

Original Research Article

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Influence of Culture Media and Temperature on Growth and Sporulation of *Lasiodiplodia theobromae*, *Pestalotiopsis microspora* and *Fusarium oxysporum* Isolated from *Ricinodendron heudelotii* in Cameroon

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ABSTRACT

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The effects of mycological media and temperature on mycelial growth and spore production of three fungal pathogens recently reported on *Ricinodendron heudelotii* were investigated. The pathogens were identified on the basis of the ITS sequences of their ribosomal DNA as *Pestalotiopsis microspora* (isolate PMHP_109L), *Lasiodiplodia theobromae* (isolate LTHP_110L) and *Fusarium oxysporum* (isolates FOBR_164S, FOBR_049L and FOHP_121L). The radial growth (mm/day) of the fungi and conidia concentrations (number of conidia/ml of suspension) were assessed on three culture media: potato dextrose agar (PDA), malt extract agar (MEA) and V₈ juice agar (V₈). All media were suitable for the growth of *L. theobromae* while V₈ juice agar supported the fastest mycelial growth rate in *P. microspora* (13.4 mm/day). PDA and MEA media were appropriate for *F. oxysporum*. The growth rate and conidia concentration increased with temperature and attained their optimum at 23°C for *P. microspora* and *L. theobromae* and 28°C for *Fusarium oxysporum* strains. There was no growth of *P. microspora* and *L. theobromae* at 33°C while at this temperature, *Fusarium oxysporum* strains continued to grow and produce spores. The best temperature for spore production was 23°C for *P. microspora*, 28°C for *F. oxysporum* and 21, 23 and 28°C for *L. theobromae*. *F. oxysporum* isolates produced the highest concentration of conidia in all the culture media. These data contribute to the knowledge of the biology of these newly recognized parasitic fungi on *R. heudelotii* and show that species like *F. oxysporum* possesses a high level of phenotypic plasticity that allows it to survive and proliferate over a wide range of environmental conditions.

Introduction

Ricinodendron heudelotii Pierre ex Heckel belongs to family *Euphorbiaceae* and is endemic to Madagascar and the Congo Basin forests of Africa. Its roots, bark, leaves and

seeds are used by local people for medicine and food (Yeboah *et al.*, 2011). It was also established that the species has a high agroforestry potential (Djeugap *et al.*, 2013)

and yearly income from the sale of its edible grains in Cameroon is estimated to 1,556,280 US dollars (Perez and Ndoye, 1999). In Cameroon, many fungi cause various diseases on *Ricinodendron heudelotii* in forests, agroforestry systems and nurseries. Disease symptoms commonly encountered include leaf spots, leaf and seed rot and shoot blight (Djeugap, 2013; Djeugap *et al.*, 2016). Despite these food, medicinal and agroforestry properties, very little work has been devoted to the study of pathogens responsible to these diseases. It is known that fungi can grow and reproduce in or on diverse culture media requiring several specific nutrient elements. They are isolated on specific culture medium for cultivation, preservation, microscopic examination and biochemical and physiological characterization. Also, a wide range of media are used for isolation of different groups of fungi that influence the vegetative growth and colony morphology, pigmentation and sporulation depending upon the composition of specific culture medium, pH, temperature, light and water availability (Kuhn and Ghannoum, 2003; Kumara and Rawal, 2008). However, requirements for fungal growth are generally less restrictive than for sporulation. The aim of this work was to assess the growth and sporulation of three cosmopolitan and polyphagous fungi, namely *Pestalotiopsis microspora*, *Lasiodiplodia theobromae* and *Fusarium oxysporum*, recently isolated from *R. heudelotii* in Cameroon (Djeugap, 2013) on three culture media at five temperatures. Several studies have shown that *P. microspora* is responsible for leaf spots, dieback and fruit rots in various plant species around the world (Keith *et al.*, 2006; Djeugap *et al.*, 2009; Zhang *et al.*, 2010). The pathogen *L. theobromae* infects more than 500 plant species on which it causes root and fruit rots, shoot blight, dieback and canker (Mohali *et al.*, 2005; Gezahgne *et al.*, 2014; Djeugap *et al.*, 2016). It was established that

F. oxysporum is responsible for diseases such as seed rots and wilt in many crop and woody plant species (Tantawi and Fernandez, 1993; Hussain *et al.*, 2012). This study was conducted with a view to contribute to the knowledge of the biology of *P. microspora*, *L. theobromae* and *F. oxysporum*, three newly recorded fungi isolated from *R. heudelotii*.

Materials and Methods

Sample collection, isolation and identification of fungi

Infected leaves and stems of *R. heudelotii* seedlings were collected in natural forests and nurseries of the World Agroforestry Centre in Cameroon. Isolation of fungi was carried out on potato dextrose agar medium (PDA) supplemented with ampicillin at 250mg/ml at 22°C for 10 days (Djeugap, 2013). Then, the pure cultures were transferred on PDA plate with cellophane membrane for 10 days. The mycelia were harvested, frozen in liquid nitrogen and crushed to a fine powder. Genomic DNA was extracted using the DNA Mini Kit Plant protocol (Qiagen) (Griffin *et al.*, 2002; Levy and Mavrodieva, 2004), resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8) and the concentration of DNA was determined with a spectrophotometer. Purified diluted DNA was used as template for the amplification of the internal transcribed spacer (ITS) of nuclear ribosomal RNA gene (rDNA) repeats with the universal ITS4 (TCCTCCGCTTA TTGATATGC) and ITS5 (GGAAGTAAAA GTCGTAACAAGG) primers (White *et al.*, 1990). The total volume of the PCR reaction was 25µl made up of 1.2µl of each primer, 12.5µl of Premix ExTaq, 5µl of diluted DNA and 5.1µl of double sterile distilled water. Amplification was performed in a PTC-225 Thermal Cycler (MJ Research, MA, USA) as follows: DNA denaturation (94°C, 2min); annealing (70°C, 2min) and final extension

(72°C, 10min) with 35 cycles. Sequencing by the Sanger method was carried out in a Life Technology 3139 XL type sequencer with 16 capillaries. DNA sequences were compared to those of the Genbank collection by using the WU-BLAST (Washington University-Basic Local Alignment Search Tool) algorithm (Altschul *et al.*, 1997).

Morphological and cultural characteristics of mycelium

Morphological characteristics of the fungi were evaluated on PDA, malt extract agar (MEA) and V₈ juice agar (10%) medium. PDA and V₈ juice media were prepared based on Rodrigues *et al.*, (2010) protocol while Pradeep *et al.*, (2013) protocol was used for preparation of MEA medium. A volume of 20 ml sterilized medium was poured in each Petri plate and allowed to solidify. Then, 5mm-diameter plugs were taken with the help of a cork borer from the margin of 7-days old isolates grown on PDA, and placed at the centre of each set of Petri plates containing different media. Petri dishes were incubated at 10, 23, 28 and 33°C. The diameter (in mm) of each isolate was recorded in two directions at right angles to each other and then average colony diameter was calculated.

