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Characterization of Isolates of *Phytophthora colocasiae* Collected from Andhra Pradesh and Telangana Causing Leaf Blight of Taro

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A B S T R A C T

The oomycetous fungus *Phytophthora colocasiae* that causes taro leaf blight is one of the most devastating diseases of taro and is widely distributed in India. Characterization of morphological and cultural was used to characterize 4 isolates of *P. colocasiae* obtained from different locations of Andhra Pradesh and Telangana. Considerable differences in morphological parameters were observed. This study confirms that isolates of *P. colocasiae* are highly dynamic in nature and a considerable degree of diversity exists among them. A detailed knowledge of the morphological characters of *P. colocasiae* will help in developing suitable control strategies against the taro leaf blight disease.

Introduction

Taro [*Colocasia esculenta* (L.) Schott] a tropical aroid is an important staple crop in the developing countries especially in Africa and South East Asian countries. Leaf blight caused by *Phytophthora colocasiae* Raciborski is the most important disease of Taro and was recorded for the first time by Butler and Kulkarni (1913) in India. Leaf blight has become a limiting factor for production in all taro growing areas in India moderate to severe form causing 25% to 50% yield loss every year (Misra *et al.*, 2007).

Leaf blight disease is prevalent in almost all the major taro growing districts of Andhra Pradesh and Telangana with varying intensities on different varieties causing yield loss of 10-55 per cent (Laxmi *et al.*, 2012). The disease appears with the onset of monsoon and spreads the entire field during rainy season through zoospores and sporangia (Misra *et al.*, 2007). Reports have revealed, however, that *P. colocasiae* is relatively short lived in infected leaf tissue like any other foliar pathogen of taro. The fungus seems to

have a poor competitive saprophytic ability. This contributes to the lack of success in isolating and growing *P. colocasiae* in an artificial medium. In order to culture the fungi in the laboratory, it is necessary to supplement in the medium, those essential elements and compounds needed for their growth and other metabolic processes. Hence different media were tried in the present investigation to select the best medium suitable for the growth of the pathogen.

Also, we studied the variation in growth and sporulation among *P. colocasiae* isolates from processing taro fields. The objective of this study was to investigate cultural and morphologic variation among isolates of *P. colocasiae* isolated from taro fields of different regions.

Materials and Methods

Isolation and identification of the pathogen

Diseased leaves showing typical symptoms of Taro plants were collected from different Taro growing areas of Andhra Pradesh and Telangana (ARI, Rajendranagar, Hyderabad, East and West Godavari districts) (Table 1). These leaves were put in sterilized polythene bags and brought to the laboratory for isolation and identification of the organism involved. Taro leaves showing typical symptoms of the disease were selected and washed with sterile water.

The surface sterilized leaf bits were transferred aseptically into sterilized Petridishes containing solidified carrot agar medium and incubated at 18±2^{0}C. After 3 days of incubation mycelial growth was absorbed along with diseased leaf bits. Hyphal tips from the advancing mycelia were transferred to the carrot agar medium slants. Also studied different composition of media *viz.*, Potato Dextrose Agar (PDA), Carrot Potato Agar (CPA), V8 agar, Host Leaf Extract Agar (HLA), Corn Meal Agar (CMA), Papaya Sucrose Agar (PSA) were used along with Carrot agar medium for investigation of cheap source and suitable medium for mycelial growth of *Phytophtora colocasiae.* The isolated pathogen was identified as *Phytophthora colocasiae* based on its mycelial and sporangial characters through standard mycological keys (Waterhouse, 1963; Hemmes, 1993) and by CMI descriptions.

Pathogenicity test

The pathogenicity test was conducted in pots under glass house. The taro variety Satamukhi susceptible to *Phytophthora colocasiae* leaf blight disease was raised in pots and 20 day old culture grown on CA broth suspension containing zoospores was inoculated to taro plant at 3-5 leaf stage with hand sprayer and covered with polythene covers.

Variability studies

Morphological characteristics

The morphology of mycelium, sporangium, oospore of *Phytophthora* were studied in seven day old culture of each isolate grown on carrot potato agar medium and stained with 0.1 per cent lacto phenol cotton blue and observed under compound microscope (40X). Observations on size and shape of sporangium, presence of papillae, size of Oospore and Zoospore were recorded.

Cultural characteristics

Observation on colony colour, growth rate, was recorded at 24 hrs interval and mycelial dry weight of each isolate was recorded at 7 days after incubation at 18±2^{0}C. Characteristics like sporulation and colour of spore were observed and recorded at 10 days after incubation.

