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Characterization of *Phytophthora* de Bary inciting capsule rot/ azhukal and leaf blight disease of cardamom (*Elettaria cardamomum* Maton)

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Abstract

A detailed study was carried out to characterize the *Phytophthora* species associated with capsule rot and leaf blight diseases in cardamom. *Phytophthora* was isolated from rot affected plant parts such as leaf, capsule and pseudostem collected from different cardamom growing tracts of Idukki and Wyanad districts of Kerala during the monsoon and post monsoon periods. Morphological and physiological studies such as colony morphology, growth rate, morphology of sporangia, pedicel length, caducity, chlamydospores, sex organs, compatibility type, growth on synthetic medium and temperature relationship was studied using 25 *Phytophthora* isolates from cardamom. Two groups of *Phytophthora* could be identified from infected capsules, leaves and pseudo stem. The group one having pigmentation, chlamydospore production and growth at >35 °C where as the group two having no pigmentation, no chlamydospore production and no growth at 35 °C. The group one is identified as *P. nicotianae* var. *nicotianae* and group two as *P. meadii*. From the studies it has been confirmed that two species of *Phytophthora viz.P. meadii*, and *P. nicotianae* var. *nicotianae* are involved in initiating capsule rot occurring during the monsoon season of which *P. meadii* is the predominant one, The leaf blight occurring during the post monsoon season is due to *P. nicotianae* var. *nicotianae*. However, there is certain amount of overlapping of these in initiating the disease in cardamom. *P. palmivora* could not be isolated from the infected plant parts as reported earlier.

Key words: Azhukal, characterization, *Elettatia cardamomum. Phytophthora meadii*, pigmentation, sporangia

Introduction

Capsule rot (Azhukal) disease caused by *Phytophthora* species has been implicated as a serious problem causing extensive damage in many cardamom growing tracts in the high ranges of Kerala. The disease appears with the onset of the southwest monsoon season. It is characterized by the rotting of leaves, panicles, capsules and pseudostem. Menon *et al.*, (1972) for the first time reported the disease as caused by *Phytophthora* and proved Koch's postulates and identified the pathogen as *P. nicotianae* (Thankamma and Pillai, 1973; Liyanage *et al.*, 1983) P. *palmivora* (Radha and Joseph,1974) and *P. meadii* (Sastry, 1982., Anon 1986). Later another disease called leaf blight due to *P. meadii* was also identified (Anon 1986). This disease appears during the post monsoon period from November to February.

The involvement of various species of *Phytophthora* in causing capsule rot and leaf blight disease as reported by various workers is controversial and an elaborate study in this aspect needs utmost importance. The present study was taken up with the objective to identify the actual pathogen involved in initiating capsule rot and leaf blight disease of cardamom and the results are discussed.

Materials and Methods

Azhukal/ rot affected plant parts such as capsules, pseudostem and leaves were collected from different cardamom growing tracts during the monsoon season. Leaf blight samples were was collected during the post monsoon season. The isolation of the pathogen was done using PVPH medium (Tsao and Guy,1977). Around 12[illegible] isolates were collected from 80 different location[illegible] which 25 isolates were short listed and studied in[illegible]

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for colony morphology, growth rate on agar, sporangial morphology, ontogeny, caducity, chlamydospore production, sex organs formation, pigmentation and temperature relations (Table 1)

Table 1. Morphology of *Phytophthora* isolates of cardamom

Place of Collection	Plant part	Isolate No.	Colony morphology/Pattern
Myladumpara	Capsule	CPC 159	Chrysanthemum
Pachakkanam	Pseudostem	CPP 244	Uniform cottonwool like aerial mycelium with slight lobes
Pallivasal	Capsule	CPC 175	Uniform cottonwool like aerial mycelium with slight lobes
Udumbanchola	Capsule	CPC .109	Uniform cottonwool like aerial mycelium with slight lobes
Mali	Capsule	CPC 310	Lobed
Puttady	Capsule	CPC 311	Chrysanthemum
Vandanmedu	Leaf	CPL 312(1)	Uniform cotton wool like aerial mycelium with slight lobes
Vandanmedu	Pseudostem	CPP 312(d)	Lobed
Pulyanmala	Capsule	CPC 313	Lobed
Mettukuzhy	Capsule	CPC 317	Uniform cotton wool like aerial mycelium with slight lobes
Myladumpara	Capsule	CPC 318	Lobed
Poopara	Leaf	CPI.342	Uniform cotton wool like aerial mycelium with slight lobes
Puttady	Capsule	CPC 349	Lobed
Myladumpara	Pseudostem	CPP 350	Uniform cotton wool like aerial mycelium with slight lobes
Myladumpara	Leaf	CPL 352	Uniform cotton wool like aerial mycelium with slight lobes
Pallivasal	Capsule	CPC353	Lobed
Muthukad	Pseudostem	CPP 356	Lobed
Muthukad	Capsule	CPC 357	Lobed
Udumbanchola	Capsule	CPC 358	Lobed
Myladumpara	Capsule	CPC 363	Chrysanthemum
Parathode	Capsule	CPC 365	Lobed
Meppadi	Leaf	CPL 366	Lobed
Meppadi	Leaf	CPL 368	Chrysanthemum
Meppadi	Leaf	CPL 369	Uniform cotton wool like aerial mycelium with slight lobes
Thachungal	Capsule	CPC 375	Chrysanthemum

Colony morphology and growth rate on agar

The colony morphology of all the 25 isolates were studied in carrot agar and corn meal agar (Brasier and Griffin, 1979; Liyanage and Wheeler, 1989). Four mm discs cut from the edge of 72 h old culture (of all the 25 isolates) was inoculated centrally in 90 mm diameter plates containing15ml of the corresponding medium, covered in aluminium foil and incubated in total darkness at 24±1°C for 5 days. Colony morphology was examined against a black background.

