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Screen House and Field Resistance of Taro Cultivars to Taro Leaf Blight Disease (*Phytophtora colocasiae*)

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Authors' contributions

This work was carried out in collaboration between all authors. Author FC designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MG and ME managed the analyses of the study. Author TE managed study design, statistics and the literature searches. Author RH also designed the study. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Taro leaf blight disease cause by *Phytophtora colocasiae* has become an economic disease in Cocoyam growing regions of Cameroon.
Aims: To screen for resistance 10 improved and 4 local cultivars of taro against taro leaf blight disease.
Study Design: A randomized complete block design study.
Place of Study: Studies were conducted at the International Institute of Tropical Agriculture (IITA) Yaounde Nkolbisson from July 2013 to January 2014.
Methodology: Taro cultivars from tissue culture were planted in the screen house conditions and

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tested for virulence and pathogenicity with 4 isolates of *Phythophthora colocasiae* at spore density of 3×10^4 spores /ml of distilled water. Plants were planted in the field to assess disease incidence and severity.

Results: The results obtained on the different taro cultivars, revealed that all the 4 isolates showed variable pathogenicity. They caused lesions on inoculated leaves. There was variability in pathogenicity based on the small lesion lengths produced on cultivars, these included BL/SM132 and Red petiole. Isolate 3 showed a stronger sensitivity to leaf collapse and defoliation irrespective of the cultivar tested. There was a significant difference ($p = 0.05$) in tissue collapse and leaf defoliation on exposure to the different fungal isolates. The result of field infection rates of *P. colocasiae* at 126 DAP-154 DAP on 10 improved and 4 local cultivars indicated that there was significant variability ($p = 0.05$) in incidence and disease severity, with high incidence and severity occurring at 154 DAP in all cultivars. Improved cultivar BL/SM132 showed no classic symptoms of *P. colocasiae* and therefore it was resistant to *Phytophthora colocasiae.*

Conclusion: The results obtained on virulence and pathogenicity of *Phythophthora colocasiae* on the different taro cultivars revealed that all the 4 isolates showed variable pathogenicity. They caused lesions, on inoculated leaves. Isolate 3 showed a stronger sensitivity to leaf collapse and defoliation irrespective of the cultivar tested. The result of field infection rates of *P. colocasiae* at 126 DAP-154 DAP on 10 improved and 4 local cultivars indicated that there was a significant variability ($p = 0.05$) in disease incidence and severity, with high incidence and severity occurring at 154 DAP in all cultivars. Improved cultivar BL/SM132 showed no classic symptoms of *P. colocasiae* and therefore it was resistant to *Phytophthora colocasiae* as compared to all the other cultivars which showed high severity rates of infection of the disease and thus were susceptible to the disease.

Keywords: Screen house; field resistance; taro cultivars; taro leaf blight; Phytophtora colocasiae.

1. INTRODUCTION

Taro (*Colocasia esculenta*) is a perennial tropical starchy root crop which belongs to the Araceae family [1]. It originated from South East Asia and later spread into other parts of the continent and Africa of tropical climatic settings [2]. Taro cultivation is high in Nigeria, China, Cameroon and Ghana, where the annual rainfall exceeds 2000 mm and it grows best under hot and wet conditions with temperatures above 21°C. It is sensitive to frost and it is therefore a lowland crop [3]. Taro is grown as an important economic food crop and vegetable in West Africa, particularly in Ghana, Nigeria and Cameroon [4].

Taro has both medicinal and nutritional uses as it is used as food for man and animal feed [5]. Taro storage roots form the basic carbohydrate element of the diet and can be eaten in many forms: roasted, boiled, fried, baked and pounded while the leaves are eaten as preferred vegetable, representing an important source of vitamins [6]. These vitamins include vitamin A, vitamin B, vitamin C, folate, thiamine and riboflavin. The petioles and flowers are consumed in certain parts of the world. It is also rich in proteins, sugars and minerals such as calcium, manganese, phosphorus, potassium and zinc [7]. From an ethno medicinal point of view, the uncooked taro root is applied to cuts to stop the bleeding of wounds and the washed fresh leaves are used to treat tooth ache [8]. The crop is a good source of income to its producers to the extent that some subsistence farmers generate enough revenue from taro production to take care of basic family needs [9].

Despite the importance of taro, the major constraints to its production in Cameroon are diseases and pests [10]. The crop is susceptible to fungal, bacterial, viral and nematode infections [11]. Among these various diseases, taro leaf blight disease is caused by *Phytophthora colocasiae* (Raciborski). It is one of the major important economic diseases of taro because it reduces corm yield of up to 50% [12] and leaf yield of up to 95% in susceptible genotypes [13]. *Phytophthora colocasiae* causes corms to rot both in the field and in storage, and this has led to heavy storage lost [14]. In 2010 taro leaf blight disease was reported in Cameroon and it caused between 50-100% yields lost of taro in most of the crop growing regions. This has led to a reduction in food, house hold income, increase poverty and some farmers have abandoned their farms and are now growing other crops [15,16].

Taro leaf blight disease (TLBD) is characterized by large necrotic zonates spot on the leaves

often coalescing to destroy large areas of leaf [17]. The margin of the lesion is marked by a white powdery band of sporangia and numerous droplets of orange or reddish exudates [18]. *Phytophthora colocasiae* originated in South East Asia [17] and is widely distributed throughout the tropical regions of the world [19].

This study was conducted to investigate test for virulence and pathogenicity of *P. colocasiae* under screen house and field conditions.