Colony diameter and growth rate per day

Twenty ml of carrot potato agar medium was poured in sterilized Petriplates and allowed to solidify. Mycelial disc of 5mm diameter were cut from the margin of the 7 day old culture of *Phytophthora colocasiae* placed in the center of the Petriplate under aseptic conditions. The plates were incubated for 8 days in an incubator at 18 ± 2^0C and the diameter of the colony was measured and recorded. Three replications of each isolate were maintained for the study. Growth rate per day of each isolate was calculated by dividing the colony diameter with number of days kept for incubation.

Dry weight of mycelium

The dry weight of the mycelium of each isolate fifty ml of carrot potato agar both was poured in 150 ml conical flask, plugged and sterilized. Mycelial disc of 5 mm diameter was cut from the margin of the seven day old culture of each isolate of the pathogen and transferred to the conical flask containing the sterilized medium under aseptic condition.

The flasks were incubated at 18 ± 2^0C in an incubator for 8 days. The mycelial mat was removed aseptically, washed thoroughly with distilled water and dried in blotters and kept in an oven at 50^0C for 12 hours. Dry weight of the mycelium of each isolate was taken and recorded using digital electronic balance.

Length, width and size of sporangium

Morphological characters such as length, width and the size of sporangium (L x B) were measured by using micrometer.

Results and Discussion

The disease samples of leaf blight of Taro were collected from farmer's fields at Kovvuru (West Godavari), Bahadurguda, (Ranga Reddy), Rajendranagar (Hyderabad), and Thiruvananthapuram (Kerala) (Table 1). The leaves showing typical symptoms of leaf blight were surface sterilized and kept on carrot agar medium for isolation of the pathogen. The pathogen isolated on carrot agar (CA) medium was identified with the help of descriptions given by Waterhouse (1963). The colony was submerged or fluffy and rosette in its growth and the colour was whitish or dull white. Mycelium was aseptate, hyaline, 1µm in width. Sporangiophore was simple, aseptate, hyaline and the sporangia were hyaline, ovoid, and semipapillate. Based on colony character and sporangial nature the pathogen isolated was identified as *Phytophthora colocasiae*. The characteristics of the pathogen were similar to the descriptions given by Waterhouse (1963).

The pathogen was further sub cultured on carrot agar medium and the culture was purified by using hyphal tip method and kept on carrot agar medium slants and preserved at $18 + 2^0$C for further studies

Effect of different media on the growth of *Phytophthora colocasiae*

In order to culture the fungi in the laboratory, it is necessary to supplement in the medium, those essential elements and compounds needed for their growth and other metabolic processes. Neither all media are equally good for all fungi nor there will be an artificial medium on which all fungi grow. Hence different media were tried in the present investigation to select the best medium suitable for the growth of the pathogen. Eight different media *viz*., Potato Dextrose Agar (PDA), Carrot Potato Agar (CPA), Carrot Agar (CA), Papaya Sucrose Agar (PSA), Oat Meal Agar (OMA), Host Leaf Extract Agar (HLEA), Corn Meal Agar (CMA) and Water Agar (WA) were used.

The growth of the pathogen *Phytophthora colocasiae* on different media was recorded 7 days after inoculation and the results are presented in Table 2. The results revealed that among all the media tested, maximum growth of the pathogen was recorded on Carrot Agar (86 mm), followed by Carrot Potato Agar (CPA) medium (79 mm), whereas minimum growth of the pathogen was recorded on PDA medium (22 mm) and Corn Meal Agar medium (29 mm) Table 2. Hence the pathogen was maintained on carrot agar medium for conducting further studies.

Palomar *et al.*, 1999 was studied different artificial media for sporsngisl production of *Phytophthora colocasiae* they reported V-8 juice agar was best medium for growth and reproduction. In their study V8 juice agar was given 83.47 mm of mycelia growth compare to other media *viz.,* V8 juice agar II(79.90 mm), Onion agar (OA)(76.90 mm), Potato dextrose agar (PDA) (51.46 mm)

Pathogenicity

The pathogenicity test was conducted in pots in glasshouse as described under materials and methods. The taro variety Satamukhi susceptible to leaf blight disease was raised in pots and 20-day-old culture grown on CA broth suspension containing zoospores was inoculated on taro plant at 3-5 leaf stage with hand sprayer and covered with polythene covers. The plants showed typical symptoms of leaf blight 5 days after inoculation.

