



## Morphological and molecular identification of fungi associated with corm rot and blight symptoms on plantain (*Musa paradisiaca*) in macro-propagators

Eneke Tambe BECHEM\* and Yannick Afanga AFANGA

Department of Botany and Plant Physiology, Faculty of Science, University of Buea P.O. Box 63 Buea, Cameroon.

\*Corresponding author; E-mail: [tamenekeso@yahoo.co.uk](mailto:tamenekeso@yahoo.co.uk); Tel: +237 677358523

### ABSTRACT

Plantain is a major staple food crop for millions of people in Cameroon and contributes significantly to food security. A limiting factor to large-scale production of plantains is the difficulty in obtaining healthy planting materials. Macro-propagation technology, which has the potential to produce 50-60 shoots per sucker in 4-5 months, was introduced to bypass this difficulty. The aim of this study was to isolate and identify the fungi growing in plantain propagators in Buea municipality, which could probably be responsible for the corm rot and blight symptoms observed. Fungi were isolated from the corm, root, pseudostem, leaves and from fruiting bodies on Potato dextrose agar. Isolates were characterized using cultural, micro-morphological and molecular techniques. A total of 5 isolates were obtained, most of which were whitish in colour and all had septate hyphae, belonging to the Ascomycota. The molecular weight of the DNA of the isolates ranged between 500-800 bp. Based on sequence similarity, these isolates were identified to belong to five species viz: *Alternaria burnsii*, *Fusarium oxysporum*, *Chaetomium globosum*, *Hypocrea lixii* and *Trichoderma harzianum*. Of these five isolates, *F. oxysporum* was the only one that had been reported to be the causal agent of *Fusarium* wilt (Panama) disease of plantain. *Fusarium* and *Chaetomium* are likely responsible for the diseased symptoms observed. Proper management and control of these fungal species in propagators will permit the production of healthy seedlings, thus enabling large scale production of plantains.

© 2017 International Formulae Group. All rights reserved.

**Keywords:** Plantain, *Fusarium*, corm rot, blight, *Chaetomium*.

### INTRODUCTION

Bananas and plantains are major staple food crops for millions of people in Cameroon and do contribute significantly to food security. They serve as raw materials for many popular delicacies and snacks (Ngoh et al., 2005). However, the productivity and lifespan of banana and plantain fields have drastically reduced due to pest and disease pressure. The decline was attributed mainly to

an increase in the prevalence of pests and diseases combined with lack of effective control strategies (Kahangi et al., 2004). The major diseases have been *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *cubense* (Foc); Black and Yellow sigatoka leaf spot caused by *Mycosphaerella fijiensis* and *Mycosphaerella musicola* respectively, weevils (*Cosmopolites sordidus*) and plant parasitic nematodes. This problem is made

intense because farmers usually depend on natural regeneration of plants for the supply of planting materials, which are often contaminated by pests and diseases.

A common limiting factor to large-scale production of bananas and plantains and/or expansion of existing plantation is the difficulty in obtaining planting materials (Baiyeri and Ajayi, 2000), which may be due to several diseases which are easily spread during the production of seedlings by vegetative propagation (Gandonou et al., 2012). Demand for pest free and high quality planting materials has been on the increase since they are required for the development of new plantations and expansion of the existing ones. As a result, tissue culture which was introduced in 1997, has been poorly adopted due to high costs of seedlings, capital requirements and skills involved (Njau et al., 2011).

As a consequence, macro-propagation technology was introduced as an alternative to tissue culture. The technology requires little capital and skill for implementation. Macro-propagation has the potential to produce 50-60 shoots per sucker in 4-5 months (Singh et al., 2011). Fungi especially those belonging to *Mycosphaerella* cause the most important leaf diseases of banana and plantain worldwide (Koné, 2008).

An unidentified species of macroscopic fungus has been observed to infest propagators in the Buea municipality, South West Region, Cameroon. Propagators in which this fungus was found were characterized by corm rot as well as blight symptoms on the leaves of the plantlets, indicating a possible relationship between this macroscopic fungus and the symptoms observed.

Corm rot and blight have caused great losses in the production of suckers and plantlets, thus resulting to the scarcity of planting material. This has made some farmers who produced and sold planting materials for a living to abandon the business.

There is urgent need to identify the fungus and develop proper control measures. The objective of the study reported here was therefore to isolate, characterize and identify the macroscopic fungus as well as other fungi associated with diseased suckers in propagators.

## MATERIALS AND METHODS

### Study area

This study was carried out in Small Soppo and Mile 14 in Buea municipality, Fako Division, South West Region, Cameroon. Cameroon is located between latitude 4° 8" 40N – 4° 11" North of the equator and between longitude 9° 16" 90E – 9° 17" 45E of the Greenwich meridian.

### Sample collection

The propagation sites were visited; pictures of the symptoms on diseased plants and that of the macroscopic fungus were taken. This was followed by collection of samples. Samples were collected after. Samples were collected by cutting infected portions of the plantain suckers using a sterilized knife. Collections were from the roots, corms, pseudo-stems, leaves and the fruiting body of the macro-fungus. They were stored in damp tissue paper and transported in plastic bags to the University of Buea Life Science Laboratory, where they were immediately processed for fungal isolation.

### **Media preparation**

Potato dextrose agar (SIGMA) was used for the isolation of fungi, and was prepared following the manufacturer's instructions, in which 42 g of potato dextrose agar (PDA) was weighed and distilled water added to make 1000ml. The medium was autoclaved at 121 °C for 15 minutes, allowed to cool to about 50 °C before the addition of 0.1 g/L of streptomycin. The mixture was stirred by gently swirling the flask, before pouring into 9 mm diameter sterile Petri dishes. The medium was allowed to solidify before inoculation.

### **Isolation of fungi**

#### ***Sample preparation***

Isolation of fungi was based on the method described by Hallman et al. (2007). The five samples from the corm, the root, the pseudostem, the leaves and the fruiting body were washed with running tap water to remove dust and debris adhering to them and surface sterilized with 70% ethanol for 1 min. The infected portions were cut into small pieces of 1x1 cm under aseptic conditions using a sterile scalpel. They were then immersed in 4% sodium hypochlorite solution for 3 mins. This was followed by rinsing with 70% alcohol for 1 min. They were finally rinsed in several changes of sterile distilled water and blotted dry on sterile tissue paper. Each plate was inoculated with 4 pieces of the surface sterilized plant part and fruiting body. Inoculation was done in triplicates, giving a total of 30 plates. Plates were sealed with parafilm wax and incubated at room temperature (25 °C) in the dark for 7 days. Fungi growing out of the inoculants were sub-cultured on fresh PDA plates at room temperature.