2. MATERIALS AND METHODS

2.1 Location and Experimental Sites

The study was carried out in the field, screen house and Laboratory of Phytopathology at the International Institute of Tropical Agriculture (IITA) Nkolbisson, Yaounde, Cameroon. IITA is located at the North of Yaoundé latitude 3°86' N and longitude 11°5' E. The altitude of the institution is 754 m above sea level.

2.2 Collection, Isolation and Identification of Fungi Isolates

Infected taro leaves with young lesions of blight were collected from the field at IITA Yaoundé from four local cultivars, Dark green petiole with small leaves, Red petiole with small leaves, White petiole with large leaves, Red and white petiole with small leaves. These leaves were preserved in separate plastic bags and transported to Phytopathology Laboratory. These leaves were cut with razor blade in to small fragments of 2 mm from the advancing edges of the disease and surface-sterilized in 5% diluted solution of sodium hypochlorite for 30 seconds and rinsed in three successive changes of sterile distilled water for 3 minute. The leaf fragments were dried on sterilized filter paper and four fragments placed on solidified cool V6 juice agar containing culture medium in each Petri dish. These dishes were labeled and put in an incubator at room temperature of 22-26°C (Brunt et al., 2001). After 2-3 days extensive mycelia formed around the leaf fragment was aseptically collected and sub cultured in Petri dishes containing freshly prepared V6 juice agar medium that contains Ampiciline (250 mg/l), penicillin (250 mg/l) and nystatine (20 mg/l) (antibiotics) to inhibit bacterial growth. This transfer was carried out 2-3 times to obtain an axenic culture. Identification of fungus was carried out under the microscope and fungi isolates were determined based on morphological characteristics such as the type of mycelia and fruiting structure, the shape/size of spores as described by Nelson et al. [13].

2.3 Preparation of Inoculum

Spore suspension was prepared from 21 days old culture of different isolates, by flooding the surface of the growing colonies in each Petri dish with 5ml of sterile distilled water and dislodging the spores with a small brush. The suspension was centrifuged for 3 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 estimated as an average of the spores counted in 10 standard heamocytometer fields. The number of spores / ml was calculated using the formula adopted from Duncan and Torrance [20].

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

Where

S = Number of spores per milliliter
N = Mean number of spores in 10 large squares counted
V = 1 ml =1000 mm^3
v = volume of spore suspension under glass cover.

A spore suspension (inoculum) of each isolate was adjusted with the aid of haemocytometer to 3×10^4 spores / ml of distilled water. The four inocula were put in a refrigerator at a temperature of 4°C for 30 minutes to stimulate liberation of zoospores and a drop of Tween 80 (25 µl) was added to each spore suspension as a surface wetting agent. The control was made up of 20 ml of sterilized distilled water [21].

2.4 Virulence and Pathogenicity Test of *P. colocasiae* under Screen House and Field Conditions

Ten improved cultivar of taro, BL/SM132, BL/SM120, BL/SM152, BL/SM144, CE/MAL07, CE/MAL14, CE/MAL08, CE/IND13, CE/IND126, CE/THA09 and four local cultivars, Dark green petiole with small leaves, Red petiole with small leaves, White petiole with large leaves, Red and white petiole with small leaves, obtained from tissue culture were planted in plastic pots filled with sterilized soils in a screen house. These

plants were arranged in a complete randomized design with four replicate of four plants per replicate. The taro was inoculated 49 days after planting with spore suspension of *P. colocasiae* from the local taro cultivars which was adjusted with a haemocytometer to a spore density of 3×10^4 spores / ml of distilled water. Inoculation was done by using a syringe to inject the spore suspension on three spots on the leaves. Observations were carried out and lesion diameter was measured using a ruler. Data for average lesion diameter, tissue collapse and defoliation was recorded for 14 days. Temperature and humidity were also recorded with the Hobo metre [22].

2.5 Field Experiment

Ten improved and four local cultivars of taro were used for this experiment namely BL/SM132, BL/SM120, BL/SM152, BL/SM144, CE/MAL07, CE/MAL14, CE/MAL08, CE/IND13, CE/IND126, CE/THA09 and Dark green petiole with small leaves, Red petiole with small leaves, White petiole with large leaves, Red and white petiole with small leaves, respectively. These cultivars were cultured in tissue culture laboratory and transplanted after five months .The cultivars were planted in a randomized complete block design, in 8 ridges which consisted of 80 cm wide and 18.82 m long on the 8th of July 2013. These plants were transplanted by putting one plant per hole at 75 cm spacing. Ridges were weeded monthly after transplanting. Data on disease incidence and severity of *P. colocasiae* on the different infected plants were recorded at two weeks interval from the first appearance of symptoms for one month and numbers of infected plants were recorded.

2.5.1 Determination of Disease Incidence of *P. colocasiae*

Percentage incidence was calculated using the formula:

$$Incidence = \frac{Number\ of\ infected\ plant}{Total\ number\ of\ plant} \times 100$$

2.5.2 Determination of Disease Severity of *P. colocasiae*

Severity of symptom on each variety was scored using the syndrome scale below: 0= No symptom, 1= Presence of lesions less than 10 cm^2 of leaf area, 2= Presence of lesions 11- 30 cm^2 of leaf area, 3= Presence of lesions 31- 60 cm^2 of leaf area, 4= Presence of lesions 61- 90 cm^2 of leaf area, 5= Presence of lesions more than 90 cm^2 up to 25% of leaf area, 6= Coalesce of spots more than 25% of leaf covered, 7= Coalesce of spots more than 50% of leaf covered, 8= Coalesce of spots more than 75% of leaf covered, 9=Collapse of petiole accompanied by complete leaf blight [4].

$$Disease\ severity = \frac{Area\ of\ leaves\ infected}{Total\ area\ of\ leaves} \times 100$$

2.6 Statistical Analysis

All data collected from taro infection, severity and incidence were subjected to analysis of Variance (ANOVA) as described by Wichura [23] using statistical software [24]. Mean variability amongst the cultivars were determined. Their treatment means were separated using Duncan Multiply Range Test (DMRT) and the Least Significant Difference (LSD) at statistical significance of 95% confidence interval.

