


# 1 Screen house and field resistance of taro cultivars to taro leaf blight 2 disease (~~*Phytophthora colocasiae*~~)

## 4 Abstract

5 **Introduction:** Taro leaf blight disease cause by *Phytophthora colocasiae* has become an economic  
6 disease in Cocoyam growing regions of Cameroon.

7 **Aims:** to screen for resistance, 10 improved and 4 local cultivars of taro against taro leaf blight diseases. 

8 **Study design:** A randomized complete block design study

9 **Place of study:** Studies were conducted at the International Institute of Tropical Agriculture (IITA)  
10 Yaounde Nkolbisson from July 2013 to January 2014.

11 **Methodology:** Taro cultivars from tissue culture were planted in the screen house conditions and tested  
12 for virulence and pathogenicity with 4 isolates of *Phytophthora colocasiae* at spore density of  $3 \times 10^4$   
13 spores /ml of distilled water. Plants were planted in the field to assess disease incidence and severity.

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

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

33  
34 **Key words:** Screen house, field resistance, taro cultivars, ~~taro leaf blight~~, *Phytophthora colocasiae*)  
35

## 36 INTRODUCTION

37 Taro (*Colocasia esculenta*) is a perennial tropical starchy root crop which belongs to the Araceae family  
38 [1]. It originated from South East Asia and later spread into other parts of the continent and Africa of  
39 tropical climatic settings [2]. Taro cultivation is high in Nigeria, China, Cameroon and Ghana, where the  
40 annual rainfall exceeds 2000 mm and it grows best under hot and wet conditions with temperatures  
41 above  $21^{\circ}$  C. It is sensitive to frost and it is therefore a lowland crop [3]. Taro is grown as an important  
42 economic food crop and vegetable in West Africa, particularly in Ghana, Nigeria and Cameroon [4].

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

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

62 Taro leaf blight disease (TLBD) is characterized by large necrotic zones spot on the leaves often  
63 coalescing to destroy large areas of leaf [17]. The margin of the lesion is marked by a white powdery  
64 band of sporangia and numerous droplets of orange or reddish exudates [18]. *Phytophthora colocasiae*  
65 originated in South East Asia [17] and is widely distributed throughout the tropical regions of the world  
66 [19].

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

## 69 **MATERIALS AND METHODS**

### 70 **Location and experimental sites**

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

### 74 **Collection, isolation and identification of fungi isolates**

75 Infected taro leaves with young lesions of blight were collected from the field at IITA Yaounde from four  
 76 local cultivars, Dark green petiole with small leaves, Red petiole with small leaves, White petiole with  
 77 large leaves, Red and white petiole with small leaves. These leaves were preserved in separate plastic  
 78 bags and transported to Phytopathology Laboratory. These leaves were cut with razor blade in- to small  
 79 fragments of 2 mm from the advancing edges of the disease and surface-sterilized in 5 % diluted solution  
 80 of sodium hypochlorite for 30 seconds and rinsed in three successive changes of sterile distilled water for  
 81 3 minute. The leaf fragments were dried on sterilized filter paper and four fragments placed on solidified  
 82 cool V6 juice agar containing culture medium in each Petri dish. These dishes were labeled and put in an  
 83 incubator at room temperature of 22-26 °C (Brunt *et al.*, 2001). After 2-3 days extensive mycelia formed  
 84 around the leaf fragment was aseptically collected and sub cultured in Petri dishes containing freshly  
 85 prepared V6 juice agar medium that contains Ampiciline (250 mg/l), penicillin (250 mg/l) and nystatine (20  
 86 mg/l) (antibiotics) to inhibit bacterial growth. This transfer was carried out 2-3 times to obtain an axenic  
 87 culture. Identification of fungus was carried out under the microscope and fungi isolates were determined  
 88 based on morphological characteristics such as the type of mycelia and fruiting structure, the shape/size  
 89 of spores as described by Nelson *et al.* [13].