### ***Sub-culture***

Fresh plates of PDA were prepared, distinct fungi colonies from primary cultures were cut out using a sterile scalpel, and then transferred to the fresh PDA plates to obtain pure cultures. Inoculated plates were sealed with parafilm wax and incubated at room temperature (25 °C), in the dark for 7 days.

### ***Cultural characterization***

From each fungus isolated in pure culture, 5 mm mycelia discs was transferred to the centre of sterile Petri dishes containing fresh PDA. Inoculated plates were incubated for 7 days. Colony diameter was measured using a graduated 30 cm ruler and the mean diameter was obtained through the following formula:

$$\text{Mean colony diameter} = \frac{\text{Sum of colony diameter in the 3 plates (1)}}{\text{Total number of replicates}}$$

### ***Morphological characterization***

For macroscopic observation, the cultural appearances (colony colour, texture, margin, form, elevation and aerial hyphae) were noted on PDA. Colony colours were determined using Methuen handbook of colour chart (Kornerup and Wanscher, 1978). A laboratory manual and a pictorial atlas for identification of fungi by Watanabe (2002) were equally used in the description of colony morphology.

### ***Micro-morphological characterization***

For micro-morphological characterization, all isolates were cultured on PDA for seven days; hyphae were observed for the presence or absence of septa in order to determine their taxonomic classes.

**Molecular characterization**

**DNA extraction**

All isolates were grown in PDA for 7 days at 25 °C. A sterile tooth pick was used to collect and transfer a small lump of mycelia from the pure culture into a 1.5ml eppendorf tube containing a lysis buffer (400 mM Tris-HCL (pH 8), 60 mM EDTA- pH 8.0, 150 mM NaCl and 1% sodium dodecyl sulphate). The tube was briefly vortexed to disrupt the mycelia, after which it was maintained at room temperature for 10 min. 150 µl of potassium acetate was added into the eppendorf tube and this was followed by brief vortexing. The tube and contents were finally centrifuged at >13,000 x g for 1 min. The supernatant was transferred to another 1.5 ml eppendorf tube and centrifuged again as described above. The resulting supernatant was transferred into a new 1.5 ml eppendorf tube and an equal volume of isopropyl alcohol was added. The tube was thoroughly mixed by inversion and centrifuged at >13,000 x g for 2 minutes and the supernatant was discarded. The resultant DNA pellet was washed in 300 µl of 70% ethanol, after which it was spun at 10,000 rpm for 1 min and the supernatant was discarded. The DNA pellet was air dried and dissolved in 50 µl of 1xTris- EDTA. 1 µl of purified DNA was used in 24 µl of PCR mixture (Liu et al., 2000).

**PCR amplification**

Fungi were characterized by the amplification of their Internal Transcribed

Spacers (ITS). The forward (ITS-1F) and reverse (ITS-4R) primers (Table 1) used in the PCR reactions to amplify the ITS region of the rRNA operon have been described in Michaelsen et al. (2006).

Each PCR mixture contained 10 µl of Red taq ready mix, 0.5 µl of each primer pair, 8 µl of analytical grade sterile water (Sigma-Aldrich) and 5 µl of genomic DNA in a total volume of 24 µl. The thermocycling program used was an initial denaturation (94 °C for 5 minutes), 30 cycles of denaturation (94 °C for 1 min), annealing (60 °C for 1 minute) and elongation (72 °C for 1 minute), then a stabilization (72 °C for 5 minutes) (Michaelsen et al., 2006). The genetic materials were electrophoresed on 2% agarose gel in Tris acetate- EDTA buffer and the gel was stained with ethidium bromide before observation in UV detector (Okoro et al., 2009). Approximated molecular sizes of the amplicons were determined using molecular weight marker 1 kb Plus DNA ladder (Invitrogen Carlsbad, California, USA).

**DNA sequencing**

Five amplicons were sent to Inqaba Biotechnical Industries (Pty) Ltd, Pretoria – South Africa for sequencing. The sequences were then blasted against known sequences in the GenBank using BLAST (Basic alignment search tool) to find regions of local similarity between sequences in order to identify the species.

**Table 1:** The ITS primer pair used in this study.

Primer	Sequence 5' → 3'
ITS1 (forward)	TCCGTAGGTGAACCTGCGG
ITS4 (reverse)	TCCTCCGCTTATTGATATGC

## RESULTS

### Macroscopic fungus, corm rot and blight symptoms observed

The fungus was dark-brown in colour with hair-like structures (Figure 1). The leaves of the plantlets were yellowish with dark spots, all being symptoms of the blight (Figure 2a). When some of the plantlets were uprooted, we observed that the corms of some were rotten (Figure 2b).

A total of 5 fungal isolates were obtained in pure culture from the root, the corm, the pseudostem, the leaf and the fruiting body. It is worth noting that isolate F was obtained from all the plant parts as well as from the fruiting body (Table 2). Isolate R was present only in the roots; P was present only in the pseudostem and L only in the leaf. Isolate C was obtained from the corm, pseudostem and leaf (Table 2). The isolates and their codes are presented in Table 2.

### Morphological characterization

The cultures of all isolates were whitish in colour with septate hyphae (Table 3).

Isolate C grew best, with an average colony diameter of 53.7 mm in 3 days. The margin was filiform, elevation was flat and the form was filamentous. Aerial hyphae were present and mycelia were whitish both on the surface and the reverse view (Table 3, Figure 3a).

Isolate L had an average colony diameter of 53.2 mm. The margin was filiform, elevation was crateriform with a filamentous form. Aerial hyphae were present and the colour tint on the surface was white, while the reverse side showed a colour change from white to pale yellow (Table 3, Figure 4a).

Isolate R showed the least growth with a colony diameter of 18.4 mm. The margin of the isolate was entire, with a flat elevation and irregular form. Aerial hyphae were absent. The surface colour of mycelia was white just like the reverse colour (Table 3, Figure 5a).

Isolate F had an average colony diameter of 22.7 mm, margin was filiform,

and elevation was umbonate, with filamentous form. Aerial hyphae were present, with a surface colour tint which was white to grey while the reverse was brown to red (Table 3, Figure 6a).

Isolate P had an average colony diameter of 27.4 mm, margin was filiform, elevation was raised, form was filamentous and aerial hyphae were present. Mycelia were whitish but gradually turned to light brown (Table 3, Figure 7a).

### Identification of isolates

Based on micro-morphological characters (Figures 3b-7b), all isolates were believed to belong to Phylum Ascomycota, since they all had septate hyphae which lacked clamp connections.

