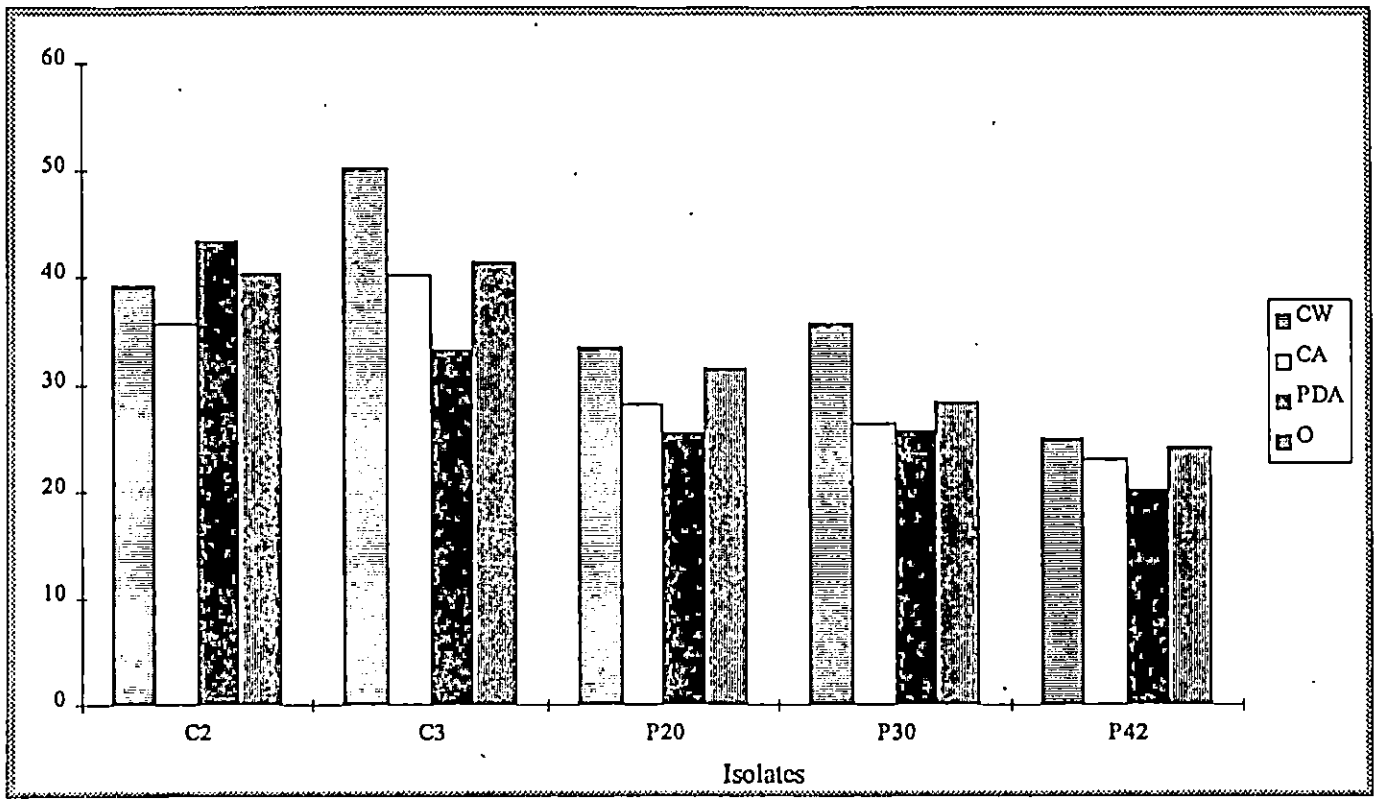
CD	Sporangial length	Breadth
Isolate (A)	1.25	0.682
Media (B)	0.626	0.341
AB	0.560	0.305

Fig-5 **Effect of media on Sporangial length of selected *Phytophthora* isolates**



CW - Coconut Water
CA - Carrot Agar
PDA - Potato Dextrose Agar
O - Oats

Fig. 6 **Effect of media on Sporangial breadth of selected *Phytophthora* isolates**



CW - Coconut Water
CA - Carrot Agar
PDA - Potato Dextrose Agar
O - Oats

Table 14 Effect of different media on pedicel length and caducity of *Phytophthora* isolates

Isolate number	Coconut water		Carrot agar		PDA		Oats	
	Pedicel length (um)	Caducity (%)	Pedicel length (um)	caducity (%)	Pedicel length (um)	Caducity (%)	Pedicel length (um)	Caducity (%)
C_2	22.4±10.0 (8.3-33.3)	33	25.7±8.05 (12.5-37.4)	21.8	16.64±5.2 (18.3-24.9)	15.3	19.13±8.5 (8.3-29.1)	20
C_3	23.29±6.7 (16.6-29.1)	16.6	21.6±5.4 (12.5-29.1)	18.5	15.2±1.96 (12.48-16.6)	14.2	12.48±3.33 (8.3-16.6)	15.3
P_{20}	106.4±4.4 (62.4-133.1)	71.4	48.6±6.2 (37.4-58.2)	25	76.96±20.9 (45.7-99.8)	49	85.2±22.8 (49.9-112.3)	28.5
P_{30}	98±16.2 (54-99.8)	45.8	68.64±26.06 (29.1-120.6)	45.4	63.4±17.2 (41.6-87.3)	29.16	88.4±26.6 (62.4-128.6)	46.15

CD	Sporangial length	Caducity
Isolate (A)	1.548	0.307
Media (B)	0.774	0.275
AB	0.692	0.615

on coconut water followed by oat meal agar, while, it was least on carrot agar (Fig. 7). There was no significant difference among the three isolates of pepper. Similarly coconut isolate also did not differ significantly. However, the coconut isolate had shorter pedicel length compared to the pepper isolates. Maximum pedicel length in coconut isolate was noticed when grown on coconut water agar media. However, there was no preference for a particular media.

L/B ratio

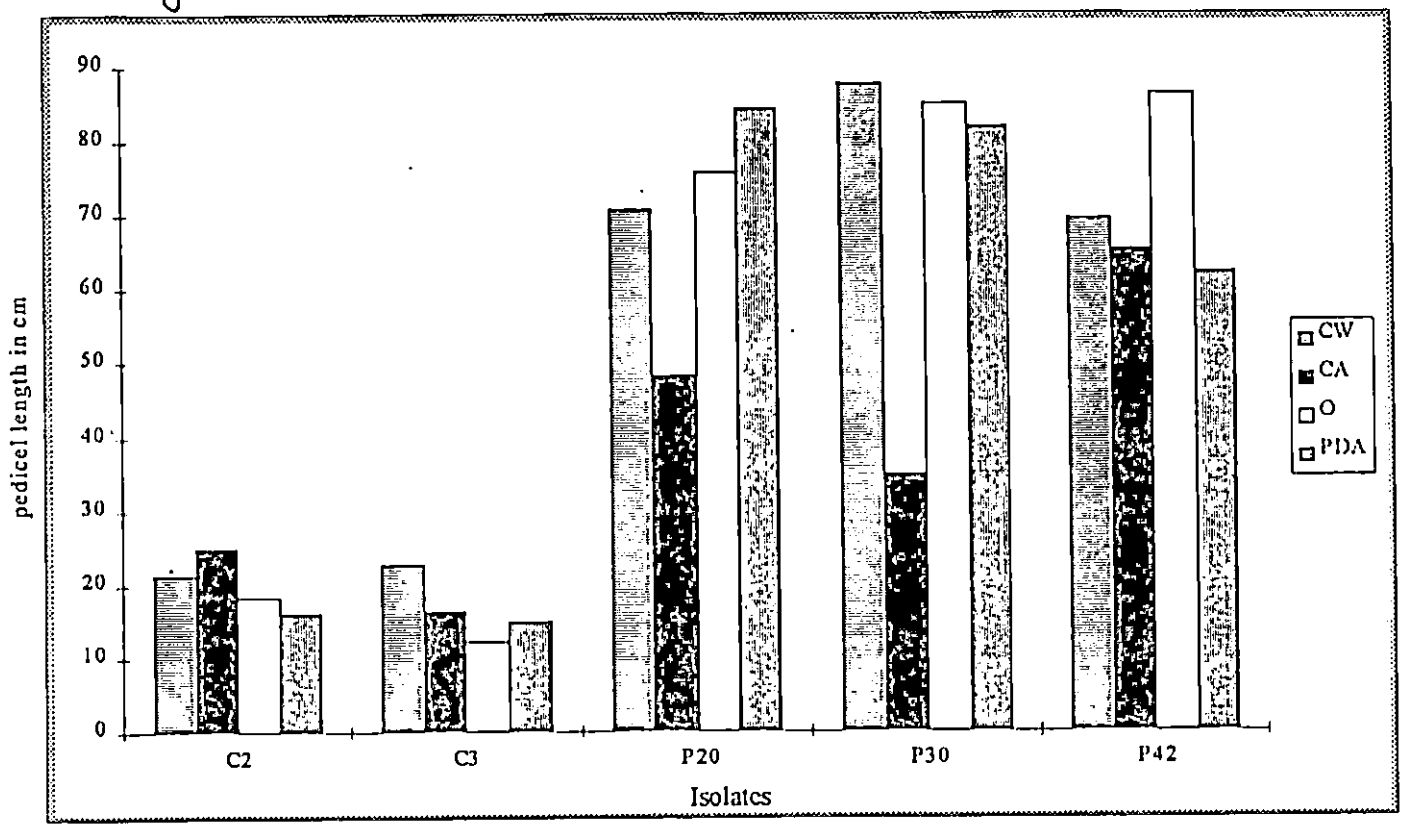
The variation in the L/B ratio of sporangia on different media was less pronounced than in pepper isolates (Table 15). Among the coconut isolates maximum L/B ratio of 1.57 ± 0.31 was noticed with C_2 isolates grown on carrot agar medium while minimum L/B ratio of 1.01 ± 0.4 observed in C_3 isolate grown on PDA.

In all the pepper isolates maximum L/B ratio was noticed in coconut water medium and the minimum in oat meal agar. Compared to a L/B ratio of 2.44 for P_{20} in coconut water, the corresponding value was only 1.24 in oat meal agar. A similar trend was also noticed in other isolates of pepper.

Caducity

Caducity of *Phytophthora* isolates from coconut and pepper showed wide variability when grown on different

Fig-7 **Effect of media on pedicel length of selected *Phytophthora* isolates**



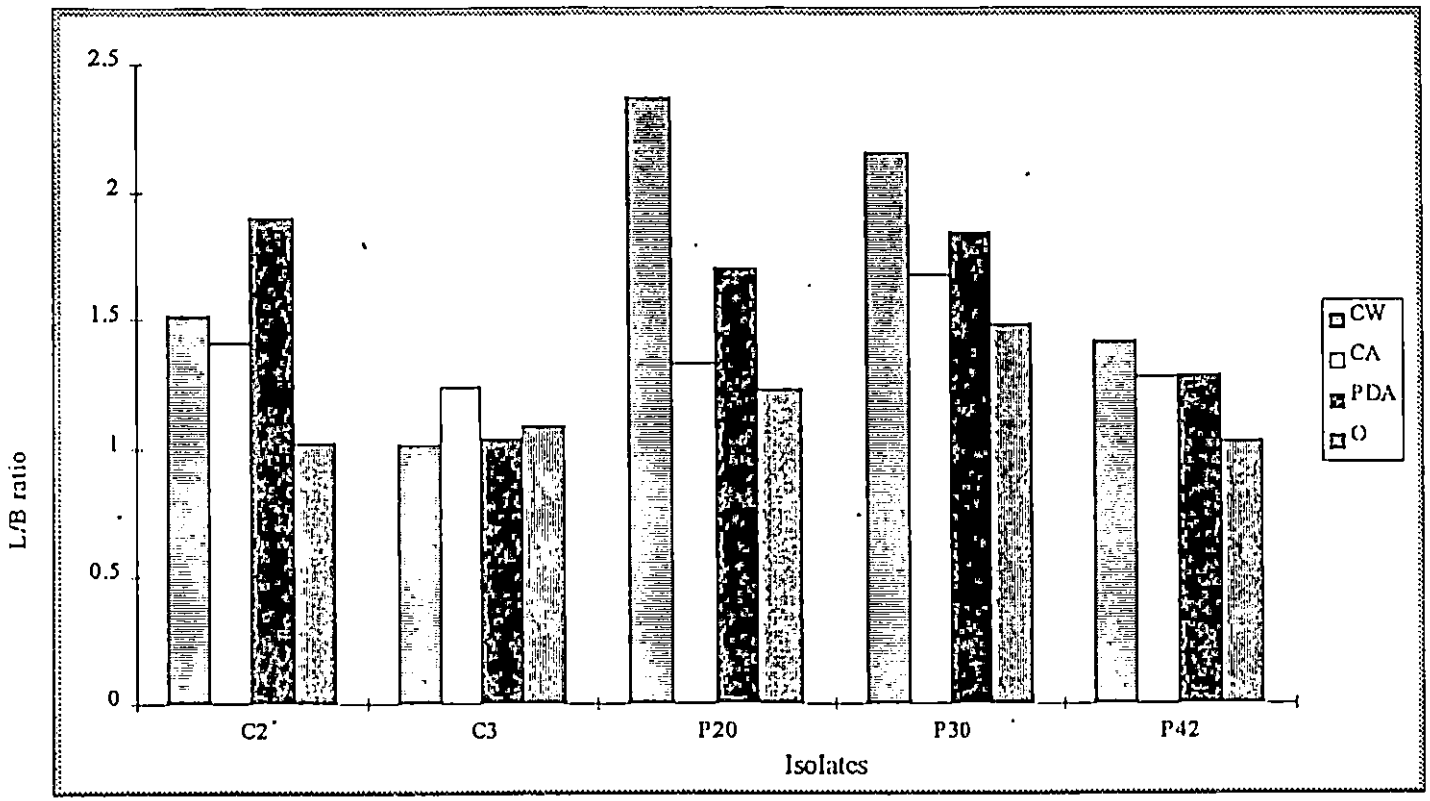
CW - Coconut Water
CA - Carrot Agar
PDA - Potato Dextrose-Agar
O - Oats

Table 15 Effect of different media on L/B ratio of *Phytophthora* isolates

Cultures used	Coconut water	Carrot agar	P.D.A.	Oatmeal agar
C_2	1.53 ± 0.36 (1.0 - 2.0)	1.57 ± 0.31 (1.0 - 2.1)	1.2 ± 0.25 (1.0 - 1.8)	1.03 ± 0.04 (1.0 - 1.1)
C_3	1.02 ± 0.05 (1.0 - 1.13)	1.26 ± 0.28 (1.0 - 1.85)	1.01 ± 0.04 (1.0 - 1.14)	1.1 ± 0.2 (1 - 1.5)
P_{20}	2.44 ± 1.04 (1.7 - 4.5)	1.34 ± 0.29 (1.0 - 1.85)	1.71 ± 0.24 (1.5 - 2.16)	1.24 ± 0.15 (1.13 - 1.55)
P_{30}	2.19 ± 0.75 (1.0 - 3.13)	1.73 ± 0.49 (1.16 - 3.2)	1.85 ± 0.21 (1.66 - 2.2)	1.51 ±0.34 (1.14 - 2.16)
P_{42}	1.45 ± 0.84 (1.0 - 3.5)	1.29 ± 0.23 (1.0 - 1.7)	1.3 ± 0.18 (1.0 - 1.5)	1.03 ± 0.06 (1.0 - 1.16)