The pathogen was isolated on CA medium and the culture characteristics were similar as that of the original culture. Hence the pathogenicity was proved.

Morphological and colony characteristics of *Phytophthora colocasiae* of Taro

The isolates were morphologically characterized by measuring the shape, size, length and width of 100 sporangia at a magnification of 40X.

Colony characteristics of different isolates of *Phytophthora colocasiae*

Colony characters of 4 isolates of *Phytophthora colocasiae* designated as PC_1 (Rajendranagar), PC_2 (Bahadurguda), PC_3 (Thiruvananthapuram), PC_4 (Kovvur) grown on carrot agar medium were used for recording the data radial growth of the mycelium at 7 days after inoculation (DAI), growth rate (mm/day), dry weight (mg) and the data is presented in Table 3.

Colour of the colony

The colour of the colony varied from dull white to white. Out of the 4 isolates, the colour of the isolate PC_1 and isolate PC_4 was white whereas the colony of isolate PC_2 and isolate PC_3 was dull white in colour.

Growth of the colony

The growth of the mycelium was measured at 24 hrs interval and a variation was observed among the isolates which ranged from 72 mm to 88 mm.

The maximum radial growth (88 mm) was recorded in isolate PC_1 and minimum growth was observed in isolate PC_2 (72 mm). The growth of the colony was petal shaped in PC_1 isolate whereas this character was not observed in other isolates.

Colony growth rate

All the four isolates of *Phytophthora colocasiae* differed in colony growth rate (mm/day) and it ranged from 10.3 to 12.6 mm/day. The isolate PC_1 recorded mean maximum growth rate of 12.6 mm/day and minimum growth rate of 10.3 was observed in isolate PC_2.

Abundance of mycelium

Four isolates of *Phytophthora colocasiae* were grouped into 2 based on abundance of mycelium produced by *Phytophthora colocasiae*. The isolate PC_2 from Bahadurguda produced profuse growth of mycelium, whereas isolates PC_1, PC_3 and PC_4 from Rajendranagar, Thiruvanantapuram and Kovvuru produced slightly sparse mycelial growth.

Colony texture

Based on the texture and appearance of the colony, the isolates were categorized into two groups *i.e.* fluffy and slightly fluffy appearance of the colony on carrot agar medium. The isolates PC_1 (Rajendranagar), PC_3 (Thiruvanantapura), PC_4 (Kovvuru) produced slightly fluffy colony whereas PC_2 (Bahadurguda) produced thick fluffy colony

Dry weight

For dry weight of mycelium of *Phytophthora colocasiae* the pure culture was inoculated on Carrot agar broth (CAB) and 10 days after inoculation the dry weight of the mycelium was recorded and the data was presented in Table 3. Isolate PC_1 recorded maximum dry weight of (282 mg) followed by isolate PC_4 (256 mg) and PC_3 (181 mg). Minimum dry weight was recorded by isolate PC_2 (152 mg).

Table.1 Isolates of *Phytophthora colocasiae* collected from different parts of Andhra Pradesh and Kerala

S. No.	Isolates	Place and Mandal	District and state
1.	PC_1	Rajendranagar	Hyderabad, Andhra Pradesh
2.	PC_2	Bahadurguda, Moinabad	Ranga Reddy, Andhra Pradesh
3.	PC_3	Sreekariyam, Thiruvananthapuram	Thiruvananthapuram, Kerala
4.	PC_4	Kovvuru	West Godavari, Andhra Pradesh

Table.2 Effect of different media on radial growth of *Phytophthora colocasiae*

S. No.	Name of the media	Radial growth (mm)
1.	Carrot agar	86.0
2.	Carrot potato agar	79.6
3.	Papaya sucrose agar	80.6
4.	Host leaf extract agar	72.0
5.	Oat meal agar	52.0
6.	Potato dextrose agar	22.0
7.	Corn meal agar	28.6
8.	Water agar	50.6
	CD at 5%	5.92
	SEm±	11.70
	CV%	5.79

Table.3 Colony characteristics of different isolates of *Phytophthora colocasiae*

S. No.	Name of the Place	Name of the isolate	Colour of the colony	Radial growth of the colony (mm)	Growth rate/day (mm)	Texture of colony	Abundance of mycelium	Dry weight of mycelium (mg)
1.	Hyderabad	PC_1	White	88.0	12.6	Slightly fluffy	Sparse	282
2.	Ranga Reddy	PC_2	Dull white	72.0	10.3	Fluffy	Profuse	152
3.	Thiruvananthapuram	PC_3	Dull white	80.0	11.4	Slightly fluffy	Sparse	181
4.	West Godavari	PC_4	White	82.0	11.7	Slightly fluffy	Sparse	256