Isolate C produced large numbers of tiny spores (Figure 3b) just like isolate L (Figure 4b). Isolate R produced ascospores (Figure 5b) while isolate F showed an ascoma releasing spores (Figure 6b). Isolate P also had prominent hyphae as well as spore mass (Figure 7b).

### Molecular characterization

#### DNA extraction

DNA was successfully extracted from all the five isolates as indicated by the electrophoregram of genomic DNA which showed distinct bands for each isolate (lanes R, L, F, C, and P) as shown in Figure 8.

#### Polymerase chain reaction (PCR)

From the PCR products, the approximated molecular sizes of the amplicons were between 500 bp and 800 bp (Figure 9)

#### Sequencing of PCR amplicons

Sequencing of the five amplicons was successfully performed by Inqaba Biotechnical Industries (Pty) Ltd, Pretoria – South Africa. Sequence analysis of the nuclear encoded rDNA showed significant alignments with 541/545 (99%) identity in isolate P for *Alternaria burnsii*, 437/456 (96%) identity in isolate C for *Fusarium oxysporum*, 359/361

(99%) identity in isolate F for *Chaetomium globosum*, 561/563 (99%) identity in isolate L for *Trichoderma harzianum*, and 573/576 (99%) identity in isolate R for *Hypocrea lixii*.

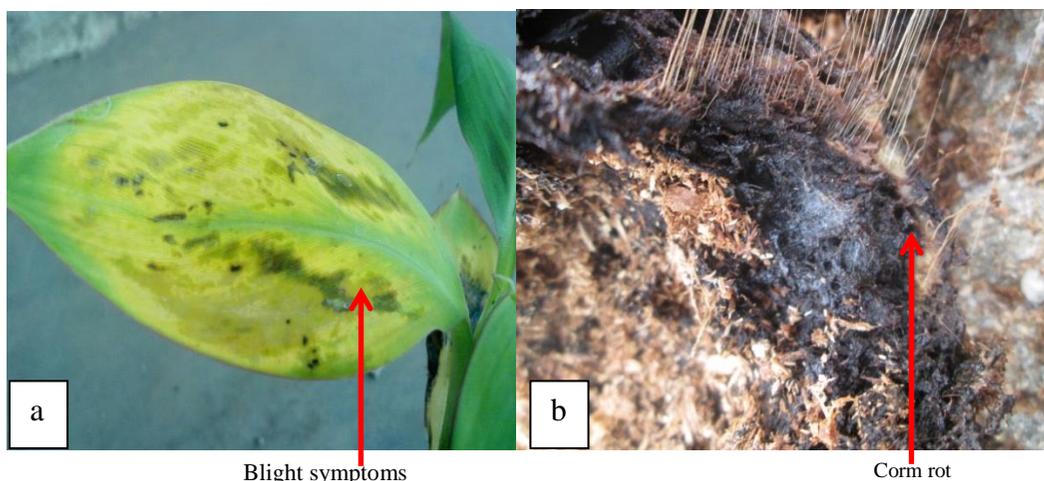
Based on sequence similarity to corresponding sequences in the GenBank, all the five isolates were identified to species as follows: *Alternaria burnsii* (KR604839.1) for isolate P, *Hypocrea lixii* (FJ461566.1) for isolate R, *Trichoderma harzianum*

(KJ028794.1) for isolate L, *Chaetomium globosum* (KT780353.1) for isolate F and *Fusarium oxysporum* (KT877646.1) for isolate C.

Overall, *T. harzianum*, *H. lixii*, and *A. burnsii* were each isolated from one sample only; *Fusarium oxysporum* was isolated from three samples while *Chaetomium globosum* was isolated from all the five samples (Table 4).



**Figure 1:** Macroscopic fungus observed on plantain corm.



**Figure 2:** a- Blight symptoms observed on diseased plantlets. b- Corm rot observed in propagators.

**Table 2:** Isolates obtained from the different plant parts and the fruiting body.

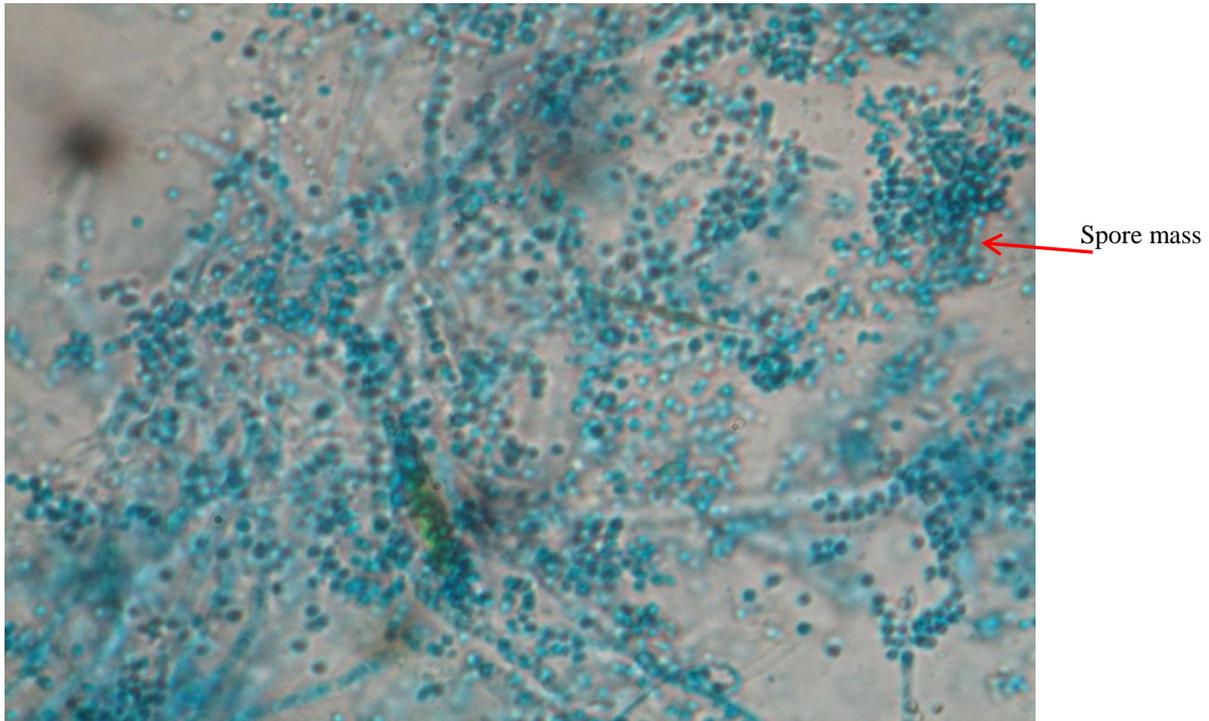
Serial number	Plant parts	Isolate code
1	Root	R, F
2	Corm	C, F
3	Pseudostem	P, F, C
4	Leaf	L, F, C
5	Fruiting body	F

