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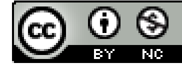

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CULTURAL, MORPHOLOGICAL AND PATHOGENIC VARIABILITY OF *Phytophthora colocasiae* ISOLATE FROM TARO IN CAMEROON

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ABSTRACT

There exist 124 species of *Phytophthora* worldwide that cause serious diseases in natural ecosystems. These species have been identified using molecular methods. This study was conducted to determine the cultural, morphological and pathogenic variability of isolates of *P. colocasiae* processed from cultivars of Taro from Cameroon.The study was conducted in three agroecological regions, namely, Yaoundé, Bambui and Ekona where the disease was prevalent. Morphology showed that the mycelia were circular on all the isolates, in the three study zones. Mycelia colours ranged from white to cotton white. The colours of the culture media, after inoculation and mycelia growth, were the same. There was a significant difference ($P<0.05$) in mycelial growth among the isolate; with the longest of 5.5 cm in V6 juice agar media. Spore morphology was either spherical or ovoid in all the Taro isolates, in all culture media, except water medium. All four isolates were pathogenic to the four cultivars of Taro, causing lesions on leaves on inoculation. The most virulent fungi isolates were L1 (dark green petiole with small leaves) and L2 (red petiole with small leaves), which caused the largest lesion (>10 mm) in Taro cultivars during 14 days of inoculation.

Key Words: Mycelial growth, pathogenicity, virulence

RÉSUMÉ

Il existe 124 espèces de *Phytophthora* dans le monde qui causent des maladies graves dans les écosystèmes naturels. Ces espèces ont été identifiées par des méthodes moléculaires. Cette étude a été menée pour déterminer la variabilité culturelle, morphologique et pathogénique des isolats de *P. colocasiae* transformés à partir de cultivars de Taro du Cameroun. L'étude a été menée dans trois régions agroécologiques, à savoir, Yaoundé, Bambui et Ekona où la maladie était prévalente. La morphologie a montré que les mycéliums étaient circulaires sur tous les isolats, dans les trois zones

d'étude. Les couleurs du mycélium variaient du blanc au blanc coton. Les couleurs des milieux de culture, après inoculation et croissance mycélienne, étaient les mêmes. Il y avait une différence significative (P<0,05) dans la croissance mycélienne parmi l'isolat ; avec le plus long de 5,5 cm dans un milieu de gélose au jus V6. La morphologie des spores était soit sphérique soit ovoïde chez tous les isolats de Taro, dans tous les milieux de culture, sauf le milieu aqueux. Les quatre isolats étaient pathogènes pour les quatre cultivars de Taro, causant des lésions sur les feuilles lors de l'inoculation. Les isolats de champignons les plus virulents étaient L1 (pétiole vert foncé avec de petites feuilles) et L2 (pétiole rouge avec de petites feuilles), qui ont causé la plus grande lésion (> 10 mm) chez les cultivars de Taro pendant 14 jours d'inoculation.

Mots Clés : Croissance mycélienne, pathogénicité, virulence

INTRODUCTION

There exist 124 species of *Phytophthora* worldwide that cause serious diseases in different crop production and natural ecosystems. These species have been identified using molecular methods (Martin *et al*., 2014). Among all the *Phytophthora* species*, Phytophthora cinnamomi* has a wider host range and is an aggressive forest pathogen (Robin *et al*., 2012). Over 2000 plant species are reportedly susceptible to *P. cinnamomi* (Shearer *et al*., 2004). The effect of *P. cinnamomi* is considerable in natural communities and has been blamed for the loss of animal species in the South-West and South-East of Australia (Cahill *et al.,* 2008), apart from causing root rots in many host plants (Hardham, 2005). In Cameroon, *P. infestans* and *P. colocasiae* (Racib) are common and cause blight disease in tubers, corms, and fruits. Late blight disease has been observed to cause up to 100 % yield loss in tomatoes and potatoes (Fontem *et al*., 2005).

Phytophthora colocasiae is a host specific pathogen to Colocasia spp. (*C. Esculenta* var., *C. antiquorum*) and *Alocasiamacrorhiza* (giant Taro) (Nelson *et al*., 2011). Although colocasia (Taro) can be infected by the pathogen, the ability of the disease to become epidemic on this host is restricted by very low inoculum production (Brunt *et al*., 2001). Taro leaf blight disease is one of the most prominent diseases in Taro growing agro-ecological zones of Cameroon; causing up to 100 % leaf and corm losses in susceptible genotypes (Fontem and Mbong, 2011; Mbong *et al*., 2013). At harvest, *P. colocasiae* spores invade Taro corms and cause considerable deterioration (Jackson, 1999).