CD	Isolate (A)	0.165
	Media (B)	0.827
	AB	0.073

Fig-8 **Effect of media on L/B ratio of selected *Phytophthora* isolates**



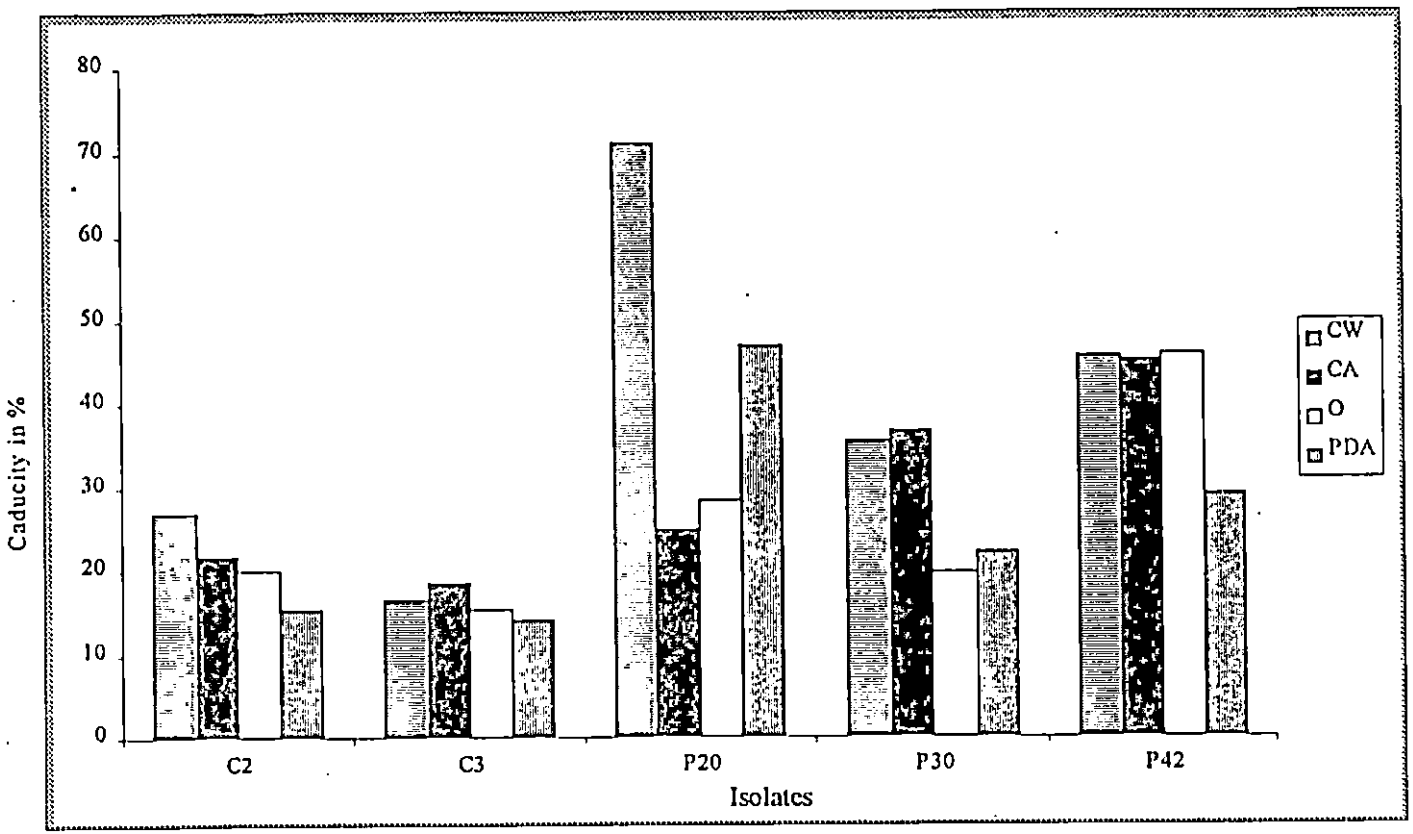
CW - Coconut Water
CA - Carrot Agar
PDA - Potato Dextrose Agar
O - Oats

media (Table 14). Caducity of the isolates was more pronounced when grown on coconut water medium. The percentage variation in caducity of C_3 isolate ranged from 14.2 to 16.6 when grown on different media while the range for P_{20} isolates was from 25 to 71.4. In general, the caducity of the sporangia was highest on coconut water followed by carrot agar (Fig. 9). Higher caducity was observed for pepper isolates. Different isolates showed different response to media. In coconut water and PDA, P_{20} exhibited the maximum caducity while for C_3, the media supported the least caducity. Irrespective of the media and isolate, C_3 was least caducous.

Effect of light on growth and sporulaton

Two coconut isolates (C_2, C_3) and three pepper isolates (P_{20}, P_{30} and P_{42}) were used for this study. The cultures in carrot agar media were exposed to three light conditions i.e., continuous dark, continuous light and intermittent dark and light. All the five cultures were incubated at room temperature and the growth variation recorded over a period of 144 hrs. No significant difference was noticed between C_2 and C_3. Both the coconut isolates completely filled a 9 cm petri dish with in 144 hrs when exposed to continuous light and intermittent light and dark, While these isolates kept in continuous dark it covered 8.4 - 8.5 cm during this period (Table 16).

Fig-9 Effect of media on caducity of selected *Phytophthora* isolates



CW - Coconut Water
CA - Carrot Agar
PDA - Potato Dextrose Agar
O - Oats

Table 16 Effect of light on growth of phytophthora isolates from coconut and pepper (in cm)

Cultures used	Growth after 48hrs			Growth after 96hrs			Growth after 144hrs		
	Complete Dark	Dark/ Light	Complete Light	Complete Dark	Dark/ Light	Complete Light	Complete Dark	Dark/ Light	Complete Light
C_2	2.7	3.7	3.7	5.5	6.7	7.6	8.5	9.0	9.0
C_3	2.6	3.2	3.5	5.9	6.3	7.8	8.4	9.0	9.0
P_{20}	2.0	2.7	3.5	4.2	5.1	5.6	6.0	8.6	8.5
P_{30}	2.0	3.0	2.5	4.6	5.4	6.4	6.3	8.0	8.5
P_{42}	6.5	7.0	7.4	9.0	9.0	9.0	9.0	9.0	9.0

CD - 0.107

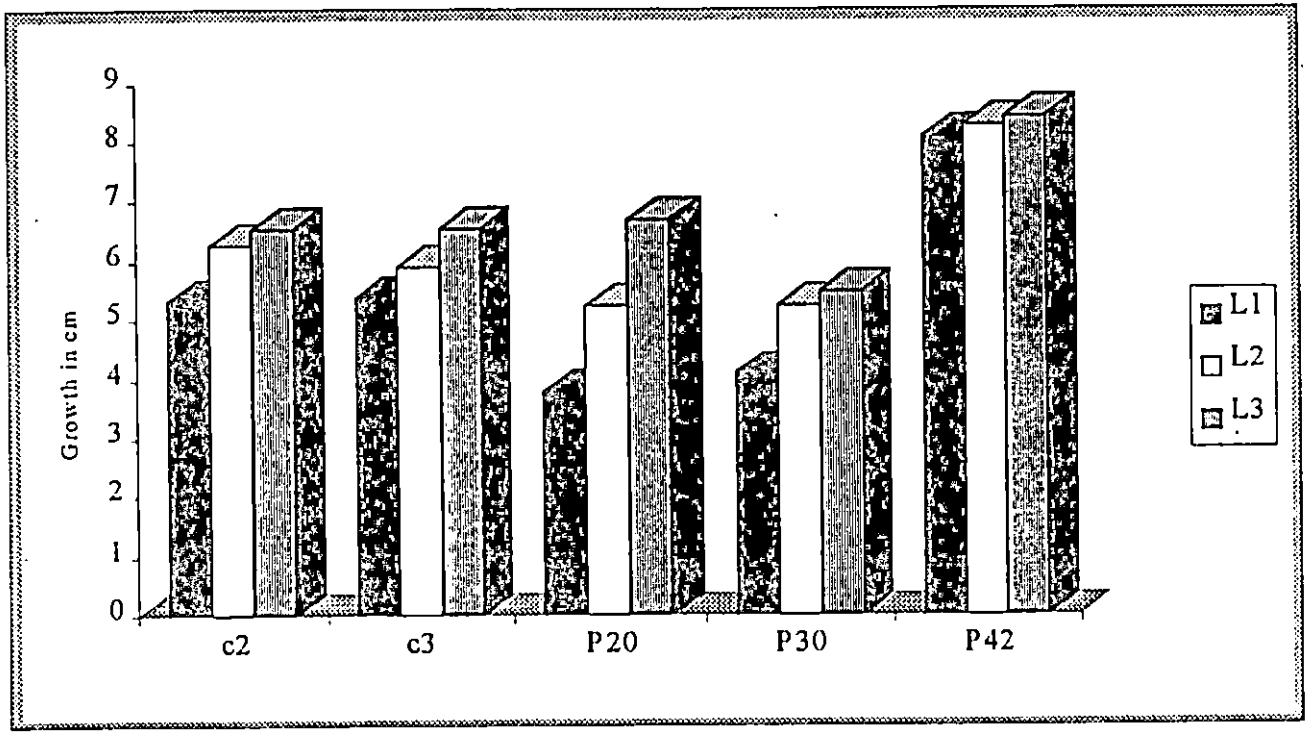
Irrespective of the intensity of light, among pepper isolates, maximum growth was observed in P_{42}. While the minimum was recorded with P_{20} and P_{30} cultures (Fig. 10). Growth of P_{42} was not influenced by light . In all the isolates, higher growth rate was observed by continuous light followed by intermittent light and complete darkness. Only with C_3, the complete light and alternate light and dark condition had significant difference.

Light influenced sporangial production (Table 17). When the C_2 and P_{42} cultures were kept in continuous darkness, they failed to produce sporangia. Even the other cultures (C_3, P_{20} and P_{30}) produced only very few sporangia when kept in dark. However, there was no marked variation in sporangial production when the cultures were exposed to alternate light and dark or to continuous light. C_2 and P_{20} isolates produced sporangia within 3 days with the continuous and alternate dark and light condition and the other cultures produced sporangia within 5 days of incubation.

Effect of pH on growth of *Phytophthora*

Differential response was observed with cultures under different pH levels (Fig. 11). The isolate P_{42}, growth rate did not differ significantly when grown on media with a pH range of six to nine, while it was significantly higher compared to the growth observed at pH 5 (Table 18). In P_{20} and P_{30}, slightly

Fig-10 **Effect of Light on growth of *Phytophthora* isolates**



L1 - Complete Darkness
L2 - Dark/Light
L3 - Complete Light

Table 17 Effect of light on sporangium production (in days) of selected *Phytophthora* isolates from coconut and pepper

Cultures used	Dark	Dark/Light	Light
C_2	No sporangia	3rd day	3rd day
C_3	6th day (very few)	5th day	4th day
P_{20}	6th day (very few)	3rd day	3rd day
P_{30}	6th day (very few)	5th day	5th day
P_{42}	No sporangia	5th day	5th day

Fig-11 **Effect of pH on growth of selectd *Phytophthora* isolates**

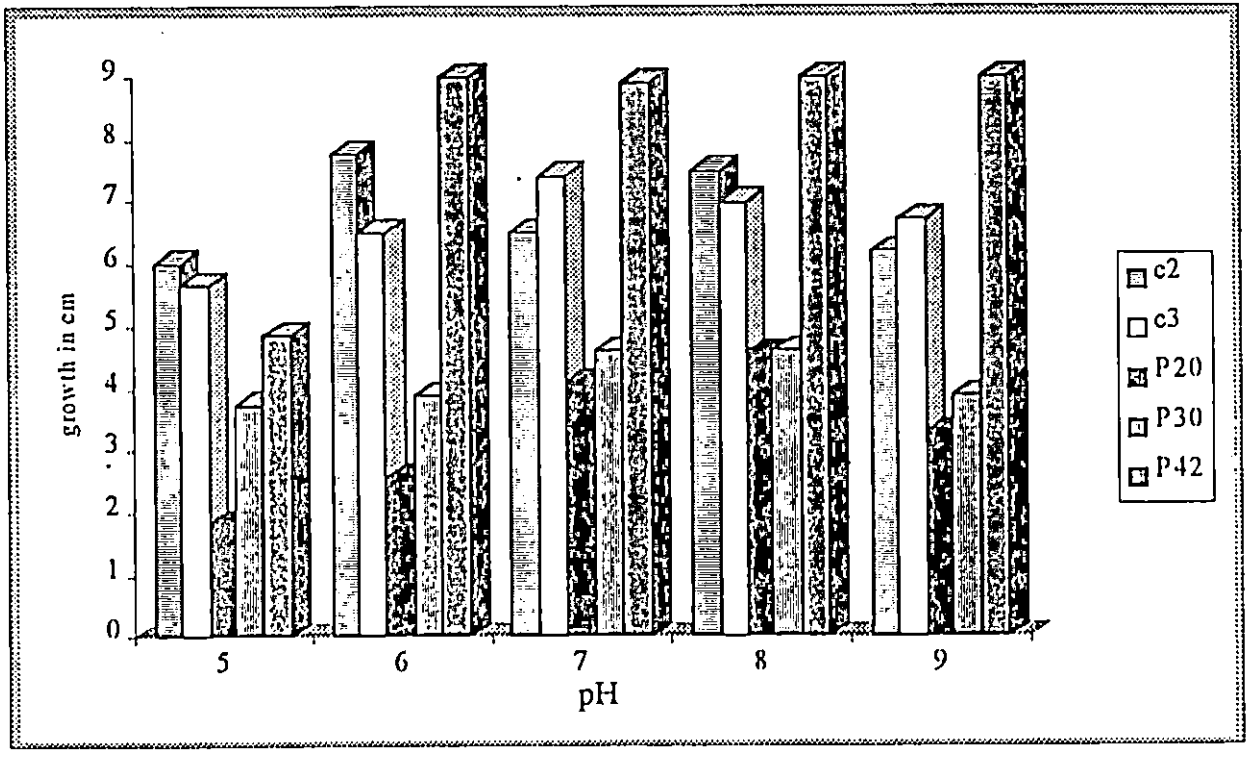


Table 18 Effect pH on growth of selected *Phytophthora* isolates from coconut and pepper (in cm)

Isolates used	pH of the medium	Growth after (hrs)			
		48	72	96	120
	5	3.3	5.6	5.1	8.6
	6	6.0	8.0	8.5	8.7
C_2	7	4.8	6.0	6.6	9.0
	8	5.5	7.2	8.6	9.0
	9	4.5	5.2	7.0	8.5
	5	3.6	4.9	6.0	8.7
	6	4.6	5.0	6.9	8.4
C_3	7	5.5	7.2	8.2	9.0
	8	4.7	6.0	8.0	8.7
	9	5.8	7.7	8.8	9.0
	5	0	2.0	2.6	3.4
	6	1.0	2.1	3.2	4.7
P_{20}	7	2.5	3.7	4.2	6.5
	8	2.2	4.0	4.8	8.2
	9	2.0	2.3	3.2	6.2
	5	2.3	3.4	3.0	6.5
	6	2.6	2.6	4.2	6.8
P_{30}	7	2.8	3.6	5.3	7.0
	8	2.8	4.0	5.0	7.2
	9	2.4	3.3	4.5	6.0
	5	2.1	3.8	5.5	9.0
	6	9.0	9.0	9.0	9.0
P_{42}	7	9.0	9.0	9.0	9.0
	8	9.0	9.0	9.0	9.0
	9	9.0	9.0	9.0	9.0

CD - 0.142

alkaline (pH 8) to neutral pH (pH 7) was significantly superior to other pH levels. For the culture C_2, maximum growth was at six and eight and the least was at pH 5. No significant difference in growth rate was noticed in pH 5, 7 and 9 while in C_3, growth was maximum at pH 7 and minimum at pH 6.

Pathogenicity tests

Pathogenicity tests were carried out using all the 62 isolates of *Phytophthora* on leaves, nuts and seedlings of coconut and rooted cuttings of pepper.