#### 90 Preparation of inoculum

91 Spore suspension was prepared from 21 days old culture of different isolates, by flooding the surface of  
 92 the growing colonies in each Petri dish with 5ml of sterile distilled water and dislodging the spores with a  
 93 small brush. The suspension was centrifuged for 3 minutes and the supernatant was filtered through a 2  
 94 layered sterile muslin cheesed cloth. A drop of spore suspension was placed on the haemocytometer  
 95 chamber, covered with a slide and the number of spores per ml estimated as an average of the spores  
 96 counted in 10 standard heamocytometer fields. The number of spores/ml was calculated using the  
 97 formula adopted from Duncan and Torrance [20].

$$S = NV/v$$

98 Where S = Number of spores per milliliter

99 N = Mean number of spores in 10 large squares counted

100 V = 1 ml = 1000 mm<sup>3</sup>

101 v = volume of spore suspension under glass cover.

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

106 **Virulence and pathogenicity test of *P. colocasiae* under screen house and field conditions**

107 Ten improved cultivar of taro, BL/SM132, BL/SM120, BL/SM152, BL/SM144, CE/MAL07, CE/MAL14,  
 108 CE/MAL08, CE/IND13, CE/IND126, CE/THA09 and four local cultivars, Dark green petiole with small  
 109 leaves, Red petiole with small leaves, White petiole with large leaves, Red and white petiole with small  
 110 leaves, obtained from tissue culture were planted in plastic pots filled with sterilized soils in a screen  
 111 house. These plants were arranged in a complete randomized design with four replicate of four plants per  
 112 replicate. The taro was inoculated 49 days after planting with spore suspension of *P. colocasiae* from the  
 113 local taro cultivars which was adjusted with a haemocytometer to a spore density of  $3 \times 10^4$  spores / ml of  
 114 distilled water. Inoculation was done by using a syringe to inject the spore suspension on three spots on  
 115 the leaves. Observations were carried out and lesion diameter was measured using a ruler. Data for  
 116 average lesion diameter, tissue collapse and defoliation was recorded for 14 days. Temperature and  
 117 humidity were also recorded with the Hobo metre [22].

118 **Field experiment.**

119 Ten improved and four local cultivars of taro were used for this experiment namely BL/SM132,  
 120 BL/SM120, BL/SM152, BL/SM144, CE/MAL07, CE/MAL14, CE/MAL08, CE/IND13, CE/IND126,  
 121 CE/THA09 and Dark green petiole with small leaves, Red petiole with small leaves, White petiole with  
 122 large leaves, Red and white petiole with small leaves, respectively. These cultivars were cultured in tissue  
 123 culture laboratory and transplanted after five months. The cultivars were planted in a randomized  
 124 complete block design, in 8 ridges which consisted of 80 cm wide and 18.82 m long on the 8<sup>th</sup> of July  
 125 2013. These plants were transplanted by putting one plant per hole at 75 cm spacing. Ridges were  
 126 weeded monthly after transplanting. Data on disease incidence and severity of *P. colocasiae* on the  
 127 different infected plants were recorded at two weeks interval from the first appearance of symptoms for  
 128 one month and numbers of infected plants were recorded.

129 **Determination of disease incidence of *P. colocasiae*.**

130 Percentage incidence was calculated using the formula:

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

131 **3.6.3.2. Determination of disease severity of *P. colocasiae***

132 Severity of symptom on each variety was scored using the syndrome scale below: 0= No symptom, 1=  
 133 Presence of lesions less than 10 cm<sup>2</sup> of leaf area, 2= Presence of lesions 11- 30 cm<sup>2</sup> of leaf area, 3=  
 134 Presence of lesions 31- 60 cm<sup>2</sup> of leaf area, 4= Presence of lesions 61- 90 cm<sup>2</sup> of leaf area, 5= Presence  
 135 of lesions more than 90 cm<sup>2</sup> up to 25 % of leaf area, 6= Coalesce of spots more than 25 % of leaf

136 covered, 7= Coalesce of spots more than 50 % of leaf covered, 8= Coalesce of spots more than 75 % of  
 137 leaf covered, 9=Collapse of petiole accompanied by complete leaf blight [4].