The growth rate was studied using five different types of media viz., carrot agar (Brasier *et al.*, 1993), capsule extract agar, oat meal agar and casein hydrolysate tyrosine agar or Timmer's medium (Timmer *et al.*, 1970). Capsule extract agar was prepared by smashing 200 g tender capsules and boiling in 1 litre of distilled water for 10 min. The juice was squeezed through two layers of cheese cloth and volume made up to one 1iter. Agar was added @ 20g /1 and sterilized. For studying the growth rate, discs were inoculated centrally on plates (90mm diameter) containing 15ml of the medium and incubated in dark at 24±1°C and the growth marked at 24hrs Interval for 120 hrs. After the incubation period two measurements of the colony diameter were made at right angles to each other and the growth rate was calculated and expressed as mm day^{-1}

Sporangial morphology, ontogeny and measurements

Sporangial morphology and sporulation was studied using slide culture technique in carrot agar medium (Dhantanarayana *et al.*, 1984). Inoculum plugs of 3 mm size were cut from the edge of 72 h old culture and placed on the agar-coated slide and incubated for 5-7days at 24±1°C and then fixed in lactophenol. These slides were microscopically examined for sporangial morphology and ontogeny.

Sporangial measurements were taken as per Tsao *et al* (1985). Discs of 4mm cut from the edge of 72hrs old actively growing culture incubated in the dark was placed in 5ml of sterile distilled water in petri-dishes and incubated under continuous light for five days. The sporangia bearing mycelium that had grown out into the surrounding water during the incubation period were cut with a scalpel and mounted on a drop of lactophenol. The length, breadth and pedicel length of 50 randomly chosen sporangia was measured under 15x X 40x magnification using an ocular micrometer.

Caducity

Among the discs incubated under light, one disc each was transferred into a test tube with 4ml of sterile water and shaken for 1 min to dislodge the sporangia. This water was poured into a counting dish and the total number of dislodged sporangia was counted under 4x magnification. The disc after dislodging the sporangia was pressed against a slide and the number of attached sporangia/ microscopic field was counted. The total number of sporangia /disc was calculated and percent caducity was calculated using the formula

$$\frac{\text{No. of detached sporangia/disc}}{\text{Total no. of sporangia / disc}} \times 100$$

Chlamydospore production

Ribeiros liquid medium (Ribeiro *et al.*, 1975) @ of 50ml/ 100ml flask was sterilized at 120°C for 20 min. These were inoculated with 5mm discs of 72 h old culture and incubated in the dark at 24±1°C for one month. Similarly 5mm discs were inoculated in carrot agar and Ribeiros solidasar medium and incubated in the dark for one month at 24±1°C. Infected plant parts were also stored in the refrigerator at 10°C and observed one month after incubation

Sexual reproduction and mating type

Dual culture method was used for sex organ production. Test isolates of cardamom were plated against known A1 isolate of black pepper in carrot agar with thiamine (Brasier *et al.*, 1993) and also in Ribeiros medium. For pairing, 3mm discs cut from the edge of 72 h old culture of both A1 and test isolates of cardamom were placed on opposite sides of the petri dish at a distance of 3cm apart. The plates were incubated in the dark at 21±1°C. Observations were taken at 5 day intervals for 21 days. Measurements of oogonia and antheridia were also taken from these paired plate cultures.

Pigmentation

Timmers's medium containing casein hydrolysate and tyrosine were used for pigment production. Agar slopes of the medium were made and inoculated with 3 mm discs of 72 h old culture and incubated in the dark at 24±1°C for 15-21 days (Kennedy and Duncan, 1995). Pigment production was observed on the 21st day

Temperature relationship

Inoculum discs of 3mm were cut from the margin of 72 h old culture and inoculated centrally in 90 mm petri dishes containing 15 ml of the medium. These plates were incubated in a BOD incubator at temperatures ranging from to 10-35°C and observations on the radial growth was measured at 24 hn interval for 96 hr and the average growth rate was calculated.

Results

Colony morphology and growth rate

Three distinct type of colony morphology was mainly observed among the isolates. They were uniform cotton wool like aerial mycelium over the entire colony, stellate or petalloid pattern and lobed pattern (Plate 1). No particular pattern could be assigned to capsule, leaf or pseudo stem isolates.

Plate 1. Colony morphology of *Phytophthora* isolates of cardamom

Among the five media tested viz. carrot agar, corn meal agar, capsule extract agar, casein hydrolysate tyrosine agar and oat meal agar, carrot agar was found to be the best medium for the growth (7-9 mm/d) followed by oat meal agar. In casein hydrolysate tyrosine agar and capsule extract agar, the growth rates of the isolates were very poor (1.5 mm/d). Pseudostem isolates showed comparatively faster growth (4.35 mm/d) followed by leaf isolates (3.9mm/d) and capsule isolates (Table 2).