Phytophthora colocasiae causes corm rots, both in the field and in storage, and this has led to post-harvest major losses (Brunt *et al*., 2001). Manju *et al.* (2017a) reported that bio-deterioration of Taro cultivars in Cameroon decomposed by 30 % after one month of storage. This pathogen is transmitted through infected planting material, and through water carrying the spores, to susceptible cultivars among plants (Nelson *et al*., 2011). Lesions appeared on leaves after transmission, as water-soaked, increased, coalesced, and destroys the leaves. Lesions tend to have distinctive water-soaked margins on newly invaded tissues, bearing a white mass of spores and orange liquid droplets. Under dry conditions, the water-soaked margin dries out during the day and during cooler rainy days the lesions continue to expand during the day (Manju *et al*., 2017b).

Phytophthora colocasiae is relatively short-lived in infected leaf tissue, and the fungus seems to have a poor competitive saprobic ability (Narula and Meherotera, 1984). This pathogen is host specific and causes Taro leaf blight disease infection which remains a major problem in the Taro growing regions of the country. This study was conducted to determine the cultural, morphological and pathogenic variability of four isolates of *P. colocasiae* processed from cultivars of Taro from Cameroon.

MATERIALS AND METHODS

Collection, isolation and identification of *Phytophthora colocasiae.* Three hundred infected Taro leaves, with young lesions of Taro blight, were collected from four local cultivars of Taro, namely Dark green petiole with small leaves (L1), Red petiole with small leaves (L2), Light green petiole with large leaves (L3), and Light green petiole with small leaves (L4) from fields of three agro-ecological regions (Yaounde, Bambui, Ekona) of Cameroon. Each of these cultivar leaves was preserved in a separate large envelope, and transported to the phytopathology laboratory at the International Institute of Tropical Agriculture (IITA), Yaounde. These leaves were cut into small fragments (about 2 mm), from the advancing edges of the disease, and surface-sterilised in a 5 % diluted solution of sodium hypochlorite, for 30 seconds and then rinsed three times in sterile distilled water for two minutes. The leaf fragments were dried on sterilised filter paper and four fragments were placed in each petri dish containing solidified cool V6 juice agar culture medium that contained antibiotics (Ampicillin (250 mg l^{-1})), Penicillin (250 mg l^{-1}), and Nystatin (20 mg l^{-1}) to prevent bacteria growth. These dishes were labelled and incubated at room temperature of 21-23ÚC (Brunt *et al*., 2001). After 2-3 days, extensive mycelia formed around the leaf fragment, was aseptically collected and sub-cultured three times in Petri dishes containing freshly prepared V6 juice agar medium to obtain axenic culture.

For fungal growth, mycelia discs were cut from an 8-day old axenic culture of *P. colocasiae,* from four local Taro cultivars isolated using a flame-sterilised 4 mm diameter cork- borer. Each of the mycelia discs was aseptically transferred with the aid of a flame-sterilised mounted needle to the centre of different media (V6 agar, V8 agar, tomatoes 8 agar, and PDA), contained in Petri dishes. The bottom of the Petri dishes was marked by two perpendicular lines passing through the centre. Each of the Petri dishes was replicated four times for each isolate, and incubated at 21±2 ^{O}C at pH 6, and placed in a randomised complete block design. Mycelia growth was measured along the perpendicular lines, using a ruler, for 8 days. Mean of mycelia growth was calculated from the different treatments for 8 days. Data on mycelia colour, growth pattern, and media colour were also recorded (Mbong *et al*., 2015).

Spore density. Spore density of *P. colocasiae* was assessed by preparing spore suspension from a 22-day old culture of different isolates. This was done by flooding the surface of the growing colonies in each Petri dish with 5 ml of sterile distilled water, mixed with a drop of tween 80 and dislodging the spores with a small brush. The suspension was centrifuged for three minutes and the supernatant was filtered through a 2-layered sterile muslin cheesed cloth. A drop of spore suspension was placed on the haemocytometer chamber, covered with a slide, and the number of spores per ml was estimated as the average of the spores counted in 10 standard haemocytometer fields. The number of spores per ml was calculated using the formula adopted by Duncan and Torrance (1992), *viz*:

$$S = {}^{NV}/_{v}$$

Where:

S = Number of spores per millilitre, N = Mean number of spores in 10 large squares counted, V = 1 ml =1000 mm^3, and $_v$ = volume of spore suspension under glass cover = 0.0004 mm^3 (4 X10^{-4} mm^3).

The mean spore count was obtained from four local Taro cultivars isolates and data on spore density among the different fungal isolates were collected (Fokunang *et al.,* 1995). For Pathogenicity testing, four isolates of *P. colocasiae* were used to test four cultivars of Taro (Dark green petiole with small leaves

(L1), Red petiole with small leaves (L2), Light green petiole with large leaves (L3), and Light green petiole with small leaves (L4)) from the three agro-ecological regions of Cameroon. Taro corms of 30 g, were planted in the greenhouse at IITA Yaounde, in sterilised soils, in 40-cm diameter plastic pots. Six weeks after planting, four spots on the leaves of the plants were inoculated, using a syringe to inject a standardised spore suspension of 3x104 spores per ml of distilled water. Control plants received distilled water, and watering was done at one-day intervals to maintain high relative humidity around the inoculated plants.