Coconut

Detached tender leaves of coconut were susceptible to infection by all the 62 isolates of *Phytophthora*, but the intensity of infection was high in Kannur and Kasaragod isolates (C_7 - C_{16}) compared to other coconut and pepper isolates (Table 19). The lesion size of Kannur and Kasaragodu isolates, 72 hrs after inoculation, ranged from 4.2 to 7 cm compared to 1.6 to 5.7 for other coconut isolates and 1.3 to 6.0 for pepper isolates. Among the pepper isolates the maximum lesion size of 6.0 cm was recorded from an isolate (P_4 collected from a pure pepper garden in Kozhikode. Marked variability in the lesion shape on coconut leaves were noticed when inoculated with different isolates. Six lesion shapes were noticed, viz. spindle round, oval, irregular, linear with round ends and square.

Table 19 Differential response of various *Phytophthora* isolates on coconut and pepper

Isolate Number	On pepper plants lesion size after (hrs)				Coconut leaf lesion Size after			Days required for infection	
	24	48	72	96	24	48	72	Tender coconut	Coconut seedlings
P_1	-	1.0	3.2	6.2 (Full leaf)	-	0.7	2.4	5	9
P_2	-	0.9	1.8	4.6	-	0.8	1.5	5	24
P_3	-	0.8	1.7	3.4	-	1.1	3.6	7	-
P_4	-	0.7	1.6	3.5	0.4	1.7	6.0	8	-
P_5	-	0.7	1.3	2.7	-	0.6	1.8	8	24
P_6	-	-	1.0	2.7	-	1.0	2.2	8	-
P_7	-	-	1.0	5.6	0.5	1.1	2.6	7	-
P_8	0.9	1.6	7.0	Full leaf	-	0.7	1.3	8	16
P_9	-	0.6	1.8	5.2	-	0.6	1.8	8	-
P_{10}	-	-	0.5	5.4	-	0.7	1.7	8	-
P_{11}	-	1.1	2.5	4.5	-	1.0	3.0	6	-
P_{12}	-	0.6	2.3	6.0	0.3	1.0	3.1	6	-
P_{13}	-	0.8	2.1	4.3	-	0.7	1.9	7	-
P_{14}	-	1.0	2.4	5.1	-	0.8	5.2	6	-
P_{15}	-	1.0	2.0	5.3	-	0.9	4.0	6	-
P_{16}	-	0.9	1.7	3.6	0.4	1.1	3.6	7	-
P_{17}	-	1.1	3.0	5.0	0.3	1.0	3.1	7	-
P_{18}	-	0.6	1.3	3.7	0.3	1.0	3.0	7	-
P_{19}	-	1.2	2.7	6.2 (full leaf)	0.8	2.2	5.7	8	-

Isolate Number	On pepper plants lesion size after (hrs)				Coconut leaf lesion Size after			Days required for infection	
	24	48	72	96	24	48	72	Tender coconut	Coconut seedlings
P_{20}	0.5	1.1	6.0 (full leaf)	(full leaf)	-	0.7	1.6	7	-
P_{21}	-	0.7	1.9	6.3 (full leaf)	-	0.6	1.2	7	-
P_{22}	0.6	1.7	3.0	6.0 (full leaf)	-	0.8	1.7	7	-
P_{23}	-	1.5	3.0	6.0 (full leaf)	0.5	1.0	4.0	8	-
P_{24}	-	0.8	2.2	4.5	0.5	1.3	4.0	7	-
P_{25}	0.8	1.9	4.0	7.0 (full leaf)	0.4	1.0	2.5	8	-
P_{26}	0.6	1.4	3.2	6.5 (full leaf)	0.3	1.1	3.4	7	-
P_{27}	-	1.0	2.5	6.2 (full leaf)	-	0.8	1.8	7	-
P_{28}	0.6	1.7	3.7	6.4 (full leaf)	-	0.6	1.6	7	-
P_{29}	-	0.6	2.0	6.4 (full leaf)	-	0.5	3.2	6	-
P_{30}	-	0.9	2.3	5.6 (full leaf)	0.3	0.9	3.4	8	-
P_{31}	0.4	1.1	3.7	6.0 (full leaf)	0.4	1.0	3.0	7	-

Isolate Number	On pepper plants lesion size after (hrs)				Coconut leaf lesion Size after			Days required for infection	
	24	48	72	96	24	48	72	Tender coconut	Coconut seedlings
P_{32}	-	1.0	3.0	6.5 (full leaf)	0.3	1.0	2.7	8	-
P_{33}	-	0.9	2.2	1.6	0.4	1.0	2.9	8	16
P_{34}	0.7	1.4	2.8	5.2	0.6	1.4	4.1	6	16
P_{35}	0.4	1.3	2.8	6.1 (full leaf)	-	0.9	4.0	6	-
P_{36}	0.5	1.2	2.6	6.0 (full leaf)	-	0.7	3.8	6	-
P_{37}	-	0.9	2.8	4.6	-	0.8	3.5	6	-
P_{38}	-	1.2	2.5	5.5	0.3	1.0	3.5	6	-
P_{39}	1.8	3.2	5.4	full leaf	-	0.8	4.6	6	-
P_{40}	0.4	1.2	2.6	6.0 (full leaf)	-	0.7	4.2	6	-
P_{41}	0.5	1.3	2.7	6.1 (full leaf)	-	0.6	1.3	6	-
P_{42}	-	0.3	0.7	6.3 (full leaf)	-	0.8	1.6	6	-
P_{43}	-	0.3	0.5	1.7	-	0.5	2.0	7	-
P_{44}	0.4	1.0	2.0	4.3	-	0.4	1.7	6	-
P_{45}	-	0.4	1.0	2.2	-	0.5	2.0	7	-
P_{46}	0.6	2.3	5.0	6.1 (full leaf)	-	0.6	4.0	7	-
C_1	-	0.4	1.6	3.2	0.3	1.0	3.2	6	16
C_2	-	0.5	1.3	2.5	0.5	1.2	1.6	6	17

Isolate Number	On pepper plants lesion size after (hrs)				Coconut leaf lesion Size after			Days required for infection	
	24	48	72	96	24	48	72	Tender coconut	Coconut seedlings
C_3	-	0.5	1.2	3.0	-	0.8	3.2	6	16
C_4	-	1.0	2.6	5.4	0.4	1.1	4.2	6	12
C_5	-	0.7	2.7	4.6	-	0.8	2.1	7	16
C_6	0.8	2.4	5.2	6.1 (full leaf)	0.3	1.0	5.7	6	12
C_7	1.3	3.1	6.2	Full leaf	-	0.6	4.6	6	9
C_8	1.2	3.0	6.1	Full leaf	0.4	1.1	6.1	5	10
C_9	1.2	3.0	6.1	Full leaf	0.3	1.0	5.2	5	9
C_{10}	1.3	3.0	5.1	6.0 (Full leaf)	0.7	1.8	6.3	6	10
C_{11}	1.0	2.8	5.3	6.0 (Full leaf)	0.6	1.9	5.2	5	11
C_{12}	0.9	3.7	6.0	Full leaf	1.0	2.4	6.7	6	12
C_{13}	1.5	3.0	5.3	6.1 (Full leaf)	0.8	1.5	4.2	5	9
C_{14}	2.0	3.6	6.0 (Full leaf)	Full leaf	0.8	2.2	6.3	5	9
C_{15}	2.1	3.5	6.0 (Full leaf)	Full	0.5	1.2	4.9	6	10
C_{16}	1.8	3.1	6.0 (Full leaf)	Full leaf	0.4	1.5	7.0	5	11

Spindle shape was the most commonly observed lesion shape. Eighteen pepper isolates and eight coconut isolates produced spindle shaped lesions on coconut leaves. Eleven pepper isolate produced round lesions and seven pepper isolates and three coconut isolate produced irregular lesions. Only 2 pepper isolates produced square lesions.

Lesion shape	Isolate number
Spindle shaped lesions	P_{14}, P_{15}, P_{16}, P_{18}, P_{19}, P_{23}, P_{24}, P_{30}, P_{32}, P_{33}, P_{35}, P_{38}, P_{39}, P_{40}, P_{43}, P_{45}, P_{46}, C_1, C_3, C_6, C_9, C_{10}, C_{11}, C_{12}, and C_{14}
Round lesions	P_2, P_8, P_9, P_{10}, P_{11}, P_{20}, P_{22}, P_{25}, P_{27}, P_{41} and P_{42}
Irregular lesions	P_1, P_3, P_7, P_{31}, P_{34}, P_{37}, P_{45}, C_7, C_{13} and C_{15}
Oval shaped	P_5, P_{12}, P_{13}, P_{28}, C_4 and C_5
Linear lesions with Smooth ends	P_4, P_{17}, P_{21}, P_{29}, C_2, C_8 and C_{16}
Square lesions	P_6 and P_{26}

Nut rot symptoms on large green tender coconut appeared 5-8 days following artificial inoculation with both coconut and

pepper isolates (Plate 9). Elongate water soaked lesions appeared near the perianth region. Irregular expansion of necrotic or dark brown areas produced mottled green-brown pattern and circular patches of green tissues. As much as half of the external nut surface became necrotic in 8-10 days. Internally husks and endocarp turned to reddish brown (Plate 10). No difference in the symptom expression was noticed with coconut or pepper isolates.

One year old seedlings grown in cement troughs were inoculated with mycelial mats of *Phytophthora*, carrot bits covered with fungal mycelia or zoospore suspensions. All the isolates produced the characteristic bud rot symptoms within one to three weeks depending upon the climatic conditions. The pepper isolates required 9-24 days (Plate 11) to produce bud rot compared to 9-16 days with coconut isolates, when both the isolates were inoculated simultaneously.

Pepper

Unlike in coconut, variation in the symptom was not noticed when the different *Phytophthora* isolates were inoculated on the pepper leaves, but there were differences in the time required for symptom expression and intensity (Table 19).

The coconut isolates of Kannur and Kasargod (C_7 - C_{16}) were most potent pathogens (Plate 12). Even with in 24 hrs these

Plate 9.

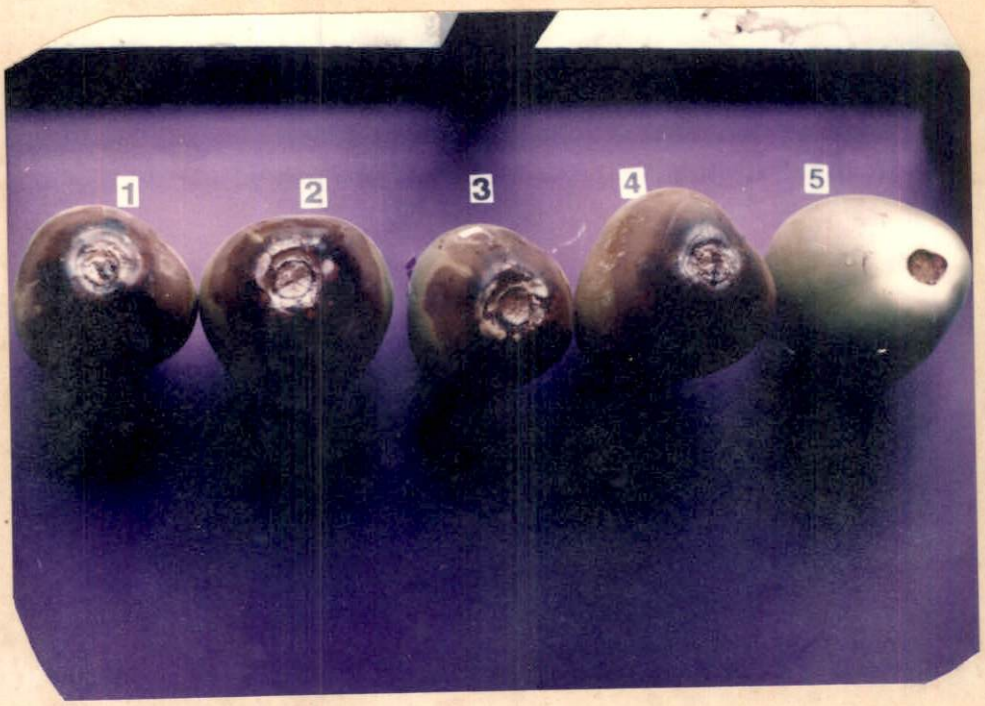
Nut rot symptom produced by coconut and pepper isolate

1 - P_{44} 2 - P_{34} 3 - C_2 4 - C_7

5. Control

Plate 10. Nut rot symptom - internal decay

1 - Control 2 - P_{39} 3 - C_7


1
2
3
4
5

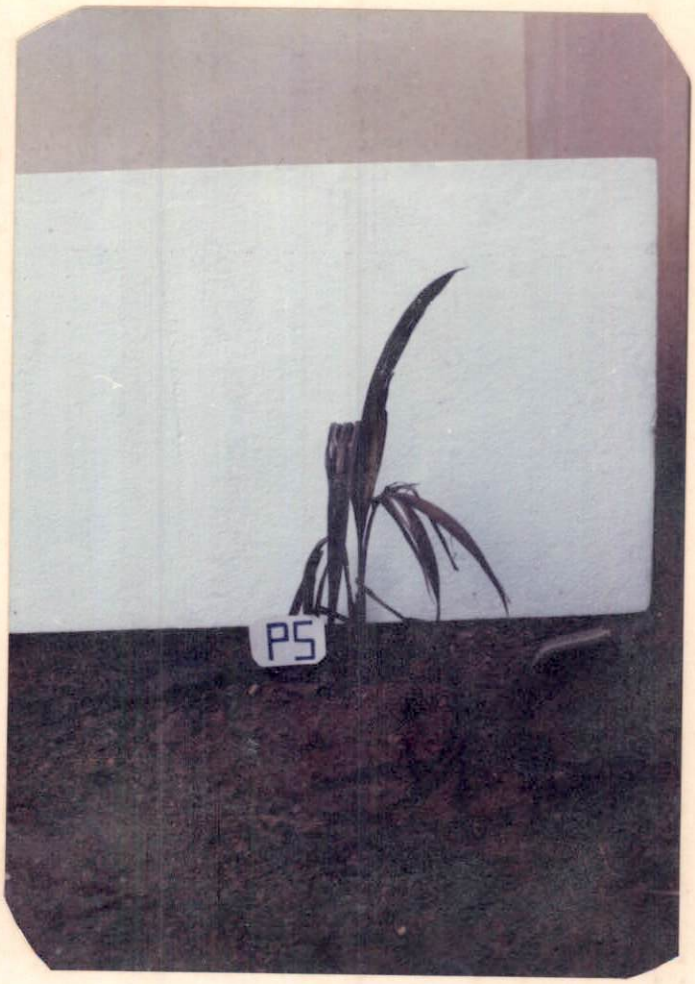

1
2
3

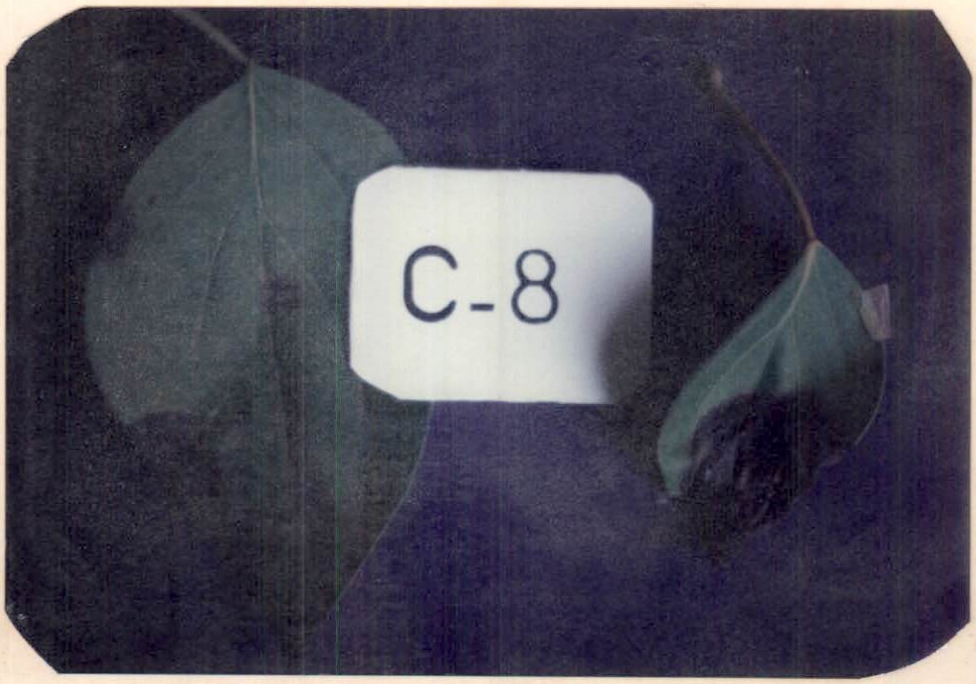
Plate 11.