Growth was measured daily until the full expansion of the culture using the formula: $DG = [(MDd_1 - MDd_0) + (MDd_2 - MDd_1) + \dots + (MDd_n - MDd_{n-1})] / n$, where DG is the daily growth rate, MDd_n is the mean diameter growth of the nth measurement day, MDd_n = $(d_1 + d_2) / 2$ where d₁ is the first diameter measure on Petri dish and d₂, the second. MDd₀ = initial diameter of the mycelium disk which is 5 mm (Pandey *et al.*, 1985; Singh *et al.*, 1993). The appearance of colonies and pigmentation, the vegetative and reproductive structures were described after 10 days of incubation. The experiment was repeated three times for each culture medium and isolate.

Microscopic characteristics of mycelium and conidia

Microscopic observations of mycelium and conidia of each fungus on each culture medium and temperature was made using a Olympus BX-41 microscope (Carsen, Markham, ON, Canada) and photographs were taken with a digital camera (Media Cybernetics, Evolution model VF) connected to a computer. A 10 ml conidial suspension was prepared by pouring sterile distilled water on 10-days old culture in Petri dishes. The concentration of spores (number of conidia/ml of suspension) was assessed for each culture medium and temperature from a drop of conidia suspension deposited on the hemocytometer. This activity was repeated three times. The size (width and length) of spores was measured on 30 conidia of each species randomly selected using the graduated dial of the ocular of a light microscope and the number of cells per conidia was counted (Bakry *et al.*, 2010). Morphological characteristics of spores were compared with those described in the literature (Pavlic *et al.*, 2004; Hussain *et al.*, 2012; Rahman *et al.*, 2012).

Statistical analysis

Data obtained were subjected to one way analysis of variance (ANOVA) and means compared by Student Least Significant Difference (LSD) test at 5% using SAS software (version 9.2).

Results and Discussion

Sequencing and BLAST

Data obtained from sequencing are presented in table 1. Isolates used are from bimodal rain forest and high plateau agroecological zones collected either on infected leaves or stem of *R. heudelotii* in crop farm, ICRAF plantation,

cocoa farms and Applied Research Farm of the Faculty of Agronomy and Agricultural Science.

Effect of culture media and temperature on morphological characteristics of fungi

The culture medium influenced the radial growth of fungi. *F. oxysporum* isolates grew faster on MEA and PDA media compared to V₈juice agar except isolate FOBR_164S. No significant difference was observed in the growth of *L. theobromae* on the three culture media. The growth rate of *P. microspora* was high on V₈juice medium (Table 2). Among the isolates of *F. oxysporum*, the colour and the physical aspect of the mycelium varied from one medium to another; the mycelium was whitish and abundant on PDA and MEA compared to V₈juice agar (Fig. 1A, B and C). Mycelium of *P. microspora* and *L. theobromae* produced black pycnidia on PDA and MEA (Fig. 1D) and on PDA (Fig. 1E), respectively. It was observed that all the isolates of *F. oxysporum* growth at all the temperatures tested. At 10 and 33°C, the mycelial growth was localized around the inoculum plugs, showing a whitish colour and a rosette appearance while at 23 and 28°C, the mycelium is abundant. At 28°C, the colour of the mycelium of *F. oxysporum* varied from one isolate to another; it was whitish to purple in FOBR_049L, whitish in FOBR_164S and purple to violet (highly pigmented) in FOHP_121L (Fig. 2A, B and C). Fungal growth was lower at 10 and 33°C in all culture media for all the fungi. In this experiment, the optimal growth temperature for *F. oxysporum* isolates was 28°C in the three culture media (Figure 2). Fungal growth rate was lowest at 10 and 33°C in all the culture media and for all the isolates. In *L. theobromae*, the maximum growth rate was at 23°C in all the culture media while in *P. microspora* and *F. oxysporum* isolates (FOZB049F, FOZB164T and FOZH121F),

the optimum growth rate was at 28°C in the three culture media (Figure 3).

The size of conidia varied among isolates of *F. oxysporum*. The width and length of microconidia and macroconidia for FOBR_049L isolate on PDA varied from 2.2-3.9 µm x 3-17.4 µm and 3.7-4.2 µm x 26-28.5 µm, respectively. Isolate FOHP_121L produced longer macroconidia than other isolates (Table 3). Microconidia in *F. oxysporum* are mono or multicellular (2-3 cells) while macro conidia are multicellular (4-10 cells). There was abundant production of chlamydospores in FOBR_049L isolate in V₈ juice agar (Figure 3). The number of cells reached 10 macroconidia in FOHP_121L while the maximum was 7 in FOBR_049L and FOBR_164S (Figures 4, 5 and 6). Isolate FOHP_121L produced microconidia and macroconidia that were longer and wider than those of FOBR_049L and FOBR_164S. The width and length of microconidia of FOHP_121L ranged from 2.2-4.4 µm x 6-12.3 µm, 2.3-4.6 µm x 7-23.5 µm and 2-3.6 µm x 6.3-11 µm on PDA, MEA and V₈ juice agar media, respectively. The size of macroconidia ranged from 3.1-5.3 µm x 26-42.2 µm, 4.2-6.5 µm x 25.6-43 µm and 2.9-4.9 µm x 26.8-39 µm on PDA, MEA and V₈ juice agar media, respectively (Table 3). On PDA, conidia of *P. microspora* were hyaline, bi- to tetra cellular with a cylindrical carrot-like shape; the tapered lower end terminated in a filamentous appendage while the more rounded upper end was extended by 2, 3 or 4 filaments (Fig. 7A). Conidia of *L. theobromae* were larger and brown with a peanut pod-shaped (Fig. 7B). The width and length of the conidia of *L. theobromae* and *P. microspora* are relatively thicker and longer on MEA medium compared to other media. In fact, conidia size of *L. theobromae* varied from 10.4 to 15.7 µm x 25.3 to 30.2 µm, 11 to 17 µm x 22.1-35.3 µm and from 9.9-14.7 µm x 18-27.8 µm on PDA, MEA and V₈

respectively while in *P. microspora*, they varied from 6.6 -7.8 µm x 18.7 -29 µm, 8 - 12.9 µm x 22.1 - 38.6 µm and from 6.5 - 8.8µm x 20.5 - 28 µmon PDA, MEA and V₈juice agar media, respectively (Table 4).