Table.5 Morphological variability of sporangium, oospore and zoospore among four isolates of *Phytophthora colocasiae*

S. No.	Isolate	Sporangium length (µm)			Sporangium width (µm)			L:B ratio	Size of the sporangium ($µm^2$)	Oospore		Size of the Zoospore ($µm^2$)
		Min.	Max.	Mean	Min.	Max.	Mean			Mean diameter (µm)	Size of oospore ($µm^2$)	
1.	PC_1	15.21	25.35	21.8	10.14	20.28	15.7	1.4:1	342	27.8	772.5	44
2.	PC_2	15.21	25.35	21.2	10.14	20.28	14.9	1.4:1	312	27.0	731.6	44
3.	PC_3	20.28	30.42	24.3	15.21	20.28	17.2	1.4:1	417	19.2	486.7	48
4.	PC_4	15.21	23.8	23.8	10.14	20.28	15.7	1.5:1	373	22.8	519	52

Table.4 Morphological characteristics of sporangia and sporangiophores of different isolates of *Phytophthora colocasiae*

S. No.	Isolate	Sporangia	Stalked / sessile	Shape of sporangium	Sporangiophores	Hyphal swellings	Hyphal width (µm)
1.	PC_1	Semi papillate	Stalked	Globose	Simple sympodial	Absent	1
2.	PC_2	Semi papillate	Stalked	Globose	Branched sympodial	Absent	1
3.	PC_3	Semi papillate	Stalked	Globose	Simple sympodial	Absent	1
4.	PC_4	Papillate	Stalked	Ovoid	Branched sympodial	Absent	1