Bud rot of coconut seedlings - induced by pepper isolate P_5

Plate 12.

Foliar symptom of foot rot on pepper artificially induced by coconut isolate C_8


P5


C-8

could produce 0.9 to 2.1 cm long lesions. Out of 46 pepper isolates only 13 could produce measurable lesions within 24 hrs. None of the pepper isolates except P_{20} isolated from Maruthonkara from a pepper and coconut mixed garden could completely cover the leaf surface within 72 hrs. At the end of 96 hrs of inoculation all the Kannur and Kasaragodu isolates, C_6 (Vellanikkara, pure coconut) isolate and 21 pepper isolates completely covered the leaf area.

Variation in symptom expression and severity were not noticed when the different coconut and pepper isolates were inoculated at the collar region of 3 month old rooted cuttings of pepper. Depending upon the season, the collar region got infected and plants succumbed to the disease within 2-7 days.

Host range

Twenty nine plants commonly seen growing in coconut and pepper gardens were artificially inoculated with C_3 isolate from coconut and P_1 isolate by pepper. Out of the 29 plants 8 were crop plants, nine were ornamental plants and 12 were weeds. The time taken for the appearance of initial symptoms varied mainly upon the hosts. Six out of the eight crop plants took infection when P_1 isolate was artificially inoculated on them (Table 20). Jack and papaya leaves failed to take up infection. Even though cucumber and *Piper longum* leaves took infection with pepper isolate, coconut isolate failed to infect them. Of the nine

Table 20 Days required for the expression of symptoms on various hosts by *Phytophthora* isolates

Name of the plant	Isolate number		
	P_1	C_3	
Cocoa (fruits)	2 days	2 days	
Colocasia (leaves)	4 days	5 days	
Cucumber (fruits, leaves)	3 days	3 days	(fruits)
	3 days		(leaves)
Jack (leaves)	-	-	
Papaya (leaves)	-	-	
Rubber (leaves)	2 days	2 days	
Tapioca (leaves)	2 days	2 days	
Thippali	6 days	-	
Acalypha	8 days	13 days	
Aglaonema	-	-	
Alocasia	-	-	
Bougainvillea	2 days	2 days	
Clitoria	6 days	-	
Croton	-	-	
Dieffenbachia	-	-	
Hibiscus	-	-	
Mirabilus	3 days	3 days	
Abutilon	-	6 days	
Andrographis	6 days	6 days	
Antagonum	-	8 days	
Castor	2 days	2 days	
Cycla	3 days	3 days	
Eupatorium	10 days	-	
Lantana	-	-	
Morinda	7 days	-	
Quisgalis	-	-	
Spermocea	7 days	6 days	
Synendrella	-	-	
Tridax	2 days	2 days	

ornamental plants, typical symptoms were noticed on Bougainvillea (Plate 13), Acalypha and Mirabilis with both coconut and pepper isolates while on clitoria only pepper isolate could produce symptom.

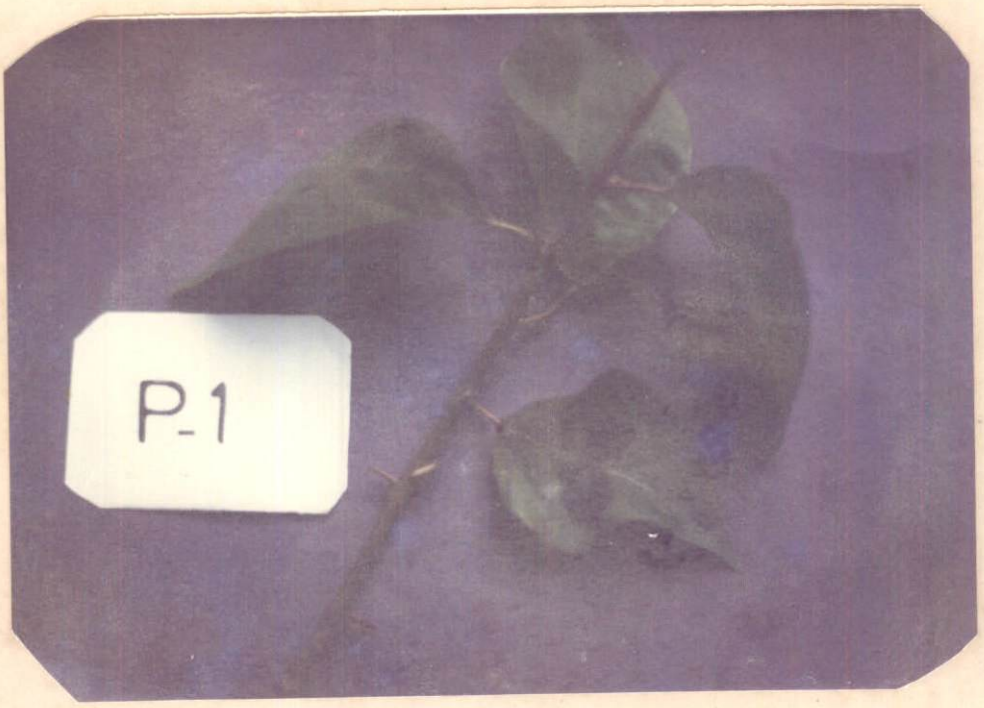
Among the weeds Synendrella, Lantana and Quisgalis failed to take up infection when inoculated with both coconut and pepper isolates. Eupatorium (Plate 14) and Morinda took infection with pepper isolates and not with coconut isolates while, Antagonum and Abutilon took infection with coconut isolates only Koch's postulates were also proved in these plants. The plants that normally attacked by *Phytophthora* took only 2-3 days for symptom development (eg. Cocoa, rubber, bougainvillea etc.) while others took longer period (Plate 15).

Effect of botanicals on growth and symptom development

In general, the growth of all the *Phytophthora* isolates were inhibited by botanicals (Fig. 12). The inhibitory effect of ocimum was more pronounced in coconut isolates (Table 21). While, the neem extract was more inhibitory to pepper isolates. For the isolate C_2 the inhibitory effect of bougainvillea and neem was on par. However, for the C_2 isolates, the inhibitory effect of bougainvillea extract did not differ significantly from that of control. The maximum inhibitory effect on P_{20} and P_{30} was exhibited by neem extract. While, the other botanicals did not significantly inhibit the growth of the isolates compared to

Plate 13. Host range of *Phytophthora* - Bougainvillea

Plate 14. Host range of *Phytophthora* - Eupatorium


P-1

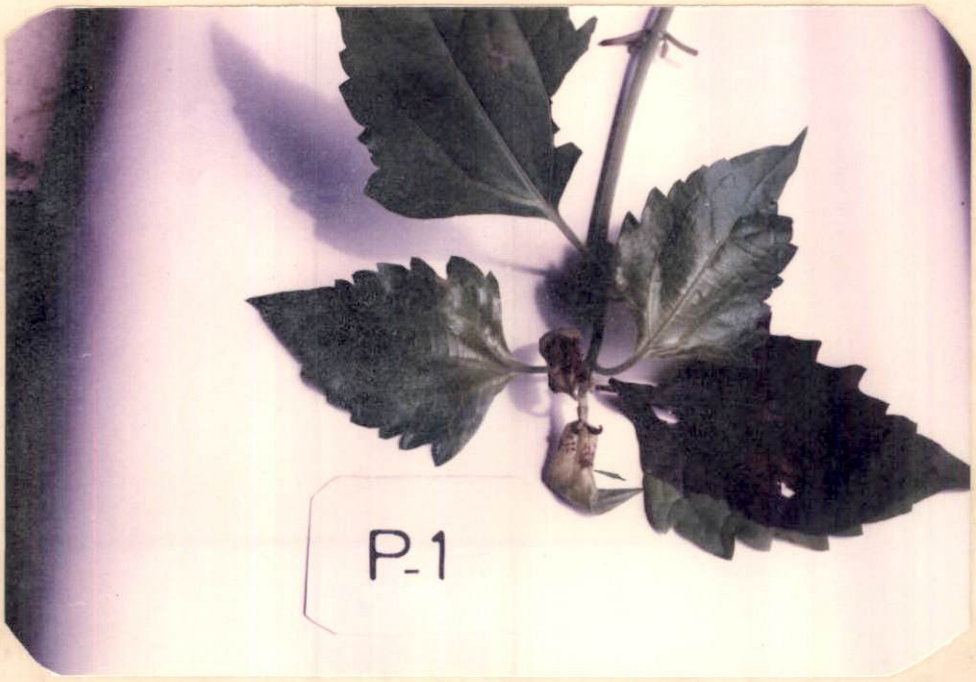

P-1

Plate 15. Host range of *Phytophthora* - Cocoa


P-1

Fig-12 **Effect of botanicals on growth of selected *Phytophthora* isolates**

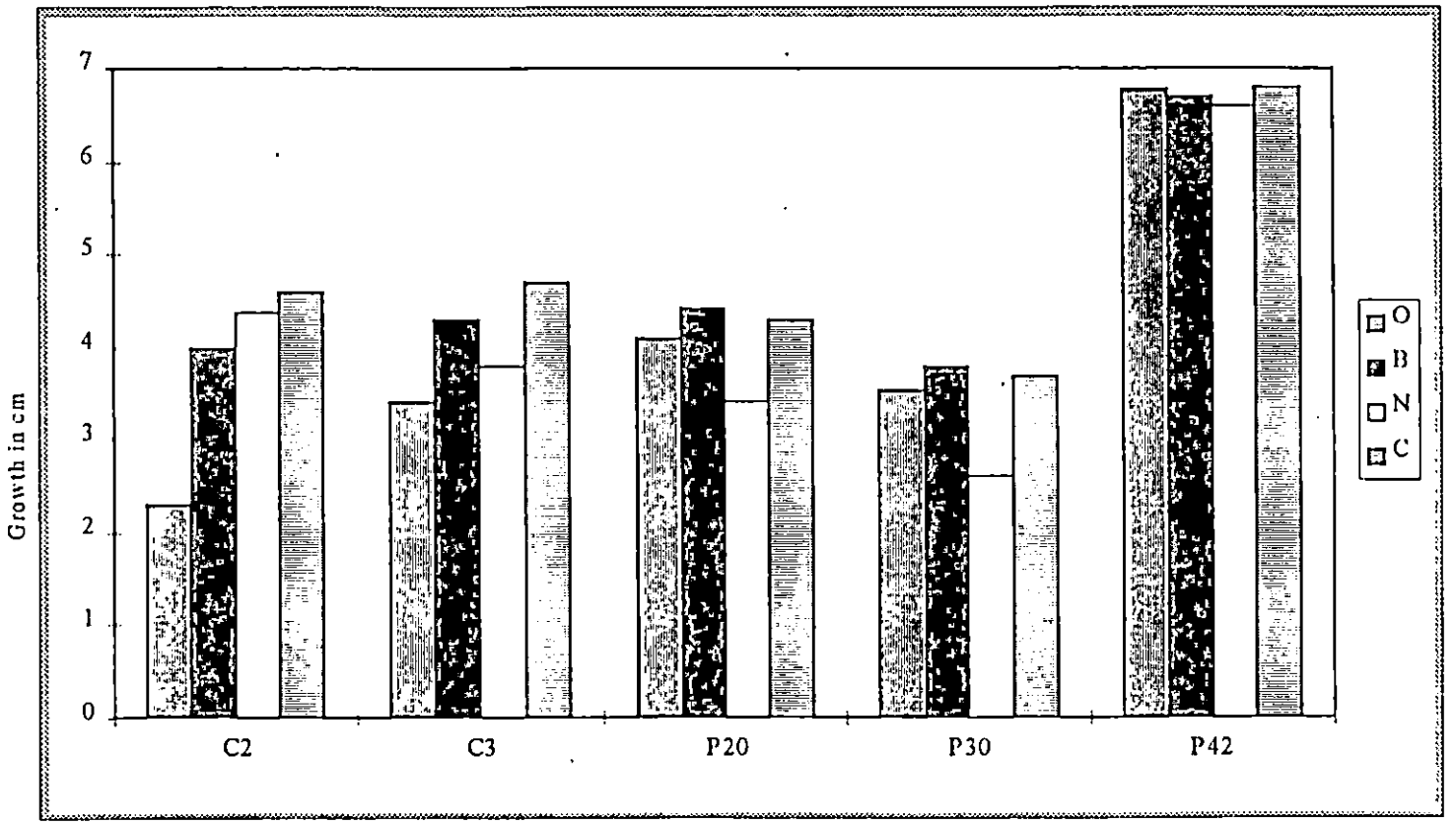


Table 21 Effect of botanicals on growth of selected *Phytophthora* isolates from coconut and pepper

Isolate Number	Growth in cm after															
	24 hrs				48 hrs				72 hrs				144 hrs			
	Ocimum	Bougainvillea	Neem	Control	Ocimum	Bougainvillea	Neem	Control	Ocimum	Bougainvillea	Neem	Control	Ocimum	Bougainvillea	Neem	Control
C_2	0	1.4	1.8	1.8	2.1	2.9	3.3	3.7	2.9	4.4	4.6	5.4	5.5	8.6	9.0	9.0
C_3	1.4	1.8	1.2	2.0	3.5	3.8	3.0	4.1	5.5	5.4	5.1	6.0	6.8	7.2	7.0	7.7
P_{20}	1.3	1.2	1.1	1.5	3.0	3.8	2.9	3.3	4.6	5.2	3.8	4.8	9.0	9.0	7.1	9.0
P_{30}	1.0	1.0	1.0	1.1	2.7	2.7	1.7	2.7	3.2	4.5	2.3	4.0	9.0	9.0	6.6	9.0
P_{42}	3.0	3.0	3.0	3.4	7.0	6.9	6.3	7.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0

CD - 0.104

control. None of the botanicals significantly inhibit the growth of P_{42} compared to control.

The effect of botanicals on the symptom expression was studied by spraying the crude and sterilized extract on the coconut leaves and inoculating with the pathogen. In general, sterilized leaf extracts had significantly higher inhibitory effect against the coconut isolates (C_2 and C_{12}) than crude extracts, when lesion size was measured 24 hrs after inoculation (Table 22). Compared to control, inhibition was better in plants sprayed with botanicals. With C_2 isolate, maximum inhibition was observed with crude and sterilized ocimum extract followed by sterilized extracts of neem and bougainvillea. For C_{12} also, the maximum inhibition was observed with extracts of ocimum and sterilized neem. Least inhibitory effect for both C_2 and C_{12} was recorded with bougainvillea extract.

When the lesion size was measured 48 hrs after inoculation of the pathogen, no significant difference was observed in size of lesions sprayed with both crude and sterilized botanicals and there was no significant difference among the botanicals. Evenafter 72 hrs, the inhibitory effect of botanicals was observed. Maximum inhibition of C_2 isolate was observed with sterilized ocimum followed by sterilized bougainvillea extract. While for C_{12}, the maximum inhibition was recorded with crude ocimum followed by sterilized bougainvillea.