Effect of temperature on the concentration of fungal spores

Temperature affected the concentration of fungal spores produced on all three culture media. In general, spore concentration increased with temperature until an optimum (28°C), and then decreased until no more spore production occurred at 33°C for *P.*

microspora and *L. theobromae*. However, *F. oxysporum* isolates continued to grow and produce spores at 33°C. Significant differences in spore concentration were observed between 28°C and the other temperatures (10, 23 and 33°C) for isolates FOBR_049L and FOHP_121L of *F. oxysporum*. In contrast, no significant difference was observed in spore's concentration of FOHP_164S at 23 and 28°C on the MEA and V₈ juice agar media. There was also no significant difference in spore concentration between 21, 23 and 28°C for *P. microspora* on PDA and for *L. theobromae* on PDA, MEA and V₈ juice agar media.

Table.1 Ecological and molecular characteristics of fungal isolates used in the study

Isolate code	Agro ecological zone	Locality	Ecological niche	Plant organ	ITS/BLAST ^a
LTHP_110L	High Plateau	Bantoum III	Crop farm	Leaves	FJ904838.1
PMHP_109L	High Plateau	Bantoum III	Crop farm	Leaves	AF377292.1
FOBR_049L	Bimodal Rainforest	Yaoundé 7e	ICRAF plantation	Leaves	HM179532.1
FOBR_164S	Bimodal Rainforest	Yokadouma	Cocoa farm	Stem	HQ658965.1
FOHP_121L	High Plateau	Dschang	ARF, FASA	Leaves	GU724513.1

^aSequences from public genbank (NCBI) with highest similarity with the sequence of fungal isolates from *R. heudelotii*. L=isolated from leaves and S= isolated from stem (seedlings). AFR, FASA= Applied Research Farm of the Faculty of Agronomy and Agricultural Science.

Table.2 Effect of culture media on the daily growth rate (mm/d) of fungal isolates incubated at 23°C

Isolate code and fungal species	Culture media		
	PDA	MEA	V ₈ (10%)
LTHP_110L (<i>Lasiodiplodia theobromae</i>)	21.6 ± 2.3 ^a	20.6 ± 1.2 ^a	19.8 ± 0.9 ^a
PMHP_109L (<i>Pestalotiopsis microspora</i>)	10.0 ± 1.1 ^b	9.7 ± 1.4 ^b	13.4 ± 1.7 ^a
FOBR_049L (<i>Fusarium oxysporum</i>)	8.4 ± 1.2 ^a	8.1 ± 0.8 ^a	6.3 ± 0.8 ^b
FOBR_164S (<i>Fusarium oxysporum</i>)	7.8 ± 0.9 ^a	8.6 ± 1.6 ^a	6.9 ± 0.6 ^a
FOHP_121L (<i>Fusarium oxysporum</i>)	9.8 ± 1.9 ^a	9.1 ± 0.7 ^a	7.0 ± 1.2 ^b

^{a,b}Means follow by the same letter in the line are not significantly different based on Student's LSD at 5%. LT: *L. theobromae*, PM: *P. microspora* and FO: *F. oxysporum*. HP: High Plateau, and BR: Bimodal Rainforest. L: isolate from leaf and S: isolate from stem.

Table.3 Micro and macro conidia sizes (μm) of isolates of *F. oxysporum* on PDA milieu incubated at $21\pm 1^\circ\text{C}$ from 10-days old culture

Isolates of <i>F. oxysporum</i>	PDA				MEA				V_8			
	Microconidia		Macroconidia		Microconidia		Macroconidia		Microconidia		Macroconidia	
	Width	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width	Length
FOBR-049L	2.2-3.9	3-17.4	3.7-4.2	26-28.5	1.2-3.1	4.4-20	4.3-4.6	26.7-29	2.1-3.8	4.8-21	3.3-4.4	25-26.7
FOBR-164S	1.7-3.1	4.6-19	3- 4.4	21-30.4	1.9-3.5	4.8-21	3.3-5.1	20.6-33	1.9-2.8	4.3-19	2.6-4.1	21-29.3
FOHP-121L	2.2-4.4	6-12.3	3.1-5.3	26-42.2	2.3-4.6	7-23.5	4.2-6.5	25.6-43	2-3.6	6.3-11	2.9-4.9	26.8-39

Table.4 Conidia sizes (μm) of *Lasiodiplodia theobromae* and *Pestalotiopsis microspora* on PDA, MEA and V_8 media incubated at $21\pm 1^\circ\text{C}$ from 10 days-old- culture

Isolates	PDA		MEA		V_8	
	Width	Length	Width	Length	Width	Length
LTHP-110 L (<i>Lasiodiplodia theobromae</i>)	10.4-15.7	25.3-30.2	11-17	22.1-35.3	9.9-14.7	18-27.8
PMHP-109L (<i>Pestalotiopsis microspora</i>)	6.6-7.8	18.7-29	8-12.9	22.1-38.6	6.5-8.8	20.5-28

Figure.1 Variation of morphological characteristics of fungi on different culture media. A: FOBR_049L isolate (*F. oxysporum*), B: FO_BR164S isolate (*F. oxysporum*), C: FOHP_121L isolate (*F. oxysporum*), D: PMHP_109L isolate (*P. microspora*) and E: LTHP_110L isolate (*L. theobroma*). Fungi were incubated at 23°C during 6 days; arrows indicate fructifications