**Table 3:** Cultural characteristics of fungi isolated from plantain parts and from fungi fruiting body.

Isolate code	Average colony diameter (3days)	Margin	Elevation	Form	Aerial hyphae	Colony tint	
						Surface	Reverse
C	53.7mm	Filiform	Flat	Filamentous	Present	Transparent to white	White
L	53.2mm	Filiform	Crateriform	Filamentous	Present	White	White to pale yellow
R	18.4mm	Entire	Flat	Irregular	Absent	White	White
F	22.7mm	Filiform	Umbonate	Filamentous	Present	White to grey	Brown to red
P	27.4mm	Filiform	Raised	Filamentous	Present	White to brown	White



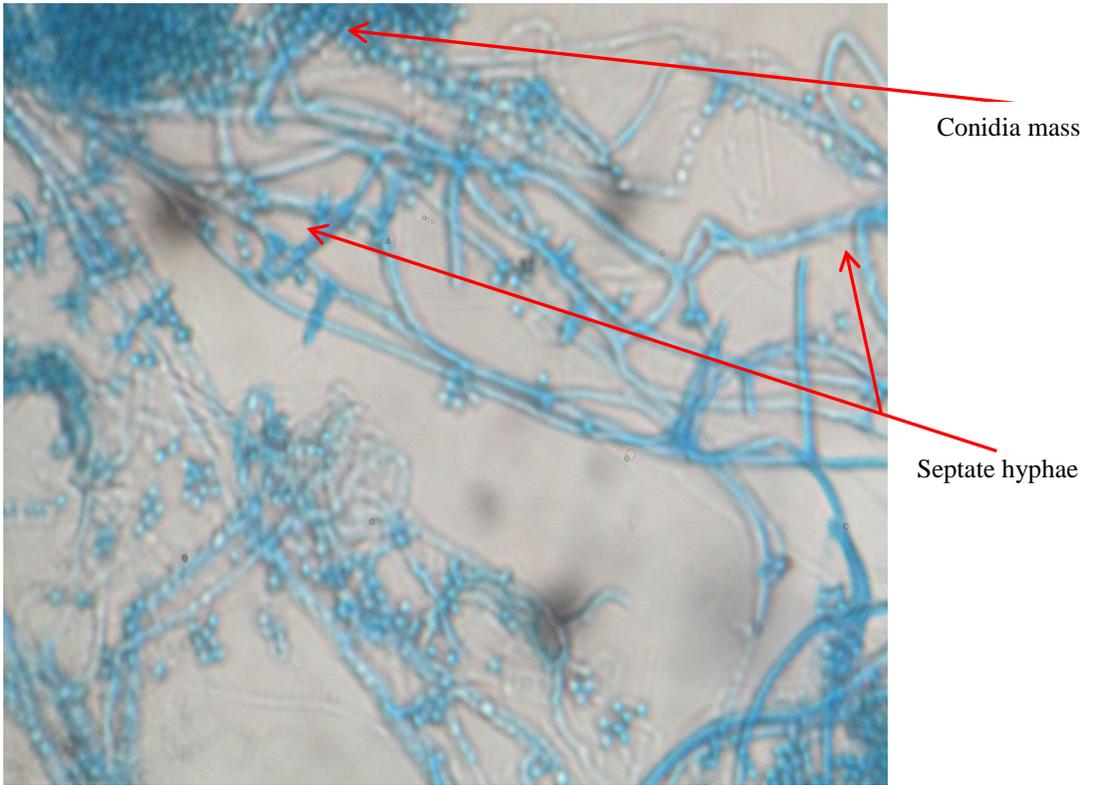
**Figure 3a:** Culture of isolate C on Potatoes Dextrose Agar (i) front view (ii) reverse view.



**Figure 3b:** Micro-morphological presentation of isolate C.



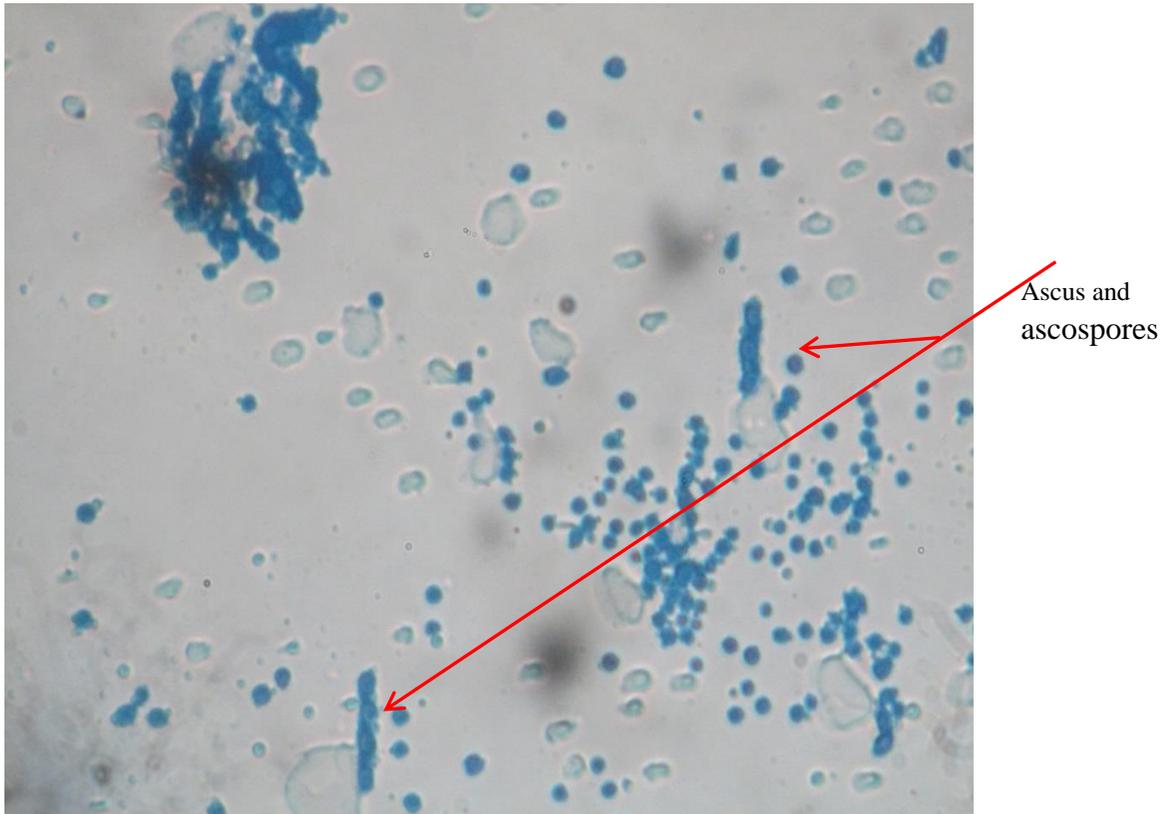
**Figure 4a:** Culture of isolate L on Potatoes Dextrose Agar (i) front view (ii) reverse view.