3. RESULTS

3.1 Virulence and Pathogenicity Test of *P. colocasiae* under Screen House Conditions

The results of virulence and pathogenicity of *P. colocasiae* (4 isolates) on 10 improved and 4 local cultivars of taro under screen house are shown on Tables 2, 3, 4 and 5. All the four isolates were all pathogenic to the ten improved and four local cultivars of taro causing lesions on leaves after they were inoculated (Tables 2, 3, 4 and 5). There was no symptom expression of lesion on the control treatment. 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 sporangia, and orange liquid droplets. There was variability in pathogenicity based on the small lesion lengths produced on cultivars, this included BL/SM132 and Red petiole where leaves collapse and defoliation were not observed on the 14th day. Holes were also observed on most of the cultivars of BL/SM132 on the 14th day.

Table 1. Virulence of isolate 1 (Dark green petiole cultivar) of *P. colocasiae* on 10 improved and 4 local cultivars of taro after leaf inoculation

Days	Cultivars and lesion length (mm)													
	BL/ SM132	BL/SM 144	BL\SM 152	BL\SM 120	CE/ IND13	CE/ MAL08	CE/ MAL14	CE\ IND126	CE\ MAL07	CE\ MAL09	Dark green petiole	Red petiole	WHITE Petiole	Red/ White petiole
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	2.5	3.7	4.3	5.0	8.3	12.3	11.7	16.3	5.0	7.3	5.0	3.3	7.3	5.7
4	6.7	13.7	14.0	10.0	17.3	22.3	20.7	24.3	18.3	14.3	14.0	11.7	20.0	16.3
5	7.3	31.7	24.3	13.0	23.3	22.5	31.0	30.3	31.7	22.3	21.0	14.7	27.7	22.7
6	8.3	35.0	31.7	14.7	31.7	LDO	37.0	35.0	36.0	29.0	26.7	15.7	25.0	31.3
7	9.3	44.5	36.0	20.0	37.3	LDO	LDO	LDO	37.0	30.0	45.0	18.0	30.0	LDO
8	10.3	LDO	38.0	28.3	42.0	LDO	LDO	LDO	LDO	37.5	LDO	21.7	35.0	LDO
9	10.3	LDO	40.0	33.3	47.0	LDO	LDO	LDO	LDO	LDO	LDO	25.3	48.0	LDO
10	10.3	LDO	LDO	38.3	57.0	LDO	LDO	LDO	LDO	LDO	LDO	29.0	50.0	LDO
11	11.0	LDO	LDO	46.0	60.5	LDO	LDO	LDO	LDO	LDO	LDO	32.0	55.0	LDO
12	11.0	LDO	LDO	52.3	LDO	LDO	LDO	LDO	LDO	LDO	LDO	34.3	58.0	LDO
13	11.0	LDO	LDO	52.0	LDO	LDO	LDO	LDO	LDO	LDO	LDO	36.3	60.0	LDO
14	11.0	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	36.5	LDO	LDO

Values are means lesion length (mm). LDO= Leaf die off

Table 2. Virulence of isolate 2 (Red petiole cultivar) of *P. colocasiae* on 10 improved and 4 local cultivars of taro after leaf inoculation

Days	Cultivars and lesion length (mm)													
	BL/ SM120	BL\ SM132	BL/ SM152	BL\ SM144	CE/ IND126	CE/ IND13	CE/ MAL07	CE\ MAL14	CE\ IND08	CE\ THA09	Dark green petiole	Red Petiole	White petiole	Red/White petiole
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	5.0	5.0	4.0	6.7	10.3	7.7	5.7	10.0	5.7	6.0	5.0	3.7	5.0	5.7
4	11.0	8.3	9.3	19.0	22.7	19.3	18.7	21.0	13.7	14.7	15.0	10.0	13.3	18.7
5	13.3	8.7	20.3	34.0	28.3	29.3	27.7	32.0	23.3	18.0	27.7	13.3	20.7	28.3
6	17.3	9.3	27.0	37.3	32.7	24.0	35.5	35.0	33.0	25.3	35.2	19.3	28.3	34.0
7	20.7	9.7	33.3	38.3	36.0	27.0	37.0	38.0	39.0	31.7	43.3	25.3	27.5	LDO
8	25.3	9.7	32.5	40.0	39.3	33.0	LDO	LDO	40.0	38.5	41.0	27.3	35.0	LDO
9	29.0	9.7	41.0	LDO	LDO	40.0	LDO	LDO	45.0	LDO	LDO	31.7	40.0	LDO
10	33.3	9.7	LDO	LDO	LDO	LDO	LDO	LDO	50.0	LDO	LDO	40.0	LDO	LDO
11	39.0	9.7	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	37.5	LDO	LDO
12	41.7	9.7	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	42.5	LDO	LDO
13	45.0	9.7	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	45.0	LDO	LDO
14	LDO	9.7	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	46.0	LDO	LDO

Values are means lesion length (mm). LDO= Leaf die off

Table 3. Virulence of isolate 3 (White petiole cultivar) of *P. colocasiae* on 10 improved and 4 local cultivars of taro after leaf inoculation