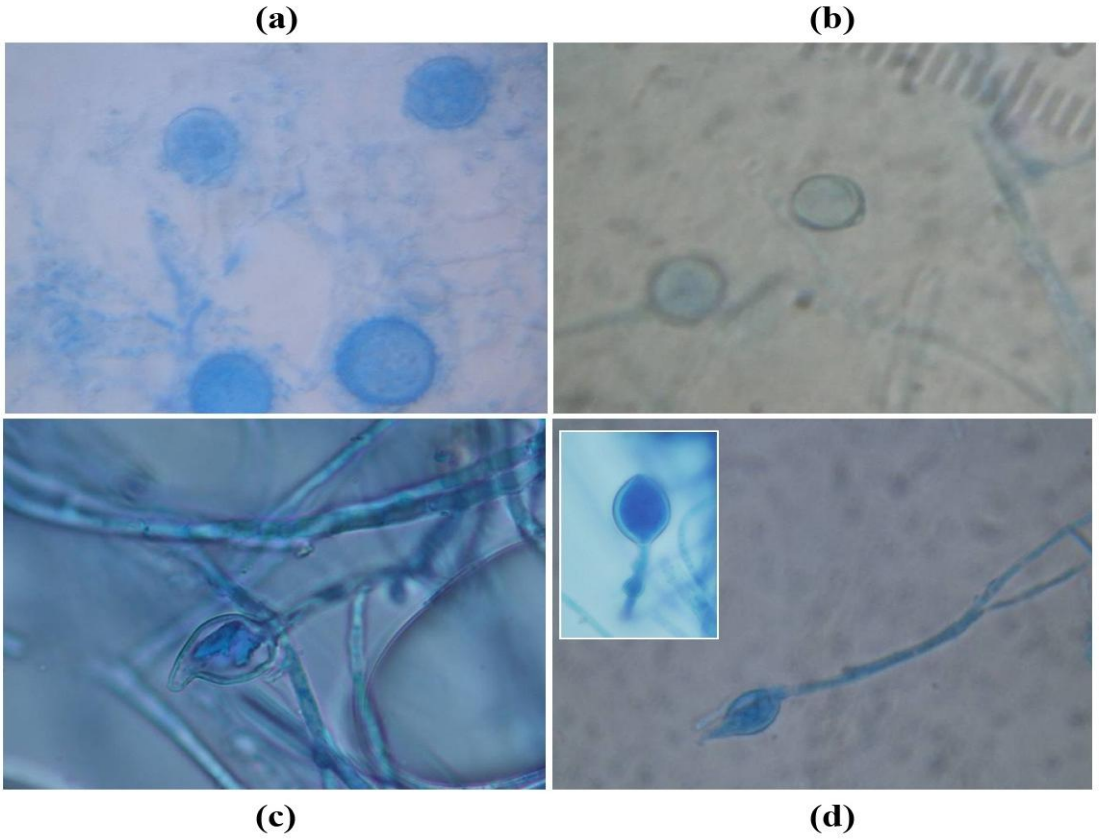
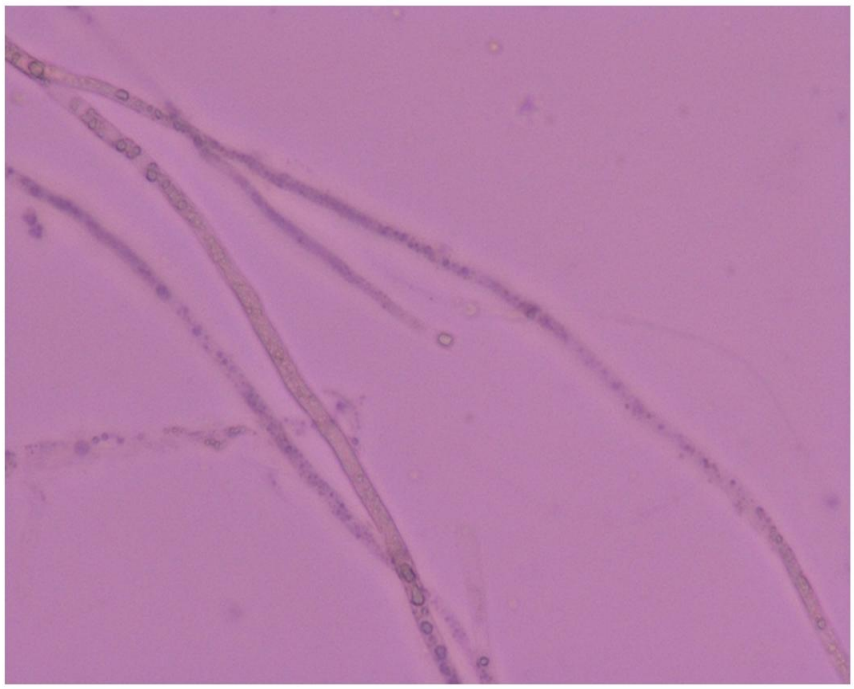
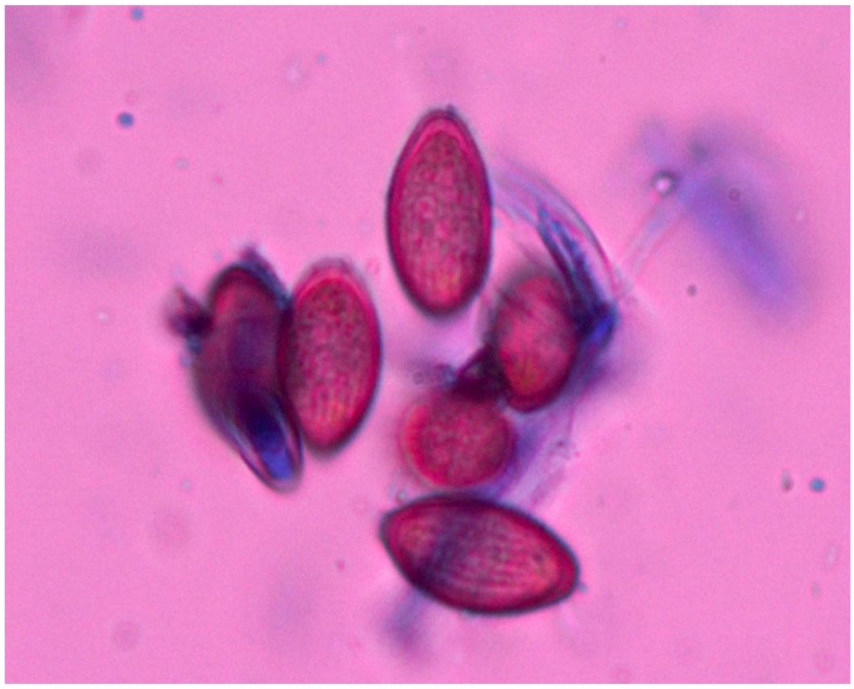