The plants were arranged in a complete randomised design, with four replicates of 10 plants per replicate. Diameters for plant lesion development were measured using a ruler, at two days intervals for 14 days (Manju *et al.*, 2020).

Preparation of V6 juice agar. Six grammes of green beans, 40 g of garden pea, 10 g of swede, 10 g of flageolets, 30 g of carrot, and 4 g of Taro leaves were washed using tap water. They were weighed using an electronic balance, boiled, put into a blender and 500 ml of distilled water added to it and crushed. This mixture was filtered twice with muslin clothes and 150 ml of Macedoine juice was added to it. This extract was put in a conical flask and sterilised distilled water was added to make 1000 ml of solution. Then, 20 g of Agar, 3 g of calcium carbonate ($CaC0_3$), and 1 ml of vegetable oil were added to the mixture. The mixture was shaken for three. minutes to homogenise; while the mouth of the flask was covered with non-absorbent cotton wool and wrapped with aluminium foil. This flask was placed in an autoclave and sterilised at a temperature of 121 ^{O}C and pressure of 1.05 Pa for 20 minutes. Ampicillin (250 mg l^{-1}), Penicillin (250 mg l^{-1}), and Nystatin (20 mg l^{-1}) were added to the culture medium after sterilisation, when the temperature of V6 juice agar was cooled to 400 ^{O}C and mixed well. Twenty millilitres of this media were poured into sterilised Petri dishes.

Preparation of V8 juice agar. Two grammes of celery, 8 g of lettuce, 12 g of waterleaf (*Talinum triangulare*), 12 g of green beans, 40 g of potatoes, 40 g of beetroot, 50 g of carrot, and 166 g of tomatoes were washed using tap water, weighed using an electronic balance, sliced, put into a blender and 500 ml of distilled water was added to it and crushed. This extract was filtered twice with muslin clothes and then homogenised in 1000 ml of sterile distilled water. Two hundred millilitres of V8 juice were put in a conical flask and sterilised distilled water was added to make 1000 ml of solution. The mixture was heated in a water bath for 10 minutes and 20 g of Agar, 3 g of calcium carbonate (CaCO3), and 1 ml of vegetable oil were added to the mixture and then heated at 100 ^{o}C for 3 minutes. The mixture was again shaken to homogenise and the mouth of the flask again covered with non-absorbent cotton wool and wrapped with aluminium foil. The flask was placed in an autoclave and sterilised at the temperature of 121 ÚC and pressure of 1, 05 Pa for 20 minutes. Ampicillin (250 mg l^{-1}), Penicillin (250 mg l^{-1}), and Nystatin (20 an mg l^{-1}d) were added to the culture medium after sterilisation. The temperature of V8 juice agar was cooled to 40 ^{O}C and mixed well. Twenty millilitres of this medium were poured into sterilised Petri dishes.

Preparation of water agar. Twenty grammes of agar-agar and 1000 ml, of sterilised distilled water, were put in a conical flask. The solution was heated in a water bath for 20 minutes and the mixture homogenised by shaking and stirring with a magnetic stirrer. The mouth of the flask was covered with non-absorbent cotton wool and wrapped with aluminium foil. The flask was placed in an autoclave and sterilised at the temperature of 121 ^{O}C and pressure of 1.05 Pa for 20 minutes. Ampicillin (250 mg l^{-1}), Penicillin (250 mg l^{-1}), and Nystatin (20 mg l^{-1}) were added to the culture medium after sterilisation and allowed to cool to 40 ^{O}C and mixed well. Twenty millilitres of

this medium were poured into sterilised Petri dishes.

Preparation of tomatoes- 8- agar. Twenty grammes of agar, 3 g of sodium carbonate, 20 g of sterilised tomato paste, and 1000 ml of sterilised distilled water were put in a conical flask. The solution was heated in a water bath for 20 minutes. The mixture was homogenised by shaking and stirring with a magnetic stirrer. The mouth of the flask was covered with non-absorbent cotton wool and wrapped with aluminium foil. The flask was placed in an autoclave and sterilised at the temperature of 121 ^{O}C and pressure of 1.05 Pa for 20 minutes. Ampicillin (250 mg l^{-1}), Penicillin (250 mg l^{-1}), and Nystatin (20 mg l^{-1}) were added to the culture medium, after sterilisation when the temperature of the medium cooled to 400 ^{O}C and mixed well. Twenty millilitres of this medium were poured into sterilised Petri dishes (Fokunang, 1995).

Preparation of potatoes dextrose agar (PDA). Twenty grammes of PDA and 1000 ml of sterilised distilled water were put in a conical flask. The solution was heated in a water bath for 20 minutes and the mixture was homogenised by shaking and stirring with a magnetic stirrer. The mouth of the flask was covered with non-absorbent cotton wool and wrapped with aluminium foil. The flask was placed in an autoclave and sterilised at the temperature of 121 ^{O}C and pressure of 1.05 Pa for 20 minutes. Ampicillin (250 mg l^{-1}), Penicillin (250 mg l^{-1}), and Nystatin (20 mg l^{-1}) were added to the culture medium after sterilisation when the temperature of PDA is cooled to 40 ^{O}C and mixed well. Twenty millilitres of this medium were poured into sterilised Petri dishes.