Days	Cultivars and lesion length (mm)													
	BL/SM 132	BL/SM 144	BL\SM 120	BL\SM 152	CE/ IND13	CE/ IND126	CE/ MAL07	CE\ MAL08	CE\ MAL14	CE\ MAL09	Dark green petiole	Red petiole	White petiole	Red/ White petiole
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	6.0	3.7	5.0	5.0	5.0	5.0	5.7	15.0	7.3	5.0	5.0	5.0	7.7	5.7
4	10.7	9.0	15.0	15.0	15.0	18.3	20.0	30.7	16.7	14.3	14.3	19.3	18.3	20.3
5	13.3	21.7	25.7	25.7	25.7	25.7	29.7	37.0	36.0	24.7	23.7	22.0	32.3	28.3
6	15.0	25.0	33.3	33.3	30.0	27.3	LDO	LDO	LDO	32.0	32.0	24.0	30.0	38.3
7	19.3	31.3	27.0	38.0	35.7	35.0	LDO	LDO	LDO	36.7	47.3	30.5	51.0	LDO
8	19.3	34.3	31.0	40.0	LDO	38.0	LDO	LDO	LDO	LDO	LDO	37.5	56.0	LDO
9	19.5	25.0	33.0	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	40.0	65.0	LDO
10	19.5	27.0	37.0	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	41.0	65.0	LDO
11	19.5	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	41.0	LDO	LDO
12	19.5	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	45.0	LDO	LDO
13	19.5	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	50.0	LDO	LDO
14	19.5	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	55.0	LDO	LDO

Values are means lesion length (mm). LDO= Leaf die off

Table 4. Virulence of isolate 4 (Red/ white petiole cultivar) of *P. colocasiae* on 10 improved and 4 local cultivars of taro after leaf inoculation

Days	Cultivars and lesion length (mm)													
	BL/ SM132	BL/ SM144	BL\ SM120	BL\ SM152	CE/ IND13	CE/ IND126	CE/ MAL07	CE\ MAL08	CE\ MAL14	CE\ MAL09	Dark green petiole	Red Petiole	White petiole	Red/ White petiole
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	3.7	3.7	5.0	5.0	15.0	5.0	9.3	5.0	15.0	5.0	5.0	5.0	1.7	5.7
4	10.0	13.7	15.0	15.0	25.0	16.7	20.3	19.7	25.0	17.0	14.3	14.3	6.7	20.3
5	21.7	31.7	25.0	28.0	30.3	23.3	30.7	28.5	33.0	21.3	20.7	18.0	20.0	29.0
6	27.5	37.5	30.7	30.0	32.7	31.7	LDO	35.0	37.0	29.3	27.3	20.3	28.3	38.3
7	33.3	37.0	45.0	41.0	LDO	37.3	LDO	LDO	LDO	34.0	39.3	25.7	31.0	LDO
8	35.0	LDO	47.0	40.0	LDO	49.5	LDO	LDO	LDO	37.0	45.3	29.7	42.5	LDO
9	20.0	LDO	50.0	LDO	LDO	61.5	LDO	LDO	LDO	LDO	LDO	21.5	50.0	LDO
10	20.0	LDO	56.0	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	25.5	55.0	LDO
11	20.0	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	26.0	LDO	LDO
12	20.0	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	26.5	LDO	LDO
13	20.0	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	32.5	LDO	LDO
14	20.0	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	27.0	LDO	LDO

Values are means lesion length (mm). LDO= Leaf die off

There was variation in the size of lesion length among cultivars. In isolate 1, the susceptible cultivars recorded lesion lengths of 22.5 mm, 37 mm, 35 mm, 31.3 mm 5 and 6 days after inoculation. These lengths were recorded in cultivars CE/MAL08, CE/MAL14, CE/IND126, Red/white petiole, respectively. The other cultivars were moderately susceptible. The highest lesion length was 60.5 mm recorded on cultivar CE/IND 13, 11 days after inoculation and the lowest length of 11 mm was observed on BL/SM132.

Similar results were obtained in isolate 2, the various susceptible ones recorded lesion length of 34, 37, 38 mm respectively, 6 and 7 days after inoculation. These were expressed in cultivars Red/white petiole, CE/MAL07, CE/MAL14. The other cultivars were moderately susceptible. The highest lesion length was 46.0 mm on cultivar Red petiole, 14 days after inoculation and the lowest length of 9.7 mm was observed on BL/SM 132.

In isolate 3, the highly susceptible ones recorded lesion length of 29.7, 37 and 36 mm respectively 5 days after inoculation. These were expressed in cultivars, CE/MAL07, CE/MAL08, CE/MAL14. The other cultivars were moderately susceptible except cultivar BL/SM132 and red petiole which were resistant, where tissue collapse and leaf defoliation was not observed on the 14th day. The highest lesion length of 65.0 mm was recorded on cultivar White petiole, 10 days after inoculation and the lowest length of 19.5 mm was observed on BL/SM 132, 14 days after inoculation.

In isolate 4, the various susceptible cultivars recorded lesion length of 30.7 mm, 38.3 mm, 35 mm, 37 mm, 32.7 mm, 37 mm respectively, at 7 days after inoculation. These were expressed in cultivars, CE/MAL07, Red/white petiole, CE/MAL08, CE/MAL14, CE/IND 13, and BL/SM144. The other cultivars were moderately susceptible. The highest lesion length was 61.5 mm on cultivar CE/IND 126, 9 days after inoculation and the lowest length of 20 mm was observed on BL/SM132, 14 days after inoculation.