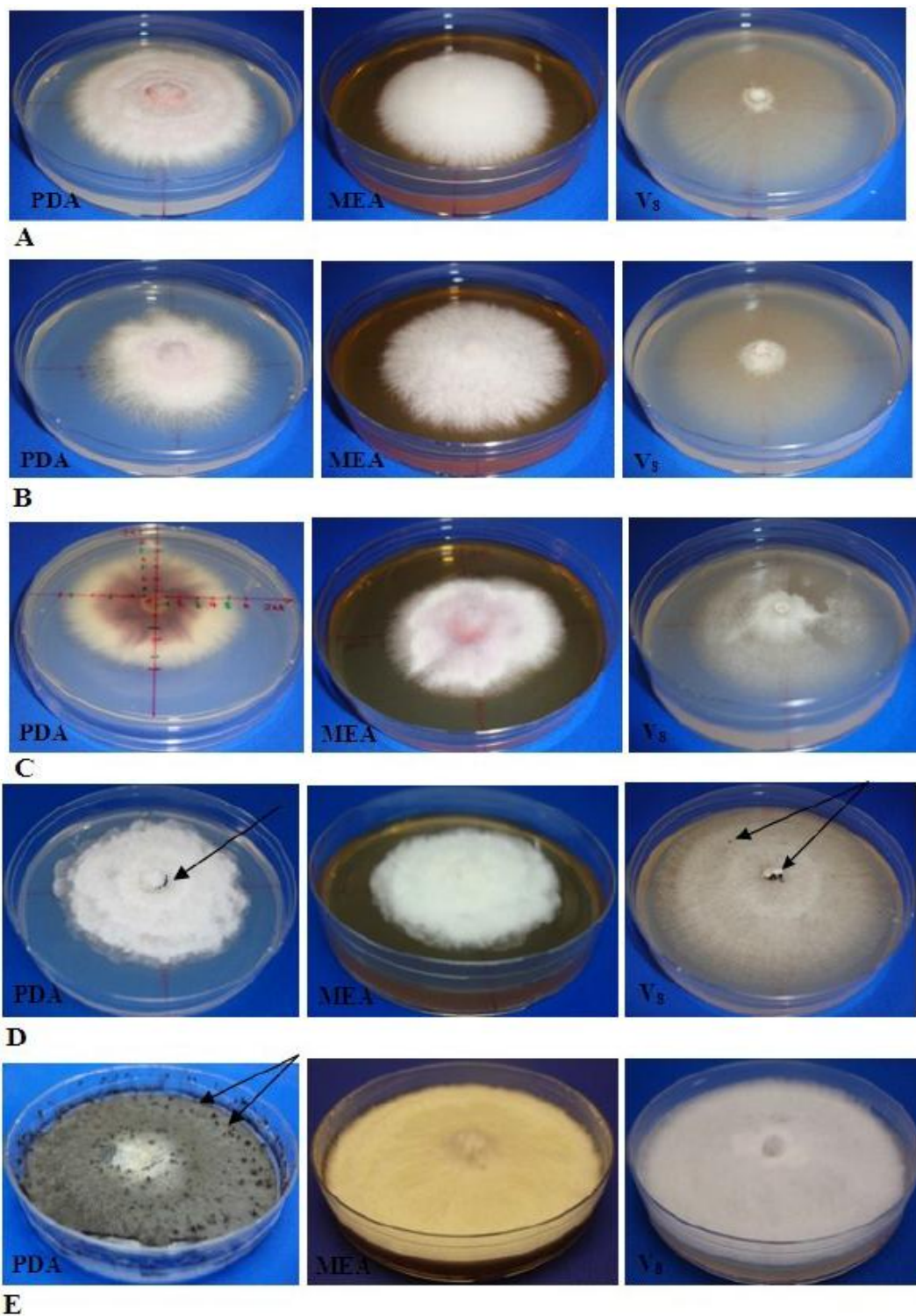


Figure.2 Variation of morphological characteristics among *Fusarium oxysporum* isolates based on temperature of incubation. A: FOBR_049L, B: FOBR_164SandC: FOBR_121L. The culture medium in the 1st and last column is PDA while the medium in the 2nd and 3rd column is MEA

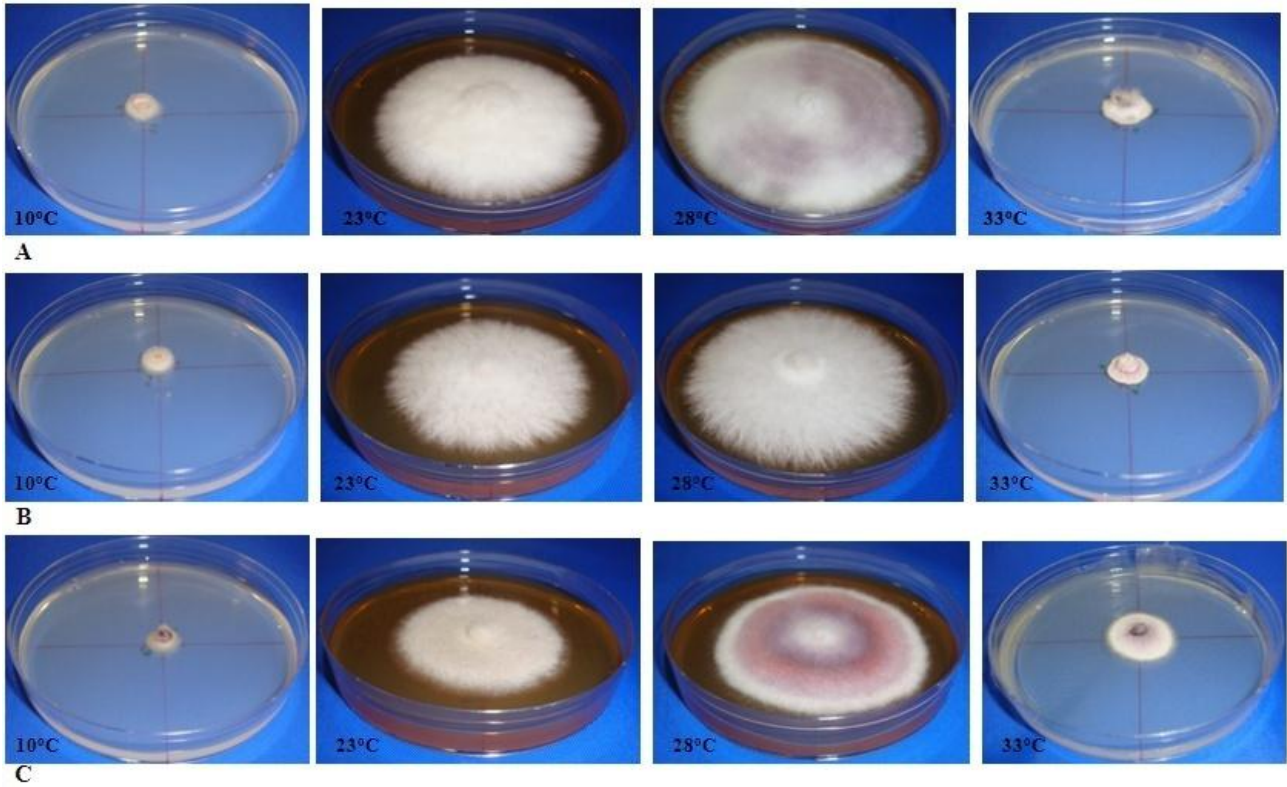


Figure.3 Evolution of growth rate (mm/day) of fungi newly recorded from *Ricinus dendron heudelotii* on PDA, MEA and V₈ media at different incubation temperatures