Table 22 Effect of crude and sterilized leaf extract on symptom development on coconut leaves

Botanicals	Isolate number	Lesion size (in cm) after 24 hrs (cm)		48 hrs (cm)		72 hrs (cm)	
		Crude	Sterilized	Crude	Sterilized	Crude	Sterilized
Ocimum	C_2	0	0.2	1.0	2.0	3.3	2.0
	C_{12}	0.3	0.2	1.4	1.5	1.7	3.5
Neem	C_2	1.0	0.6	2.0	1.5	3.0	3.6
	C_{12}	0.5	0.2	1.3	0.6	3.0	3.4
Bougainvillea	C_2	1.0	0.5	2.0	1.4	3.4	2.4
	C_2	1.0	1.0	2.0	1.4	3.2	2.5
Control	C_2	1.3	1.3	2.6	2.6	4.6	4.6
	C_{12}	1.0	1.0	2.0	2.0	4.3	4.3

CD after 24 hrs - 0.06
48 hrs - 0.13
72 hrs - 0.06

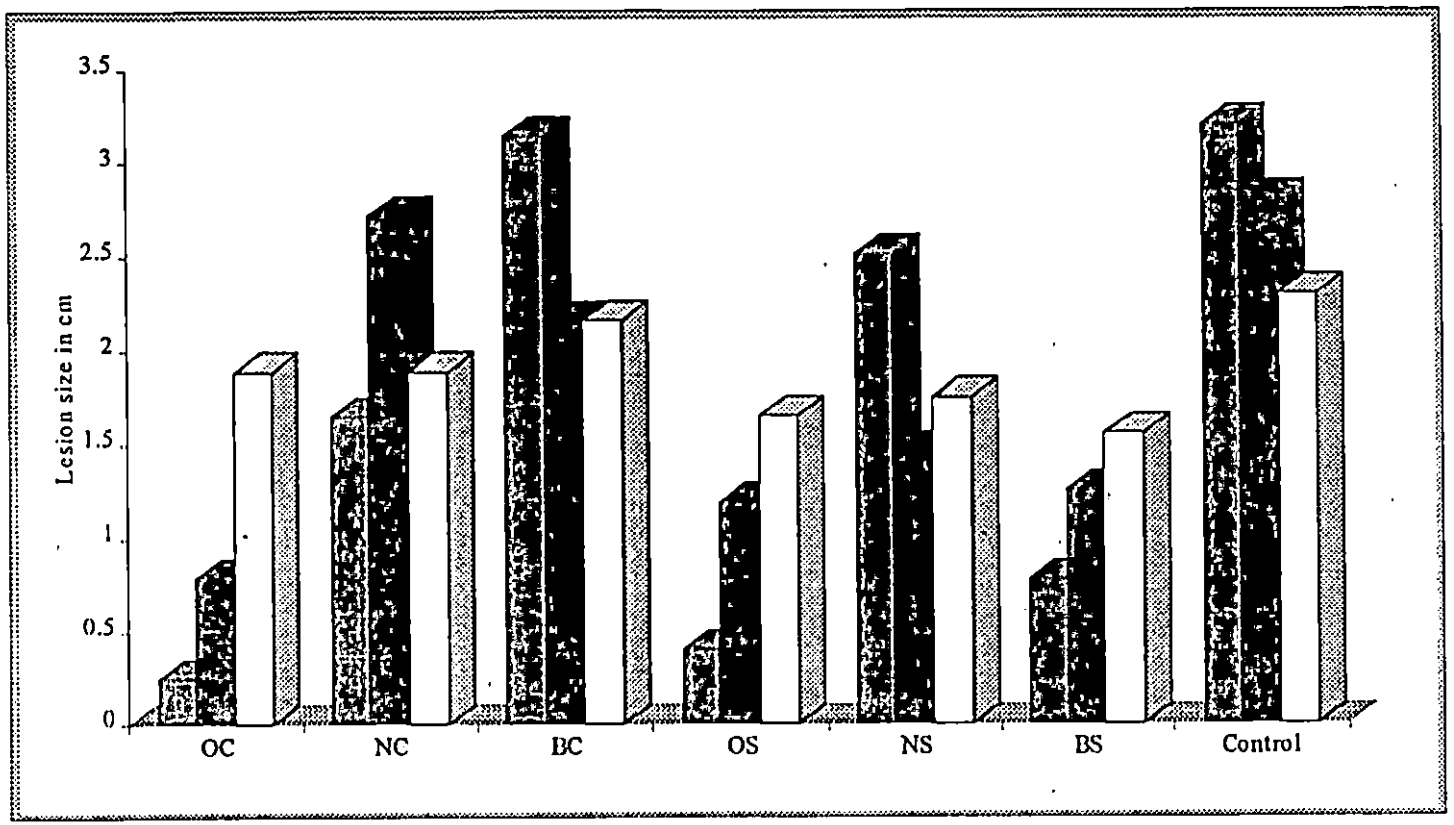
When pepper leaves were inoculated with *Phytophthora* isolate, P_{30} immediately after spraying with extracts of ocimum, neem and bougainvillea, the symptom development was inhibited maximum when crude ocimum sprayed (Table 23). This was followed by sterilized ocimum extract and sterilized bougainvillea extract. However, the symptom development in plants sprayed with crude bougainvillea extract was similar to that observed in control plants. The inhibitory action of botanicals declined with days (Fig. 13). Maximum inhibitory effect of all the botanicals was noticed when the observations were made 48 hrs after spraying, while the inhibitory effect was minimum at the end of 120 hrs. Further there was significant difference in the intensity of infection. For example, at the end of 48 hrs, the lesion size in sterilized neem extract sprayed pepper leaves was 1.0 cm compared to 2.0 cm with control. While, the corresponding figures at the end of 120 hrs were 4.6 and 4.5 cm respectively. When lesion size at the end of 48, 72, 96 and 120 hrs were examined, a significant difference was noticed among the treatments. At 48 hrs after spraying, crude ocimum and crude neem extract were superior to other treatments. In all other intervals, least infection was observed on plants sprayed with sterilized ocimum and crude ocimum. The inhibitory effect of botanicals was also noticed when the botanicals sprayed plants were inoculated after 3 or 7 days. When the inoculation was done 3 days after spraying the botanicals, the highest inhibition was

Table 23 Effect of crude and sterilized leaf extract on symptom development on pepper leaves

Botanicals		Lesion size (in cm) after spraying														
		Immediately					3 days					7 days				
		After hours					After hours					After hours				
		24	48	72	96	120	24	48	72	96	120	24	48	72	96	120
Ocimum	Crude extract	0	0	0.2	0.4	0.5	0	0.3	0.8	1.0	1.2	0	0.6	1.3	2.8	3.3
	Sterilized extract	0	0	0.1	0.2	0.4	0	0.4	1.0	1.7	2.0	0	1.0	1.3	1.9	2.6
Neem	Crude extract	0	0.9	1.7	2.4	3.1	0	1.0	2.5	4.1	Shed	0	0.6	1.4	2.8	3.2
	Sterilized extract	0	1.0	1.8	2.0	Shed	0	0.4	1.3	2.0	2.5	0	1.0	1.5	2.0	2.7
Bougain villea	Crude extract	0	2.0	3.7	4.1	Shed	0	0.7	1.6	3.0	4.0	0	0.8	1.5	3.0	4.0 Shed
	Sterilized extract	0	0.5	0.6	0.9	1.2	0	0.4	1.1	1.7	2.1	0	0.9	1.5	1.8	2.2
Control		0	2.0	2.6	4.0	Shed	0	1.3	2.0	4.0	Shed	0	1.0	2.5	4.0	Shed

CD	Treatment	Period	Treatment x period
Immediately	0.054	0.037	0.099
After 3 days	0.106	0.075	0.197
After 7 days	0.139	0.092	0.244

Fig-13 **Effect of botanicals on symptom development on pepper leaves**



OC - Ocimum Crude
NC - Neem Crude
BC - Bougainvilla Crude
OS - Ocimum Sterilized
NS - Neem Sterilized
BS - Bougainvilla Sterilized

noticed in treatments receiving crude ocimum followed by sterilized ocimum and sterilized bougainvillea extract. However, these crude neem extract did not show significant inhibition compared to control. When the efficacy of the different extracts were compared after 48, 72, 96 and 120 hrs after inoculation, there was a reduction in the inhibitory effect of the botanicals. Crude ocimum sprayed plants showed higher rate of inhibition in different periods, while, the lesion size in plants sprayed with sterilized ocimum, bougainvillea and neem were on par.

The efficacy of botanicals was markedly reduced when the plants were inoculated 7 days after spraying with the botanicals compared to that inoculated immediately after spraying. Except crude bougainvillea extract all other treatments were superior to control, while, there was no marked difference among the treatments when plants were inoculated with pathogen after seven days of spraying.

Effect of antibiotics

Sensitivity of five antibiotics viz. Streptomycin, Negadix. Ampicillin. Erythromycin, Cephalaxin at two concentrations (50 ppm and 800 ppm) against two isolates of *Phytophthora* (P_2 and C_2) was tested under *in vitro* conditions. Complete inhibition of the growth of the coconut isolate C_2 was observed when streptomycin was incorporated into the medium irrespective of the concentration (Table 24). Complete inhibition of the

Table 24 Effect of antibiotics on the growth of *Phytophthora* isolates from coconut and pepper

Name of the antibiotic with concentration in ppm		Growth in cm after					
		48 hrs		96 hrs		144 hrs	
		Isolate number		Isolate number		Isolate number	
		C_2	P_2	C_2	P_2	C_2	P_2
Streptomycin	50	0	3.1	0	7.2	0	9.0
	800	0	3.0	0	7.2	0	9.0
Negadiz	50	2.7	3.8	5.5	7.7	9.0	9.0
	800	0	0	0	0	0	0
Ampicillin	50	4.0	4.0	9.0	9.0	9.0	9.0
	800	4.1	4.3	9.0	9.0	9.0	9.0
Erythromycin	50	1.86	1.8	4.5	2.0	5.8	2.5
	800	0	0	0	0	0	0
Cephalaxin	50	4.3	4.2	9.0	9.0	9.0	9.0
	800	4.0	3.9	9.0	9.0	9.0	9.0
Control		4.0	4.1	8.5	9.0	9.0	9.0

CD					
	Antibiotics	(A)	0.025	AB -	0.036
	Isolate	(B)	0.010	AC -	0.039
	Period	(C)	0.019	BC -	0.016

isolate was also recorded when the antibiotics erythromycin and negadix at 800 ppm was incorporated in the medium. When the growth of C_2 in the remaining treatments was compared. Maximum inhibition was observed in media incorporated with 50 ppm of erythromycin and negadix. While significant difference in growth was not observed between cephalaxin 50 ppm and control. Further, cephalaxin and ampicillin at 800 ppm supported significantly more growth of the isolate C_2 compared to control.

As observed with the coconut isolate C_2, complete inhibition of the growth of the pepper isolate P_2 was noticed when 800 ppm of negadix and erythromycin were incorporated in the medium. While, unlike in C_2, complete inhibition of the growth of P_2 was not observed when streptomycin was incorporated. The least inhibition of the isolate was by the antibiotics ampicillin (800 ppm) followed by cephalaxin (50 and 800 ppm). Both streptomycin and ampicillin supported more growth of higher compared to lower concentrations.

In order to find out whether this differential action is present with other isolates, a detailed study was conducted using eight concentrations of streptomycin (at 50, 200, 400, 600, 800, 1000, 1500 and 2000 ppm) and all the 62 isolates of *Phytophthora*.

Eight pepper isolates (P_1 to P_6 and P_9 and P_{10}) and 10 coconut isolates (C_7 to C_{16}) were insensitive to streptomycin

upto a concentration of 2000 ppm (Table 25). Among these except P_3 all the pepper isolates were from Kozhikode district collected either from a pure pepper or a mixed garden where pepper was interplanted with coconut, arecanut or cocoa. All the streptomycin insensitive coconut isolates were from coconut or coconut-pepper mixed gardens from Kannur and Kasaragod districts. The coconut isolates C_1 to C_6 and pepper isolates P_{13} to P_{15}. P_{18} to P_{28}, P_{30} to P_{38} and P_{46} failed to grow even in 50 ppm streptomycin containing media. P_{43}, an isolate from Malapuram with a coconut pepper cropping system could grow in media containing streptomycin upto 800 ppm. In all the streptomycin insensitive *Phytophthora* isolates sporangia were produced.

In vitro evaluation of fungicides

The efficacy of four fungicides viz. Copper oxy chloride, Ridomil, Bavistin and Bordeux mixture at three concentrations (500, 1000 and 2000 ppm) were tested against one isolate each of pepper (P_2) and coconut (C_2) under *in vitro* conditions. Copper oxy chloride, Ridomil and Bordeux mixture at all the three concentrations, completely inhibited the growth of the isolates (Table 26). However, Bavistin even at highest concentration of 2000 ppm was not inhibitory to both the isolates and it completely covered a 9 cm petri dish with in 4 days compared to 5 days in absolute control.

In order to study the variabilities in the sensitivity of Ridomil to all the isolates of pepper and coconut a study was

Table 25 Sensitivity of different concentrations of Streptomycin on different isolates of *Phytophthora* from coconut and pepper

Isolate number	50 ppm	200 ppm	400 ppm	600 ppm	800 ppm	1000 ppm	1500 ppm	2000 ppm
P_1	+	+	+	+	+	+	+	+
P_2	+	+	+	+	+	+	+	+
P_3	+	+	+	+	+	+	+	+
P_4	+	+	+	+	+	+	+	+
P_5	+	+	+	+	+	+	+	+
P_6	+	+	+	+	+	+	+	+
P_7	+	+	-	-	-	-	-	-
P_8	+	-	-	-	-	-	-	-
P_9	+	+	+	+	+	+	+	+
P_{10}	+	+	+	+	+	+	+	+
P_{11}	-	-	-	-	-	-	-	-
P_{12}	+	-	-	-	-	-	-	-
P_{13}	-	-	-	-	-	-	-	-
P_{14}	-	-	-	-	-	-	-	-
P_{15}	-	-	-	-	-	-	-	-
P_{16}	+	-	-	-	-	-	-	-
P_{17}	+	-	-	-	-	-	-	-
P_{18}	-	-	-	-	-	-	-	-
P_{19}	-	-	-	-	-	-	-	-
P_{20}	-	-	-	-	-	-	-	-

Isolate number	50 ppm	200 ppm	400 ppm	600 ppm	800 ppm	1000 ppm	1500 ppm	2000 ppm
P_{21}	-	-	-	-	-	-	-	-
P_{22}	-	-	-	-	-	-	-	-
P_{23}	-	-	-	-	-	-	-	-
P_{24}	-	-	-	-	-	-	-	-
P_{25}	-	-	-	-	-	-	-	-
P_{26}	-	-	-	-	-	-	-	-
P_{27}	-	-	-	-	-	-	-	-
P_{28}	-	-	-	-	-	-	-	-
P_{29}	+	-	-	-	-	-	-	-
P_{30}	-	-	-	-	-	-	-	-
P_{31}	-	-	-	-	-	-	-	-
P_{32}	-	-	-	-	-	-	-	-
P_{33}	-	-	-	-	-	-	-	-
P_{34}	-	-	-	-	-	-	-	-
P_{35}	-	-	-	-	-	-	-	-
P_{36}	-	-	-	-	-	-	-	-
P_{37}	-	-	-	-	-	-	-	-
P_{38}	-	-	-	-	-	-	-	-
P_{39}	+	-	-	-	-	-	-	-
P_{40}	+	-	-	-	-	-	-	-
P_{41}	+	-	-	-	-	-	-	-
P_{42}	+	+	-	-	-	-	-	-
P_{43}	+	+	+	+	+	-	-	-