3.2 Time Taken for Tissue to Collapse on 14 Different Cultivars

The studies conducted to investigate the duration of tissue collapse of infected cultivars showed variability's amongst the improved and local cultivars as shown in Table 6. From the results at 14 days after inoculation of leaves with the isolates (Table 5), there was a significant difference of tissue collapse at $p = 0.5$ within different isolate on cultivars. Improved cultivar BL/SM132 leaves did not collapse 14 days after inoculation; instead lesions dried off and holes were observed with isolate 1 and isolate 2. With isolate 3 and isolate 4 very few plant leaves collapse with mean tissue collapse days of 3±3.0 and 5.3±2.7, respectively. Cultivar Red petiole, BL/SM120 recorded longer mean days tissue collapse of 13.7±0.3 and 12.3±0.3, respectively as compared with cultivar BL/MAL8 with short mean day tissue collapse of 4.7±0.3 with isolate 1. For isolate 2 the longest mean day's tissue collapse of 12.7±0.3 and 12.7±1.3 were recorded with BL/SM 120 and Red petiole while short mean day of tissue collapse of 5.7±0.7 was recorded with CE/MAL14. Isolate 3 and isolate 4 recorded longer mean days of tissue collapse of 9.0±2.6 and 10.0±1.5 respectively with cultivar Red petiole whereas CE/MAL8 and CE/MAL7 recorded shorter mean days of tissue collapse of 4.3±0.6 and 5.0±0.0, respectively. Isolate 3 showed a stronger sensitivity to leaf collapse irrespective of the cultivar tested.

3.3 Time Taken for Leaf Defoliation on 14 Different Cultivars after 14 Days of Inoculation

Effect of field survival of cultivar was determined by assessing leaf defoliation of both the improved and local cultivars. There was a significant difference in leaf defoliation on exposure to the different fungal isolates as shown in Table 6. Cultivar BL/SM120 took longer mean days (13.3±0.3, 13.7±0.3, 8.3±1.3 and 9.7±0.9) for leaves to defoliate on all the isolates 1, 2, 3 and 4, respectively whereas cultivar BL/SM144 took mean day of 9.7±0.7 with isolate 3. The shortest mean day's leaf defoliation of 4.0±2.0, 3.7±3.7, 5.0±2.6, was observed with White petiole (isolate 1), Red petiole (isolate2), and Red petiole (isolate3), respectively while CE/MAL 7, CE/MAL8 had short mean day defoliation of 6.00±0.6 with isolate 4. There was no defoliation on BL/SM132 with isolate 1 and 2 while isolate 3 and 4 showed very little defoliation and mean days of 3.3±3.3 and 6.0±3.0 were recorded. Isolate 3 was more sensitive to leaf defoliation in all the cultivars tested. Maximum and minimum humidity (103.8% and 74.4%) and temperature (34.43°C and 20.57°C), respectively were recorded from hobo meter during this experiment.

Table 5. Time taken for tissue collapse on 10 improved and 4 local cultivars of taro after leaf inoculation

Cultivars	Isolate and tissue collapse in days			
	Isolate 1	Isolate 2	Isolate 3	Isolate 4
Red petiole	13.7±0.3a	12.7±1.3a	9.0±2.6a	10.0±1.5a
BL/SM120	12.3±0.3a	12.7±0.3a	7.3±1.3ab	8.7±0.9b
CE/IND13	8.3±1.3b	6.3±1.3b	7.0±0.0bc	8.3±0.7ab
WHITE	8.0±3.0b	7.7±0.9b	6.7±1.7bc	7.7±1.3bc
BLS/SM152	7.7±0.7bc	8.0±0.6b	6.7±0.7bc	7.0±0.6bc
CE/MAL09	7.3±0.7bc	7.7±0.6b	7.0±0.0bc	7.0±0.6bc
Dark green Petiole	7.0±0.0bc	7.7±0.3b	7.0±0.0bc	8.0±0.0bc
BL/SM144	6.3±0.7bc	7.0±0.0b	8.7±0.7a	6.0±0.6bc
CE/IND126	6.0±6.0bc	8.0±0.0b	8.0±0.0ab	6.0±0.0bc
Red/white petiole	6.0±0.0bc	6.0±0.0b	6.0±0.0bc	6.0±0.0bc
CE/MALO7	6.0±0.6bc	6.0±0.6b	5.7±0.3bc	5.0±0.0c
CE/MAL14	5.3±0.3bc	5.7±0.7b	5.0±0.0bc	5.7±0.3bc
CE/MAL8	4.7±0.3b	7.7±1.2b	4.3±0.3c	5.0±0.6c
BL/SM132	0.0±0.0d	0. 0±0.0c	3±3.0d	5.3±2.7bc

Means followed by the same letter (s) within the same column are not significantly different at p = 0.05 (DMRT). Values are means days followed by standard error

Table 6. Time taken for leaf defoliation on 10 improved and 4 local cultivars of taro after 14 days of leaf inoculation