138

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

### 139 **Statistical analysis**

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

## 144 **RESULTS**

### 145 **Virulence and pathogenicity test of *P. colocasiae* under screen house conditions**

146 The results of virulence and pathogenicity of *P. colocasiae* (4 isolates) on 10 improved and 4 local  
 147 cultivars of taro under screen house are shown on Table 2, 3, 4 and 5. All the four isolates were all  
 148 pathogenic to the ten improved and four local cultivars of taro causing lesions on leaves after they were  
 149 inoculated (Table 2, 3, 4 and 5). There was no symptom expression of lesion on the control treatment.  
 150 Lesions appeared on all the cultivars two days after inoculation and had a distinctive water-soaked  
 151 margin of newly invaded tissue bearing a white mass of sporangia, and orange liquid droplets. There was  
 152 variability in pathogenicity based on the small lesion lengths produced on cultivars, this included  
 153 BL/SM132 and Red petiole where leaves collapse and defoliation were not observed on the 14<sup>th</sup> day.  
 154 Holes were also observed on most of the cultivars of BL/SM132 on the 14<sup>th</sup> day.

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

160 Similar results were obtained in isolate 2, the various susceptible ones recorded lesion length of 34, 37,  
 161 38 mm respectively, 6 and 7 days after inoculation. These were expressed in cultivars Red/white petiole,  
 162 CE/MAL07, CE/MAL14. The other cultivars were moderately susceptible. The highest lesion length was  
 163 46.0 mm on cultivar Red petiole, 14 days after inoculation and the lowest length of 9.7 mm was observed  
 164 on BL/SM 132.

165 In isolate 3, the highly susceptible ones recorded lesion length of 29.7, 37 and 36 mm respectively 5 days  
166 after inoculation. These were expressed in cultivars, CE/MAL07, CE/MAL08, CE/MAL14. The other  
167 cultivars were moderately susceptible except cultivar BL/SM132 and red petiole which were resistant,  
168 where tissue collapse and leaf defoliation was not observed on the 14<sup>th</sup> day. The highest lesion length of  
169 65.0 mm was recorded on cultivar White petiole, 10 days after inoculation and the lowest length of 19.5  
170 mm was observed on BL/SM 132, 14 days after inoculation.

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

176

Days	Cultivars and lesion length (mm)													
	BL/ SM1 32	BL/ SM144	BL\ SM 152	BL\ SM120	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

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

Days	Cultivars and lesion length (mm)													
	BL/S M	BL\S M	BL/SM 152	BL\SM 144	CE/IND 126	CE\IND 13	CE/MAL 07	CE\MAL 14	CE\IND 08	CE\THA 09	Dark green petiole	Red Petiol e	White petiole	Red/Whi te 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

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

180

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



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

Cultivars and lesion length (mm)														
Days	BL/SM 132	BL/SM 144	BL\SM 120	BL\SM 152	CE/ IND13	CE/ IND126	CE/ MAL0	CE\ MAL08	CE\ MAL14	CE\ MAL09	Dark green petiole	Red petiole	White petiole	Red/ White petiole
<b>1</b>	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
<b>2</b>	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
<b>3</b>	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
<b>4</b>	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
<b>5</b>	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
<b>6</b>	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
<b>7</b>	19.3	31.3	27.0	38.0	35.7	35.0	LDO	LDO	LDO	36.7	47.3	30.5	51.0	LDO
<b>8</b>	19.3	34.3	31.0	40.0	LDO	38.0	LDO	LDO	LDO	LDO	LDO	37.5	56.0	LDO
<b>9</b>	19.5	25.0	33.0	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	40.0	65.0	LDO
<b>10</b>	19.5	27.0	37.0	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	41.0	65.0	LDO
<b>11</b>	19.5	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	41.0	LDO	LDO
<b>12</b>	19.5	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	45.0	LDO	LDO
<b>13</b>	19.5	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	50.0	LDO	LDO
<b>14</b>	19.5	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	LDO	55.0	LDO	LDO

183

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

**Cultivars and lesion length (mm)**

Days	BL/S	BL/SM	BL\SM	BL\SM	CE/	CE/	CE/	CE\	CE\	CE\	Dark	Red	White	Red/
	M	144	120	152	IND13	IND126	MAL07	MAL08	MAL14	MAL09	green	Petiole	petiole	White petiole
	132										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

