Mycelia growth and sporulation of *Phytophthora colocasiae* isolates under selected conditions

ABSTRACT

This work was carried out at the International Institute of Tropical Agriculture (IITA) YaoundeNkolbisson. 10 improved and 4 local cultivars of taro were used to carry out a pathogenicity test from which 1 virulent and 1 less virulent isolate from both improved (BL/SM123, BL/SM120) and local cultivars (Dark green petiole, White petiole) were selected and subsequently used in determining the effect of media, temperature, pH, light on growth parameters- mycelia growth and spores density.The most appropriate media for mycelia growth and spores production were V6 and V8 juice agar. The most suitable temperature for mycelia growth and spore density was 24^0C and also the best pH for spores to be produced was pH 6. Incubation in both light and dark was best for mycelia growth and sporulation.

Key words: taro cultivars, *Phytophthoracolocasiae,* mycelia growth, sporulation.

INTRODUCTION

The major constraints of taro production in Cameroon are diseases and pests[1]. The crop is susceptible to fungal, bacterial, viral and nematode infections [2]. Among these various diseases, taro leaf blight disease is caused by *Phytophthoracolocasiae* (Raciborski). It is one of the major important economic diseases of taro because it reduces corm yield of up to 50 % [3] and leaf yield of up to 95% in susceptible genotypes [4].*Phytophthoracolocasiae*causes corms to rot both in the field and in storage, and this has led to heavy storage lost [5]. 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 [6, 7).

Taro leaf blight disease (TLBD) is characterized by large necrotic zonates spot on the leaves often coalescing to destroy large areas of leaf [8]. The margin of the lesion is marked by a white powdery band of sporangia and numerous droplets of orange or reddish exudates [9].

Phytophthoracolocasiae originated in South East Asia [8] and is widely distributed throughout the tropical regions of the world [10].

In order to understand the epidemiology of *Phytophthoracolocasiae* and to obtain necessary information that could be useful for in-vitro screening in the genetic improvement for resistance totaro leaf blight disease, we studied the effect of media, temperature, pH, light on growth parameters- mycelia growth and spores density of the fungus.

Materials and methods

Four of the fungi isolate from two improved (BL/SM123, BL/SM120) and two local cultivars (Dark green petiole, White petiole) of taro were collected from the field at IITA Yaounde. Using a pathogenenicity test on these 4 hosts plants, their virulence was identified based on their necrotic lesion production [11] and subsequently used in determining the effect of media, temperature, pH, light on growth parameters- mycelia growth and spores density of the fungus.

Effect *P. Colocasiae* growth parameters on culture media

Mycelial growth of fungus

The following five culture media used were V8 juice agar, V6 juice agar, water agar, tomatoes-8-agar and potatoes dextrose agar (PDA). With the aid of a flame-sterilized 4 mm diameter cork-borer, mycelia discs were cut from an 8 day old axenic culture of *P.colocasiae*fromtwo improved (BL/SM123, BL/SM120) and two local taro cultivars (Dark green petiole, White petiole). Each of the mycelia disc was aseptically transferred with the aid of a flame-sterilized mounted needle to the centre of different media 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 cultivar and incubated at 24 ±2 ^{0}C at pH 6, and their mycelia growth was measured along the perpendicular lines using a ruler. The means of mycelia growth wasthen calculated from the different treatments on the 8 day.

Sporulation density of*P.colocasiae*

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 3minutes 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 [12].

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

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[13].

3.7.2. Effect of temperature on growth parameters of *P.colocasiae*

Four of the fungi isolate from two improved (BL/SM123, BL/SM120) and two local cultivars (Dark green petiole, White petiole) were grown on V6 juice agar media in Petri dishes. Four of each isolate was then incubated at different temperatures of 15 ^{0}C, 17 ^{0}C, 24 ^{0}C and 33 ^{0}C with a constant pH of 6. Mycelia growth was measured with a ruler on 8th day of incubation and sporulation density was measuredon the 21st day of incubation using a haemocytometer[13].

