# An Improved Slide Culture Technique for the Microscopic Identification of Fungal Species

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#### ABSTRACT

Several phyto-pathogenic fungi have been discovered by numerous researchers who continue to be saddled with the problem of proper identification of these fungal agents. The conventional method requires plating out the diseased tissues of such plant materials onto culture media and observing their gross morphological features on agar plates. As soon as their colonial characteristics have been studied, the microscopic examination of fungal reproductive structures (spores) and mycelia must be done as a confirmative method of identification which must be followed by Molecular identification in order to ensure complete identification. This step often disturbs the fragile spore-hypha arrangements, thus, leading difficulties in interpretation of morphological results owing to the teasing effect in the preparation of wet mounts. The slide culture method of identification developed by Riddel in 1950 which uses an agar block of medium transferred to a glass slide and put in a moist petri dish have since been used with various modifications that have not really taken care of the slight disturbance of the mycelial arrangement of the study fungi that occurs during removal and replacement of coverslips during lactophenol cotton blue stain. We have developed a rapid slide-culture method that can diminsh this problem to the barest minimum.

KEYWORDS: Slide Culture, Microscopy, Pathogen, Fungal Species

# INTRODUCTION

Colonial and microscopic identification of fungi remains the most employed method for the identification of fungi even with the discovery of molecular procedures, due to its high cost (Wijedasa and Livanapathirana, 2012). Slide culture remains the backbone of morphological identification of fungi in any mycological laboratory. This technique sustains the basic morphological characteristics relatively intact compared with tease mounts and cello-tape mounts. However, slide cultures are also associated with problems. Taking away of the cover slip and agar block results in changes in the morphological features, such as the angle of branching and attachment of macro and micro spores to sporangiophores, which leads problem to characterizing organisms down to species level.

Also repeated viewing of same slide over a period of time to study different growth stages of the fungi and examination of structures implanted in agar is not *How to cite this paper:* Agu, Kingsley Chukwuebuka | Chidozie, Chiamaka Perpetua "An Improved Slide Culture Technique for the Microscopic

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feasible with traditional Slide culture technique (Smith, 1960). The nonexistence of a customary technique for performing submerged sporulation has precluded the development of a suitable general slide culture method based on sporulation of moulds in 1969). liquids (Madelin, The microscopic identification of many pathogenic fungi still relies heavily on morphology, location, and arrangement of individual spores or groups of spores as well as the shape of the columella for those with sporangiospores (Johnson, 1946). The teased wet-mount preparation is becoming outdated since it disrupts the hyphal and mycelia arrangements as is the case with the fungi Blastomyces dermatitidis or Sporotrichum schenkii. Slide cultures, in contrast, when suitably performed, fosters the absolute recognition of these genera, and also aid substantially with the categorization of other varieties of fungi. The available slide culture methods, however, is plagued with several intricacies

(Henrici, 1930; Brown, 1942; Lewis and Hopper, 1943).

Numerous traditional slide-culture methods exist that have been developed by various researchers. Nonetheless, these methods regularly disrupt the feeble cellular architecture thus, resulting in difficulties in elucidation of the key morphological features under the microscope (Larsen and Covey, 1979). Several fungi form very feeble propagative arrangement (spores) which are at easily disturbed by even the most meticulous operation. This famous feature has led to the formulation of assorted slide culture methods (Anthony and Walkes, 1962; Cole and Kendrick, 1968; Ellis and Ajello, 1982). Each of these was developed to solve the problems of the fragility of the fungi been evaluated and to meet the investigator's options.

This paper is aimed at explicating an improved slide culture technique which is easy to perform, inexpensive, time-saving and labour-saving in which sporulating cultures could be grown for slide mounts. It also provides important guide for microscopic recognition of fungi, as well as for pedagogic activities.

#### Materials and Methods Test Fungi

The test fungi viz. Aspergillus tamarii, Aspergillus niger, Aspergillus flavus, Mucor circinelloides, Lasiodiplodia theobromae and Fusarium solani were obtained from a previous study (Ogbo and Agu, 2015) and used for this study.