Sporangial morphology and ontogeny

Sporangia, irrespective of whether capsule, pseudostem or leaf isolate, were ovoid to ellipsoidal with basal plug conspicuous in some. The papillae were very

prominent with wide exit pores. Sporangia were mostly symmetrical. Although asymmetrical types were also noticed occasionally. In some isolates swelling at the point of sporangial formation was observed. Sporangia with round base and some times with slight tapering was also noticed. Sporangia were produced in simple sympodia and well defined in agar. They were formed in abundance when the culture disc was kept in water under continuous fluorescent light (Plate 2). Certain isolates showed the presence of hyphal thickenings and sporangial outgrowths (Plate 2a & 2b).

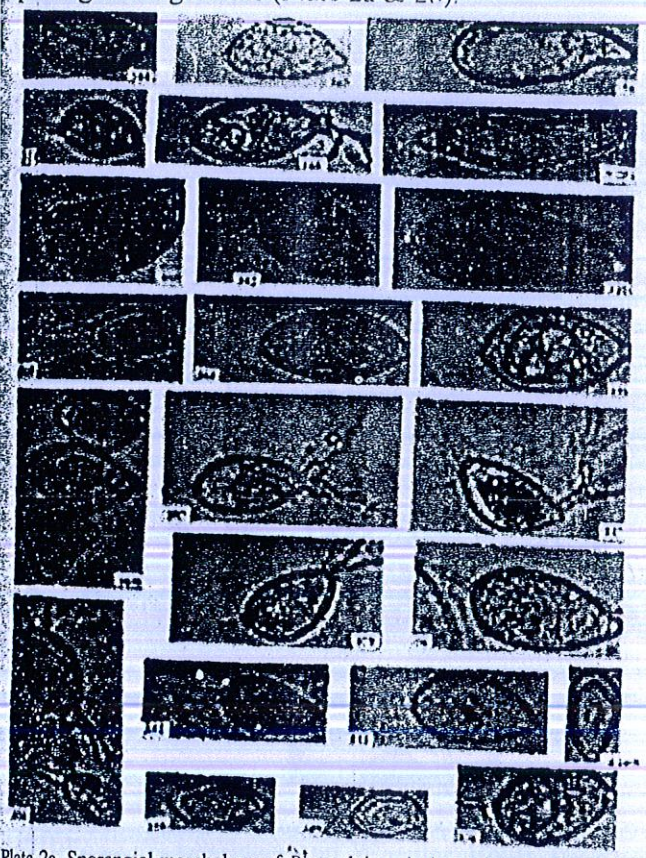

Plate 2a. Sporangial morphology of *Phytophthora* isolates of cardamom

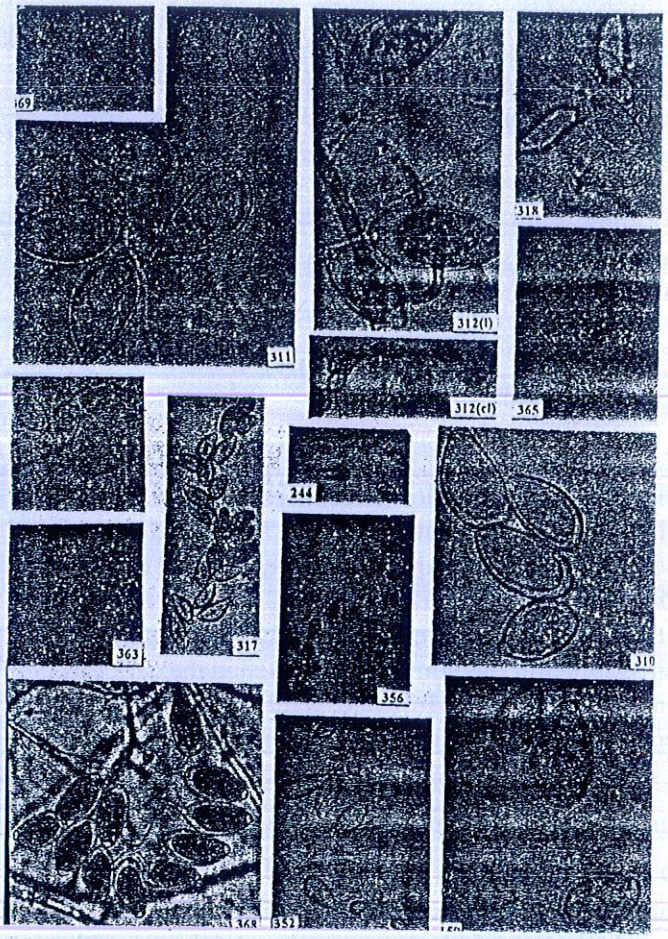

Plate 2b. Sporangial ontogeny of *Phytophthora* isolates of cardamom

Sporangial measurements:-

Length, breadth and l/b ratio

The size of the sporangia differs considerably in solid agar and in water culture. Sporangia were generally larger in solid culture. There was no considerable variation in sporangial size between capsule, leaf and pseudostem isolates. The mean sporangial length: breadth ratio of the three group of isolates were almost same and were more than 1.7 (1.7-2.09). The sporangial dimensions of individual cultures in solid and water culture is given in Table 3.

The mean sporangial length in solid culture ranged from 51.39-56.82 μm and breadth ranged from 27.43-28.45 μm. In water culture it ranged from 42.22-45.40 μm and 24.99-25.09 μm respectively. The l/b ratio in solid culture ranged from 1.84-2.09 as compared to 1.75-1.82 in water (Table 3).