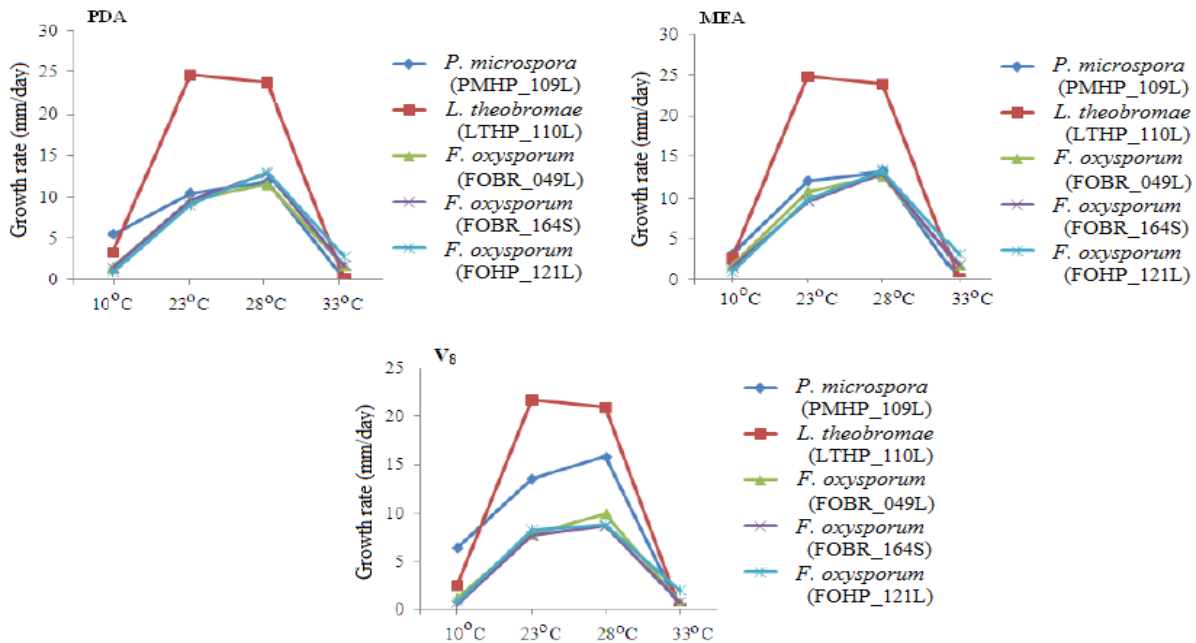


Figure.4 Micro and macro conidia of *Fusarium oxysporum* (isolate FOBR_049L) on PDA and MEA media. Chlamydospores on V₈ Agar medium from 14-day-old culture, 400X; gentian violet as mounting liquid

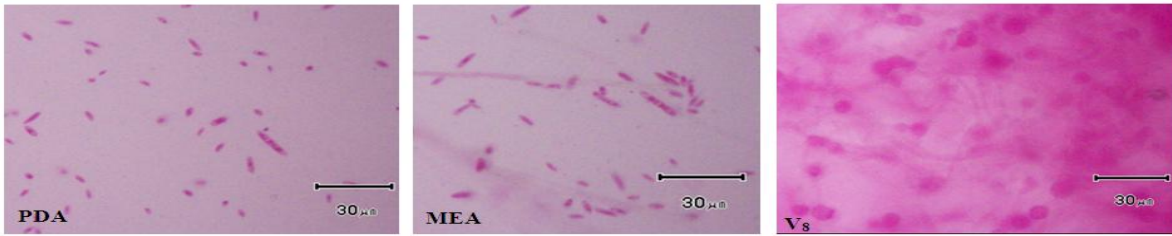


Figure.5 Micro and macro conidia of *Fusarium oxysporum* (isolate FOBR_164S), from infected stem of seedlings of *Ricinodendron heudelotii* incubated at 21oC on PDA, 400X; water as mounting liquid



Figure.6 Micro and macro conidia of *Fusarium oxysporum* FOHP_121L from infected leaves of seedlings of *Ricinodendron heudelotii* incubated at 10, 28 and 33oC on PDA, 400X; water as mounting liquid

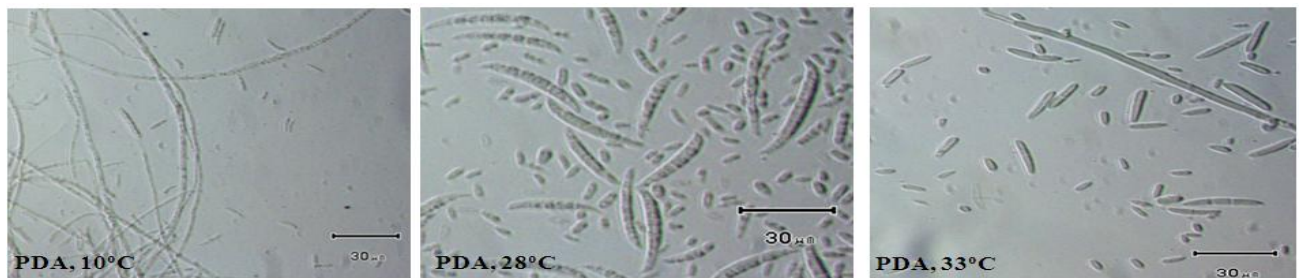


Figure.7 Conidia of *Pestalotiopsis microspora* (A) and *Lasiodiplodia theobromae* (B) isolated from *Ricinodendron heudelotii* incubated at 23°C on PDA medium, 400X; water as mounting liquid