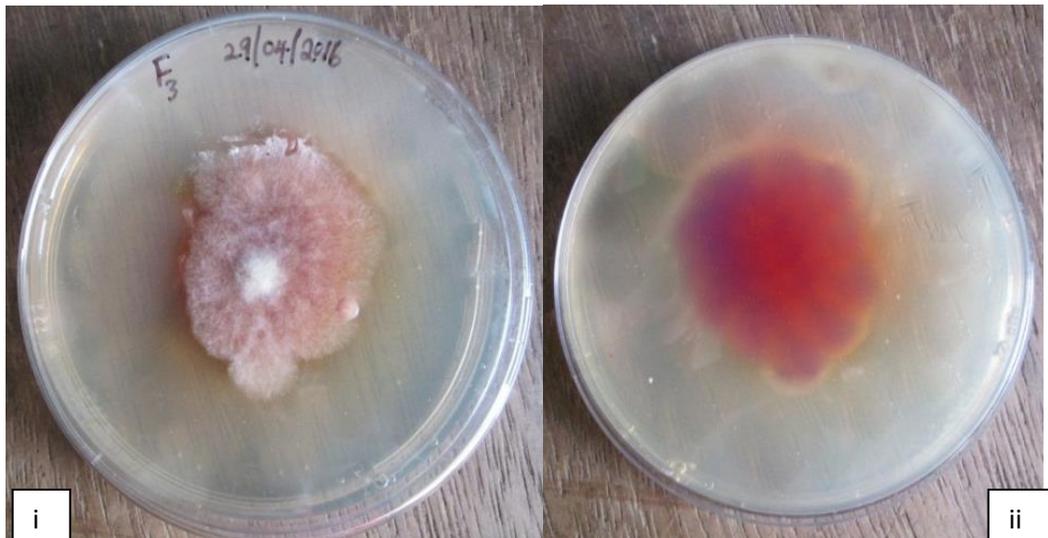
**Figure 4b:** Micro-morphological presentation of isolate L.



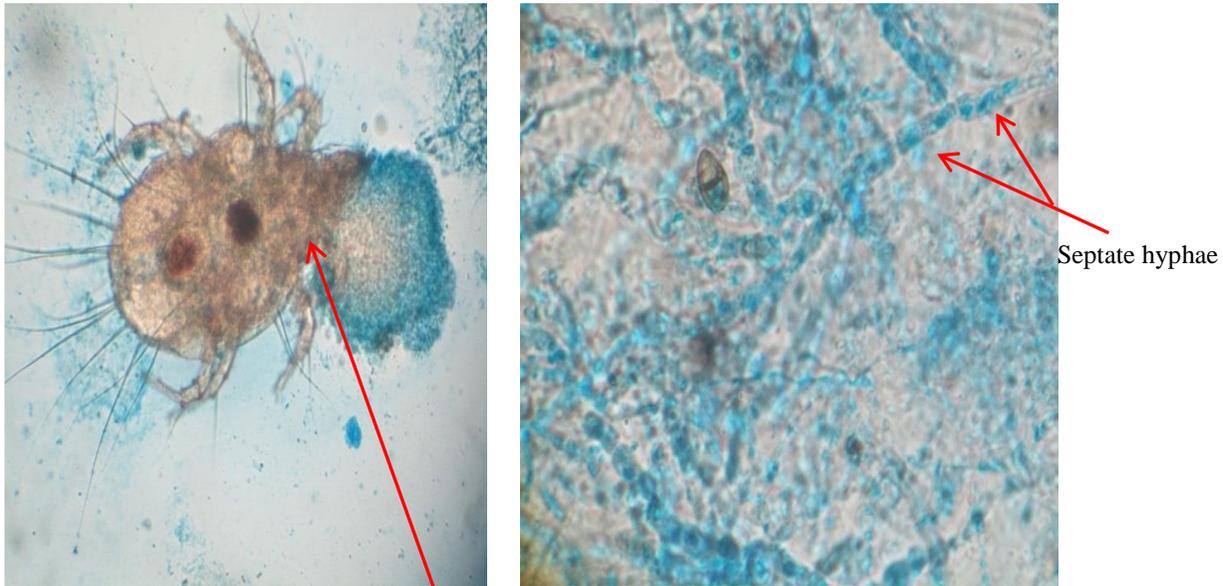
**Figure 5a:** Culture of isolate R on Potatoes Dextrose Agar (i) front view (ii) reverse view.