Effect of pH on growth parameters of *P.colocasiae*

The medium of V6 juice agar was used. Two improved (BL/SM123, BL/SM120) and two local taro cultivars (Dark green petiole, White petiole) were cultured at different pH levels ranges 4, 6, 7, 8 and 9 on V6 growth media at temperatures of 24± 2 ^{0}C. To prepare the different media 200 ml of V6 juice solution without agar was prepared as above and put in 5 conical flasks. The pH of the mixture was measured with the aid of electronic pH meter mark Thermo Orion. The pH of this mixture in the flask was 6.4 and two of the flasks content was adjusted by adding 10 %

dilute hydrochloric acid progressively, until the required pH was observed on the pH meter 4 and 6. Ten percent of dilute sodium hydroxide (NaOH) was added to the other three mixtures as above to obtain the pH 7, 8 and 9. 4 g of agar was added to each flask and well agitated. These mixtures were sterilized before adding antibiotics. From each of these media, 20 ml were placed in 5 Petri dishes each per cultivar. 4 mm diameter fragment of mycelia obtained from an axenic culture was aseptically transferred with the aid of the sterilized wire lobe and place at the center of solidified culture medium. Each of these Petri dishes containing the fungi fragment was incubated and two perpendicular lines were drawn at the bottom of the Petri dishes.Mycelia growth was measured with a graduated ruler on 8 day of incubation following the method of Fokunang *et al.* [13]. Data for sporulation density was recorded on the 21st day as described earlier.

Effect of light on growth parameters of *P.colocasiae*

Petri dishes with V6 juice agar were inoculated with 4mm diameter mycelia disc of two improved (BL/SM123, BL/SM120) and two local cultivars (Dark green petiole, White petiole) of an axenic cultures. Four Petri dishes each of both local and improved cultivar were incubated in a dark cupboard and four placed under light. These were incubated for 21 days at pH 6 and temperatures of 24±2 ^{0}C. Mycelia growth was measured on the 8th day and spore density was determined on the 21st day as above.

Statistical analysis

Data on the effect of media,temperature, pH and lightfungal were subjected to analysis of Variance (ANOVA) as described by Wichura [14]using statistical software [15]. 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.

Results

Effect of culture media on fungal growth parameter

Mycelial growth of fungus

Fungal growth parameter of mycelia growth was determined for the fungal isolates as shown in Table 1. Mycelia growth was observed on all the culture media 3 days after incubation for both the improved (BL/SM132, BL/SM120) and the local cultivars (Dark green petiole, White petiole) respectively. The surface of the Petri dishes was covered with whitish mycelia 8 days after inoculation as shown in Figure 1. There was a significant difference ($p = 0.05$) in mean mycelia growth among the cultivars with culture media V8 Juice agar and Potatoes Dextrose Agar. The maximummeanmycelia growth length of 86.0±0.0 mm was observed in local cultivar white petiole withV6 Juice agar and V8 Juice agar media as opposed to minimum mean mycelia growth length of 27.7±1.7 mm observed in BL/ SM120, Dark green petiole and white petiole with water media after 8 days of incubation. Fungal growth performance was significant with culture media V6 juice and V8 juice Agar, respectively.

Table 1: Mycelia growth (mm) of *P. colocasiae* 8 days after incubation in different culture media.

Cultivars	Mycelia growth (mm)				
	V6 juice agar	**V8 juice agar**	**PDA agar**	**Tomato 8 agar**	**Water agar**
BL/SM 132	79.3±3.3a	70.0±5.8b	43.0±1.0ba	49.3±3.3a	29.3±1.7a
BL/SM120	80.7±2.9a	76.0±0.0ba	40.3±2.3b	51.0±2.9a	27.7±1.7a
Dark green petiole	82.7±3.3a	79.3±3.3ba	46.0±0.0a	39.3±13.3a	27.7±1.7a
White petiole	86.0±0.0a	86.0±0.0a	46.0±0.0a	52.7±13.3a	27.7±1.7a

Means followed by the same letters in the same column are not significantly different at $p = 0.05$ (DMRT). Values are means of mycelia growth followed by standard error.