Plate 4.6. Sporangial characteristics of *Phytophthora colocasiae* observed by compound microscope (40X)

a) Semi papillate sporangia in isolate PC 1
b) Semi papillate sporangia in isolate PC 2
c) Semi papillate sporangia in isolate PC 3
d) Papillate sporangia in isolate PC 4



a) Aseptate Mycelium



b) Semi papillate sporangia

Plate 4.7. Morphological characterstics of *Phytophthora colocasiae* observed by compound microscope (400X)
a) Aseptate mycelium
b) Semi papillate sporangia

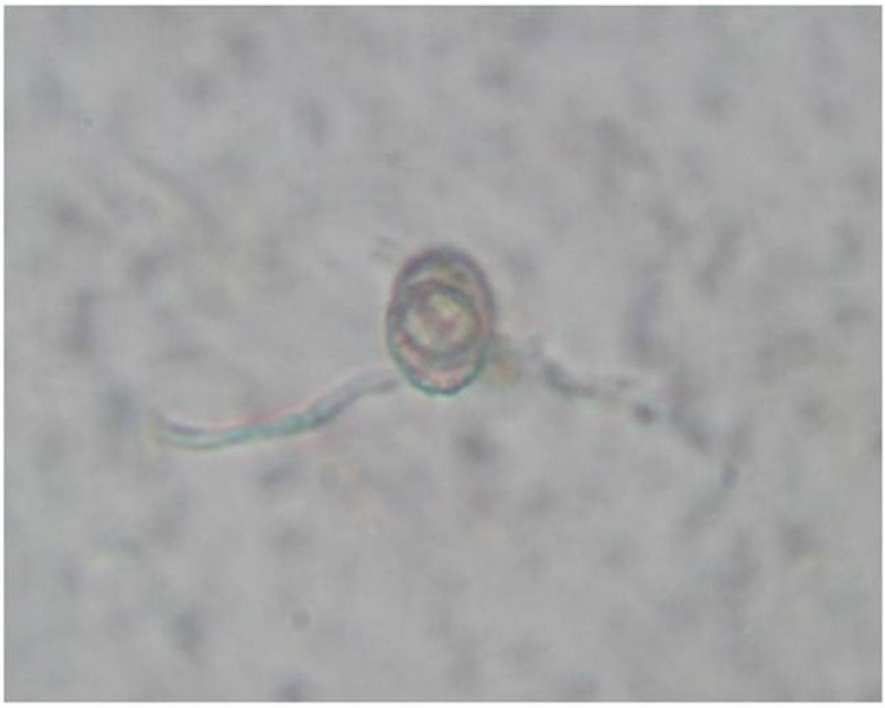


Plate 4.9. Formation of oospore in *Phytophthora colocasiae* observed under compound microscope (40X)

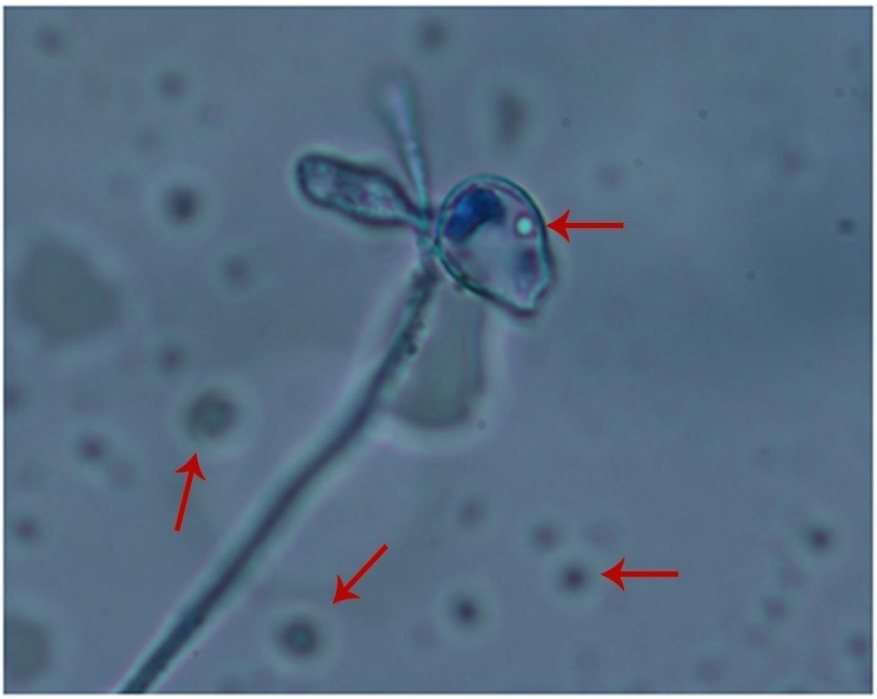


Plate 4.10. Release of zoospores from sporangium of *Phytophthora colocasiae* observed under compound microscope (400X)