Statistical analysis. The data for mycelia growth, spore density and lesion diameter were subjected to analysis of variance (ANOVA), using statistical software JPM version 8, 2007. Means were separated using the student T-test (STT) at 5% level of significance.

RESULTS

The isolates showed two colours (white and cotton white) of mycelia in the various media (Table 1). There was no evidence of growth of mycelia in water media. All the isolates showed circular growth patterns of mycelia in all three localities (Fig. 1). The colours of the growth media of the isolates were normal as the initial non-inoculated culture plate after the growth of mycelia (Light brown, dark brown, cream white, and white) in the three agro-ecological regions of Cameroon.

Spore density in different media. Spores were observed in V6 agar, V8 agar, tomatoes 8 agar and PDA (Fig. 2). There was no observable spore produced in water media, which served as the control media in the three field sites. Two types of spores were observed (spherical and round) with a significant number of round spores, compared to spherical spores in all three study sites (Fig. 2).

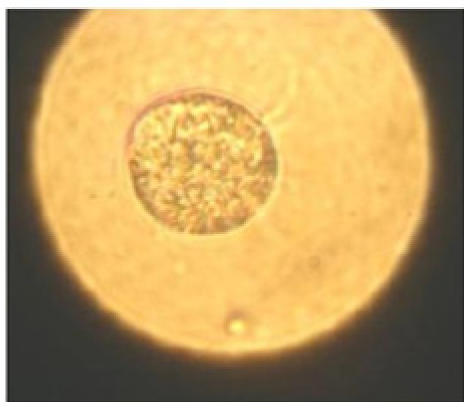
The spore density of dark green petiole with small leaves (L1), isolate twenty-two days after incubation on different culture media in the three field sites (Yaounde, Bambui, and Ekona) showed variability among media (Fig. 3). Under the haemocytometer, isolate L1 recorded high density of round spore in V6 juice agar media and low density of round spore in tomato agar in the three field sites. Round spores of isolate L1 were recorded in V6 juice agar, V8 juice agar media, tomatoes agar, and potatoes dextrose agar in the three study field sites. Ovoid spores were absent in tomatoes and V6 juice agar in Ekona, tomatoes agar in Bambui and V8 juice agar in Yaounde. The highest mean round spore density of 25x 106 spores per ml of sterile distilled water on isolate L1, was recorded in V6 juice agar medium in Yaounde; while the least in tomatoes and potatoes dextrose agar were recorded in Yaounde and Bambui, respectively (Fig. 3).

TABLE 1. Morphological characteristics of *Phytophthora colocasiae* isolates in different media from three agro-ecological regions

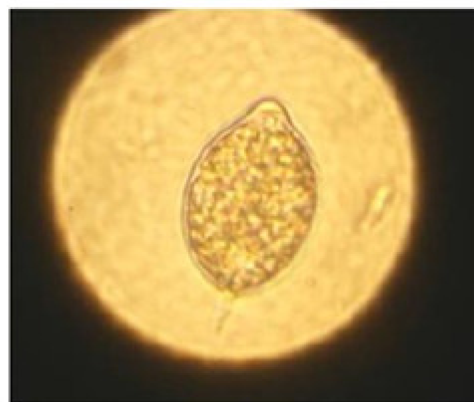
Isolate	Field sites	Media	Media colour	Mycelia colour	Growth pattern
Ll	Yaounde	V6	Light brown	Whitish	Circular
Ll	Bambui	V6	Light brown	Whitish	Circular
Ll	Ekona	V6	Light brown	Whitish	Circular
Ll	Yaounde	V8	Dark brown	Whitish	Circular
Ll	Bambui	V8	Dark brown	Whitish	Circular
Ll	Ekona	V8	Dark brown	Whitish	Circular
Ll	Yaounde	T	Cream white	Whitish	Circular
Ll	Bambui	T	Cream white	Whitish	Circular
Ll	Ekona	T	Cream white	Whitish	Circular
Ll	Yaounde	PDA	White	Cotton white	Circular
Ll	Bambui	PDA	White	Cotton white	Circular
Ll	Ekona	PDA	White	Cotton white	Circular
Ll	Yaounde	H2O	White	Absent	Absent
Ll	Bambui	H2O	White	Absent	Absent
Ll	Ekona	H2O	White	Absent	Absent

V6 = V6 juice agar; V8 = V8 juice agar; T = Tomatoes -8- agar; PDA = Potatoes dextrose agar; H2O = Water agar media. Isolate L1= Dark green petiole with small leaves

Figure 1. *Phytophthora colocasiae* on different media with circular growth pattern on cultivar L1 (dark green petiole with small leaves).