Cultivars	Isolate and defoliation in days			
	Isolate 1	Isolate 2	Isolate 3	Isolate 4
BL/SM120	13.3±0.3a	13.7±0.3a	8.3±1.3b	9.7±0.9b
CE/IND13	9.3±1.3ba	7.3±1.3bc	8.0±0.0b	9.3±0.7ab
BLS/SM152	8.7±0.7bc	9.0±0.6b	7.7±0.7ab	8.0±0.7ab
CE/MAL09	8.3±0.7bc	8.7±0.3b	8.0±0.0b	8.0±0.7ab
Dark green petiole	8.0±0.0bc	8.7±0.3b	8.0±0.0b	9.0±0.0ab
BL/SM144	7.3±0.7bc	8.0±0.0b	9.7±0.7a	7.0±0.7bc
CE/IND126	7.0±0.0bc	9.0±0.0b	9.0±0.0b	7.0±0.0bc
Red/ white petiole	7.0±0.0bc	7.0±0.0bc	7.0±0.0ab	7.0±0.0bc
CE/MAO7	7.0±0.6bc	7.0±0.6bc	6.7±0.3ab	6.0±0.6c
CE/MAL14	6.3±0.3bc	6.7±0.7bc	6.0±0.0ab	6.7±0.3c
CE/MAL8	5.7±0.3bc	8.7±1.2b	5.3±0.3ab	6.0±0.6c
Red petiole	4.7±4.7bc	3.7±3.7c	5.0±2.6c	11.0±1.5a
White petiole	4.0±2.0dc	8.7±0.9b	7.7±1.7ab	8.7±1.3ab
BL/SM132	0.0±0.0d	0.0±0.0d	3.3±3.3c	6.0±3.0c

Means followed by the same letter (s) within the same column are not significantly different at p = 0.05 (DMRT). Values are means days followed by standard error

3.4 Virulence and Pathogenicity Test of *P. colocasiae* under Field Conditions

3.4.1 Disease incidence of *P. colocasiae* on 10 improved and 4 local cultivars of taro at 126 DAP, 140 DAP and 154 DAP

The percentage incidence of *P. colocasiae* increased with age of the plant (126 DAP – 154 DAP) in both local and improved cultivars. The highest percentage incidence of *P. colocasiae* of 100% was recorded in most of the cultivars at 154 DAP except BL/SM114 with 25% (Fig 1). Disease was not observed at 126 DAP with cultivar BL/SM120, CE/MA 07 and CE/TH 09. This data indicated that all the cultivars were susceptible to *P. colocasiae* as compared to BL/SM132 whose leaves showed percentage incidences of 100% of another disease symptom from 126 DAP to 154 DAP.

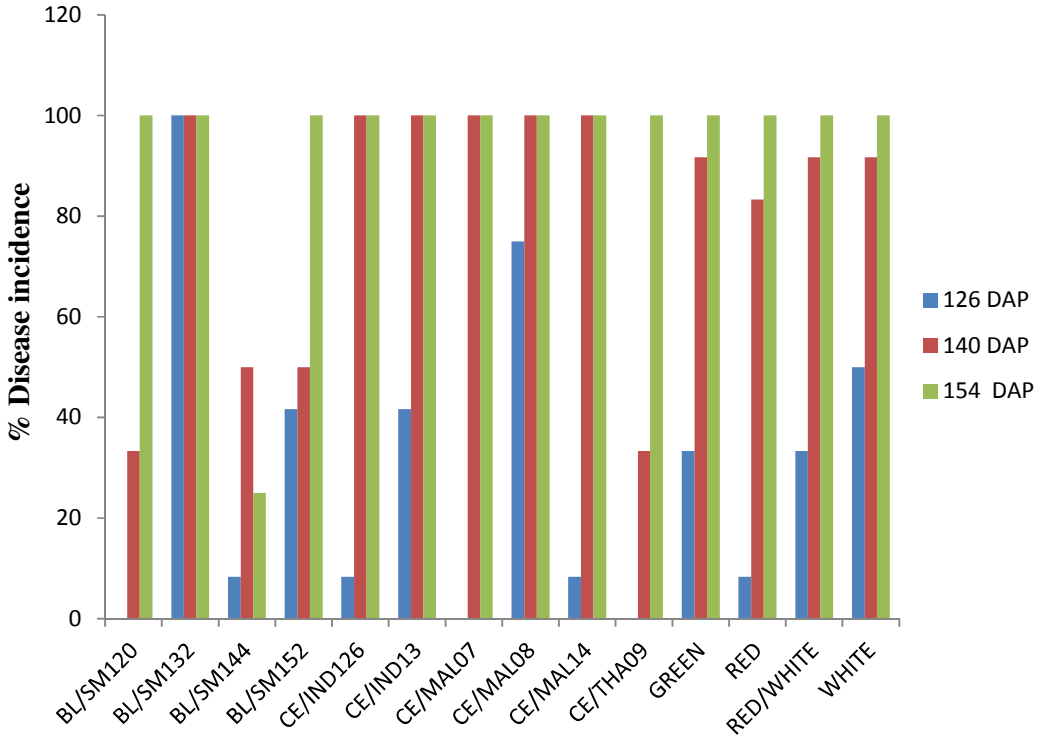

Fig. 1. Disease incidence of *P. colocasiae* on 10 improved and 4 local cultivars of taro at 126 DAP, 140 DAP and 154 DAP

DAP = Days after planting

3.4.2 Percentage of infection and severity of *P. colocasiae* on 10 improved and 4 local cultivars of taro leaves at 126 DAP, 140 DAP and 154 DAP

The taro cultivars showed significant differences (p = 0.05) in infected leaf severity with fungal isolates and taro sensitivity to infection as shown in Table 7. The mean percentage of *Phytophthora colocasiae* infection on fourteen taro cultivars' leaves showed that the number of leaves infected increased with DAP (126 days, 140 days and 154 days after planting). All the local cultivars Dark green petiole, White petiole, Red petiole, Red/white petiole were all infected with *P. colocasiae* and some of the improved cultivars were also infected, these included BL/SM144, BL/SM152, CE/IND126 CE/IND13, CE/MAL8, CE/MAL14 at low leaf infection rates at 126 days after planting. At 140 and 154 days after planting, all the cultivars leaves were infected by *P. colocasiae* with the highest mean percentage leaf infection of 79.6±2.1% observed on improved cultivar CE/MAL7 at 154 days after planting. The lowest mean percentage leaf infection of 0.0% was observed in cultivar BL/SM120, BL/SM132 and BL/SM144 at 126 days after planting. Cultivar BL/SM132 showed symptoms that were not classical for the tested fungal disease as indicated in Table 7. The mean percentage leaf infection for this cultivar was 24.9±2.9%, 60.5±3.9%, and 61.7±2.8% at 126 days, 140 days and 154 days respectively after planting.