Pedicel length

In all the cases the pedicel length was found in the intermediate group range ie. 5-20 μm. But certain isolates showed considerable variation in pedicel length in solid and water culture (Table .3). The mean pedicel length of

Table 2. Growth rate on Agar media

Media and time after inoculation		Capsule		Leaf		Pseudo-stem	
		Mean	Range	Mean	Range	Mean	Range
CA	0-120h	7.92:±0.22 (6.7-9.00)		8.06±.41 (6. 75-9.00)		8.6±0.25(7.9-9.00)	
CMA	O-120h	5.54±0.24 (4.54-6.18)		5.92±0.18(5.43-6.43)		6.04±0.20(5.86-6.43)	
CHT	0-120h	3.13± 1.54 (2.15-4.97)		3.90±0.50(3.86-4 .03)		4.35±0.25(4.00-5.08)	
CEA	0-120h	4.32±0.20 (2.82-6.13)		4.44+0.13(3.93-4.63)		4.19±0.17(3.82-4.5)	
OMA	0-120h	7.29±0.23(5.69-7.69)		7.32± 0.17(6.69-7.69)		6.83±0.12(6.57-7.07)	

Table 3. Mean sporangial dimensions of *Phytophthora* isolates of cardamom

Variate	Capsule		Leaf		Pseudostem	
	Solid culture	Liquid culture	Solid culture	Liquid culture	Solid culture	Liquid culture
Length	55.25±3.18	45.40±1.56	51.39±3.05	42.22±2.96	56.82±4.05	43.85±2.77
Range	25.8±113.52	20.64±116.1	33.54±70.2	25.8±72.24	29.7±108.36	25.8±79.98
Breadth	28.36±1.09	24.99±0.56	28.45±1.30	24.79± 1.84	27.43±0.74	25.0± 1.63
Range	16.2±46.44	10.32±41.28	18.06±43.86	51.16±41.28	154.48±36.12	15.48±38.7
L/B	1.95±0.09	1.82±0.04	1.84±0.10	1. 75±0.09	2.09±0. 16	1.77±0.53
Range	1.62-2.91	1.60-2. II	1.59-2.21	1.56-2.08	1.82-2.52	1.63-1.87
Pedicel length	15.05-1.40	21.32±1.45	15.40±3.75	21.44±0.66	14.83±2.09	23.96±3.31
Range	2.58-67.08	1.29-69.66	5.16-45.9	5.16-67.08	2.58-45.9	2.58-69.66

the three group of isolates in solid culture varied from 14.83-16.62 μm and in water culture it varied from 21.32-23.96 μm.The high frequency of mean pedicel length in the range of 5-20 μm was noticed in solid culture. The pedicel length of >20 μm was observed in the range of 4-68% .In water culture the pedicel length in the range of 5-20 μm was noticed @ 53.8% and >20 μm was noticed @ 46.2%. The capsule isolates showed a frequency of 50-96% and mean pedicel length in the range of 5-20 μm.

Caducity

The degree of caducity (expressed as % detached sporangia) varied in solid and water culture. Caducity was more in water culture (28.46-41.72%) as compared to solid culture (11.57-14.55%) (Table 4). Not much variation was observed in percent caducity between the three groups of isolates in solid culture. In water culture pseudostem isolates were found to be comparatively more caducous (41.72%) compared to capsule isolates (35.84%). Leaf isolates showed the least caducity (28.46%).

Among the capsule isolates CPC 159, CPC 309, CPC 311, CPC 313 and CPC 349 showed very low caducity (below 10%) in solid culture while others have an average caducity of 18.5%. In water culture these isolates showed very high caducity (more than 25%). But the isolate CPC 313 was found to have low caducity in water when compared to others (16.23%). In solid culture, the three groups of isolates showed almost uniform caducity (11.57-14.55%).

Chlamydospore production

Among the three sources, it was observed that capsule isolates were very poor in producing chlamydospores as compared to leaf and pseudostem isolates. Only 31.25% of the capsule isolates produced chlamydospores, whereas 60% of the leaf and 50% of the pseudostem isolates produced chlamydospores in culture. Some of the isolates produced chlamydospores in water. Both terminal and intercalary chlamydospores were present. They were spherical, hyaline and thin walled and the diameter ranged from 20-31μm with an average of 26.63 μm (Plate 3).