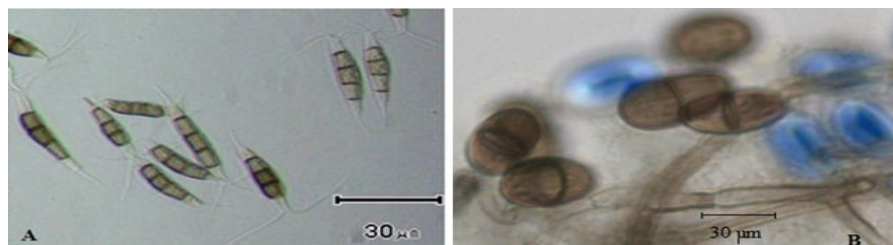
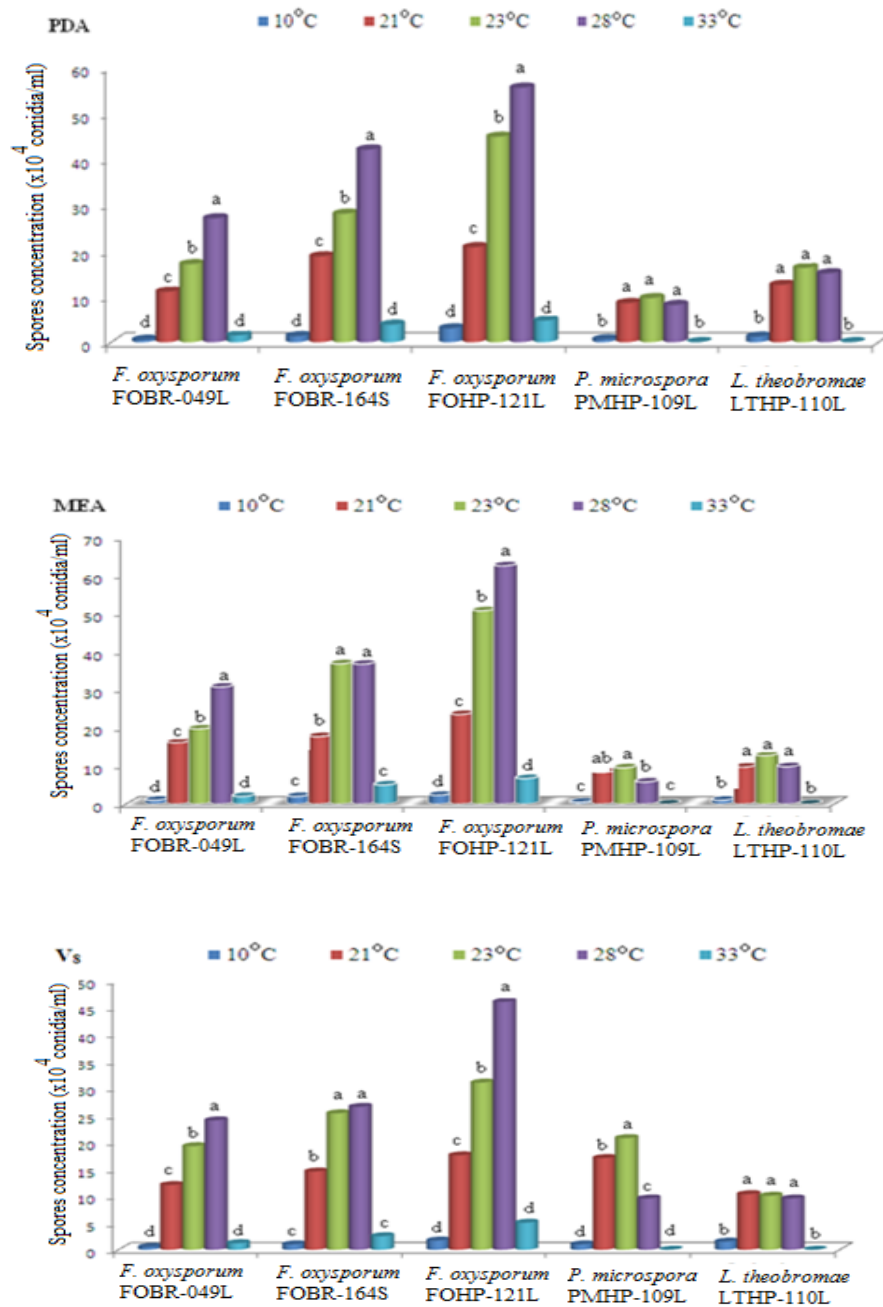


Figure.8 Concentration of spores of fungi isolated from *Ricinodendron heudelotii* and incubated during 14 days on PDA, MEA and V₈ media. Means follow by the same letter in one fungal species are not significantly different based on Student's test at 5%



However, spore concentration was significantly higher in these three temperatures and media compared to 10°C and 33°C for *L. theobromae* and *P. microspora* (Figure 8).

The study reveals marked differences in the mycelial growth and sporulation patterns for the three fungal species tested on three culture media that are routinely used in plant pathology laboratories. Culture medium and temperature affected the morphocultural

characteristics of the fungi tested. Indeed, preferences of fungi vis-a-vis the culture medium and temperature varied among species and, within *Fusarium oxysporum*, from one isolate to another. Some fungi grow and sporulate better on organic media incubated at low or high temperature, others on more selective media. It is known that temperature affects the number of spores produced on the culture medium (Agrios, 2005; Rahman *et al.*, 2012). Mycelial growth and sporulation were abundant on PDA, MEA and V₈juice agar media. These media are organic substrates rich in carbohydrates which are source of energy for cell metabolism, growth and sporulation of fungi. Several workers have recognized the importance of various organic media for mycelial growth and spore production in fungi (Kim *et al.*, 2005; Zhao *et al.*, 2010; Pradeep *et al.*, 2013). PDA is one of the most commonly used culture media because of its simple formulation and its ability to support mycelial growth of a wide range of fungi. Several researchers state that PDA is one of the best media for mycelial growth of many fungi followed by MEA (Xu *et al.*, 1984; Maheshwari *et al.*, 1999; Khadega and Al-Hussaini, 2015). V₈ juice agar was also reported as suitable for cultivation and sporulation of other fungal species like *Mycosphaerella fijiensis*, *M. musicola*, *Phomamacdon aldi* and *Phytophthora* spp (Medina and Platt, 1999; Drenth and Sendall, 2001; Abadie *et al.*, 2005; Al Fadil, 2006; Tsombeng *et al.*, 2012). Morphological traits (mycelia appearance, conidium size and pigmentation of mycelium) observed in isolates of *F. oxysporum* from *R. heudelotii* are comparable at some extent to those reported in other *F. oxysporum* (Sharma *et al.*, 2005; Hussain *et al.*, 2012). Isolates of *F. oxysporum* from wilted guava grow and sporulate abundantly with production of chlamydospores on PDA medium at 20 and 25°C (Hussain *et al.*, 2012) while corn meal

agar medium was reported to be suitable for the growth of *Fusarium oxysporum* f. sp. *cubense* on which length of microconidia and macroconidia reached 12.9 and 57.4 µm, respectively (Rahman *et al.*, 2012).

The range of temperatures (from 10 to 33°C) at which isolates of *F. oxysporum* from *R. heudelotii* could grow and sporulate indicates that this pathogen possesses a high level of phenotypic plasticity that allows it to survive and proliferate over a wide range of environmental conditions. The optimal temperature of mycelial growth for *F. oxysporum* isolates was 28°C. This was also reported in *F. moniliforme* KUMBF 1201 isolate cultivated on PDA and potato dextrose broth media and isolated from paddy field soil in India (Pradeep *et al.*, 2013) and in *Fusarium oxysporum* f. sp. *psidii* and *F. solani*, causal agents of wilt in guava cultivated on PDA (Gupta *et al.*, 2010). However, it was established that *Fusarium* species like *F. oxysporum* f. sp. *narcissi* can grow at 45°C in liquid medium (Linfield, 1986).