Isolate number	50 ppm	200 ppm	400 ppm	600 ppm	800 ppm	1000 ppm	1500 ppm	2000 ppm
P_{44}	+	-	-	-	-	-	-	-
P_{45}	+	-	-	-	-	-	-	-
P_{46}	-	-	-	-	-	-	-	-
C_1	-	-	-	-	-	-	-	-
C_2	-	-	-	-	-	-	-	-
C_3	-	-	-	-	-	-	-	-
C_4	-	-	-	-	-	-	-	-
C_5	-	-	-	-	-	-	-	-
C_6	-	-	-	-	-	-	-	-
C_7	+	+	+	+	+	+	+	+
C_8	+	+	+	+	+	+	+	+
C_9	+	+	+	+	+	+	+	+
C_{10}	+	+	+	+	+	+	+	+
C_{11}	+	+	+	+	+	+	+	+
C_{12}	+	+	+	+	+	+	+	+
C_{13}	+	+	+	+	+	+	+	+
C_{14}	+	+	+	+	+	+	+	+
C_{15}	+	+	+	+	+	+	+	+
C_{16}	+	+	+	+	+	+	+	+

+ - Insensitive

- - Sensitive

Table 26 Effect of fungicide on *Phytophthora* from coconut and pepper

Fungicides used	Concentration (ppm)	Growth after 48 hrs		Growth after 96 hrs		Growth after 144 hrs	
		C_2	P_2	C_2	P_2	C_2	P_2
Copper oxy chloride	500	-	-	-	-	-	-
	1000	-	-	-	-	-	-
	2000	-	-	-	-	-	-
Ridomil	500	-	-	-	-	-	-
	1000	-	-	-	-	-	-
	1000	-	-	-	-	-	-
Bavistin	500	5.6	5.3	9.0	9.0	9.0	9.0
	1000	5.0	5.0	6.5	7.5	9.0	9.0
	2000	4.4	4.5	9.0	6.5	9.0	9.0
Bordeaux mixture	0.25%	-	-	-	-	-	-
	0.5%	-	-	-	-	-	-
	1.1%	-	-	-	-	-	-
Control	-	5.3	4.8	8.0	8.0	9.0	9.0

conducted with three concentrations of Ridomil viz. 50, 100 and 150 ppm. All the 62 isolates failed to grow in the media containing 150 ppm of Ridomil (Table 27). All the *Phytophthora* isolates from bud rot affected coconut from Kozhikode, Wynad, Alapuzha and Thrissur were inhibited at 100 ppm. While those from Kannur and Kasaragod districts grew well on the media. These isolates were collected from pure plantations of coconut or from pepper was intercropped with coconut. Out of the 46 pepper isolates, only eight isolates (P_1, P_2, P_3, P_4, P_6, P_9, P_{10} and P_{15}) grew on media containing 100 ppm Ridomil. These isolates were from either a pure pepper garden or from a mixed garden where pepper was intercropped with coconut, cocoa and arecanut. None of the pepper isolates from Wynad district grew at this concentration. Inhibitory effect of Ridomil at 50 ppm was noticed only on two isolates of coconut (C_4 and C_5) and 30 isolates of pepper. Unlike in coconut isolates, the place of collection and cropping pattern had no role in the sensitivity of pepper isolates.

Sporangial production was not observed in all the Ridomil incorporated media.

Antagonism of phylloplane and soil organisms towards *Phytophthora*

Fungi, bacteria and actinomycetes found associated with healthy and diseased pepper and coconut plants and from the soils of pepper and coconut gardens of different parts of Kerala were

Isolate number	50 ppm	100 ppm	150 ppm	Isolate number	50 ppm	100 ppm	150 ppm
P_1	+	+	-	P_{32}	+	-	-
P_2	+	+	-	P_{33}	-	-	-
P_3	+	+	-	P_{34}	-	-	-
P_4	+	+	-	P_{35}	-	-	-
P_5	-	-	-	P_{36}	-	-	-
P_6	+	+	-	P_{37}	-	-	-
P_7	+	-	-	P_{38}	-	-	-
P_8	+	-	-	P_{39}	+	-	-
P_9	+	+	-	P_{40}	-	-	-
P_{10}	+	+	-	P_{41}	-	-	-
P_{11}	+	-	-	P_{42}	-	-	-
P_{12}	-	-	-	P_{43}	+	-	-
P_{13}	-	-	-	P_{44}	+	-	-
P_{14}	-	-	-	P_{45}	-	-	-
P_{15}	+	+	-	P_{46}	-	-	-
P_{16}	-	-	-	C_1	+	-	-
P_{17}	-	-	-	C_2	+	-	-
P_{18}	-	-	-	C_3	+	-	-
P_{19}	-	-	-	C_4	-	-	-
P_{20}	-	-	-	C_5	-	-	-
P_{21}	-	-	-	C_6	+	-	-
P_{22}	-	-	-	C_7	+	+	-
P_{23}	-	-	-	C_8	+	+	-
P_{24}	-	-	-	C_9	+	+	-
P_{25}	+	-	-	C_{10}	+	+	-
P_{26}	-	-	-	C_{11}	+	+	-
P_{27}	-	-	-	C_{12}	+	+	-
P_{28}	-	-	-	C_{13}	+	+	-
P_{29}	-	-	-	C_{14}	+	+	-
P_{30}	-	-	-	C_{15}	+	+	-
P_{31}	-	-	-	C_{16}	+	+	-

+ - insenstive - - sensitive

isolated and brought in to pure culture. These organisms were then dual cultured separately on carrot agar to study whether any antagonistic action exists between these organisms and *Phytophthora* isolates.

More than 200 fungi, bacteria and actinomycetes were isolated from the phyllosphere and soil. Out of which only 28 fungi showed marked antagonistic property against all the 62 isolates of *Phytophthora* (Table 28). None of the bacteria and actinomycetes showed antagonistic properties. Among the fungi which showed antagonistic properties were 16 species of *Aspergillus*, 2 *Chaetomium* 3 *Penicillium* and 4 *Trichoderma* and one each of *Diplodia*, *Verticillium* and *Curvularia* (Plates 16, 17, 18 and 19). All the *Aspergillus Penicillium* and *Verticillium* inhibited the growth in dual culture by restricting or inhibiting the growth of *Phytophthora*. In these cases, a clear inhibition zone was noticed. While *Curvularia Diplodia* and *Trichoderma* over grew *Phytophthora* and restricted its growth without producing an inhibition zone.

When the spore suspensions of the antagonistic fungal isolates were sprayed on the leaves of pepper plants and inoculated immediately with P_{30} isolate of *Phytophthora*, 12 out of the 28 antagonists inhibited infection by varying degree (Table 29). Even though on control leaves one cm lesions was noticed 24 hrs after inoculation none of the leaves sprayed with

Table 28. List of antagonistic organisms against *Phytophthora* isolates (*in vitro*)

Sl.No.	Place of collection	Name of the antagonistic organism
1.	Karingad. Badagara - Coconut	*Aspergillus* sp.
2.	Maruthonkara - Coconut	*Aspergillus* sp.
3.	Thottilpalam - Coconut	*Aspergillus flavus* Link.
4.	Vellayani - Pepper	*Aspergillus flavus* Link.
5.	Maruthonkara - Coconut	*A. fumigatus* Fresenius
6.	Balaramapuram - Coconut	*A. terreus* Thom.
7.	Balaramapuram - Coconut	*A. wentii* Wehmer
8.	Vellayani - Coconut	*A. fumigatus* Fresenius
9.	Vellayani - Coconut	*A. ruber* (Brem.)
10.	Manjappara - Pepper	*A. carneus* (v. Tiegh) Blockwitz
11.	Malapuram - Pepper	*A. clavatus* Desm.
12.	Vellayani - Pepper	*A. niveus* Bloch., emend
13.	Vellayani - Pepper	*A. terreus* Thom.
14.	Vellayani - Pepper	*A. fumigatus* Fresenius
15.	Balaramapuram - Coconut	*Chaetomium* sp
16.	Vellayani - Pepper	*Chaetomium* sp.
17.	Thamarasseri - Coconut	*Penicillium oxalicum* Currie Thom.
18.	Malapuram - Pepper	*P. citrinum* Thom
19.	Vellayani - Pepper	*P. nigricans* Bain ex Gray

Sl.No.	Place of collection	Name of the antagonistic organism
20.	Marunthonkara - Coconut	*Trichoderma viride* Pers. ex Gray
21.	Kulathupuzha - Forest	*T. harzianum* Ri Fai
22.	Balaramapuram - Coconut	*Aspergillus* sp
23.	Balaramapuram - Coconut	*Curvularia* sp
24.	Vellayani - Coconut	*Trichoderma viride* Pers. ex Gray
25.	Ambalavayal - Pepper	*Diplodia* sp
26.	Malapuram - Pepper	*Trichoderma harzianum* Ri Fai
27.	Vellayani - Pepper	*Verticillium chlamydosporium* Goddard
28.	Vellayani - Pepper	*Aspergillus terreus* Thom.

Plate 16 Antagonism exhibited by different fungi

C - Control *Phytophthora*

1 - *Aspergillus canescens*

2 - *Verticillium chlamydosporium*

3 - *Trichoderma viride*

4 - *A. fumigatus*

Plate 17 Antagonism exhibited by different fungi

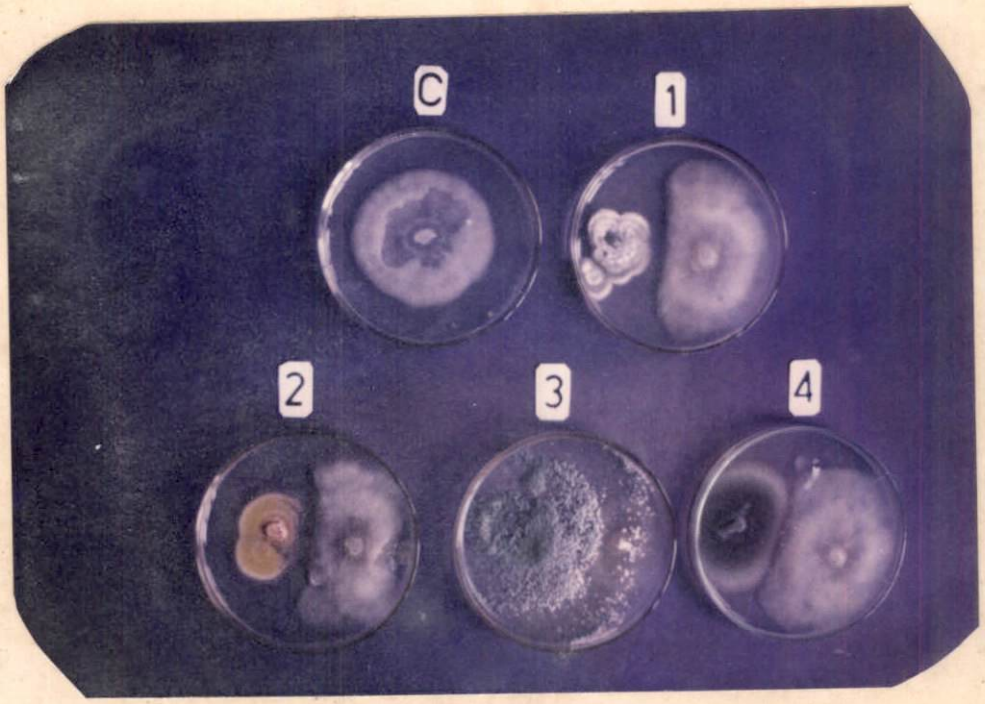
1 - *Aspergillus* sp

2 - *Trichoderma viride*

3 - *Chaetomium* sp

4 - *A. terreus*

5 - *Phytophthora*


C
1
2
3
4

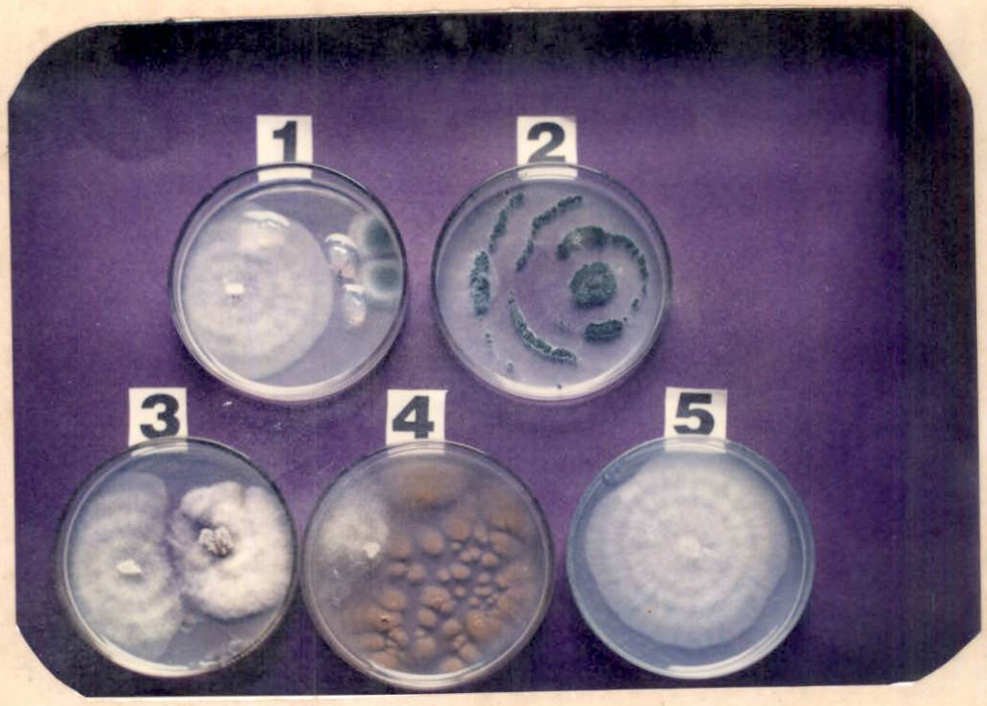

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Plate 18 Antagonism exhibited by different fungi

1 - *Penicillium citrinum*
2 - *Aspergillus flavus*
3 - *A. fumigatus*
4 - *Trichoderma*
5 - *Phytophthora*

Plate 19 Antagonism exhibited by different fungi

1 - *Trichoderma* sp
2 - *Chaetomium* sp
3 - *Aspergillus clavatus*
4 - *Penicillium nigricans*
5 - *Aspergillus* sp
6 - *Phytophthora*


1
2
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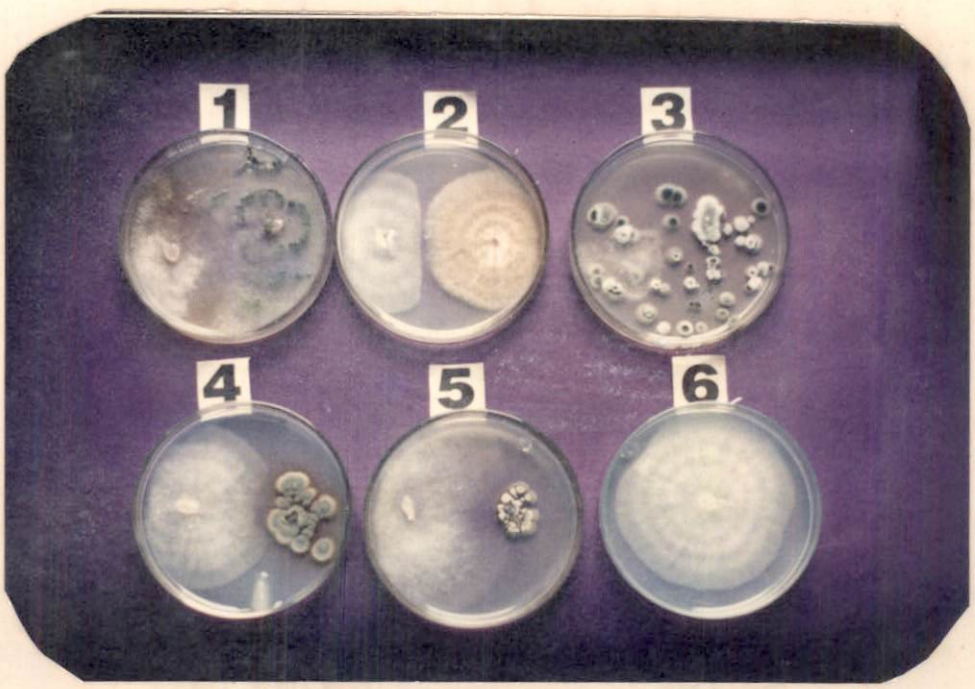
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2
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4
5
6

Table 29 Comparative efficiency of different antagonists in checking *Phytophthora* infection on pepper

Name of the antagonist	Lesion size (in cm) after			
	24 hrs	48 hrs	72 hrs	96 hrs
Penicillium citrinum	-	-	0.6	1.0
Aspergillus flavus	-	-	0.6	1.0
Chaetomium sp	-	-	-	-
Verticillium chlamydosporium	-	-	-	-
Aspergillus canescens	-	-	0.2	0.7
Chaetomium spp	-	-	0.6	1.0
Trichoderma	-	0.2	1.5	2.1
Aspergillus ruber	-	0.3	1.3	1.9
Trichoderma harzianum	-	-	1.2	2.0
Penicillium nigricans	-	-	1.3	2.2
Aspergillus terreus	-	-	-	-
Trichoderma viride	-	0.3	0.9	1.4
Control	1.0	2.3	4.1	Shed

antagonists and inoculated with P_{30} showed any infection. However, after 48 hrs of inoculation symptoms were observed on leaves sprayed with four out of 12 antagonistic organisms. Four days (96 hrs) after inoculation the leaves of the control plants were shed. None of the leaves sprayed with *Chaetomium* sp. *Verticillium Chlamydosporium* and *Aspergillus terreus* showed any disease symptoms, while the lesion size of the leaves sprayed with the other antagonists ranged from 1.0 to 2.2 cm.