(A) *Phytophthora colocasiae* round sporangia (400x)



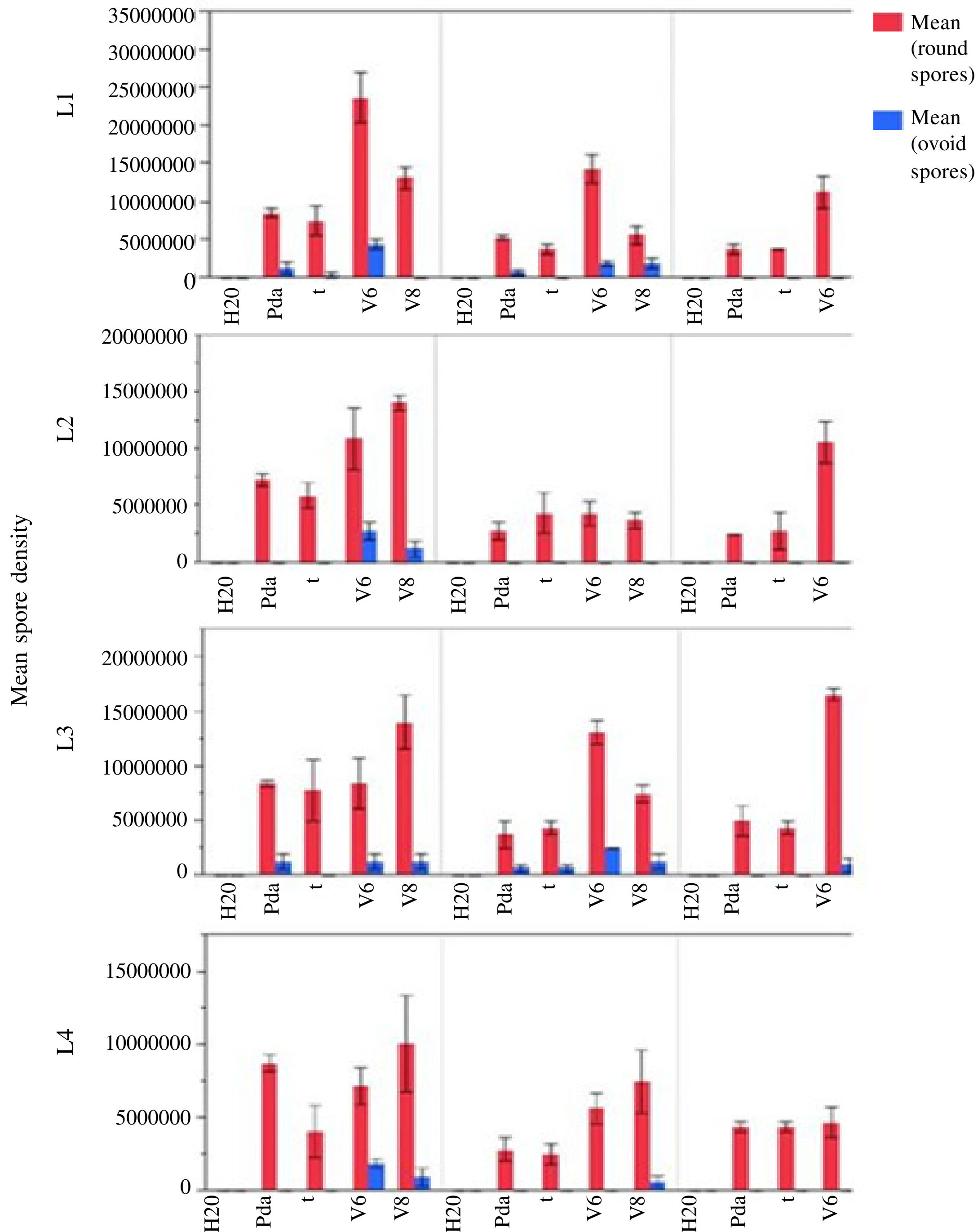
(B) *Phytophthora colocasiae* ovoid sporangia (400x)

Figure 2. *Phytophthora colocasiae* round (A) and ovoid spores (B) observed under the light microscope on V6 juice agar media in IITA Phytopathology Laboratory, Yaounde, Cameroon.

Ovoid spores were recorded on a few culture media in Bambui study site on L2 isolate 22 days, after incubation on different culture media in the three field sites (Fig. 3). In Yaounde, ovoid spore density was recorded in V6 juice agar, V8 juice agar media and in Ekona in V8 juice agar media. The highest mean ovoid spore density of 2.5× 106 spores per ml of sterile distilled water on isolate L2, was recorded in V6 juice agar in Yaounde; while the least mean ovoid spore density of 1.25× 106 spores on V8 juice agar medium in Ekona. Isolate Red petiole with small leaves (L2), recorded a high mean spore density of 15×106 spores per ml of sterile distilled water on V8 juice medium in Yaounde; and the least mean round spore density of 2.5×106 spores on potatoes dextrose agar media in Ekona. There was a significant variation ($P<0.05$) in the density of spores in the different culture media at different field sites (Fig. 3).