The severity of *P. colocasiae* was observed on leaves of taro plants 126 days, 140 days and 154 days after planting in Nkolbisson Yaounde as presented on Table 8. There was a significant variability (p = 0.05) on disease severity amongst the taro cultivars. The *Phytophthora colocasiae* severity on the different cultivars of taro increases at DAP (126-154) days after planting. All the local cultivars Dark green petiole, White petiole, Red petiole, Red/white petiole were all infected with *P. colocasiae* and some of the improved cultivars were also infected BL/SM144, BL/SM152, CE/IND126, CE/IND13, CE/MAL8,

CE/MAL14 at low severity rates at 126 DAP. At 140 and 154 days after planting, all the plants were infected by *Phytophthora colocasiae.* The highest mean severity of 9.0±0.0 mm was observed with cultivar CE/IND126 for both dates. There was a mean severity significant difference (p = 0.05) among the improved and local cultivars with age (126 DAP, 140 DAP, and 154 DAP). It was observed that improved cultivar BL/SM132 showed disease symptom that was different from *P. colocasiae.*

4. DISCUSSION

Studies on virulence and pathogenicity of *Phythopthora colocasiae* on the different taro cultivars indicated that all the 4 isolates showed variable pathogenicity. They caused lesions on inoculated leaves. There was a gradual increase in lesion as days increased except in BL/SM132. The reaction of the taro cultivar was broadly identical to all the Fungi tested. Invasion of wounded leaves by the fungus resulted in severe

Table 7. Mean percentage of infected leaves by *P. colocasiae* on 10 improved and 4 local cultivars of taro a 126 DAP, 140 DAP and 154 DAP

Cultivars	126 DAP percentage of infected leaf	140 DAP percentage of infected leaf	154 DAP percentage of infected leaf
BL/SM120	0.0±0.0b	2.8±1.2e	54.8±6.5bc
BL/SM132	24.9±2.9a	60.5±3.9b	61.7±2.8 b
BL/SM144	0.2±0.2b	4.3±1.3e	6.9±1.3e
BL/SM152	2.1±0.9b	4.7±1.5e	39.8±4.3d
CE/IND126	0.7±0.7b	76.6±0.5a	53.5±2.0bc
CE/IND13	2.1±1.0b	24.9±0.8d	42.3±3.2d
CE/MAL8	4.1±1.1b	33.1±2.3c	41.8±4.1d
CE/MAL14	1.1±1.1b	8.3±0.9e	54.6±4.5bc
CE/MAL7	0.0±0.0b	5.2±0.4e	79.6±2.1a
CE/THA9	0.0±0.0b	2.0±0.9e	59.2±4.0b
Dark green petiole	3.9±1.9b	35.1±6.5c	74.1±4.4a
Red petiole	0.9±0.9b	6.6±1.3e	46.9±4.2bc
Red/ white petiole	3.9±1.9b	22.3±3.5d	61.2±2.1b
White petiole	3.4±1.2b	33.2±4.6c	46.2±2.4cd

Means followed by the same letter (s) within the same column are not significantly different at p = 0.05 (DMRT). Values are means of percentage of infected leaves followed by standard error. DAP = Days after planting

Table 8. Mean severity of infected leaves by *P. colocasiae* on 10 improved and 4 local cultivars of taro at 126 DAP, 140 DAP and 154 DAP

Cultivars	126 DAP severity of infection	140 DAP severity of infection	154 DAP severity of infection
BL/SM120	0.0±0.0c	1.5±0.6ed	7.4±0.8c
BL/SM132	3.5±0.8a	3.6±0.4cb	8.7±0.1a
BL/SM144	0.1±0.1c	1.0±0.3ed	5.6±1.1dc
BL/SM152	0.6±0.3cb	0.8±0.3ed	8.8±0.2a
CE/IND126	0.1±0.9c	9.0±0.0a	9.0±0.0a
CE/IND13	0.3±0.1bc	4.3±0.8b	8.3±0.1ba
CE/MAL8	0.9±0.3b	4.1±0.8cb	5.3±1.1d
CE/MAL14	0.1±0.1c	1.5±0.1ed	9.0±0.0a
CE/MAL7	0.0±0.0c	1.5±0.1ed	9.0±0.0a
CE/THA9	0.0±0.0c	0.3±0.1e	9.0±0.0a
Dark green petiole	0.4±0.2bc	3.7±1.0cb	7.4±0.9c
Red petiole	0.1±0.2c	1.0±0.8ed	6.3±1. 1 bc
Red/ white petiole	0.3±0.1bc	2.3±0.7cd	9.0±0.0a
White petiole	0.6±0.2bc	5.3±1.2ed	9.0±0.0a