Chaetomium sp. *Verticillium chlamydosporium* and *Aspergillus terreus* were used for further studies in which P_{30} isolate was inoculated immediately, 3 days and 7 days after spraying with the spore suspension of the three antagonistic organisms. All the three antagonists completely inibited symptom development on pepper leaves upto 3 days (Table 30). But when the leaves were inoculated with *Phytophthora* 7 days after spraying with antagonists, symptoms were observed on *Chaetomium* sp and *Aspergillus terreus* sprayed leaves. While the *Verticillium* sprayed leaves were free from infection. Even on *Chaetomium* and *Aspergillus terreus* sprayed leaves, the lesion size was only 0.9 and 1.6 cm respectively after 96 hrs of inoculations compared to complete death of the leaves in the control.

Table 30 Comparative efficiency of selected antagonists in checking *Phytophthora* infection on pepper

Chaetomium sp	-	-	-	-	-	-	-	-	-	0.3	0.6	0.9
Verticillium chlamydosporium	-	-	-	-	-	-	-	-	-	-	-	-
Aspergillus terrcus	-	-	-	-	-	-	-	-	-	0.7	1.2	1.6
Control	1	2.6	4.2	Shed	1	2.7	4	Shed	1	3.0	4.2	Shed

DISCUSSION

DISCUSSION

Phytophthora is one of the most important plant pathogens causing diseases on a large number of cash crops in Kerala viz., coconut, pepper, arecanut, rubber, cardamom, cocoa etc. These diseases are very serious either causing heavy loss in yield or complete death of the affected plants. The *Phytophthora* species attacking these crops were named differently by different workers (Coleman, 1910; Mc Rae, 1919, Petch, 1921; Thomson, 1929; Muller, 1936; Thankamma and Pillai, 1973; Sastry and Hegde, 1982). All the confusions arising in the literature is due to the fact that it is difficult to apply a natural system of classification to the genus *Phytophthora* with the data currently available at our disposal. Therefore different workers gave importance to some morphological characters which tend to overlap species descriptions creating confusions in the identification and classification (Ribeiro, 1978). Correct identification of the causal species is a requisite for the orderly progress of the necessary studies of the diseases caused by *Phytophthora*, such as the development of root stocks, chemical control and epidemiology. The present investigation was undertaken with the objective of finding out the correct identity and host range of *Phytophthora* affecting coconut and pepper and to study the variability of the organism.

Seven districts in Kerala were selected for the study. They represented three cropping patterns viz. pure plantations of coconut, pure plantation of pepper and gardens where pepper and coconut are grown together or with other crops like cocoa, arecanut etc.

Coconut and pepper found to be infected with *Phytophthora* species in all the locations surveyed. The symptoms produced on coconut and pepper in different parts of Kerala and on artificial inoculation were similar to those reported by Radha and Joseph (1974, 1982), Holliday and Mowat (1963), Mammootty *et al.*, (1980) and Sarma *et al.* (1991).

Sixteen isolates of *Phytophthora* from bud rot affected coconut from six districts in Kerala representing three cropping patterns and similarly 46 isolates of *Phytophthora* from foot rot affected pepper plants from three districts representing three cropping systems were isolated and brought into pure culture and detailed morphological characters were studied. Since the first description of foot rot of pepper and its causal fungus in 1936 by Muller (1936) till 1990's more than ten species and variety names of *Phytophthora* have been reported as responsible for causing the disease (Tsao, 1991). With a view to correctly identify the causal organism, in depth attention was given on the sporangial ontogeny, sporangial morphology, pedicel length, caducity of sporangia and measurement of sexual structures.

Based on the characters of the 62 isolates, the *Phytophthora* found associated with all the bud rot affected coconut palms in Kerala was identified as *P. palmivora* (Butler) Butler and the one causing foot rot of pepper as *P. capsici* Leonian. This present work confirms the previous reports (Radha and Joseph, 1974; Tsao, 1991).

Variability in cultural, morphological, pathological, as well as sexual characteristics among isolates of *P. palmivora* and *P. capsici* has been reported (Idosu and Zentmyer, 1978; Dantanarayana *et al*. 1984; Mchau and Coffey, 1995). Conceptually variation is the inevitable product of our observational and experimental methods and an aftermath of imperfect attempts at taxonomic groupings. During the past few decades, time tested variability parameters including morphology, physiology, pathogenicity and fungicide resistance have generated a wealth of information which has helped in the basic classification of *Phytophthora*. There were marked differences among the coconut and pepper isolates collected from different parts of Kerala.

Six types of colony characteristics were recognised among the 16 coconut isolates when grown on carrot agar medium. Similarly with 46 pepper isolates, seven colony characteristics were observed. Eventhough there was consistency in these characters, it was difficult to correlate colony characteristics with other morphological characters of the genus so as to

differentiate them into different groups. However, all the soil isolates of pepper had almost similar characters. They were conspicuous by the absence of aerial mycelium unlike the other isolates. Idosu and Zentmyer (1978) used the colony characteristics as one of the criteria to differentiate *P. palmivora* from cocoa into 'Typical and atypical isolates.

Based on the growth rate, the coconut isolates could be grouped into two viz., fast and slow growers. All the Kannur and Kasaragod isolates were fast growers and all of them produced fluffy aerial mycelium which was lacking in the slow growing types. Similarly, the 46 pepper isolates were grouped into three viz., slow, medium and fast. All the soil isolates of pepper were fast growers and unlike the coconut isolates they lacked aerial mycelium.

The study of morphological characters of sporangia of the different isolates revealed that the *Phytophthora* attacking coconut and pepper could be divided into subgroups.

Considerable variations in the sporangial shape were noticed among the different isolates. However, based on the shape, coconut isolates could be grouped into two. All the Kannur and Kasaragod isolates had only lemon shaped and spherical sporangia, while, the others had 10-50% atypical sporangia. A similar pattern was noticed with the soil isolates of pepper (P_{39} - P_{46}). All the Wynad isolates (P_{14}- P_{17} and P_{21} - P_{38})

produced 50-88% atypical sporangia. Numerous reports in literature (Frezzi, 1950; Satour and Butler, 1968; Alizadeh and Tsao, 1985) have shown that sporangial morphology in *P. capsici* is extremely variable. The sporangia can be ovoid, ellipsoid, subspherical or of other intermediate forms. This could be due, in part, to variations in the conditions under which the cultures were grown and sporulation occured.

Based on the sporangial size, coconut isolates could be grouped into two, those with a sporangial length of more than 30 um and those above 30 um. All the fast. growing Kannur and Kasaragod isolates had small sporangia. Similarly in pepper most of the fast growing soil isolates had a sporangial length $\leq$ 30 um.

The L/B ratio of coconut and pepper isolates showed marked variability. However, all the coconut isolates could be grouped into two based on this characters. All the Kannur and Kasaragod isolates had a L/B ratio of $\leq$ 1.3:1 while it was > 1.3:1 in the other isolates. An identical pattern was noticed in pepper isolates also. Majority of the soil isolates had a L/B ratio of < 1.3:1 similar to Kannur and Kasaragod isolates of coconut. Most of the remaining pepper isolates had a L/B ratio of >1.5:1. The L/B ratio of sporangia in *Phytophthora* isolates is being used as a taxonomic criteria in identifying *P. palmivora* and *P. capsici*. However, Alizadeh and Tsao, 1985 cautioned that

this criteria could be helpful only if it is used under defined conditions. In the present investigation, the L/B ratio of all the isolates were recorded by growing the cultures under the same conditions. Thus the study clearly shows that the variations in the L/B ratio of sporangia could be used in differentiating them into groups.

The caducity and pedicel length are two characters, eventhough known to exist in *Phytophthora* since de Bary (1887), have not been studied in detail until recently, nor have been used as a criterion in identification. Failure to use sporangium caducity and pedicel length as diagnostic characters has resulted in occasional misidentifications of some *Phytophthora* isolates (Al-Hedaithy and Tsao, 1979).

The pedicel length and caducity of Kannur and Kasaragod isolates of coconut could not be taken as they failed to produce sufficient number of sporangia under normal conditions. Most of the other isolates of coconut had a pedicel length of less than 25 u . In pepper, all the isolates had a pedicel length of more than 25 u. However, even among the pepper isolates those from soil had a pedicel length more than 60 u.

The caducous nature of coconut isolates was less pronounced (less than 25%), while the caducity in pepper isolates was always more than 25 per cent. Those isolate with a higher pedicel length in general had a higher caducity. Even in highly

caducous isolates, the detachment was never higher than 62 per cent. One reason for this, perhaps, is that the method employed is not sufficient enough to recover all detachable sporangia. Another reason may be the high percentage of immature sporangia in test cultures. Only mature sporangia are detachable (Waterhouse, 1974; Al-Hedaithy and Tsao, 1979) percentage detachment by itself, under any given culture condition cannot be used as a determinant of sporangium caducity in most *Phytophthora* species or isolates unless the uniformity of pedicel length is also taken into account. The result of the study support the findings of Al Hedaithy (1979) that the pedicel length of a species under the genus *Phytophthora* appears to be fixed under normal conditions.

Chlamydospores were produced in all the coconut isolates while only five pepper isolates collected from Wynad produced it.

D^2 analysis was employed to find out the extent of variability in the coconut and pepper isolates. Four important morphological characters of sporangia viz., sporangial length, sporangial breadth, L/B ratio and pedicel length were used to cluster the isolates. Based on D^2 analysis, all the *Phytophthora* isolates were grouped into eight clusters. However, the isolates C_1 and C_5 from coconut could not be included in any of the above clusters. All the coconut isolates from Kannur and Kasaragod

districts were in cluster I along with the isolate C_4. The remaining three isolates were in cluster V with three other pepper isolates. Except the isolate C_4, all other isolates in cluster I were homothallic in nature. This homothallic nature may be one of the factors for their uniformity in morphological characters. Isolate C_1 - C_6 were highly heterogenous in their characters with the result they were included in cluster I, Cluster V and two as indepenent. This variability may be due to the heterothallic nature of the isolates.

The pepper isolates were highly heterogenous in nature and they were grouped into seven clusters. This high degree of variability may be due to the heterothallic nature of the isolates. It's possible to effect a better clustering of different isolates of *Phytophthora*, with more morphological characters.

Phytophthora species are either homothallic or potentially homothallic, but functionally heterothallic (Agarwal *et al*. 1990). *P. palmivora* isolates from coconut were homothallic or heterothallic. All the isolates of coconut from Kannur and Kasaragod were homothallic, and produced large number of typical oospores. All other isolates from different parts of Kerala produced oospores only by mating with typical A_1 mating types showing that they are of the A_2 mating type. The present observation clearly indicates that *Phytophthora* isolates of

coconut from Kerala have two distinct mating behaviour. That is some are homothallic and others are heterothallic with A_2 mating type. This is the first report of varied mating behaviour of *P. palmivora* isolates from coconut in Kerala. Sastry and Hegde working with *P. palmivora* of different crop plants from Karnataka observed both A_1 and A_2 mating types. Aged cultures of *Phytophthora* may give rise to colonies bearing abundant oospores (Tsao *et al.* 1980). However, in the present study cultures from Kannur and Kasaragod produced characteristic oospores even in young cultures and those of the heterothallic group failed to produce oospores even in aged cultures. This clearly indicates their homothallic nature.

All the *Phytophthora capsici* isolates from different parts of Kerala failed to produce oospores in cultures. However, on mating with A_2 types, it produced oospores indicating that all the *P. capsici* in Kerala are of the A_1 mating type. Sastry and Hegde (1982) have shown the presence of both A_1 and A_2 mating types from Karnataka, while Sarma *et al.* (1991) observed only A_1 mating type of *P. capsici* in Kerala. Thus the result of the present investigation supports the findings of Sarma *et al.* (1991). Sex organ formation in *P. palmivora* from pepper was reported in single spore cultures, stored for 2 to 3 months (Turner, 1962; Tsao, 1991). However, in the present investigation sex organ formation in cultures on prolonged storage or when crossed with A_1 mating types were not noticed.

This clearly indicates the heterothallic nature of *P. capsici* isolates. Based on the abundance of oospore production, the pepper isolates could be grouped into two categories, all the soil isolates of *P. capsici* produced large number of oospores while the other isolates produced only sparse oospores.

Several natural and synthetic media were used for studying the growth characteristics of *Phytophthora* isolates from coconut and pepper. In general, all the growth characters were better in carrot agar, oats and coconut water. This suggests that these media may be richer in certain nutrients (eg. Minerals, vitamins, natural sterols) as observed by Kaosiri *et al.* (1980).

Variability in the morphological characters like sporangial dimensions, pedicel length and caducity of *Phytophthora* isolates varied with the composition of the media, pH and light intensity. Morphological characters especially pedicel length and caducity are considered as two important diagnostic characters. Being a subjective character whenever these characters are used for identification, use of standard media and growing conditions are of atmost importance as suggested by Alizadeh and Tsao (1985); Brasier and Griffin (1979) and Al-Hedaithy and Tsao (1979).

Light influenced the sporangial production in media and in dark the sporangial production was inhibited, while, much variation in the quantity of sporangial production was not noticed when the culture was exposed to either continuous light or to intermittent light and darkness (Brasier, 1969, Rocha and Machadu, 1973; Cohen *et al.*, 1975; Sagir *et al.*, 1982 and Alizadeh and Tsao (1985). However, the result was not in full agreement with the work of Narula and Mehrotra (1984) on *P. colocasiae* where, they showed that darkness favoured vegetative growth while for sporangial production, continuous light was necessary. This variation in the results may be due to the difference in the species of *Phytophthora* studied.

Variability in the symptom expression on coconut and pepper was observed when different *Phytophthora* isolates were used for artificial inoculation. All the coconut isolates induced typical bud rot symptoms on healthy seedlings. But the intensity of infection varied with isolates. The Kannur and Kasaragod isolates were highly pathogenic. All the coconut isolates also produced typical foot rot symptoms on pepper plants. Similarly, six pepper isolates collected from different parts of Kerala could induce bud rot symptoms. This indicates that all the coconut isolates in Kerala can cause foot rot disease of pepper while, only some of the isolates of pepper are potent enough to induce bud rot symptoms on coconut.