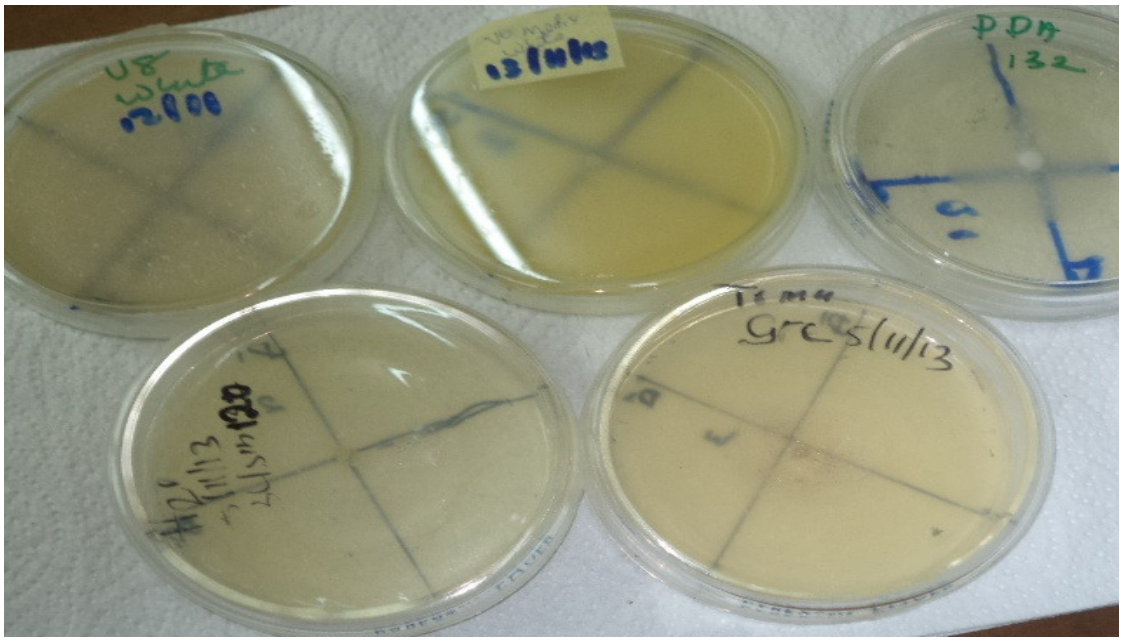

Figure 1: Growth of mycelia (mm) in different culture media

Sporulation density of*P.colocasiae*

There was a significant variation in spore density with respect to the culture media on the taro cultivars as indicated in Table 2. Spores were not observed in all the cultivars with tomatoes and water agar. There was sporulation in all the cultivars with V6 Juice, V8 juice and potatoes dextrose agar at 21days after incubation. There was a significant difference ($p = 0.05$) in sporulation density among the cultivars with culture media V8 Juice agar and potatoes dextrose agar. A maximum mean sporulation density of 1.6×10^9 spores /ml of sterile distilled water wasobserved in local cultivar White petiole with V6 Juice agar media whereasminimummean sporulation densities of $0.6\times 10^9$and 0.7×10^9spores /ml of sterile distilled water were observed in BL/ SM132, Dark green petiole, with V8 Juice agar and potatoes dextrose agar media respectively, after 21days of incubation.

Table 2: Sporulation density of *P.colocasiae* 21 days after incubation in culture media.

Cultivars	Sporulation x 10^9 spores /ml				
	V6 juice agar	**V8 juice agar**	**PDA agar**	**Tomato 8 agar**	**Water agar**
BL/SM 132	1.0±0.0a	0.6±0.1b	0.0±0.0b	0.0±0.0a	0.0±0.0a
BL/SM120	1.1±0.0a	0.8±0.0b	0.0±0.0b	0.0±0.0a	0.0±0.0a

Dark green petiole	1.5±0.1a	1.2±0.0a	0.7±0.3a	0..0±0.0a	0.0±0.0a
White petiole	1.6±0.0a	1.3±0.0a	1.0±0.0a	0.0±0.0a	0.0±0.0a

Means followed by the same letters in the same column are not significantly different at p = 0.05 (DMRT). Values are means spore density followed by standard error.

Effect of temperature on fungal growth parameters

Studies on the effect of temperature of growth parameters were conducted and the result showed that temperature variation has an influence of mycelial growth parameters as shown in Table 3. The four cultivars tested in V6 media had mycelia growth in all the different temperatures with excellent growth on all the cultivars at temperature 24 ^{0}C. The highest mycelia growth of 73.3 mm at temperature 24 ^{0}C was attained by cultivar BL/SM120 and White petiole. The least mycelia growth on all the cultivars was observed at temperature 33 ^{0}C with cultivars BL/SM132 and BL/SM120 of 23.3±2.3 mm, 25.7±2.3mm, respectively. There was a significant difference (p = 0.05) of mycelia growth between the improved and local cultivars at temperature15 ^{0}C and 17 ^{0}C, respectively.

Table 3: Effect of temperature on mycelia growth (mm) of *P. colocasiae*after 8 days of incubation.