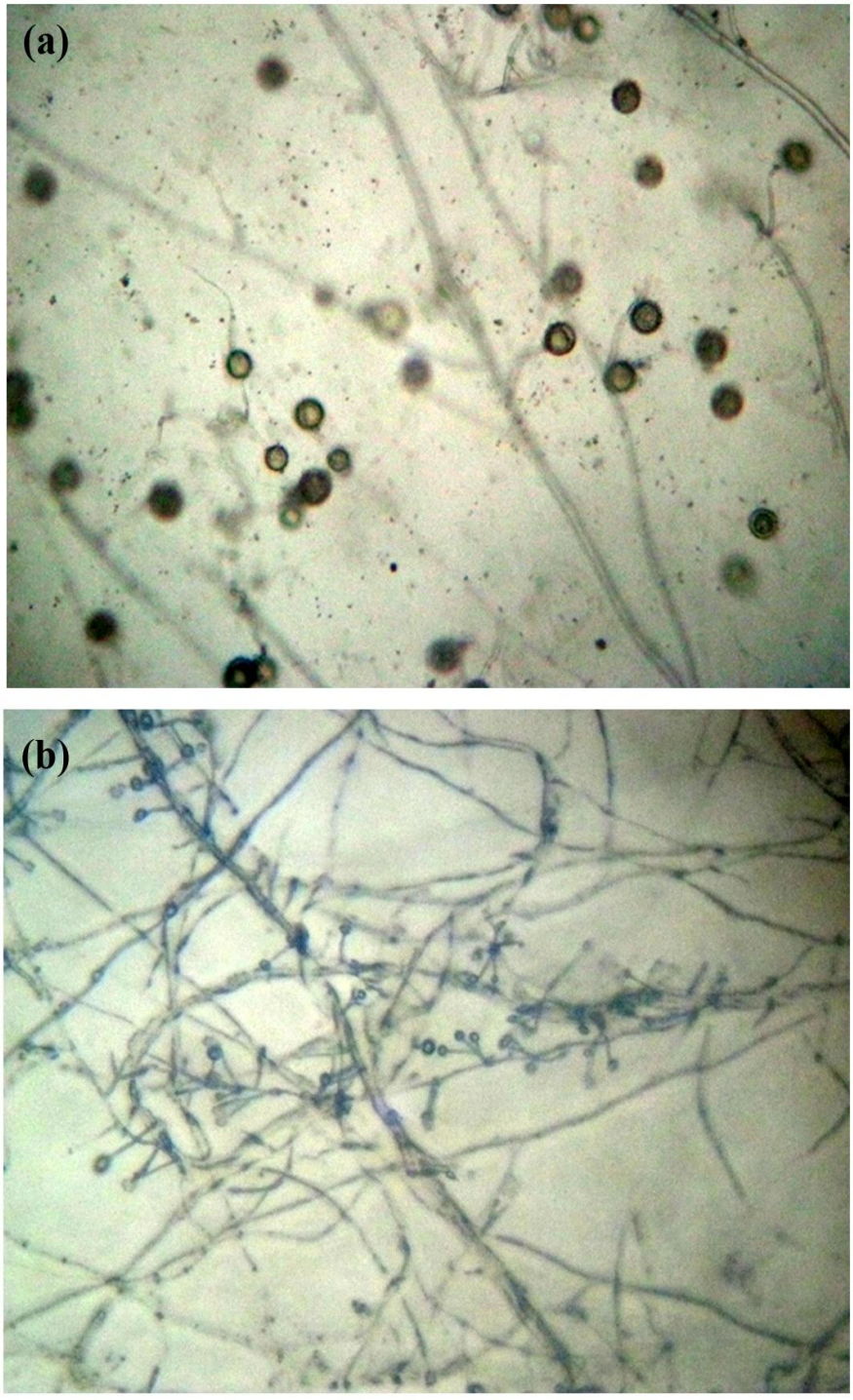


Plate 4.8. **Sporangiophore characteristics of *Phytophthora colocasiae* observed by compound microscope (40X)**

a) Simple sympodial sporangiophore of isolate PC 1 and PC 3
b) Branched sympodial sporangiophore of isolate PC 2 and PC 4

Morphological characteristics of different isolates of *Phytophthora colocasiae*

Sporangial characters

Sporangial characters like size of sporangium, shape, presence or absence of papillae, variation in length, width and their ratio and size of oospore was studied for all the isolates of *Phytophthora colocasiae* using Olympus microscope at a magnification of 40X and the data pertaining to sporangial characters are indicated in Tables 4 and 5.