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When the different isolates of coconut and pepper were inoculated on coconut leaves, six different and distinguishable symptoms were observed. However, such a variation was not observed on pepper leaves. This differential symptom expression of coconut leaf may be used in grouping the various isolates of *Phytophthora*. According to Manomohan Das (1982), the pathogen responsible for bud rot of coconut, foot rot of pepper, azhukal disease of cardamom, abnormal leaf fall of rubber etc. are all *P. palmivora*. While Sarma and Nambiar (1982) and Sarma *et al*. (1991) reported that the pathogen responsible for foot rot of pepper is *P. capsici* and is different from the bud rot pathogen. The present study shows that eventhough the pathogen responsible for bud rot is *P. palmivora*, it could produce symptoms on pepper similar to that produced by *P. capsici*. This indicates that in Kerala, the foot rot of pepper is caused both by *P. palmivora* and *P. capsici*.

The *Phytophthora* isolates from coconut and pepper were found to have a wide host range, comprising of crop plants, ornamentals and weeds and they could serve as collateral hosts, there by perpetuating the pathogen during the off season. A wide host range of *Phytophthora* spp was reported by several workers (Manomohan Das, 1982; Sastry and Hegde, 1987 and Dodan and Shyam, 1995).

Leaf extracts of bougainvillea, neem and ocimum showed varying degrees of inhibition of symptom expression on pepper.

Bougainvillea extract was not effective when applied as a crude extract while it was effective when exposed to high temperature and pressure. Such variation in inhibition was not observed with ocimum extract. The release of a toxic principle from bougainvillea extract under high tempeature and pressure may be responsible for this. A similar observation was also made by Agarwal (1990) while working with inhibition nature of crude distillate of *Ocimum gratissimum* against *P. palmivora* of cocoa pods.

Phytophthora isolates from coconut and pepper responded differently against different antibiotics and fungicides. Kannur and Kasaragod isolates of coconut and eight pepper isolates from different parts of Kozhikode and Thiruvananthapuram were insensitive to streptomycin even at a concentration of 2000 ppm. While, many other isolates were completely checked under *in vitro* condition even at a concentration of 50 ppm. Although, most fungi, are insensitive to bacterial antibiotics like streptomycin, members of perenosporales behaved like prokaryotic organisms in that they are sensitive to antibacterial antibiotics especially to those with broad spectra (Tsao, 1983). The inhibitory nature of streptomycin against different species of *Phytophthora* was also recorded by Eckert and Tsao (1962); Leary *et al.* 1982 and Agarwal *et al.* 1990.

Considerable variability in the sensitivity of *Phytophthora* isolates to Ridomil was observed in the present

study. In general, all the isolates which showed high degree of insensitivity to streptomycin also to an extent, showed resistance to higher concentration of Ridomil. It is known that various species of *Phytophthora* differ in their response to Ridomil (metalaxyl) (Fuller and Gisy, 1985; Ramachandran *et al.*, 1988). Wide variability is also reported even among the isolates (Shew, 1984) and races (Bruck *et al.* 1980) within a single species. This information will help in determining the concentration of Ridomil for controlling *Phytophthora* diseases of crop plants.

Of the several phyllosphere and soil microorganisms tested 28 organisms were found to be inhibitory, under *in vitro* conditions against *Phytophthora* spp. Among these organisms, *Verticillium chlamydosporium*, *Aspergillus terreus* and *Chaetomium* sp were found effective in checking foot rot disease of pepper. *V. chlamydosporium* could inhibit foliar infection on pepper plants even when the plants were artificially inoculated with pathogen seven days after spraying with the biocontrol agent. While *Aspergillus terreus* and *Chaetomium* sp were effective upto a period of 3 days. Under *in vitro* conditions both *Verticillium chlamydosporium* and *Aspergillus terreus* produced an inhibition zone, while Chaetomium overgrew *Phytophthora* cultures indicating the possible toxin/inhibitory chemical production by *Verticillium* and *Aspergillus terreus*. The inhibitory effect of *V. chlamydosporium* was reported by Domsch *et al.* 1980).

Table 31 Comparison of characters of homothallic and heterothallic isolates of coconut

Characters	Kannur and Kasaragod isolates	Other isolates
1. Isolate number	C_7 to C_{16}	C_1 - C_6
2. Growth rate in Carrot agar medium	Fast	Slow
3. Sporangial production	Nil to sparse	Profuse
4. Shape of sporangium (%)	Spherical (20-75%) or Lemon (25 - 80%)	Spherical (20-48.8%) Lemon (30 - 57.1%) Irregular (10-50%)
5. Size	Small	Large
6. Length	22.2 to 29.5 um	49.9 to 54.8 um
7. Breadth	19.4 to 29.1 um	14.5 to 36.6
8. L/B	1:1 to 1:13	1:1.12 to 1:1.8
9. Pedicel length	-	5.4 to 25.7 um
10. Caducity	-	11.1 to 23.6
11. Sexuality	Homothallic	Heterothallic
12. Mating type	-	A_2
13. Antheridia		
Length	12.48 to 24.26 um	9.36 to 17.67 um
Breadth	9.15 to 21.60 um	7.2 to 17.60 um
14. Oogonia	15.0 to 29.9 um	38.6 to 46.8 um
15. Oospore	13.0 to 25.2 um	33.7 to 42.6
16. Streptomycin tolerance	Insensitive $\geq$ 2000 ppm	Sensitive $\geq$ 50 ppm
17. Metalaxyl tolerance	Insensitive $\leq$ 100 ppm	Sensitive $\leq$ 50 ppm
18. Pathogenicity	Highly virulent on coconut, pepper and other plants	Less virulant on coconut and other plants

Table 32 Comparison of characters of soil and foliar isolates of pepper

Characters	Soil isolate	Foliar isolates
1. Isolate number	P_{39} to P_{46}	P_1 - P_{38}
2. Growth rate in Carrot agar medium	Fast	Slow
3. Sporangial production	Produced	Produced
4. Shape of sporangium (%)	Spherical (Nil-50%) Lemon (40-91.6%) Others (Nil to 10)	Spherical (0-43.5%) Lemon (11.8-80%) Others (0-88.2%)
5. Size		
Length	20.8 to 54.9 um	29.1 to 67.8 um
6. Breadth	20.3 to 32.8 um	17.8 to 36.6 um
7. L/B	1:1.02 to 1.59	1:1.26 to 2.03 um
8. Pedicel length	40.8 to 73.2 um	24.9 to 74 um
9. Caducity	45.4 to 61.5%	16.6 to 68.4%
10. Mating type	A_1	A_1
11. Oospore production in carrot agar medium	Abundant	Sparse
12. Antheridia		
Length	8.32 - 9.8 um	6.24 - 15.25 um
13. Breadth	5.9 - 10.7 um	6.6 - 14.56 um
14. Oogonia	29.4 - 35.7 um	26.6 - 46.9 um
15. Oospore	24.3 - 31.0 um	22.3 - 41.8 um
16. Streptomycin tolerance	Sensitive	Some are insensitive
17. Metalaxyl tolerance	Sensitive	Sensitive
18. Pathogenicity	Virulent	Virulent

Based on the study conducted with 16 isolates of *Phytophthora* causing bud rot of coconut and 46 causing foot rot of pepper, the following conclusion could be made. The characters of Kannur and Kasaragod isolates were different from the other isolates (Table 31). From this observations, it is clear that there exists two sub populations among the *P. palmivora* causing bud rot of coconut.

Similarly, based on the characters of 46 pepper isolates, *P. capsici* could be grouped into two as per the details given below (Table 32).

The variations in the cropping pattern did not appreciably influence the variability of the isolates. However, wide range of variation could be noticed among the isolates of pepper and coconut. Presence of both A_1 (Pepper) and A_2 (coconut) mating types may be one of the possible reasons for the abundance of varied isolates in Kerala.

SUMMARY

The genus *Phytophthora* is a serious pathogen causing severe economic losses to several important plantation crops of Kerala. The present investigation was taken up to study the distribution of species of *Phytophthora* among coconut and pepper gardens in Kerala and to assess their comparative role in causing diseases on coconut and pepper. It also aimed at isolating viable antagonistic organisms against the pathogen so as to utilise them in future for biological control. The study was conducted at Department of Plant Pathology, College of Agriculture, Vellayani during 1992-96.

Periodic collections were made from bud rot affected coconut and foot rot affected pepper from seven districts of Kerala representing three cropping systems viz., pure coconut garden, pure pepper garden, and mixed gardens of coconut and pepper. Sixteen *Phytophthora* isolates were made from bud rot infected coconut palms and 46 from foot rot affected pepper plants.

Morphology of all the 62 isolates were studied in detail. The sixteen coconut isolates exhibited six types colony morphology. The length, breadth, L/B ratio, pedicel length, caducity, zoospore production and Chlamydospore production were

studied. Sporangia of pepper isolates, irrespective of place of collection were bigger than the coconut isolates. They also had higher L/B ratio, pedicel length and caducity. Based on the morphological characters the *Phytophthora* isolates from pepper were identified as *P. capsici* and coconut isolates as *P. palmivora*. Ten *Phytophthora* isolates from Kannur and Kasaragod districts (representing pure coconut garden and mixed gardens of coconut and pepper (C_7-C_{16}) - failed to produce sporangia in carrot agar medium, while it produced sexual structures. The sporangia when produced by cold treatment were smaller in size compared to the other isolates and had a L/B ratio of 1-1.3.

The 62 isolates were grouped based on D^2 analysis with respect to the morphological characters. Based on the analysis the isolates could be clustered into 8 groups and two isolates remained independently.

All the isolates of coconut and pepper (except C_7-C_{16} were heterothallic in nature. The isolates were mated with known A_1 and A_2 mating types. Coconut isolates produced oospores when mated with known A_1 mating type and pepper isolates produced oospores when mated with known A_2 mating type. Even on mating with opposite mating group, the abundance of sexual structures varied among isolates. Only nine pepper isolates produced abundant number of oogonia, and out of these nine isolates, eight were soil isolates collected from Wynad, Kozhikode and Trivandrum

district. All the Kannur and Kasaragod isolates (C_7 - C_{16}) produced abundant number of sexual structures on carrot agar even without mating indicating their homothallic nature. This is the first report of a homothallic *Phytophthora* causing bud rot in Kerala.

Seven media were tried to find out the best media suitable for the growth and sporulation of coconut and pepper isolates. Based on the study, oat meal was found to be the best media for isolation and carrot agar for the culturing and maintenance of the isolates. Sporangial length, breadth, L/B ratio, pedicel length and caducity of the isolates varied with media.

Light was found necessary for the production of abundant sporangia in cultures.

Pathogenicity of the *Phytophthora* isolates was tested using coconut leaves, tender coconut, coconut seedlings and rooted cuttings of pepper. All the 16 coconut isolates produced water soaked lesions on pepper plants. All the pepper isolates produced lesions on coconut leaves and tender coconut nut, but only six isolates could produce typical bud rot on coconut seedlings. Typical symptoms of bud rot on coconut leaves, tender coconut and seedlings were observed with artificial inoculation within one to two hours, five to seven days and nine to sixteen days respectively. While pepper leaves were infected within two

to four days. Six cultivated plants, four ornamental plants and nine weeds found in coconut and pepper plantations were infected by pepper and coconut isolates on artificial inoculation.

The inhibitory effect of crude and sterilized extract of ocimum, neem and bougainvillea on *Phytophthora* isolates were tested. Ocimum extract showed maximum inhibitory effect compared to neem and bougainvillea both under laboratory condition and on pepper plants.

Isolates showed variability in their response to streptomycin and Ridomil. Sensitivity of all the 62 isolates were tested against 50, 200, 400, 600, 800, 1000, 1500 and 2000 ppm. Eight pepper isolates and coconut isolates of Kannur and Kasaragod districts were insensitive even at 2000 ppm while 25 pepper isolates and six coconut isolates were sensitive at a low concentration of 50 ppm. In the case Ridomil, the concentrations tested were 50, 100 and 150 ppm. At 150 ppm, none of the 62 isolates could grow. Eight pepper isolates and 10 coconut isolates (Kannur and Kasaragod districts) were insensitive at 100 ppm concentration, while all other isolates failed to grow even at 50 ppm.

Microorganisms found associated with healthy and diseased pepper and coconut plantations and microorganisms from soils of pepper and coconut gardens of different parts of Kerala were isolated and tested for its inhibitory effect on

Phytophthora using dual culturing technique. Twenty eight fungal species inhibited the growth of *Phytophthora* isolates under *in vitro* condition. Out of these 28 organisms, 12 inhibited leaf infection by *Phytophthora* on pepper plants to varying degree. Three organisms viz., *Verticillium chlamydosporium*, *Aspergillus terreus* and *Chaetomium* sp completely protected the leaves from the pathogen infection even after seven days of spraying.

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DISTRIBUTION OF SPECIES OF PHYTOPHTHORA AFFECTING COCONUT AND PEPPER IN KERALA

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ABSTRACT OF A THESIS

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ABSTRACT

The present investigation was undertaken to correctly identify the species of *Phytophthora* causing bud rot of coconut and foot rot of pepper in Kerala and to find out their comparative role in causing diseases on coconut and pepper. The study also aimed at isolation of viable antagonistic agents against the pathogen, so as to utilize them in future for biological control.

The study was conducted during 1992-96 at College of Agriculture, Vellayani and Indian Institute of Spices Research, Kozhikode. Detailed symptomatology of both the diseases at different locations were studied. Periodic collections were made from diseased coconut and pepper from seven districts of Kerala.

The morphological characters of the isolates were studied in detail. The length, breadth, L/B ratio, pedicel length and caducity of sporangia of coconut isolates were smaller than pepper isolates. The sporangial shapes differed considerably between and among the isolates. All the isolates except those collected from Kannur and Kasaragod districts (C_7 - C_{16}), produced abundant number of sporangia on carrot agar.

Phytophthora are either heterothallic or homothallic. Generally *P. palmivora* and *P. capsici* are heterothallic. But ten

coconut isolates obtained from Kannur and Kasaragodu districts produced abundant number of sexual structures on carrot agar, indicating their homothallic nature and this is the first report of homothallic *Phytophthora* causing bud rot of coconut in Kerala. All other 52 isolates were mated with known A_1 and A_2 mating type and all the coconut isolates produced oospores with A_1 and all the pepper isolates produced oospores when mated with A_2.

Zoospore production was induced from sporangia using cold treatment. The number of zoospores in sporangia ranged from 6-15 in number. Chlamydlospore production was a rare phenomenon in pepper isolates while all the coconut isolates and five pepper isolates produced chlamydospores.

All the isolates were inoculated into pepper seedlings, coconut leaves, tender coconut and coconut seedlings and the time required for infection, variation in symptom expression by different isolates etc. were studied. All the sixteen coconut isolates produced foot rot in pepper. Fourty six isolates of pepper produced lesions on coconut leaves and nut rot in tender coconut, while, only six isolates of pepper produced bud rot in coconut plants. The cross infectivity of pepper isolates needed further confirmation to prove the point beyond doubt. Eight cultivated plants, 9 ornamental plants and 12 weed plants were tested for the host range of coconut and pepper isolates. Many plants belonged to the above three groups took infection successfully.

The microorganisms found associated with healthy and diseased pepper and coconut plantations and microorganisms from soils of pepper and coconut gardens of different parts of Kerala were isolated. Out of more than 200 microorganisms, only 28 fungi inhibited the growth of *Phytophthora in vitro*. On pepper plant, 12 isolates had inhibitory action. Three organisms, viz., *Verticillium chlamydosporium*, *Aspergillus terreus* and *Chaetomium* sp checked the pathogen completely even after seven days of spraying with the organisms.