Isolate light green petiole with large leaves (L3), 22 days after incubation on different culture media in the three field sites, showed that the highest mean spore density of 17.5x 106 spores per ml of sterile distilled water was recorded in V6 juice agar medium in Ekona; while the lowest mean round spore density of 3.75x 106 spores per ml of sterile distilled water was found on potato dextrose agar in Bambui. There was a significant variation of mean round spore on isolate L3 in the different culture media, in the different study sites and within the study sites. Ovoid spores were produced on some of the culture media, potato dextrose agar and tomato agar in Ekona, and tomato agar in Yaounde (Fig. 3). The highest mean ovoid spore density of 2.5x 106 spores per ml of sterile distilled water were observed on V6 juice agar media in Bambui; and the least mean ovoid spore density of 1.25x 106 spores per ml of sterile distilled water on V8 juice agar in Ekona and potato dextrose agar and tomatoes agar in Bambui (Fig. 3).

Spore density of light green petiole with small leaves (L4) isolate observed 22 days after incubation, on different culture media (from the three field sites), showed that V8 juice agar had the highest mean round spore density of 13.75 x 106 spores per ml of sterile distilled water in Ekona; and the least mean round spore density of 2.5 x 106 spores per ml of sterile distilled water on tomatoes agar in Bambui. A significant mean round spore density variation was observed on isolate L4 in the culture media at the different study sites and within the experimental sites (Bambui). No ovoid spores were observed on isolate L4 on



Phytophthora colocaciae isolate from cultivars L1, L2, L3 and L4 in different media at different regions

Bars represent mean of spore density with standard errors. H2O = Water agar; PDA = Potatoes dextrose agar; T= Tomatoes -8- agar; V6 = V6 juice agar; V8 = V8 juice agar media. Isolate L1= Dark green petiole with small leaves; isolate L2= Red petiole with small leaves; isolate L3= Light green petiole with large leaves; isolate L4= Light green petiole with small leaves cultivars.

Figure 3. Spore density of L1, L2, L3 and L4 isolate 22 days after incubation on different culture media in the three field sites.

any of the culture media in Ekona. In Bambui, ovoid spores were observed on V8 juice agar media; and in Yaounde on V6 juice agar and V8 agar media. The highest mean ovoid spore density of 2.5 x 106 spores per ml of sterile distilled water was observed on V6 juice agar in Yaounde, and the lowest of 1.25 x 106 spores per ml of sterile distilled water in V8 juice agar in Bambui. There was a significant mean ovoid spore density variation observed on isolate L4 in the culture media at the different study sites (Fig. 3).

Mycelia growth in different media. Mycelia were observed in V6 agar, V8 agar, tomatoes 8 agar, and PDA two days after incubation (Fig. 4). There was no observable mycelia growth in water media, which was the control study, in all three field sites (Fig. 4). But there was a high mycelia growth of all isolates on all the culture media. All 4 isolates (L1, L2, L3, and L4) grew best in V6 juice agar and V8 juice agar media.

Growth of *P. colocasiae* on L1 isolates for 8 days in a culture media at different study sites indicated that mycelia grew on all culture media in the three study sites, with the longest mycelia growth rate of 5.5 cm in V6 juice agar media at Ekona. The shortest mycelia growth of 3 cm was recorded on tomato agar in Ekona. There was a significant difference in culture media on isolate L1 in Ekona and no significant difference within the isolates in Bambui and Yaounde (Fig. 4).

The growth of *P. colocasiae* mycelia on L2 isolates for 8 days in culture media at different study sites, showed that a significant difference in mycelia growth on the culture media in Yaounde and Bambuistudy sites (Fig. 4). Minimum mycelia growth of 4.5 cm was observed in tomatoes agar in Yaounde; and maximum mycelia of 5.2 cm were observed in Bambui in V6 juice agar media.

Maximum mycelia growth of 5 cm was recorded in V6 juice agar media in Ekona and Bambui, on L3 isolates for 8 days of culture. A minimum mycelia growth of 3 cm was recorded on tomato agar in Ekona. There was a significant variation in isolate L3, among the different culture media in Yaounde, Bambui and Ekona study sites (Fig. 4).