Cultivars	**Mycelia growth (mm)**			
	15^{0}C	**17^{0}C**	**24^{0}C**	**33^{0}C**
BL/SM 132	26.0±0.0b	46.0±0.0b	71.3±0.3a	23.3±2.3a
BL/SM120	26.0±0.0b	46.0±0.0b	73.3±0.7a	25.7±2.3a
Dark green petiole	32.7±1.7a	50.3±1.2a	72.3±0.9a	28.3±2.7a
White petiole	36.0±0.0a	50.3±0.3a	73.3±0.3a	33.0±4.0a

Means followed by the same letters in the same column are not significantly different at p = 0.05 (DMRT). Values are means of mycelia growth followed by standard error.

There was a significant difference (p = 0.05) in sporulation density among all the cultivars with temperature17 ^{0}C and 24 ^{0}C , where cultivar Dark green petiole had highest sporulation density of 1.5 × 10^9 spores /ml on sterile distilled water at temperature of 24 ^{0}C (Table 4). The lowest sporulation of 0.2× 10^9 spores /ml on sterile distilled waterat 17 ^{0}C were recorded for BL/SM132and BL/SM120 cultivars. There was no sporulation of all the cultivars at temperature 15 ^{0}C and 33 ^{0}C respectively.

Table 1: Effect of temperature on sporulation of *P. colocasiae*after 21 days of incubation

Cultivars	Sporulation x 10^9 spores /ml			
	15^{0}C	**17^{0}C**	**24^{0}C**	**33^{0}C**
BL/SM 132	0.0±0.0a	0.2±0.0b	1.0±0.0b	0.0±0.0a
BL/SM120	0.0±0.0a	0.2±0.0b	1.1±0.0b	0.0±0.0a
Dark green petiole	0.0±0.0a	0.5±0.0a	1.5±0.1a	0.0±0.0a
White petiole	0.0±0.0a	0.5±0.0a	1.4±0.0	0.0±0.0a

Means followed by the same letters in the same column are not significantly different at p = 0.05 (DMRT). Values are means spore density followed by standard error.

4.3.3. Effect of pH on fungal growth parameter.

Studies of the effect of pH on fungal growth parameters,mycelial growth and sporulation showed no significant variation in mycelial growth (p =0.05) amongst the cultivars under incubation with the fungal isolates at pH 4, pH 6, and pH 7 as shown in Table 5. High mycelia growth was observed on all the cultivars in all the pH media. There was a significant difference (p = 0.5) in mycelia growth at pH 8 and pH 9 among the cultivars. The lowest mycelia growth was recorded with all the cultivars with mean value of 66.0±0.0 mm at pH 4 and the maximum on cultivars BL/SM120 and BL/SM132 with mean values of 84.67±0.7 mm at pH 7.

Table 5: Effect of pH on mycelia growth (mm) of *P. colocasiae* after 8 days of incubation

Cultivars	Mycelia growth (mm)				
	pH4	**pH6**	**pH7**	**pH8**	**pH9**
BL/SM 132	66.0±0.0a	74.7±2.9a	84.7±0.7a	70.0±0.0b	66.7±3.3b
BL/SM120	66.0±0.0a	76.7±3.3a	84.7±0.7a	82.0±2.0a	73.3±3.3ab
Dark green petiole	66.0±0.0a	81.7±4.3a	85.3±0.7a	81.0±0.0a	77.3±3.7a
White petiole	66.0±0.0a	81.0±5.0a	85.7±0.3a	81.0±0.0a	81.0±0.0a

Means followed by the same letters in the same column are not significantly different at p = 0.05 (DMRT). Values are means of mycelia growth followed by standard error.

There was significant difference (p = 0.05) in spore's density at pH 7 and pH8 among the local and improved cultivars (Table 6). Spores were not observed at pH 4 and pH 9 in all the cultivars. Cultivars Dark green petiole and White petiole had high sporulation density with mean values of 1.2±0.5× 10^9 and 1.5±0.0× 10^9 spores /ml of sterile distilled waterat pH 6, respectively. The lowest sporulation density values were recorded with cultivars BL/SM 132 and BL/SM120 with mean value of 0.1±0.0× 10^9 spores /ml of sterile distilled water at pH 8.

Table 6: Effect of pH on sporulation density of *P. colocasiae* after 21 days of incubation

Cultivars	Sporulation x 10^9 spores /ml				
	pH4	**pH6**	**pH7**	**pH8**	**pH9**
BL/SM 132	0.0±0.0a	1.0±0.6a	0.4±0.1b	0.1±0.0b	0.0±0.0a
BL/SM120	0.0±0.0a	1.1±0.0a	0.5±0.0b	0.1±0.0b	0.0±0.0a
Dark green petiole	0.0±0.0a	1.2±0.5a	1.1±0.7a	0.4±0.0a	0.0±0.0a
White petiole	0.0±0.0a	1.5±0.0a	1.1±0.1a	0.4±0.0a	0.0±0.0a

Means followed by the same letters in the same column are not significantly different at p = 0.05 (DMRT). Values are means spore density followed by standard error.