**Figure 5b:** Micro-morphological presentation of isolate R.



**Figure 6a:** Culture of isolate F on Potatoes Dextrose Agar (i) front view (ii) reverse view.

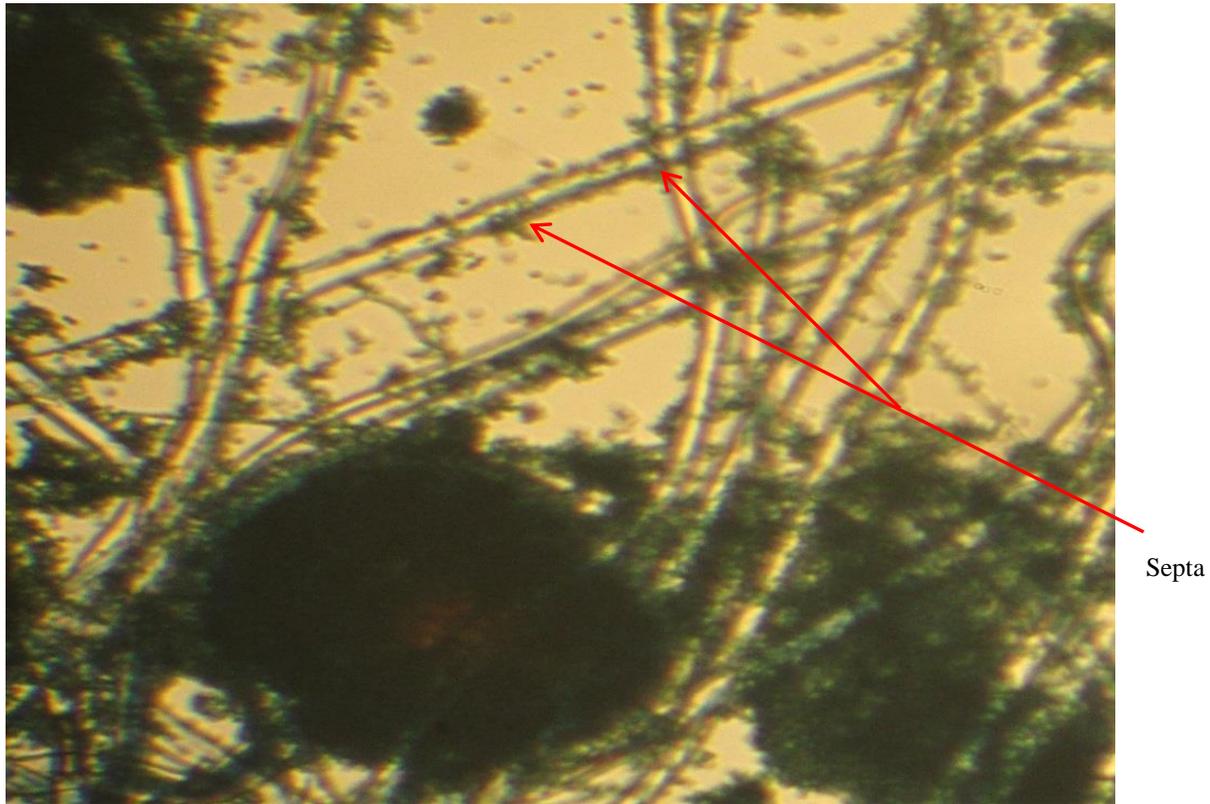


Ascoma releasing spores

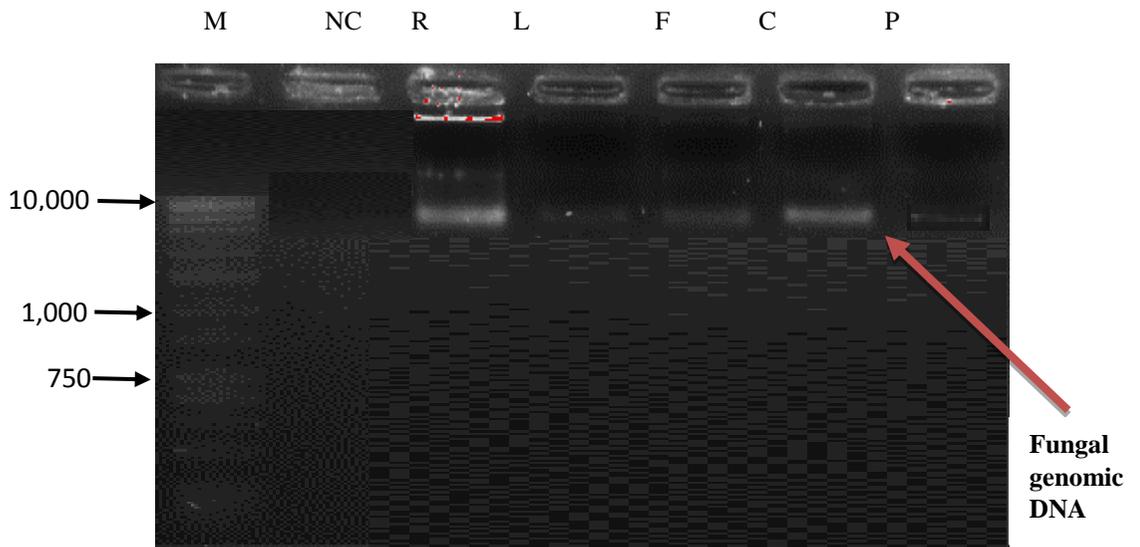
**Figure 6b:** Micro-morphological presentation of isolate F.



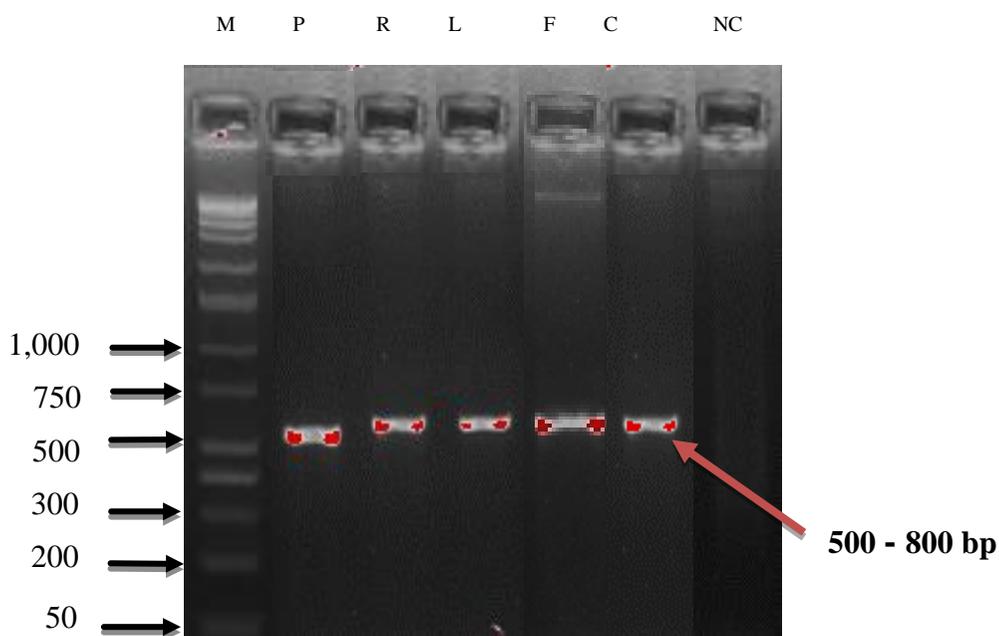
**Figure 7a:** Culture of isolate P on Potatoes Dextrose Agar (i) front view (ii) reverse view.



**Figure 7b:** Micro-morphological presentation of isolate P.



**Figure 8:** Electrophoregram of genomic DNA. Lane M: Molecular weight markers, NC: Negative control, P, R, L, F, and C are the PCR products of isolates P, R, L, F, and C. Samples were run on 2% agarose gel.



**Figure 9:** PCR products obtained from fungi isolates using ITS primer pair. Lane M: Molecular weight markers, NC: Negative control, P, R, L, F, and C are the PCR products of isolates P, R, L, F, and C. Samples were run on 2% agarose gel.