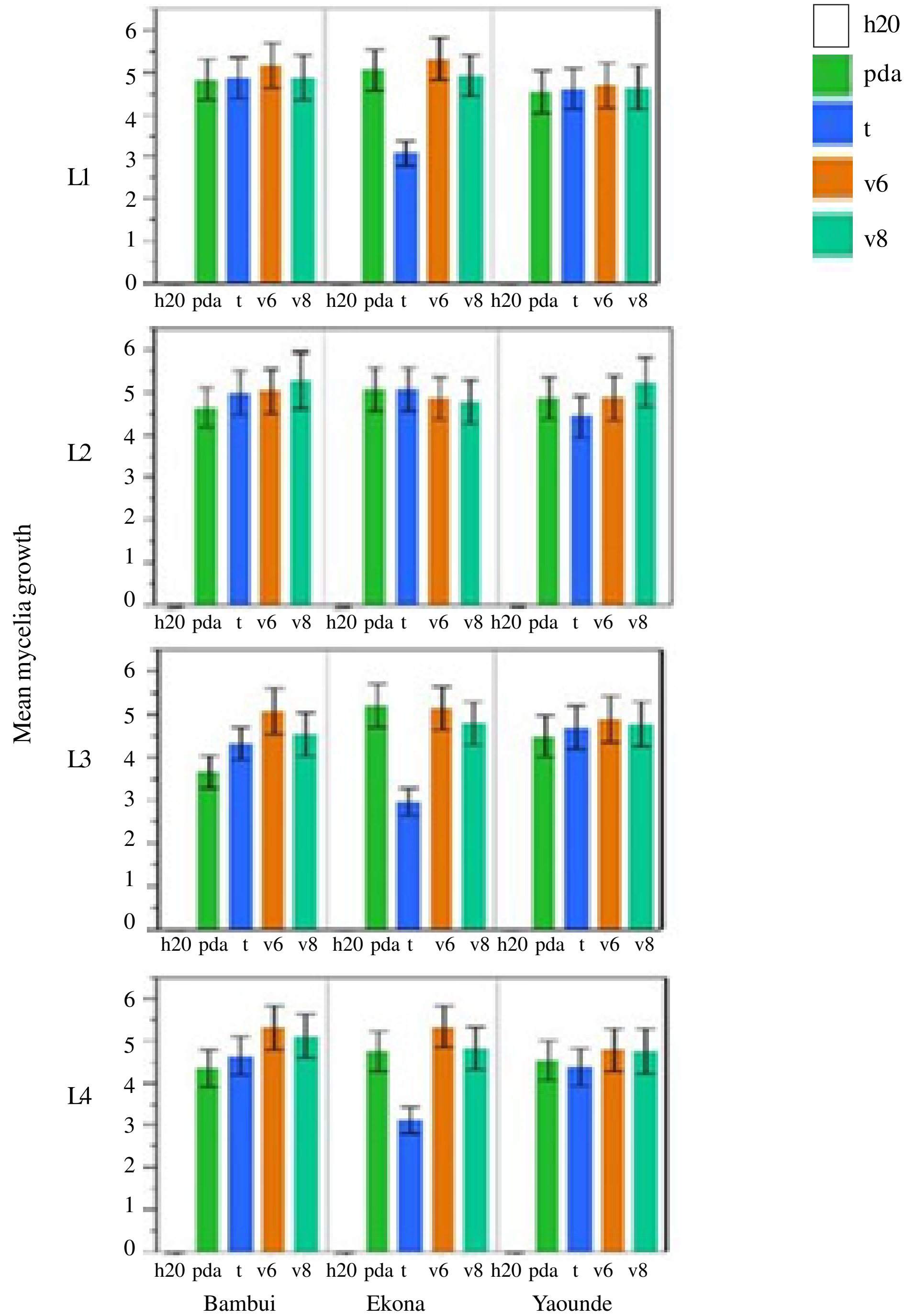
There was also a maximum mycelia growth of 5.5 cm recorded on V6 juice agar media in two field sites (Bambui and Ekona) on cultivar L4 isolate for 8 days in a culture media. Minimum mycelia growth of 3.2 cm was recorded on tomato agar media in Ekona. There was a significant difference within the culture media on isolate L4 in the three different study sites (Fig. 4).

All four isolates were pathogenic to the four local cultivars of Taro, causing lesions on leaves after they were inoculated (Table 2). However, there was no symptom expression of lesion on the leaves in the control plants. Lesions appeared on all the cultivars, two days after inoculation; and had a distinctive water-soaked margin of newly invaded tissue bearing a white mass of spores, and orange liquid droplets. There was a gradual increase in the size of the lesion at 14 days of inoculation. Isolates L1 and L2 were more virulent, causing lesions greater than 10 mm in all the tested Taro cultivars, 14 days after inoculation. There was a significant variability in pathogenicity at ($P< 0.05$) within different isolate on cultivars.

DISCUSSION

The cultural and morphological characteristics of four isolates collected from Taro infected leaves from the three field sites (Bambui, Ekona, Yaounde) showed no significant variations ($P<0.05$) in mycelia growth pattern in different culture media (Fig. 4), indicating that climatic and edaphic factors have no influence on the morphology of *P. colocasiae* growing on Taro landraces in the field. The growth pattern of mycelia was circular on all the isolates, but there was variation in mycelia colour from white to cotton white in the three study sites.

Two types of spores were observed, spherical and round spores, with more round spores produced in the various culture media



Phytophthora colocaciae isolates in different media at regions

Bars represent mean of mycelia with standard errors. H2O = Water agar; PDA = Potatoesdextrose agar; T= Tomatoes -8- agar; V6 = V6juice agar; V8 = V8 juice agar media, Isolate L1= Dark green petiole with small leaves; isolateL2= Red petiole with small leaves; isolate L3= Light green petiole with large leaves; isolate L4= Light green petiole with small leaves cultivars.