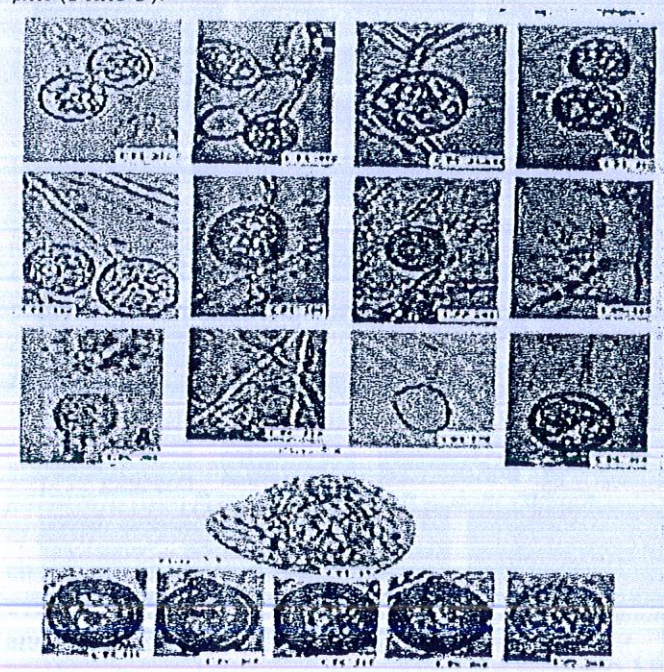

Plate-3 Chlamydospores of *Phytophthora* isolates of cardamom

Sexual Reproduction

All the isolates except two produced sex organs on pairing with A1 isolate of *P. capsici* of black pepper. Certain isolates produced sex organs on a band formed in the junction of meeting the two isolates. Oogonia were spherical, hyaline and thin walled but in some isolates it became golden yellow in colour (Plate 4). Antheridia are purely amphigynous. Some of the isolates produced sex organs within 5-8 days whereas others took 15-21 days. Among the three sources it was observed that pseudostem isolates showed the biggest oogonia (33.23μm) and the smallest antheridia (12.35μm) whereas the leaf isolates showed the smallest oogonia and the biggest antheridia(17.13μm). In capsule isolates the l/b of antheridia was almost equal to one (0.97) whereas it was

1.19 and 0.85 for leaf and pseudostem isolates respectively (Table 5). The oogonial diameter of capsule isolates ranged from 24.04-39.69 μm and the antheridial length ranged from 11.29 μm-16.77μm. Of the 25 isolates examined, 24 belonged to the A2 compatibility type and one isolate was found sterile with out producing any oogonia or antheridia even in pairing (CPC 375). The mating behaviour was also variable with respect to isolates. Some of the isolates produced few oogonia while others produced oogonia in abundance.

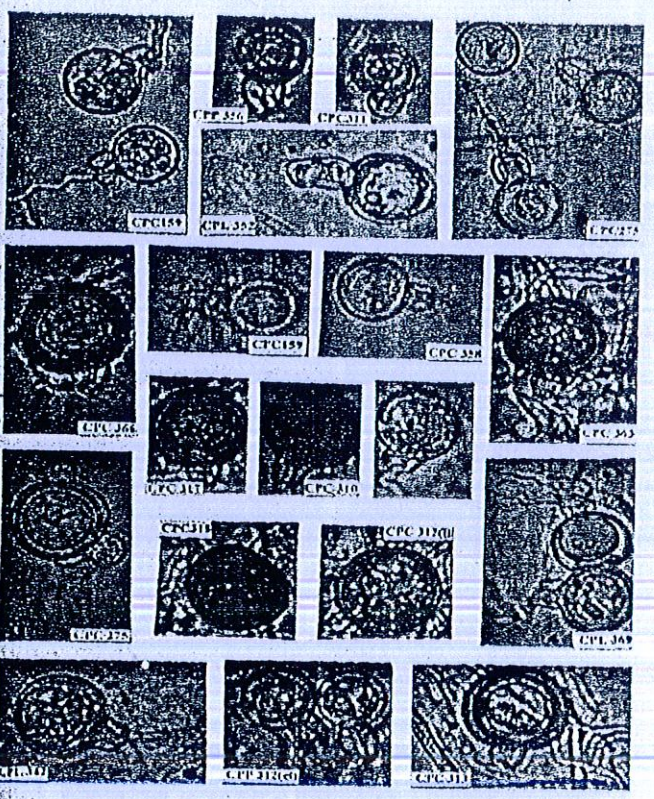

Plate-4 Oogonia and oospores of *Phytophthora* isolates of cardamom

Table 4. Mean caducity and pedicel length of *Phytophthora* isolates of cardamom

Isolates	Caducity %		Pedicel length(μm)	
	Solid culture	Water culture	Solid culture	Water culture
Capsule	14.55± 1.82	35.34±3.13	16.62±1.06 (7.74-24.04)	21.32± 1.47 (11.54-81.825)
Leaf	11.57±3.04	28.46±2.89	15.40±3.74 (8.31-24.95)	21.43±0.66 (19.24-22.53)
Pseudo-stem	13.49±4.23	41.72±4.62	14.83±2.33 (9.06-19.37)	23.96±3.31 (17.33-32.8)

Pigment production

Chocolate brown pigmentation was observed in Timmer's medium (CHT) by some of the isolates when incubated for 21 days at 24-28°C whereas others had no pigmentation in the medium. (Plate-5).

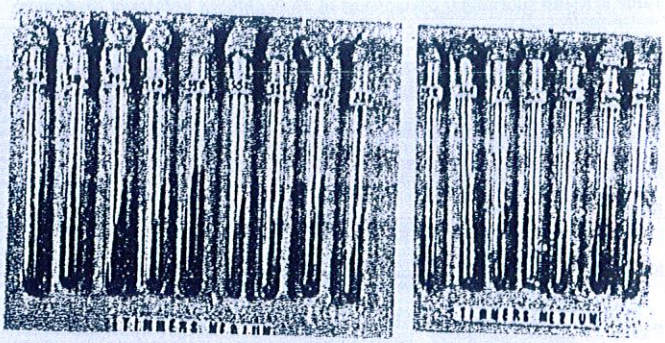

Plate-5 Pigmentation in Timmers medium

Temperature relationship

All the isolates were found to be growing between 10-32°C whereas they failed to grow at 5°C or at 40°C. Most of the pseudo stem and leaf isolates grew well at 36°C. The optimum temperature range for growth of most of the isolates has been found to be between 20°C and 28°C. Irrespective of the source of isolation, maximum growth (8. 9mm/day) of the isolates occurred at 20-28°C and grew up to 32°C. At 32°C growth was observed at the rate of 1-6mm/d and the capsule isolates showed the minimum growth (1.89mm/d) as compared to leaf and pseudostem isolates (4.54 mm/d).Minimum growth was observed at 10°C (1-1.73 mm/d) for all the three groups.