Effect of light on fungal growth parameter

There was no significant difference in mycelia growth in light and dark condition among the cultivars. High mycelia growth was observed in all the cultivars in both light and dark exposure conditions. (Table 7).

Table 7: Effect of light on mycelia growth (mm) of *P. Colocasiae*after 8 days of incubation

Cultivars	Exposure conditions	
	Light	Dark
BL/SM 132	72.0±0.0a	75.3±5.3a
BL/SM 120	76.6±4.8a	78.7±4.7a
Dark green petiole	72.3±0.8a	77.3±4.7a
White petiole	73.3±0.3a	82.7±3.3a

Means followed by the same letters in the same column are not significantly different at p = 0.05 (DMRT). Values are means of mycelia growth followed by standard error.

There was a significant difference (p = 0.5) in sporulation density in light and dark conditions among the cultivars. High sporulation density of 1.5±0.0was observed with cultivar White petiole and Dark green petiole in light and low sporulation density of 0.9±0.3 was observed with cultivar BL/SM 132 in dark exposure conditions. (Table 8).

Table 82: Effect of light on sporulation density (x 10^9 spores /ml)of *P. colocasiae* after 21 days of incubation.

Cultivars	Exposure conditions	
	Light	Dark
BL/SM 132	1.0±0.0c	0.9±0.0b
BL/SM 120	1.2±1.3b	1.0±0.0b
Dark green petiole	1.5±0.0a	1.4±0.0a
White petiole	1.50±0a	1.4±0.0a

Means followed by the same letters in the same column are not significantly different at p = 0.05 (DMRT). Values are means spore density followed by standard error.

Discussion

From the studies carried out on two improved and two local cultivars BL/SM132, BL/SM120,Dark green petiole and White petiole, mycelia growth was observed in all the media with maximum mycelia growth observed in V6 and V8 juice agar media as opposed to poor mycelia growth in water and tomatoes agar media. This was in accordance with results of Tsopmbeng*et al*. [16] who stated that both V6 and V8 Juice agar media were the most suitable for *Phytophthoracolocasiae* cultivation in Cameroon. The poor development of fungus on water and tomatoes agar medium may be due to its nutrient. Nutrient is very important in the expression of the fungus in the culture media. The absence of spores and slow mycelia growth in water and tomatoes culture media could be due to the absence of some minerals which were necessary for the growth and development of the Fungus. The most appropriate media where spores were produced was V6 and V8 juice agar, with little spores produced on local cultivar in potatoes dextrose agar.

Optimum sporulation and mycelia growth was observed at temperature 24 ^{0}C in all the cultivars. This result agree with the works of Fullerton and Tyson [17] who reported that the optimum temperature for growth *in vitro* is approximately 25 ^{0}C in detached leaf tissues, the rate of symptom development is greatest at temperatures 25-30 ^{0}C and at 35 ^{0}C symptom development is halted. Under optimum conditions (relative humidity approaching 100 %, temperatures of 20-25 ^{0}C) sporulation can take place at the margin of lesion in less than 3 hours. This study had shown that temperature at 24 ^{0}C was the best for mycelia growth and sporulation.

As concerns pH, there was high mycelia growth on all the cultivars in all the pH media both acidic and basic. Spores were not produced at very low pH 4 (highly acidic) and at very high pH 9 (highly basic). High sporulation density 1.5× 10^9 spores /ml of sterile distilled waterwere observed at pH 6. This was in accordance with report by Sahu*et al.* [18] who stated that pH 6, 5 and temperature of 28 ^{0}C is favorable for the growth of *P. colocasiae.*

Results on light showed that there was no effect on sporulation and mycelia growth because there were spores and mycelia growth in all the cultivars both improved and local.

Conclusion

The assessment of growth parameters on media, temperature, pH and light has confirmed that culture media, temperature and pH had a great influence on mycelia growth and sporulation density but light had no impact. Mycelia growth and sporulation density were dependent on the growth medium. The most appropriate media for mycelia growth and spores production were V6 and V8 juice agar. The most suitable temperature for mycelia growth and spore density was 24 ^{0}C and also the best pH for spores to be produced was pH 6.