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

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

188 **Time taken for tissue to collapse on 14 different cultivars.**

189 The studies conducted to investigate the duration of tissue collapse of infected cultivars showed  
 190 variability's amongst the improved and local cultivars as shown in Table 6. From the results at 14 days  
 191 after inoculation of leaves with the isolates (Table 5), there was a significant difference of tissue collapse  
 192 at  $p = 0.5$  within different isolate on cultivars. Improved cultivar BL/SM132 leaves did not collapse 14 days  
 193 after inoculation; instead lesions dried off and holes were observed with isolate 1 and isolate 2. With  
 194 isolate 3 and isolate 4 very few plant leaves collapse with mean tissue collapse days of  $3 \pm 3.0$  and  
 195  $5.3 \pm 2.7$ , respectively. Cultivar Red petiole, BL/SM120 recorded longer mean days tissue collapse of  
 196  $13.7 \pm 0.3$  and  $12.3 \pm 0.3$ , respectively as compared with cultivar BL/MAL8 with short mean day tissue  
 197 collapse of  $4.7 \pm 0.3$  with isolate 1. For isolate 2 the longest mean day's tissue collapse of  $12.7 \pm 0.3$  and  
 198  $12.7 \pm 1.3$  were recorded with BL/SM 120 and Red petiole while short mean day of tissue collapse of  
 199  $5.7 \pm 0.7$  was recorded with CE/MAL14. Isolate 3 and isolate 4 recorded longer mean days of tissue  
 200 collapse of  $9.0 \pm 2.6$  and  $10.0 \pm 1.5$  respectively with cultivar Red petiole whereas CE/MAL8 and CE/MAL7  
 201 recorded shorter mean days of tissue collapse of  $4.3 \pm 0.6$  and  $5.0 \pm 0.0$ , respectively. Isolate 3 showed a  
 202 stronger sensitivity to leaf collapse irrespective of the cultivar tested.

203

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

Cultivars	Isolate and tissue collapse in days			
	Isolate 1	Isolate 2	Isolate 3	Isolate 4
Red petiole	$13.7 \pm 0.3a$	$12.7 \pm 1.3a$	$9.0 \pm 2.6a$	$10.0 \pm 1.5a$
BL/SM120	$12.3 \pm 0.3a$	$12.7 \pm 0.3a$	$7.3 \pm 1.3ab$	$8.7 \pm 0.9b$
CE/IND13	$8.3 \pm 1.3b$	$6.3 \pm 1.3b$	$7.0 \pm 0.0bc$	$8.3 \pm 0.7ab$
WHITE	$8.0 \pm 3.0b$	$7.7 \pm 0.9b$	$6.7 \pm 1.7 bc$	$7.7 \pm 1.3bc$
BLS/SM152	$7.7 \pm 0.7bc$	$8.0 \pm 0.6b$	$6.7 \pm 0.7 bc$	$7.0 \pm 0.6bc$
CE/MAL09	$7.3 \pm 0.7bc$	$7.7 \pm 0.6b$	$7.0 \pm 0.0 bc$	$7.0 \pm 0.6bc$
Dark green Petiole	$7.0 \pm 0.0bc$	$7.7 \pm 0.3b$	$7.0 \pm 0.0 bc$	$8.0 \pm 0.0bc$
BL/SM144	$6.3 \pm 0.7bc$	$7.0 \pm 0.0b$	$8.7 \pm 0.7a$	$6.0 \pm 0.6 bc$
CE/IND126	$6.0 \pm 6.0bc$	$8.0 \pm 0.0b$	$8.0 \pm 0.0 ab$	$6.0 \pm 0.0 bc$
Red/white petiole	$6.0 \pm 0.0bc$	$6.0 \pm 0.0b$	$6.0 \pm 0.0 bc$	$6.0 \pm 0.0 bc$
CE/MAL07	$6.0 \pm 0.6bc$	$6.0 \pm 0.6b$	$5.7 \pm 0.3 bc$	$5.0 \pm 0.0c$
CE/MAL14	$5.3 \pm 0.3bc$	$5.7 \pm 0.7b$	$5.0 \pm 0.0 bc$	$5.7 \pm 0.3 bc$
CE/MAL8	$4.7 \pm 0.3b$	$7.7 \pm 1.2b$	$4.3 \pm 0.3 c$	$5.0 \pm 0.6c$
BL/SM132	$0.0 \pm 0.0d$	$0.0 \pm 0.0c$	$3 \pm 3.0d$	$5.3 \pm 2.7 bc$

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

208 **Time taken for leaf defoliation on 14 different cultivars after 14 days of inoculation.**