Table 5. Measurements of oogonia and antheridia of *Phytophthora* isolates cardamom

Isolates	Antheridia (μm)			Oogonia μm
	Length	Breadth	l/b	Diameter
Capsule	14.32 ± 0.42	14.72±0.25	0.97	31.16± 1.29
Leaf	17.13 ± 3.48	14.37± 1.08	1.19	29.94:±1.18
Pseudostem	12.35± 0.85	14. 59 ±0.72	0.85	33.23±2.56

Discussion

Successful isolations of pure cultures of *Phytophthora* from infected tissues of cardamom using PVPH and BNPRA+HMI medium (Nair and Menon, 1980) and Lima Bean Agar medium (Thankamma *et al.*, 1968) was reported earlier. In cardamom three distinct types of colony morphology were observed among the isolates. They were uniform cotton wool like aerial mycelium over entire colony with vague lobed pattern, stellate or petalloid pattern and lobed pattern. The result is in accordance with those obtained for colonies of M isolates of rubber described by Dantanarayana *et al*, (1984)and also S & L types described by Brasier and Griffin (1979). The variability in colony morphology is found to be a distinctive character among the isolates of cardamom and it also varied with type of media used. No definite pattern was noticed specifically for capsule, leaf or pseudostem isolates. Incidentally the isolates exhibited variability in colony morphology and hence could not be used as an important taxonomic character.

In the present study the growth rate of isolates showed considerable variation in different media used. Variability in growth rate was reported among *P. meadii* and *P. palmivora* (M&P) isolates of rubber and *P. palmivora* and P. *megakarya* (M&P) isolates of Cocoa (Brasier and Griiffin, 1979, Dantanarayana *et al.*, 1984). Variability in growth rate was also reported for isolates collected from various diseased parts of rubber (Liyanage and Wheeler, 1989. Thus the variation in growth rate with respect to media, a condition under which it was grown and the parts from which isolations have been made was true in the case of cardamom isolates also.

The pedicel length of the isolates is in agreement with the key's of Waterhouse (1963), Newhook *et al* (1978) Stamps *et al* (1990), and Ho (1981). Waterhouse (1965) described *P. meadii* as having deciduous sporangia with pedicel 2-10 μm. Newhook *et al* (1978) and Stamps *et al.* (1990) described the pedicel as caducous small, medium or long. Cardamom isolates came under the second category of medium pedicel length. According to the key of Ho (1981) caducous sporangium having pedicel length of 5-20 μm which sheds in water was reported for species such as *P. botryos, P. colocasiae* Raciborshi, *P. illicis* Buddenhagen and Young, *P. meadii* Mc Rae and *P. megakarya* (Brassier and Griffin 1979). The mean pedicel length fell a between 15-24μm which corresponded to the intermediate pedicel length group as described by Zentmyer *et al*,(1977) and Al-Hedaithy and Tsao (1979a). The mean l/b ratio ranged from 1.77-2.09. The l/b ratio of cardamom isolates were>1.6 which included both *P. meadii* and *P. nicotianae* var. *nicotianae*. The l/b ratio of *P. nicotianae* as described by Garreston-Cornell (1989) was1.3-1.5. All the isolates sporulate heavily in water in light whereas sporulation in the dark in both solid and water culture was very scanty. Only a few sporangia were produced in solid agar in dark except one isolate (CPC342) which produced plenty of sporangia in solid agar in dark with intercalary chalmydospores and junctional thickenings. The present study was similar to those reported by Rajalekshmy and Annakutty Joseph (1986).They observed scanty sporangial production in plate cultures of *P. meadii* incubated in the dark.

The caducity in water (28.46-41.72%) was double than that in solid agar plate method (ll.57-14.%) which corresponded with the increase in pedicel length. The resu lts obtained in the present study is in contrast to that of *P. palmivora* where the percentage detached sporangia in solid agar plate method was higher than in agar disc in water method. The degree of caducity (high or low detachment of sporangium) was stated as a variable character since it can be affected by the medium and method of sporangium production, while the pedicel length was a stable character since neither the medium nor the sporangium production has any effect on the sporangia with short and intermediate pedicel length (Al-Hedaithy and Tsao 1979b). The result of the present study differs from the earlier reports by the fact that cardamom isolates being intermediate in pedicel length, the pedicel length showed variation in solid and water culture and it was found to be directly proportional to caducity. The percentage of detached sporangia varied in solid and water culture. The description was comparable to those described for M isolates *(P. meadii)* of rubber and *Pricptiaral var. parasitice isolates of Cordyline terminalis* isolates *of* (Trichilo and Aragaki, 1982). Similarly analogous radial projections as outgrowths of the wall occur as a unique character on sporangia of many isolates of *P. nicotianae* var. *nicotianae* (Waterhouse *et al.*, 1983). These characters exhibited by some of the isolates of cardamom confirmed the presence of *P. nicotianae* var. *nicotianae* among cardamom isolates. Swelling along the sporangiophore was also characteristic of *P. nicotianae* var. *nicotianae* whereas swelling at the point of branching was characteristic of *P. meadii, P. citrophthora, P. citricola,. P. colocasiae. P. infestans* and *P. phaseoli.*