**Table 4:** Frequency of occurrence and percentage of the different fungi species, as isolated from the plant parts and fruiting body, on PDA containing streptomycin (100mg/L).

Species	Plant parts	Frequency	% Frequency
<i>Hypocrea lixii</i>	Root	1	20
<i>Chaetomium globosum</i>	Root	5	100
	Corm		
	Pseudostem		
	Leaf		
	Fruiting body		
<i>Fusarium oxysporum</i>	Corm	3	60
	Pseudostem		
	Leaf		
<i>Alternaria burnsii</i>	Pseudostem	1	20
<i>Trichoderma harzianum</i>	Leaf	1	20

## DISCUSSION

Of all isolates identified, *Fusarium oxysporum* is the only one that has been reported as the causal agent of *Fusarium* wilt (Panama) disease of Plantain. This was confirmed by Ghag et al. (2015) who reported that *Fusarium* wilt disease of plantain/banana is caused by *Fusarium oxysporum* which invades the roots and stems tissues, blocking the water conducting channels and causing leaves to turn yellow, dry and eventually collapse.

So far, no literature has been encountered associating *Chaetomium globosum* with plantain. However, Biles et al. (2012) reported that *Chaetomium globosum* decomposes plant cells using hyphal cellulase activity. More to that, they are known to cause soft rot rather than brown rot, *C. globosum* plant decomposition leaves behind lignin residues (Biles et al., 2012). Therefore, *C. globosum* could also be a contributing factor to the corm rot observed in the macro propagators. Moreover, Abou and Shebany (2012) reported that *C. globosum* are endophytic to many plants. In spite of their ability to cause plant decomposition, their asymptomatic colonization in some plants, helps the plants to tolerate metal toxicity. When maize plants were treated with *C. globosum*, they expressed less growth inhibition and increased biomass (Abou and Shebany, 2012). *C. globosum* is also known to reside in *Ginkgo biloba* plants (Zhang et al., 2013). Sudarma and Suprpta (2011) isolated *Chaetomium* sp. from soil collected in a banana habitat, where the plants showed symptoms of *Fusarium* wilt in Indonesia. Interestingly in the same study, *Chaetomium* was not present in soils from a banana habitat where the plants did not suffer from *Fusarium* wilt. It is therefore highly likely that *Chaetomium* may play an important role in the development of *Fusarium* wilt in plantains and banana. In the study reported here, it is possible that *C. globosum* had contributed towards the disease symptoms observed. This is more so because this fungus was isolated from all parts of the plant, as well as from the

fruiting body which was seen in all macro propagators during the survey.

*Alternaria* spp. have been isolated frequently during surveys of endophytes in the xylem and stem tissues of plants, in the leaves of important medicinal plants (Kurandawad and Lakshman, 2011), and in the shoots and leaves of *Vitis vinifera* (Mostert et al., 2000). Moreover, Thomma (2003) reported that most *Alternaria* species are saprophytes that are commonly found in soil or decaying plant tissues which could possibly imply an association between this species and the corm rot observed in the macro propagators in this study. *Alternaria alternata* has been associated with *Alternaria* leaf spot of ornamental dwarf banana in China (Fu et al. 2014). Although this fungus has not been associated with any disease on edible bananas in Cameroon and the rest of the world, it is likely that its activities may have deleterious effects on the growth and development of bananas and plantains.

Isolate L was identified as *Trichoderma harzianum* while isolate R was *Hypocrea lixii*. Chaverri and Samuels (2002) revealed that *Hypocrea lixii* is the teleomorph (sexual stage) of *Trichoderma harzianum* which is the anamorph (asexual stage) of the same species. Therefore, isolates L and R which were initially considered as different species based on micro-morphological characteristics were found to be different stages of the same species based on molecular techniques. Although no literature that associates *T. harzianum* and plantain has been encountered so far, it has been reported that *Trichoderma harzianum* is a common soil species and is used in biological control of a variety of plant-pathogenic fungi. It has been isolated from the rhizosphere of banana (Haryono, 2007). *T. harzianum* is effective against pathogenic fungi and diseases such as *Rhizoctonia solani* root rots, *Phytophthora megasperma* f. sp. glycine a root rot on soybean, *Gaeumannomyces graminis* take-all of wheat, *Sclerotinia sclerotiorum* rots, *Sclerotium rolfsii* Southern stem blight of tomato, and *Fusarium* wilt of tomatoes

(Gnancadja et al., 2015). In addition to the bio-control properties of *T. harzianum*, this species has potential for the enhancement of plant growth and resistance to plant pathogens (Ouedraogo and Hien, 2015). It is therefore unlikely that this species is implicated in the diseased symptoms observed.

### Conclusion

From our findings, it is very likely that *Fusarium* and *Chaetomium* were the causative organisms for the corm rot and blight symptoms observed in macro propagators in Buea. Proper management and control of these fungi in propagators will therefore permit the production of healthy seedlings, thus making them available to farmers. The availability of these seedlings will guarantee large scale production of plantains.

### COMPETING INTERESTS

The authors declare that they have no competing interests.

### AUTHORS' CONTRIBUTIONS

ETB conceived, designed and supervised the research, YAA collected data and samples from the field, analyzed the samples in lab-based experiments under the supervision of ETB, ETB and YAA contributed in drafting the manuscript.

### ACKNOWLEDGEMENTS

Authors would like to acknowledge Professor Fidelis Cho Ngwa for allowing us to use the Biotechnology Unit of the University of Buea for the Molecular aspects of this research. We are also thankful to Ms Joice Meshi for technical support. We appreciate the comments from the anonymous reviewers, which have helped us to improve the quality of the manuscript.