209 Effect of field survival of cultivar was determined by assessing leaf defoliation of both the improved and  
 210 local cultivars. There was a significant difference in leaf defoliation on exposure to the different fungal  
 211 isolates as shown in Table 6. Cultivar BL/SM120 took longer mean days ( $13.3 \pm 0.3$ ,  $13.7 \pm 0.3$ ,  $8.3 \pm 1.3$  and  
 212  $9.7 \pm 0.9$ ) for leaves to defoliate on all the isolates 1, 2, 3 and 4, respectively whereas cultivar BL/SM144  
 213 took mean day of  $9.7 \pm 0.7$  with isolate 3. The shortest mean day's leaf defoliation of  $4.0 \pm 2.0$ ,  $3.7 \pm 3.7$ ,  
 214  $5.0 \pm 2.6$ , was observed with White petiole (isolate 1), Red petiole (isolate2), and Red petiole (isolate3),  
 215 respectively while CE/MAL 7, CE/MAL8 had short mean day defoliation of  $6.00 \pm 0.6$  with isolate 4. There  
 216 was no defoliation on BL/SM132 with isolate 1 and 2 while isolate 3 and 4 showed very little defoliation  
 217 and mean days of  $3.3 \pm 3.3$  and  $6.0 \pm 3.0$  were recorded. Isolate 3 was more sensitive to leaf defoliation in  
 218 all the cultivars tested. Maximum and minimum humidity (103.8 % and 74.4 %) and temperature ( $34.43$   
 219  $^{\circ}\text{C}$  and  $20.57^{\circ}\text{C}$ ), respectively were recorded from hobo meter during this experiment.