Means followed by the same letter (s) within the same column are not significantly different at p= 0.05 (DMRT). Values are mean severity of infection followed by standard error. DAP = Days after planting

or slight disease development, depending on the cultivar and isolate. On non inoculated leaves, no disease developed. The leaves had spots which were water soaked, or dry gray appearance, as spots increased in size, coalesced and quickly destroyed the leaves. This can be supported by reports of Brooks [25] and Mbong et al. [10] who reported that on the lower leaf surface, spots have water – soak, or dry gray appearance. As spots increases in size they coalesce and quickly destroy the leaf. In BL/SM132 it was observed that the centers of lesions become papery and fall out, producing shot-hole appearance on leaves. Lebot et al. [26] also reported that in dry weather or on some resistant cultivars, the centers of lesions become papery and fall out, producing shot-hole appearance. Many of these shot-holes' expand no further; others will resume development under conditions of heavy rain in susceptible cultivars. The most rapid expansion of lesions occur when cool, showery weather allows fungal growth in tissues both night and day. This finding suggests that the pathogen most have colonized the damage tissue at the early stage to cause the disease development.

The effect of 4 isolates at spore density of 3×10^4 spores / ml of distilled water on 10 improved and 4 local cultivars showed that there was tissue collapse on all the cultivars. Cultivar Red petiole and BL/SM120 took longer days for tissues to collapse indicating that they were moderately resistant to *P. colocasiae*. Cultivar CE/MAL8, CE/MAL14 and CE/MAL7 took very few days for tissues to collapse thus were highly susceptible to the *P. colocasiae*. This idea is supported by the finding of Davinder et al. [27] who reported that leaves of susceptible cultivars collapse in about 20 days compared to 40 days of non–infected plants, therefore photosynthesis is greatly reduced in susceptible plants leading to progressively smaller leaves and corms. Cultivar BL/SM132 did not show tissue collapse with isolate 1 and 2 where as isolate 3 and 4 showed very little tissue collapse, instead lesion dried off and holes were observed on leaves which imply that it was resistant. This result was in accordance with that of Nelson et al. [13] who reported that in some resistant taro cultivars the centre of lesions become papery and break apart, which gives a conspicuous "shot-hole" appearance.

From the results, there was defoliation of leaves on most of the cultivars except BL/SM132 where there was little or no defoliation of leaves based on the fungi isolate. This defoliation of leaves could be due to maximum and minimum humidity of (103.8% and 74.4%) and temperature of (34.43℃ and 20.57℃) respectively that were recorded during the experiment that favours *P. colocasiae* development. This tie with reports from Brooks [25] who reported that *P. colocasiae* is a warm – weather pathogen, growing most rapidly at temperatures between 27- 30℃. Maximum and minimum temperatures for growth are 10℃ and 35℃ respectively. Reports from Mbong et al. [10] who stated that the pathogen can cause rapid and complete defoliation of leaves and crops destruction.

High percentage incidence of 100% of *P. colocasiae* was observed on all the cultivars of taro at 154 DAP. This result showed that the incidence of *P. colocasiae* can be very high when there is high humidity and temperatures. This idea is supported by finding of Brooks [25] who reported that the warm humid days and cool wet nights of the tropics are ideal for the reproduction and spread of *P. colocasiae*. During rainy weather, leaves of taro cultivars that are normally destroyed for 30-40 days may be destroyed in less than 20 days. Therefore a healthy plant that carries 5-7 functional leaves may have only 2-3 leaves when infected. This reduces photosynthesis resulting in reduced corm yield. Highly susceptible cultivars appear to be destroyed in the field, producing smaller and smaller leaves on shorter and shorter petioles. All the cultivars were infected with *P. colocasiae* indicating that there were susceptible to the pathogen except BL/ SM132 that was resistant to the pathogen and showed classical symptom of another disease.

The *Phytophthora colocasiae* severity and percentage leaf infection on the different cultivars of taro increases with age 126- 154 days after planting. The increase in *Phytophthora colocasiae* severity and percentage leaf infection with age of the plant could be due to environmental conditions such as increase in humidity and favorable temperatures. This result is in accordance with reports of Mbong et al. [10] who reported that when conditions are warmer 28-30℃, the sporangia germinates directly by a germ tube and infect the leaf. Nelson et al. [13] who also reported that *Phytophthora colocasiae* (Raciborski) reduced leaf yield of up to 95% in susceptible genotypes. Improved cultivar BL/SM132 did not show symptom of the taro leaf blight disease and therefore it was resistant to *Phytophthora colocasiae* as compared to all the

other cultivars which showed high severity rates of infection of the disease and thus were susceptible to the disease.

5. CONCLUSION

The results obtained on virulence and pathogenicity of *Phythophthora colocasiae* on the different taro cultivars revealed that all the 4 isolates showed variable pathogenicity. They caused lesions, on inoculated leaves. Isolate 3 showed a stronger sensitivity to leaf collapse and defoliation irrespective of the cultivar tested. There was variability in pathogenicity based on the small lesion lengths produced on cultivars, these included BL/SM132 and Red petiole where leaf collapse and defoliation were not observed on the 14th day. There was a significant difference ($p = 0.05$) in tissue collapse and leaf defoliation on exposure to the different fungal isolates.

The result of field infection rates of *P. colocasiae* at 126 DAP-154 DAP on 10 improved and 4 local cultivars indicated that there was a significant variability ($p = 0.05$) in disease incidence and severity, with high incidence and severity occurring at 154 DAP in all cultivars. Improved cultivar BL/SM132 showed no classic symptoms of *P. colocasiae* and therefore it was resistant to *Phytophthora colocasiae* as compared to all the other cultivars which showed high severity rates of infection of the disease and thus were susceptible to the disease.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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