### REFERENCES

About A, Shebany Y. 2012. Endophytic *Chaetomium globosum* enhances maize seedling copper stress tolerance. *Plant Biology*, **14**(5): 859-

863. DOI:10.1111/j.1438-8677.2012.00608.x
- Baiyeri KP, Ajayi AR. 2000. Status and Constraints of *Musa* spp. Production in a Sub-humid Zone of Nigeria. *Acta Horticulturae*, **540**: 72- 80.
- Biles C, Wright D, Fuego M, Guinn A, Cluck T, Young J, Martin M, Biles J, Poudyal S. 2012. Differential Chlorate Inhibition of *Chaetomium globosum* Germination, Hyphal Growth, and Perithecia Synthesis. *Mycopathologia*, **174**: 475-487. DOI. <http://doi.org/10.1007/s11046-012-9572-5>
- Chaverri P, Samuels G, 2002. *Hypocrea lixii* Pat., the teleomorph of *Trichoderma harzianum* Rifai. *Mycol. Prog.*, **1**: 283–286. DOI. <http://doi.org/10.1007/s11557-006-0025-8>
- Fu BZ, Zhang ZH, Wang LH, Li G, Zhang JZ, Zhang Y. 2014. First report of leaf spot caused by *Alternaria alternata* on Chinese dwarf banana in China. *Plant Disease*, **98**(5): 691. DOI: 10.1094/PDIS-08-13-0831-PDN
- Gandonou CB, Ahanhanzo C, Agbangla C, Agbidinokoun A, Doussou A, Cacaï G, Dossoukperi R. 2012. Micropropagation in vitro de la variété locale «Aloga» du bananier plantain (*Musa x paradisiaca* L.) au Bénin. *Int. J. Biol. Chem. Sci.*, **6**(3): 1102-1111. DOI: <http://dx.doi.org/10.4314/ijbcs.v6i3.16>
- Ghag SB, Shekhawat UKS, Ganapathi TR. 2015. *Fusarium* wilt of banana: biology, epidemiology and management. *International Journal of Pest Management*, **61**(3): 250-263. DOI: <https://doi.org/10.1080/09670874.2015.1043972>
- Gnancadja LS, Tonon DHE, Faton EMO, Douro Kpindou KO, Dannon E, Akoegninou A. 2015. Efficacité de l'agent antagoniste *Trichoderma harzianum* sur *Fusarium oxysporum* f. sp. *lycopersici* agent pathogène de la tomate. *Int. J. Biol. Chem. Sci.*, **9**(2): 770-782. DOI: <http://dx.doi.org/10.4314/ijbcs.v9i2.17>
- Hallman J, Berg G, Schulz B. 2007. *Isolation Procedures for Endophytic Fungi Microorganisms*. Springer Brelin Heidelberg: New York.
- Haryono J. 2007. Biological suppression of

- seedling banana heart rot at TP Nusantara Tropical Fruit, Lampung. BSc. Thesis, Jenderal Soedirman University, Indonesia.
- Kahangi EM, Muthee AM, Chege BK. 2004. Constraints and sustainable solutions for adoption of TC banana technology and marketing. *Acta Hort. (International Society for Horticultural Science)*, **638**: 441-447. DOI: [http://www.actahort.org/books/638/638\\_5\\_6.htm](http://www.actahort.org/books/638/638_5_6.htm)
- Koné D. 2008. Contribution à l'étude des champignons épiphyllés des bananiers en Côte d'Ivoire: Caractérisation morphologique et pathologique de *Mycosphaerella fijiensis* var. *difformis* Morelet, *Cladosporium musae* Mason, *Deightonella torulosa* (Syd.) Ellis et *Cordana musae* Zim.; sensibilité à quelques fongicides. Thèse de Doctorat d'Etat. Université de Cocody, Côte d'Ivoire, p. 244.
- Kornerup A, Wanscher IH. 1978. *Methuen Handbook of Colour*. Eyre Methuen Ltd.
- Kurandawad JM, Lakshman HC. 2011. Studies on endophytic fungal diversity in some important medicinal plants of the botanical garden: a report. *J Theor Exp Biol.*, **8**: 45–51.
- Liu D, Coloe S, Baird R, Pedersen J. 2000. Rapid mini-preparation of fungal DNA for PCR. *Journal of Clinical Microbiology*, **38**(1): 471. DOI: <http://jcm.asm.org/content/38/1/471>.
- Michaelsen A, Pinzari F, Ripka K, Lubitz W, Pinar G. 2006. Application of molecular techniques for identification of fungal communities colonizing paper material. *International Biodeterioration and Biodegradation*, **58**: 33-141. DOI: <http://doi.org/10.1016/j.ibiod.2006.06.019>
- Mostert L, Crous PW, Petrini O. 2000. Endophytic fungi associated with shoots and leaves of *Vitis vinifera*, with specific reference to the *Phomopsis viticola* complex. *Sydowia*, **52**: 46–58. DOI: <http://hdl.handle.net/20.500.11755/034f1e0e-ff18-40cc-868d-19f8895654ce>
- Njau N, Mwangi M, Gathu R, Mbaka J, Muasya R. 2011. Banana weevil (*Cosmopolites sordidus*) reduces availability of corms for seedling production through macropropagation technology. *J. Anim. Plant Sc.*, **12**(1): 1537-1542.
- Ngoh NG, Tchango TJ, Fokou E, Etoa FX. 2005. Processing and food uses of bananas and plantains in Cameroon. *Fruits*, **60**(4): 245-253. DOI: <http://doi.org/10.1051/fruits:2005031>
- Okoro CC, Amund OO, Eliora R. 2009. Plasmids of phenanthrene and dibenzothiophene-degrading bacteria isolated from produced water samples in oil production. *Int. J. Biol. Chem. Sci.*, **3**(2): 186-191. DOI: <http://dx.doi.org/10.4314/ijbcs.v3i2.44501>
- Ouedraogo E, Hien E (2015). Effet d'un compost enrichi par des spores du clone *Trichoderma harzianum* (rifai) sur le rendement du niébé et du maïs sous abris au Burkina Faso. *Int. J. Biol. Chem. Sci.*, **9**(3): 1330-1340. DOI: <http://dx.doi.org/10.4314/ijbcs.v9i3.18>
- Singh HP, Uma S, Selvarajan R, Karihaloo JL. 2011. *Micropropagation for Production of Quality Banana Planting Material in Asia-Pacific*. Asia-Pacific Consortium on Agricultural Biotechnology (APCoAB): New Delhi, India; 92.
- Sudarma MI, Suprpta DN. 2011. Diversity of soil microorganisms in banana habitats with and without *Fusarium* wilt symptom. *J.ISSAAS* **17**(1): 147-159.
- Thomma BP. 2003. *Alternaria* spp.: from general saprophyte to specific parasite. *Mol. Plant Pathol.*, **4**(4): 225-236. DOI: 10.1046/j.1364-3703.2003.00173.x.
- Watanabe T. 2002. *Pictorial Atlas of Soil and Seed Fungi: Morphologies of Cultured Fungi and Key to Species* (2nd edn). CRC Press LLC.
- Zhang GZ, Zhang YH, Qin JC, Qu XY, Liu JL, Li X, Pan HY. 2013. Antifungal metabolites produced by *Chaetomium globosum* No.04, an endophytic fungus isolated from *Ginkgo biloba*. *Indian J. Microbiol.*, **53**: 175-180. DOI: <https://link.springer.com/article/10.1007/s12088-013-0362-7>