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

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

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

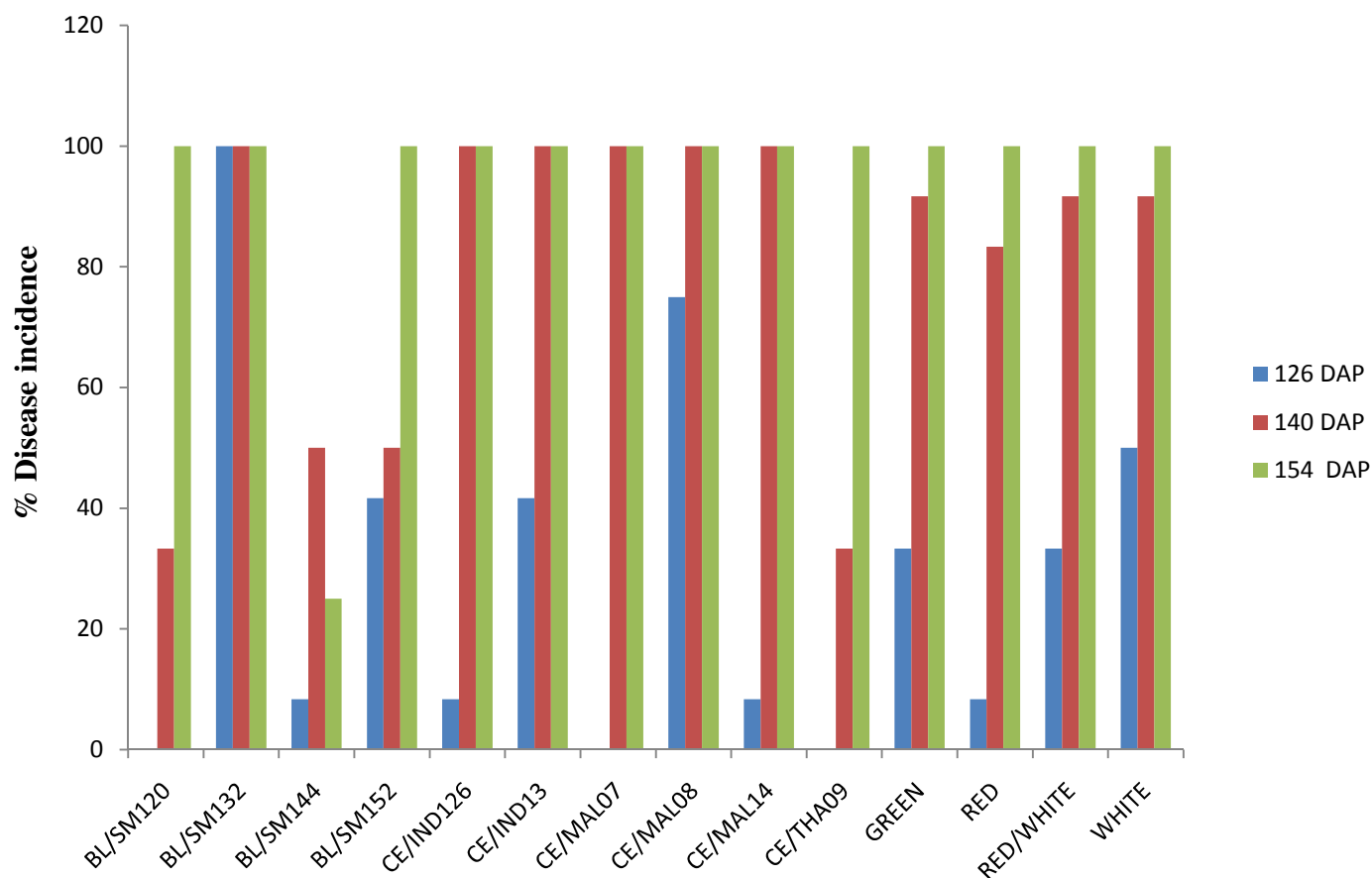
224

225 **Virulence and pathogenicity test of *P. colocasiae* under field conditions**

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

228 The percentage incidence of *P. colocasiae* increased with age of the plant (126 DAP – 154 DAP) in both  
 229 local and improved cultivars. The highest percentage incidence of *P. colocasiae* of 100 % was recorded  
 230 in most of the cultivars at 154 DAP except BL/SM114 with 25 % (Figure 1). Disease was not observed at  
 231 126 DAP with cultivar BL/SM120, CE/MA 07 and CE/TH 09. This data indicated that all the cultivars were  
 232 susceptible to *P. colocasiae* as compared to BL/SM132 whose leaves showed percentage incidences of  
 233 100 % of another disease symptom from 126 DAP to 154 DAP.

234



235

236 Figure 1: Disease incidence of *P. colocasiae* on 10 improved and 4 local cultivars of taro at 126 DAP, 140  
 237 DAP and 154 DAP. DAP = Days after planting.

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

240

241 The taro cultivars showed significant differences ( $p = 0.05$ ) in infected leaf severity with fungal isolates  
 242 and taro sensitivity to infection as shown in Table 7. The mean percentage of *Phytophthora colocasiae*  
 243 infection on fourteen taro cultivars' leaves showed that the number of leaves infected increased with DAP  
 244 (126 days, 140 days and 154 days after planting). All the local cultivars Dark green petiole, White petiole,  
 245 Red petiole, Red/white petiole were all infected with *P. colocasiae* and some of the improved cultivars  
 246 were also infected, these included BL/SM144, BL/SM152, CE/IND126 CE/IND13, CE/MAL8, CE/MAL14  
 247 at low leaf infection rates at 126 days after planting. At 140 and 154 days after planting, all the cultivars  
 248 leaves were infected by *P. colocasiae* with the highest mean percentage leaf infection of  $79.6 \pm 2.1$  %  
 249 observed on improved cultivar CE/MAL7 at 154 days after planting. The lowest mean percentage leaf  
 250 infection of 0.0 % was observed in cultivar BL/SM120, BL/SM132 and BL/SM144 at 126 days after  
 251 planting. Cultivar BL/SM132 showed symptoms that were not classical for the tested fungal disease as  
 252 indicated in table 7. The mean percentage leaf infection for this cultivar was  $24.9 \pm 2.9$  %,  $60.5 \pm 3.9$  %, and  
 253  $61.7 \pm 2.8$  % at 126 days, 140 days and 154 days respectively after planting.