Conidia sizes in *P. microspora* isolated from *R. heudelotii* are relatively similar to values reported for 16 isolates of the same fungus isolated from *Taxodium distichum* and incubated on PDA at 23°C (Li *et al.*, 1996), and for isolates from *Psidium guajava* grown on PDA at 24°C (Keith *et al.*, 2006). However, Keith *et al.* (2006) reported that all isolates of *P. Microspora* from *P. guajava* grew at temperatures ranging from 10 to 35°C and that the optimum growth temperature differed among isolates and varied between 22 and 28°C. In our study, *L. theobromae* and *P. microspora* failed to grow at 33°C probably due to the inactivation of enzymes by temperature increase with a resulting effect on metabolism which affects growth (Mishra *et al.*, 2013; Pathak *et al.*, 2014). The optimum growth temperature for

P. microspora isolate was 23°C on PDA, MEA and V₈juice agar media. Morphocultural characters and sizes of conidia in *L. theobromae* are similar to those described by Pavlic *et al.*, (2004) and Shah *et al.*, (2010) for cultures isolated from infected cocoa (*Theobromae cacao* L.) and avocado (*Perseaa mericana* L.), respectively. Conidial sizes of *L. theobromae* ranged from 11-15 µm x20-35µmon MEA medium at 25°C (Pavlic *et al.*, 2004).

For all the fungi, it was observed that as temperature increased, mycelial growth and spore production also increased but at 28°C they started to decline for *F. oxysporum* and 23°C for *P. microspora* and *L. theobromae* on PDA, MEA and V₈juice agar media. This could be attributed to increase in enzymatic activity of the fungi.

In conclusion, this study revealed that culture media differentially influence the growth, colony characteristics (physical aspect and pigmentation of the mycelium) and sporulation of *P. microspora*, *L. theobromae* and *F. oxysporum* recovered from *R. heudelotii*. Among the test media used in the present study, PDA and MEA were found to be most suitable for mycelial growth and sporulation of *F. oxysporum* while V₈juice agar was suitable for *P. microspora* and the three media were appropriate for *L. theobromae*. The optimum temperature for high production of spores was 28°C for *F. oxysporum* isolates and 23°C for *P. microspora* and *L. theobromae*. These data will be important for future large-scale studies involving cultural and morphological characterization of more isolates of these newly recorded fungi from *R. heudelotii*.

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References

- Abadie, C., L. Pignolet, A. Elhadrami, R. Habas, M-F. Zapater and Carlier J. 2005. Inoculation avec *Mycosphaerella* sp., agent de cercosporioses de fragments de feuilles de bananiers maintenus en survie. *Cahier des Techniques* 2005: 131-134.
- Agrios, G.N. 2005. *Plant pathology*. 5th edn. Elsevier Academic Press, California, USA, 922 Pp.
- Al Fadil, T.A. 2006. Déterminisme de la tolérance du tournesol à *Phoma macdonaldii* au collet et sur racines: approches génétiques et histologiques. Thèse de Doctorat, Ecole Nationale Supérieure Agronomique de Toulouse, Toulouse, France.
- Altschul, S.F., T.L. Madden, A.A. Schäffer, J. Zhang, Z. Zhang, W. Miller and Lipman, D.J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein data base search programs. *Nucleic Acids Research* 25: 3389-3402.
- Bakry, M., G. Bussièrès, M.S. Lamhamedi, H.A. Margolis, D.C. Stowe, M. Abourouh, M. Blais and Bérubé J.A. 2010. A first record of *Pestalotiopsis clavispora* in Argan mass cutting propagation: Prevalence, prevention and consequences for plant production. *Phytoprotection*, 90: 117-120.
- Djeugap, J.F., J.R. Kuate and Fontem D.A. 2009. Etat sanitaire post-récolte de la mangue commercialisée à Dschang et efficacité *in vitro* de quelques huiles essentielles contre *Colletotrichum gloeosporioides*, agent de l'antracnose. *Annales de la 9^{ème}*

- Conférence Internationale des maladies des Plantes*, Tours, France, Pp. 77-80.
- Djeugap, F.J. 2013. Contraintes de germination et diagnostic moléculaire des champignons associés aux maladies chez *Ricinodendron heudelotii* au Cameroun. Thèse de Doctorat/PhD, Université Laval, Québec, Canada, 183 Pp.
- Djeugap, F.J., L. Bernier, D. Dostaler, D. Khasa, D.A. Fontem and Nwaga, D. 2013. Opportunités et contraintes agroforestières de *Ricinodendron heudelotii* au Cameroun. *International Journal of Biological and Chemical Science* 7(1): 344-355.
- Djeugap, F.J., L. Bernier, D. Dostaler and Zena D.G.R. 2016. First report of *Lasiodiplodia theobromae* causing shoot blight of *Ricinodendron heudelotii* seedlings in Cameroon. *International J. Agronomy Agricult. Res.*, 8(1): 59-63.
- Drenth, A. and Sendall B. 2001. Practical guide to detection and identification of *Phytophthora*. Version 1.0 CRC for Tropical Plant Protection, Brisbane, Australia, 42p.
- Gezahgne, A., A. YirguandKassaH. 2014. First report of *Lasiodiplodia theobromae* causing canker on tapped *Boswellia papyrifera* trees in Ethiopia. *New Disease Reports* 29:11.
- Griffin, D.W., C.A. Kellogg, K.K. Peak and Shinn E.A. 2002. A rapid and efficient assay for extracting DNA from fungi. *Letters in Applied Microbiology* 34: 210–214.
- Gupta, V.K., A.K. Misra and Gaur R.K. 2010. Growth characteristics of *Fusarium* spp. causing wilt disease in *Psidium guajava* L. in India. *Journal of Plant Protection Research* 50(4): 452-462.
- Hussain, M.Z., M.A. Rahman, M.N. Islam, M.A. Latif and Bashar M.A. 2012. Morphological and molecular identification of *Fusarium oxysporum* Sch. isolated from guava wilt in Bangladesh. *Bangladesh Journal of Botany* 41: 49-54.
- Keith, L.M., M.E. Velasquez and Zee, F.T. 2006. Identification and characterization of *Pestalotiopsis* spp. causing scab disease of guava, *Psidium guajava*, in Hawaii. *Plant Disease* 90: 16-23.
- Kim, Y.K., C.L. Xiao and Rogers J.D. 2005. Influence of culture media and environmental factors on mycelial growth and pycnidial production of *Sphaeropsis pyriputrescens*. *Mycologia*, 97: 25-32.
- Khadega, H.K. and Al-Hussaini, I.M. 2015. Diagnostic and environmental study of *Aspergillus terreus* isolated from various varieties of apples fruits. *Journal of Contemporary Medical Sciences* 1(4): 31–35.
- Kuhn, D.M. and Ghonnoum, M.A. 2003. Indoor mold, toxigenic fungi, and *Stachybotrys chartarum*: Infectious disease perspective. *Clinical Microbiology Reviews* 16(1): 144-172.
- Kumara, K.L.W. and Rawal, R.D. 2008. Influence of carbon, nitrogen, temperature and pH on the growth and sporulation of some Indian isolates of *Colletotrichum gloeosporioides* causing anthracnose disease of papaya (*Carrica papaya* L.). *Tropical Agricultural Research and Extension* 11: 7-12.
- Levy, L. and Mavrodieva, V. 2004. PCR detection and DNA isolation methods for use in the *Phytophthora ramorum* national program. DNA and PCR Protocol elaborated at Oregon State University and the University of California at Berkeley for *P. ramorum*, 11 p.
- Li, J.Y., G. Strobel, R. Sidhu, W.M. Hess and