Length of sporangium

The mean sporangial length of 4 isolates of *Phytophthora colocasiae* ranged from 21.2 µm to 24.3 µm. The maximum length of sporangia was recorded in isolate PC_3 (24.3 µm) followed by PC_4 (23.8 µm) while minimum length of sporangium was observed in isolate PC_2 with 21.2 µm and was on par with isolate PC_1 which recorded a sporangial length of 21.8 µm.

Width of sporangium

The mean width of 4 isolates of *Phytophthora colocasiae* ranged from 14.9 µm to 17.2 µm. The maximum width of sporangium was recorded in isolate PC_3 (17.2 µm) and a minimum width of 14.9 µm was recorded in isolate PC_2, whereas isolates PC_1 and PC_4 showed a mean width of 15.7 µm.

Length and width ratio

Maximum length and width ratio of *Phytophthora colocasiae* isolates was recorded in isolate PC_4 (1.5 µm) collected from East Godavari district (Kovvuru mandal), while in other isolates *i.e.*, PC_1, PC_2 and PC_3 the length and width ratio was 1.4:1.

Size of the sporangium

The isolates of *Phytophthora colocasiae* showed variation in size of the sporangium. The sporangial size varied from 312 μm^2 to 417 μm^2. Maximum size 417 μm^2 was observed in isolate PC_3 and minimum size of the sporangium was recorded in isolate PC_2 with 312 μm^2. The size of the sporangium of isolate PC_1 was 342 μm^2 while the size was 373 μm^2 in isolate PC_4.

Shape of the sporangium

The shape of the sporangium of three isolates of *Phytophthora colocasiae* PC_1, PC_2 and PC_3 were globose, semi papillate whereas the sporangium of isolate PC_4 was ovoid and papillate (Plate 4.6).

Sporangiophore

The sporangiophore of isolates PC_1 and PC_3 were simple sympodial whereas branched sympodial sporangiophore was observed in isolates PC_2 and PC_4 (Plate 4.8).

The hyphal swellings were absent in all the four isolates of *Phytophthora colocasiae* and a hyphal width of 1 µm was also recorded in all the four isolates of *Phytophthora colocasiae.*

Size of oospore

A variation in the size of the oospore was observed among the four isolates of *Phytophthora colocasiae* (Plate 4.9). The maximum size of the oospore was recorded in isolate PC_1 with 772.5 μm^2 with mean diameter of 27.8 μm^2 followed by isolate PC_2 (731.6 μm^2) with 27.0 μm^2 diameter and the minimum size of the oospore was recorded in isolate PC_4 (519 μm^2) followed by isolate PC_3 (486.7 μm^2) with 19.2 μm^2 and 22.8 μm^2 mean diameter respectively

Zoospores

The size of the zoospores varied from 44 µm - 52 µm which were biflagellate and slender. The size of the zoospore in isolate PC_1 and PC_2 was 44 µm followed by isolate PC_3 which was 48 µm and maximum size of zoospore was observed in isolate PC_4 with 52 µm

Similar observations were made by Misra (1996) in *P. colocasiae*. The zoosporangiophores were slender, cylindrical and extremely narrow at the tip and measured up to 50 µm in length. The zoosporangia were elongated lemon or pear shaped and generally measured 38 - 60 µm × 18 - 26 µm and zoosporangial length over 100 µm and width over 50 µm were recorded.

Bandyopadhyay *et al.,* (2011) reported that the sporangia of *P. colocasiae* were hyaline, papillate and measured 25 to 55 µm × 15 to 30 µm. Zoospores encysted within 30 min. after release and size of the cyst was 9.7 µm to 19.5 µm in diameter.

Omane *et al.,* (2012) reported morphological characters of *P. colocasiae* isolates of taro from Ghana. Sporangia were ovoid, hyaline, papillate, caduceus, 30 to 60 µm × 17 to 28 µm, and pedicels were 3.5 µm to 10 µm long.

These results also confirmed the present findings that the sporangia were globose in three isolates except in isolate PC_4 which was ovoid.

This study confirms that isolates of *P. colocasiae* are highly dynamic in nature and a considerable degree of diversity exists among them. A detailed knowledge of the morphological characters of *P. colocasiae* will help in developing suitable control strategies against the taro leaf blight disease

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