Figure 4. Mycelia growth of *Phytophthora colocasiae* on L1, L2, L3 and L4 isolates for 8 days in culture media at the different study sites.

TABLE 2. Lesion lengths of isolates of *P. colocasiae* on Taro cultivars after leaf inoculation at 14 days interval)

Cultivars	Regions	Lesion length of isolates of *P. colocasiae* (mm)			
		Isolate 1	Isolate 2	Isolate 3	Isolate 4
L1	Yaounde	17.7±0.3a	16.7±1.3a	10.0±2.6a	10.0±1.5a
L2	Yaounde	16.3±0.3a	16.7±0.3a	8.3±1.3ab	8.7±0.9b
L3	Yaounde	12.3±1.3b	10.3±1.3b	8.0±0.0bc	8.3±0.7ab
L4	Yaounde	12.0±3.0b	11.7±0.9b	7.7±1.7bc	7.7±1.3bc
L1	Bambui	11.7±0.7bc	12.0±0.6b	7.7±0.7bc	7.0±0.6bc
L2	Bambui	11.3±0.7bc	11.7±0.6b	8.0±0.0bc	7.0±0.6bc
L3	Bambui	11.0±0.0bc	11.7±0.3b	8.0±0.0bc	8.0±0.0bc
L4	Bambui	10.3±0.7bc	11.0±0.0b	9.7±0.7a	6.0±0.6bc
L1	Ekona	10.0±6.0bc	12.0±0.0b	9.0±0.0ab	6.0±0.0bc
L2	Ekona	10.0±0.0bc	10.0±0.0b	7.0±0.0bc	6.0±0.0bc
L3	Ekona	10.0±0.6bc	10.0±0.6b	5.7±0.3bc	5.0±0.0c
L4	Ekona	9.3±0.3bc	9.7±0.7b	5.0±0.0bc	5.7±0.3bc

Means followed by the same letter (s) within the same column are not significantly different at p = 0.05 (STT)

than ovoid spores. These results were similar to findings several researchers (Brunt *et al.*, 2001; Brooks, 2005; Bandyopadhyay *et al.,* 2011; Scot *et al.*, 2011; Omane *et al.*, 2012), who observed ovoid to ellipsoid shaped spores of *P. colocasiae* in Taro cultivars. Nutrients are very important in the expression of the fungus in a given culture media.

Among the five-culture media, V6 and V8 culture media produced the highest number of spores (Fig. 3), and mycelium growth was vigorous (Fig. 4), indicating that they were richer in nutrients compared to potatoes dextrose agar and tomato agar. Growth media have been shown to influence spore germination and other growth parameters in virulent fungi (Fokunang *et al.,* 1995). Tsopmbeng *et al.* (2012) showed that V6 and V8 Juice agar media are the most suitable growth media for culturing *P. colocasiae.*

Results of virulence and pathogenicity of *P. colocasiae* on the different Taro cultivars indicated that all four isolates showed variable pathogenicity (Table 2) causing lesions on inoculated leaves. There was a gradual increase in the lesion as the days increased, indicating progressive spread of the pathogen to uninfected areas, through spores or mycelia. From observations, penetration of wounded leaves by the fungus resulted in severe or slight disease development, depending on the cultivar and isolate.

Spores play an important role in the transmission of diseases by initiating the development of an infection. When spores are carried by wind and rainfall to a suitable substrate (leaves and petioles) under favourable environmental conditions (rainfall, temperature, and humidity), they germinate to produce zoospores that infect Taro. Palomar *et al.* (1999) stated that mycelia and spores play an important role in inoculation; and are essential in the initiation and development of infection. In the present study, the disease was not observed on leaves inoculated with water, probably because there were no *P. colocasiae* spores in water which could cause infection and subsequently disease on the leaves. Subsequently, the lesions on the leaves became water-soaked, enlarged, coalesced and

destroyed the leaves. Earlier studies showed that lesions caused by *P. colocasiae* on the lower surface of Taro leaves progressively become water soak, increase in size, coalesce, and destroy the leaves (Brooks, 2005; Mbong *et al.,* 2013). All landraces were susceptible to *P. colocasiae* infections after inoculation with spores. This may be attributed to the conducive growth medium (soil) in which the landraces were cultured. Generally, most pathogenic fungi show maximum growth potential and virulence on host crops when cultured in a suitable growth condition of host crops (CMI, 1997).

CONCLUSION

Mycelia growth of isolates exhibit a circular growth pattern in all the culture media; with mycelia colours varying from white to cotton white in the different media, at the study sites. Round and spherical spores appeared on all the isolates, depending on type of culture media. The media in which maximum mycelia growth and spore's density was recorded were V6 and V8 juice agar media, suggesting these to be the appropriate media for *P. Colocasia* growth in the 3 study sites. Virulence and pathogenicity of *P. colocasiae,* on the different Taro cultivars, revealed that all the four isolates vary in pathogenicity.

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