# **Traditional Slide Culture Technique**

A sterile Whatmann No. 1 filter paper was placed in a sterile petri dish. A bent sterile glass rod was placed at the bottom of the petri dish, then, 2ml of sterile distilled water was dispensed into the the petri dish. A sterile glass slide was placed on top of the bent glass rod. Using a sterile scalpel, a small square (about 1 x 1 cm) of agar block from Saboraud Dextrose Agar (SDA) was cut and placed on the centre of the slide. With the aid of a sterile inoculation needle, the agar the agar block was inoculated with a small amount of fungus under test on each of the four sides of the block. Therefter, a heat-sterilized coverslip was laid over the block and pressed down gently. The procedure was repeated for the entire seven fungi used in this study. Thereafter, the plates were incubated at room temperature for 3 to 5 days

depending on the growth rate of the fungus. When desired growth was observed the coverslips were removed and few drops of Lactophenol cotton blue stain dropped on the developing cultures on the slide before the coverslips were replaced and viewed under the microscope. Referencing was done using Fungal Atlases (Barnett and Hunter, 2000; Watanabe, 2002; Ellis *et al.*, 2007).

## Improved Slide Culture Technique

A sterile glass slide was placed on the bottom of a sterile petri dish. With the aid of a sterile syringe 0.5 ml of molten Saboraud Dextrose Agar (SDA) maintained at 45 °C in a water bath was dispensed on the sterile glass slide. The cover of the petri dish was replaced and the molten agar allowed to gel. Upon gelling, a sterile inoculation needle was used to inoculate the agar bump with a small amount of fungus under test at the centre of the bump. Therefter, a heat-sterilized coverslip was laid just over the agar bump without pressure. The procedure was repeated for the seven fungi used in this study. The fungi used in this study were obtained from previous studies (Frank and Kingsley, 2013; Frank and Kingsley, 2013; Agu et al., 2014 2015; Okigbo et al., 2015; Ogbo and Agu, 2015). Thereafter, the plates were incubated at room temperature for 3 to 5 days depending on the growth rate of the fungus. When desired growth was observed, few drops of Lactophenol cotton blue stain was dropped at the interface of the developing cultures on the slide and the coverslip so as to preserve the integrity of the culture and allowed to permeate the entire culture before viewing under the microscope. Referencing was done using Fungal Atlases (Barnett and Hunter, 2000; Watanabe, 2002; Ellis et al., 2007).

#### Molecular Confirmation of Fungal Identities

The identities of the seven isolates were confirmed by the Microbial Identification Service, CABI E-UK, Bakeham Lane, Egham, Surrey, TW20 9TY, England, UK. using the partial ITS rDNA sequencing analysis and a BLAST search using the GenBank sequence database.

#### RESULTS

These test isolates were obtained from a previous study on the evaluation of a new method for testing the pathogenicity of molds to yam tubers (Ogbo and Agu, 2015).

		Microscopio	Microscopio		
		wheroscopic	wheroscopic	Partial ITS rDNA	
Isolates	Cultural	characteristics	characteristics	sequencing	Identity
	characteristics	(Traditional	(Improved slide-	analyses	
		slide-culture)	culture)	unuryses	
SC 1	On SDA,	Conidial heads	Conidial heads	A BLAST search of	Aspergillus
	colonies were	were radiate,	were radiate,	the GenBank	flavus
	powdery, flat	splitting to form	splitting to form	sequence data base	-
	with radial	loose columns	loose columns	showed 100 %	
	grooves, yellow	(200 µm in	(300- 400 µm in	Identity to multiple	
	at first, but later	diameter). Thus,	diameter). Thus,	ITS sequences	
	turned to bright	conidiophores	conidiophores	reported from A.	
	to dark vellow-	stipes were	stipes were	orvzae and A. flavus	
	green with age	hvaline and	hvaline and	The best matches	
	Colour on the	coarsely	coarsely	included sequences	
	reverse side	roughened	roughened	reported in peer-	
	was creamy	noticeable near	noticeable near	reviewed literature	
	Colonica wara	the variale and	the variale and	(Vin V at al	
	incubated at 20			(1111, 1. et al.	
	incubated at $50^{\circ}$	non-septate.	non-septate.	(2009). Molecular	
	C for 5 days.	Conidia were	Conidia were	characterization of	
		globose to	Se globose to	toxigenic and	
		subglose (2 µm in	subglose (3-6 µm	atoxigenic A. flavus	
		diameter), pale-	in diameter),	isolates collected	
		green and	pale-green and	from peanut fields in	
		conspicuously	conspicuously	China. Journal of	
		echinulate.	echinulate.	Applied	
				Microbiology 107	
			esearch and	(6): 1857- 1865).	
		NE D	evelopment 💦 🥊	This sample was	
				Sdistinguished from	
			IN. 2430-0470	A. oryzae based on	
				Lits smaller conidia	
		44		$\sim$ (up to 6 $\mu$ m,	
		10m		whereas those of A.	
		and and	TWOLES	<i>oryzae</i> are larger, up	
				to 8 µm).	
SC 2	On SDA.	Sporangiophores	Sporangiophores		Mucor
	colonies were	were hvaline.	were hvaline.		circinelloides
	floccose	erect. non-septate	erect. non-septate		
	(cottony in	and branched	and branched		
	texture) nale	sympodially and	sympodially and		
	grevish-brown	circinate	circinate		
	Growth rate	Sporangia were	Sporangia were		
	was rapid thus	terminal dark-	terminal dark-		
	colonies filled	brown finely	brown finely		
	the entire petri	echinulate to	echinulate to		
	dish in 2 days	smooth and	smooth and		
	Colour on the	should allu	should allu		
		splicitcal (20- 80	splicitcal (20- 80		
	reverse side	μπ m diameter).	μπ m diameter).		
	was yellow.	Sporangiospores	Sporangiospores		
	Colonies were	were nyaline or	were nyaline or		
	incubated at $30$	pale-brown.	pale-brown.		
	C for 5 days.	Collumellae were	Collumellae were		
		ellipsoidal and	ellipsoidal and		