The presence or absence of chlamydospore is a significant parameter separating species such as *P. palmivora* from *P. megakarya* and *P. meadii* (Waterhouse *et al,*.1983). None of the M isolates of rubber produced chlamydospores on LBA (Lima Bean Agar medium), even after three months of incubation in natural light and dark at 20-25°C supporting the present study. Kunhimotto *et al* (1976) observed chlamydospores in *P. nicotianae* var. *parasitica* in water when washed mycelial mats were kept in sterile water for 4 weeks at 16°C. M isolates of rubber produced chlamydospores. Liyange and Wheeler (1991) reported that *P. meadii* isolates of Sri Lankan soils produced chlamydospores in culture and they survived up to 12 weeks. In the present study also chlamydospores were often noticed in Azhukal affected capsules when stored for long under low temperature (10°C) and also under ordinary conditions (28-32°C) indicated the association of a chlamydospore forming species with Azhukal disease of cardamom and are considered as survival structures.

In the present investigation except one isolate, all the other isolates were found to be of A2 mating type thereby indicating the predominance of A2 mating type in cardamom. Some of the isolates were found to be bisexual /heterothallic producing sex organs in single culture. The

Table 6. Characters of *P. meadii and P. nicotianae var. nicotianae*

A. Sporangial	*As* per the key (Stamps *et al.*, 1990) *P. meadii*	*P. nicotianae* var. *nicotianae*	Isolates studied *P. meadii*	*P. nicotianae* var. *nicotianae*
Papillae pore narrow	+	Oc	+	+
> 1 apex	+	Oc	Oc	Oc
Caducous, pedicel length	M	Oc	M	M
Av.l/b ratio >1.6		*	>1.6	>1.6
± spherical	*	*		
Ellipsoidal	*		+	+
Obpyriform	*	*		
Obturbinate		*		
Elongated neck in water	+			
Distorted shapes	*	+	+	+
Hyphal projections Oc		+/-	+	+
Lateral attachment	*	*	+	+
Intercalary		+		
45-75μ) m	*	+	+	+
Residual globule	+		+	+
B. Sporangiophore				
Aerial on agar	*	+	+	+
Basal swellings Oc.	+		+Oc	
	Intercalary swellings	+	+Oc	
C. Antheridia				
Amphigynous	*	+	+	+
±spherical	*	+	+	+
Ellipsoidal or ovoid	*		+	+
Stalk often eccentric		+		
<30μm	*	+	+	+
30-40μm	*	+	+	+
40-50 μm	*			
Wall rough with age	+		+	+
D. Oospores				
formed readily in the host	+/-			
Frequent on isolation	+/-	+/-		
Often best in dual culture	+	+	+	+
<20-30m	*	+	+	+
20- 30μm	*	+	+	+
30-50μm	*	+	+	
Wall thick (rel. dia)	*	+	+	+
Markedly aplerotic	*	+	+	+
E. Chlamydospores				
Abundant	+			+
In some isolates only	+			+
<25μm	+	+		
25-35μm	+	+	+	+
>35μm		+		
F.Hyphal swellings on agar		+	Oc	+Oc
In water round or angular		+	+	\
In chains or clumps			+	+
Large spherical			+	+
Spherical with radiating hyphae		Oc	+	+
Growth at > 35°C		+Oc		+

*character present from original descriptions and figures .

+Oc character has particular importance +/- present in some isolates

± more or less < less than > more than Oc occasional M medium

heterothallic nature *of P. meadii* occurring both as Al and A2 compatibility type was in contrast to the above with some isolates showing self- fertility and one isolate sterile in pairings. Self- fertility in aging cultures (Brasier 1975; Peries and Dantanarayana, 1965) and self sterility in pairings (Brasier 1972) was also adjudgeable in the present study. Sansome *et al* (1991) reported self sterile isolates *of P. meadii,* which she attributed as due to subculturing.

Hybridization occurred between some of the cardamom isolates and *P. capsici* as shown by the sex organ formation on a band in between the two mating type. This was supported by the work of Boccas (1981). He obtained single oospore cultures from inter specific crosses *of P. megakarya* x *P. parasitica, P. parasitica* x *P. palmivora. P. parasitica* x *P. capsici, P. capsici* x *P. palmivora* and *P. cambivora* x *P. cinnamomi.* The present study showed the interspecific crosses between *P. nicotianae* x *P. capsici.*

According to Ho (1981) pigment production was characteristic of species such as *P. cactorum, P. cryptogea P. cambivora, P. capsici, P.citricola, P. citrophthora, P. drechsleri* var. *drechsleri, P. erithroseptica. P. palmivora MF4. P. parasitice* var. *parasitica, P. parasitica var. nicotianae* and *P. vignae.* Kennedy and Duncan (1995) used CHT for pigment production to compare papillate *Phytophthora* species from raspberry namely *P. cactorum, P. citricola* and *P. syringae* with isolates *of P. cactorum* from apple and strawberry and observed the production of dark pigment as compared to the usual yellow pigment by *P. cactorum,* whereas no pigment production was observed in *P. citricola* and *P. syringae.*