254 **Table 7: Mean percentage of infected leaves by *P. colocasiae* on 10 improved and 4 local cultivars**  
 255 **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

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

259 The severity of *P. colocasiae* was observed on leaves of taro plants 126 days, 140 days and 154 days  
 260 after planting in Nkolbisson Yaounde as presented on Table 8. There was a significant variability ( $p =$   
 261  $0.05$ ) on disease severity amongst the taro cultivars. The *Phytophthora colocasiae* severity on the  
 262 different cultivars of taro increases at DAP (126-154) days after planting. All the local cultivars Dark green  
 263 petiole, White petiole, Red petiole, Red/white petiole were all infected with *P. colocasiae* and some of the  
 264 improved cultivars were also infected BL/SM144, BL/SM152, CE/IND126, CE/IND13, CE/MAL8,  
 265 CE/MAL14 at low severity rates at 126 DAP. At 140 and 154 days after planting, all the plants were  
 266 infected by *Phytophthora colocasiae*. The highest mean severity of  $9.0 \pm 0.0$  mm was observed with  
 267 cultivar CE/IND126 for both dates. There was a mean severity significant difference ( $p = 0.05$ ) among the  
 268 improved and local cultivars with age (126 DAP, 140 DAP, and 154 DAP). It was observed that improved  
 269 cultivar BL/SM132 showed disease symptom that was different from *P. colocasiae*.

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

Cultivars	126 DAP	140 DAP	154 DAP
	Severity of infection	Severity of infection	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±1.1bc
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

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

275

## 276 DISCUSSION

277 Studies on virulence and pathogenicity of *Phytophthora colocasiae* on the different taro cultivars indicated  
278 that all the 4 isolates showed variable pathogenicity. They caused lesions on inoculated leaves. There  
279 was a gradual increase in lesion as days increased except in BL/SM132. The reaction of the taro cultivar  
280 was broadly identical to all the Fungi tested. Invasion of wounded leaves by the fungus resulted in severe  
281 or slight disease development, depending on the cultivar and isolate. On non inoculated leaves, no  
282 disease developed. The leaves had spots which were water soaked, or dry gray appearance, as spots  
283 increased in size, coalesced and quickly destroyed the leaves. This can be supported by reports of  
284 Brooks [25] and Mbong *et al.* [10] who reported that on the lower leaf surface, spots have water – soak, or  
285 dry gray appearance. As spots increases in size they coalesce and quickly destroy the leaf. In BL/SM132  
286 it was observed that the centers of lesions become papery and fall out, producing shot-hole appearance  
287 on leaves. Lebot *et al.* [26] also reported that in dry weather or on some resistant cultivars, the centers of  
288 lesions become papery and fall out, producing shot-hole appearance. Many of these shot-holes' expand  
289 no further; others will resume development under conditions of heavy rain in susceptible cultivars. The  
290 most rapid expansion of lesions occur when cool, showery weather allows fungal growth in tissues both  
291 night and day. This finding suggests that the pathogen most have colonized the damage tissue at the  
292 early stage to cause the disease development.

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

305 From the results, there was defoliation of leaves on most of the cultivars except BL/SM132 where there  
306 was little or no defoliation of leaves based on the fungi isolate. This defoliation of leaves could be due to



307 maximum and minimum humidity of (103.8 % and 74.4 %) and temperature of (34.43 °C and 20.57 °C)  
308 respectively that were recorded during the experiment that favours *P. colocasiae* development. This tie  
309 with reports from Brooks [25] who reported that *P. colocasiae* is a warm – weather pathogen, growing  
310 most rapidly at temperatures between 27- 30 °C. Maximum and minimum temperatures for growth are 10  
311 °C and 35 °C respectively. Reports from Mbong *et al.* [10] who stated that the pathogen can cause rapid  
312 and complete defoliation of leaves and crops destruction.

313 High percentage incidence of 100 % of *P. colocasiae* was observed on all the cultivars of taro at 154 DAP.  
314 This result showed that the incidence of *P. colocasiae* can be very high when there is high humidity and  
315 temperatures. This idea is supported by finding of Brooks [25] who reported that the warm humid days  
316 and cool wet nights of the tropics are ideal for the reproduction and spread of *P. colocasiae*. During rainy  
317 weather, leaves of taro cultivars that are normally destroyed for 30-40 days may be destroyed in less than  
318 20 days. Therefore a healthy plant that carries 5-7 functional leaves may have only 2-3 leaves when  
319 infected. This reduces photosynthesis resulting in reduced corm yield. Highly susceptible cultivars appear  
320 to be destroyed in the field, producing smaller and smaller leaves on shorter and shorter petioles. All the  
321 cultivars were infected with *P. colocasiae* indicating that there were susceptible to the pathogen except  
322 BL/ SM132 that was resistant to the pathogen and showed classical symptom of another disease.

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

### 333 CONCLUSION

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

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

347   
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