REFERENCES

1 Mbong, G.A., Fokunang C.N., Lum A., Fontem, Bambot MB, Tembe E.A. 2013. An overview of *Phytophthoracolocasiae*of cocoyams: A potential economic disease of food security in Cameroon. Vol. 1(9): 140-145. *Discourse journal of Agriculture and Foodsciences*. www.resjournals.org/JAFS.

2 Gadre ,U.A. and Joshi, M.S. 2003. Influence of weather factors on the incidence of leaf blight of Colocasia. *Annual of Plant Protection Science*, 11: 168-170.

3 Singh, D., Guaf, J., Okpul, T., Wiles, G.and Hunter, D.2006. Taro (*Colocasiaesculenta*) variety release recommendations for Papua New Guinea based on multi-location trials. *N. Z. J. Crop Horticult.Sci34*, 163–171.

4 Nelson, S., Brooks,F. and Teves, G. 2011. Taro Leaf Blight in Hawaaii; Plant Diseases Bulletin No. PD -71;University of Hawaii: Manoa,HI,USA.New Caledonia.

5 Brunt, J., Hunter, D.andDelp, C. 2001. A Bibiliogrphy of Taro Leaf Blight;Secrtariat of the Pacific Community: N ew Caledonia. Pp1-10.

6 Guarion,L.2010. Taro leaf blight in Cameroon.Agricultural Biodiversity Weblog. Available on line: http:// agro. Biodiver .se/2010/ 07/ taro-leaf- blight-in- Cameroon/ (accessed on 15 may 2012)

7 Fontem,D.A.andMbong, G.A.2011. A novel epidermic of taro (Colocasiaesculenta) blight by Phytophthoracolocasiae hits Cameroon(Abstact*). In: Science de la vie et Productions animales* .Third life Science Conference. CAFOBIOS, Universite de Dschang, Cameroun.

8 Zhang,K.M.,Zheng, F.C.,Li,Y.D., Ann,P.J. and Ko, W.H. 1994. Isolates of *PhytophthoraColocasiae* from Hainan Island in China: evidence suggesting an Asia origin of this species.*Mycologia* 86:108-112.

9 Bandyopadhyay, R.,Sarma, k.,Onyeka,T. J.,Aregbesola, A.and Kumar, P.L. 2011. First report of taro *(Colocasiaesculenta*) laef blight caused by Phytophthoracolocasiae in Nigeria.*Plant Dis* 95 , 618.

10 CMI.1997.Commonwealth Mycological Institute, D istribution Maps of Plant diseases,Map No.466, Edition 3. *Phytophthoracolocasiae*. Common wealth Agricultural Bureau,Wallingford, Oxfordshire, UK.

11 Fokunang, C. N. 1995. Evalation of cassava genotypes for resistance to anthranose ,bacterial blight and mosaic diseases through integrated control strategies. PhD thesis, University of Ibadan,Nigeria.217pp.

12 Duncan, C and Torrence,L., 1992. Techniques for rapid detection of plant pathology, Blackwell scientific publication,Oxford, London, Paris,234pp.

13 Fokunang, C. N., Ikotun, T. and Dixon, A.G.O. 1995. Mycelial growth, sporulation and spore germination of virulent *colletotrichumgloesporioides f. sp. Manihotis* isolates under selected growth conditions. *Afr. J. of root and tuber crops*, 1: 26-31.

14 Wichura,M.J.2006. The coordinate –free approach to linear models .Cambridge Series in Statistical and Probabilistic Mathematics. Cambridge: Cambridge University Press .pp xiv +199.ISBN 978-0-521-86842-6.MR2283455(htt://www.ams.org/mathscinet-getitemmr=2283455).

15 SAS.1998.SAS UsersGuide.Statistical System Institute,Cany,NC,USA.

16 Tsopmbeng, G. R., Fontem, D. A. and Yamde, K. F. 2012. Evaluation of culture media for growth and sporulation of *Phytophthoracolocasiae*Racid., causal agent of taro leaf blight. *Int. J. Biol. Chem. Sci*. 6(4): 1566-1573.

17 Fullerton, R. A. and Tyson, J. L. 2004. The biology of *Phytophthoracolocasiae*and implications for its management and control. *In*: Secretariat of the Pacific Community (Ed.). *Third Taro Symposium*, 2003.Nadi Fiji Islands, 107-111.

18 Sahu, A. K., Maheshwari, S. k., Sriram, S. and Misra, K. S. 2000. Effects of temperature and pH on growth of *phytophthoracolocasiae.* Journal Annals of Plant Protection Sciences 8(1): 112-114