A clear-cut differentiation could be obtained using the temperature relationship. The isolates which produced chlamydopsores in culture and those which showed pigmentation in casein hydrolysate tyrosine agar medium were found growing at >35°C, whereas in others little or no growth was observed at this temperature. Lin and Chang (1986) studied the fruit rot of Durian caused by *P. palmivora* and explained the failure of these isolates to grow around 36°C and distinguished them from *P. nicotianae* and *P nicotianae* var. *parasitica* which could grow well at temp above 35°C. Since Tucker (1931) many keys have used growth at minimum, maximum and optimum temperature as a useful criteria for classification at species level (Waterhouse *et al.*, 1983). Among the 57 isolates of *P. nicotianae* var. *parasitica* studied by Tucker, 7 isolates grew well at 35°C. Minimum temperature of *P. meadii* was reported as 10°C and Optimum temperature for mycelial growth was 28-30°C (Waterhouse, 1974a).

Identification of *Phytophthora* isolates of cardamom based on the Tabular key of Newhook *et al.* 1978; Ho, 1981; Stamps *et al.*, 1990

All the 25 isolates of cardamom studied were papillate with intermediate pedicel length (5-20 μm). Ho (1981) included species such as P. *botryosa P. colocasiae, P. illicis, P. meadii* and *P. megakarya* MF3 in the group having caducous sporangia which shed in water with pedicel length 5-20 μm. *P. parasitica* var. *nicotianae* or *P. nicotianae* var. *nicotianae* was included in the non-caducous group with pedicel absent or non-uniform type. Among the caducous group with pedicel 5-20 μm, P *colocasiae, P. illicis* were semi papillate type and hence the possibilities of their occurrence among cardamom isolates were ruled out. Internal proliferation is not observed in any of the cardamom isolates. The l/b ratio of the isolates were >1.6 with ovoid or ellipsoidal or obpyriform sporangia. Sex organs were produced readily and abundantly in intra and inter-specific crossings with distinct A1 and A2 compatibility types. Antheridia is entirely amphygynous, 2-20 μm long. Ability to produce chlamydospores varies with the isolates. In the first set chlamydospores were rare or absent and in the third set it is present at all temperatures. The first set included *P. botryosa* and *P. megakarya* whereas the second set included *P. meadii. P. nicotianae* var. *nicotianae* came under the third set due to the abundance of chlamydospores in culture. Thus based on the tabular key to the species of *Phytophthora* de Bary (Newhook *et al.*, 1978; Ho, 1981 and Stamps *et al.*, 1990) the isolates of cardamom were grouped into two categories. Category I having pigmentation in casein hydrolysate tyrosine agar with chlamydospore production and the ability to grow at >35°C and Category II having no pigmentation in casein hydrolysate tyrosine agar without chlamydospore production and no growth at 35°C. The pedicel length of the two groups fell in the intermediate group of 5- 20 μm. The above characters along with the other diagnostic characters in the key such as caducity, sporangial formation, nature of sex organs, hyphal swellings etc. all agreed with that of *P. nicotianae* var. *nicotianae* in the first category and *P. meadii* in the second category. Except for these characters all other characters were similar to the two categories and it was difficult to distinguish *P. meadii* from *P. nicotianae* var. *nicotianae* by simple morphological characters. Hence from the results it was found that the cultures with papillate sporangia of intermediate pedicel, hyphal swellings along sporangiophore, amphigynous antheridia spherical oogonia with chlamydospore production in culture and the ability to grow at >35°C along with

pigmentation in CHT medium were *P. nicotianae* var. *nicotianae* whereas the characters as above minus chlamydospore production, pigmentation and ability to grow at >35°C were *P. meadii.*

Characterization of the species

Based on the studies, two basic groups can be recognized in initiating capsule rot and leaf blight disease of cardamom. Group I having pigmentation in CHT whereas Group II without. Each group could again be divided into two depending on the formation of chlamydospores. Group I was found to be growing at high temp (>35°C) whereas Group II has no growth at that temperature. According to the key of Ho (1981) the Group I having pigmentation, chlamydospore formation and growth at >35°C with occasional hyphal swellings from *P. parasitica* group is *P. nicotianae* var. *nicotianae.* The non--chlamydospore forming isolates without pigmentation and no growth at >35°C were invariably *P. meadii.* Moreover both of these groups readily produced sex organs with known Al isolate of black pepper thereby establishing the predominance of A2 mating type of *P. meadii* and *P. nicotianae* in cardamom. Earlier only A1 isolate of *P. meadii* was reported (Sastry, 1989) Liyanage and Wheeler, 1989, and Rajalekshmi *et al.*, 1985). Hence in the light of the above studies it is confirmed that *P.* Mc Rae of A2 mating type is the predominant *Phytophthora* species involved in capsule rot and leaf blight occurring during the post monsoon period is due to *P. nicotianae* var. *nicotianae.* However there is certain amount of overlapping. *P. nicotianae var nicotianale* can also cause capsule rot. As the pedicel length of none of the isolates fall in the range of *<5μm,* the possibility of the occurrence of *P. palmivora* can be ruled out.

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