- Ford, E.J. 1996. Endophytic taxol-producing fungi from bald cypress, *Taxodium distichum* L. *Microbiology*, 142: 2223-2226.
- Linfield, 1986. A comparison of the effects of temperature on the growth of *Fusarium oxysporum* f. sp. *narcissi* in solid and liquid media. *Journal of Phytopathology*, 116(3): 278–281.
- Maheswari, S.K., D.V. Singh and Sahu A.K. 1999. Effect of several nutrient media, pH and carbon sources on growth and sporulation of *Alternaria alternata*. *Journal of Mycopathological Research*, 37: 21-23.
- Medina, M.V. and Platt H.W. 1999. Comparison of different culture media on the mycelial growth, sporangia and oospore production of *Phytophthora infestans*. *American Journal of Potato Research* 76: 121–125.
- Mishra, S., P. Kumar and Malik, A. 2013. Effect of process parameters on the enzyme activity of a novel *Beauveria bassiana* isolate. *International Journal of Current Microbiology Applied Science* 2(9): 49-56.
- Mohali, S., T. Burgess and Wingfield M.J. 2005. Diversity and host association of the tropical tree endophyte *Lasiodiplodia theobromae* revealed using simple sequence repeat markers. *Forest Pathology*, 35: 385-396.
- Pandey, R.R. and Dwivedi, R.S. 1985. *Fusarium oxysporum* f. sp. *psidii* a pathogen causing wilt of guava (*Psidium guajava* L.) in Varanasi district, India. *Phytopathologische Zeitschrift*, 114: 243-248.
- Pathak, S.S., S. Kumar, R.C. Rajak and Sandhu, S.S. 2014. Study of effect of temperature on amylase production by soil mycotic flora of Jabalpur region. *World Journal of Pharmacy and Pharmaceutical Sciences* 3 (9): 1448-1458.
- Pavlic, D., B. Slippers, T.A. Coutinho, M. Gryzenhout and Wingfield, M.J. 2004. *Lasiodiplodia gonubiensis* sp. nov., a new *Botryosphaeria* anamorph from native *Syzygium cordatum* in South Africa. *Studies in Mycology*, 50: 313-322.
- Pradeep, F.S., M.S. Begam, M. Palaniswamy and Pradeep, B.V. 2013. Influence of culture media on growth and pigment production by *Fusarium moniliforme* KUMBF 1201 isolated from paddy field soil. *World Applied Sciences Journal*, 22(1): 70-77.
- Rabbani, N., R. Bajwa and Javaid, A. 2011. Influence of culturing conditions on growth and sporulation of *Drechslera hawaiiensis*, the foliar blight pathogen of *Marsilea minuta*. *African Journal of Biotechnology*, 10(10): 1863-1872.
- Rahman, N.R.B., K. Ahmadi, P. Lepun, Z. Rosli, A. Abdu and King, W.S. 2012. Effects of culture media on growth and spore occurrence of *Fusarium oxysporum* f. sp. *Cubense* (E.F. Smith) W.C. Syd. and H.N. Hans. *Asia Life Sciences* 21: 133-148.
- Rodrigues, T.T.M.S., L.A. Maffia, O.D. Dhingra and Mizubuti, E.S.G. 2010. *In vitro* production of conidia of *Alternaria solani*. *Tropical Plant Pathology* 35(4): 203-212.
- Singh, G. R.K. Padhvay, G.S. Narayaman, K.P. Padmkumari and G.P. Rao (1993). Chemical and fungitoxic investigation on the essential oil of Citrus Pers. *Z. Deutsche Zeits half fur Pflangenfrankenenen und Flanzenschultz*, 100: 69-74.
- Shah, M.D., K.S. Verma, K. Singh and Kaur, R. 2010. Morphological, pathological and molecular variability in *Botryodiplodia theobromae* (Botryosphaeriaceae) isolates associated with die-back and bark canker of pear trees in Punjab, India.

- Genetic Molecular Resources* 9: 1217–1228.
- Sharma, R.L., B.P. Singh, M.P. Thakur and Thapak S.K. 2005. Effect of media, temperature, pH and light on the growth and sporulation of *Fusarium oxysporum* f.sp. *lini* (balle) Snyder and Hansen. *Annual Plant Protection Science* 13: 172-174.
- Tantaoui, A. and Fernandez, D. 1993. Comparaison entre *Fusarium oxysporum* f.sp. *albedinis* et *Fusarium oxysporum* des sols des palmeraies par l'étude du polymorphisme de longueur des fragments de restriction (RFLP). *Phytopathologie méditerranéenne*, 32: 235-244.
- Tsopmbeng, G.R., D.A. Fontem and Yamde K.F. 2012. Evaluation of culture media for growth and sporulation of *Phytophthora colocasiae* Racib., causal agent of taro leaf blight. *International Journal of Biological and Chemical Science* 6(4): 1566-1573.
- White, T.J., T.D. Bruns, S. Lee and Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp 315-322 In: *PCR Protocols: A Guide to Methods and Applications*. M.A. Innis, D.H. Gelfand, J.J. Sninsky, and T.J. White Eds. Academic Press, San Diego, Canada.
- Xu, S.O., S.Z. Yuan and Chen X.C. 1984. Studies on pathogenic fungus (*Alternaria tenuis* Nees) of popular leaf blight. *Journal of North East Forestry Institute* 12: 56-64.
- Yeboah, S.O., Y.C. Mitei, J.C. Ngila, L. Wessjohann and Schmidt J. 2011. Compositional and Structural Studies of the Major and Minor Components in three Cameroonian seed oils by GC-MS, ESI-FTICR-MS and HPLC. *Journal of American Oil Chemistry Society* 88: 1539-1549.
- Zhang, M., H. Wu, T. Tsukiboshi and Okabe, I. 2010. First report of *Pestalotiopsis microspora* causing leaf spot of hidcote (*Hypericum patulum*) in Japan. *Plant Disease* 94(8): 1064.
- Zhao, H., L. Huang, C.L. Xiao, J. Liu, J. Wei and Gao, X. 2010. Influence of culture media and environmental factors on mycelial growth and conidial production of *Diplocarpon mali*. *Letters in Applied Microbiology* 50: 639–644.

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