# Table 1: Morphological Comparisons of Test Fungi using Traditional and Improved Slide Culture Technique using Genotypic Characteristics as a Standard

		4.5-7 x3.5-5 μm	4.5-7 x3.5-5 μm		
		in size.	in size.		
		Chlamydospores	Chlamydospores		
		were absent.	were absent.		
SC 3	Colonies had	Conidiophores	Conidiophores		Fusarium
	rapid growth.	were hvaline.	were hvaline.		solani
	4.5cm in 4	simple, bearing	simple, bearing		~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
	days Aerial	snore masses at	snore masses at		
	mycelium was	the aneves As tall	the aneves As tall		
	white to groom	as the length of	as the length of		
	with orongo	as the length of	as the length of		
	with Orange-	a four times	a four times		
	yenow	a lew tilles.	a lew tilles.		
	pigmentation.				
	Colour on the	nyaline and	nyaline and		
	reverse side	phialosporous. 1-	phialosporous. 3-		
	was dark-	3 macroconidia	5 macroconidia		
	yellow.	were present,	were present,		
	Colonies were	which were	which were		
	incubated at 30	fusiform,	fusiform,		
	<sup>o</sup> C for 5 days.	cylindrical,	cylindrical,		
		moderately	moderately		
		curved with an	Scurved with an		
		indinstinctly	indinstinctly	0	
		pedicellate foot	pedicellate foot	V)	
		cell and a short	cell and a short		
		blunt apical cell,	blunt apical cell,	3 8	
		15-30 x 2-4 µm.	28-42 x 4-6 µm.	s Q	
		Microconidia	Microconidia	5 8	
		were abundant, R	were abundant,	d N	
		which were	evelwhich were		
		cylindrical to	cylindrical to	58	
		oval, one to two-	oval, one to two-	B	
		celled borne on	celled borne on	8	
		lateral phialides.	lateral phialides.	9	
		4-8 x 1-3um.	8-16 x 2-4.5um.		
		Chlamydospores	Chlamydospores		
		were hvaline.	were hvaline.		
		globose, smooth	globose, smooth		
		to rough-walled.	to rough-walled.		
		borne singly and	borne singly and		
		in pairs on short	in pairs on short		
		lateral branches.	lateral branches.		
		6- 10 um	6- 10 um		
SC 4	On SDA	Conidial heads	Conidial heads	A blast search using	Aspergillus
	colonies were	varied greatly in	varied greatly in	the GenBank	tamari
	colourless at	size in the same	size in the same	sequence database.	
	first then	fruiting area	fruiting area	showed that the	
	gradually	from more or less	from more or less	sequence from this	
	turned orange-	columnar to	columnar to	sample showed 100	
	vellow to	nearly but not	nearly but not	% identity to	
	brown or light	completely	completely	multinle ITS	
	brownish-olive	globose and up to	globose and up to	sequences reported	
	with age but	250m in diameter	300m in diameter	from A tamari Rest	
	often presenting	with	with	matches included	
	a suggestion of	radiating chains	radiating chains	sequences reported	
	a suggestion of	radiating chams	radiating chams	sequences reported	

			Ĩ		
	green that was	and columns of	and columns of	in peer-reviewed	
	transient and	conidia.	conidia.	literature (Rakeman,	
	limited to areas	Conidiophores	Conidiophores	<i>et al.</i> (2005).	
	of young heads.	arising from	arising from	Multilocus DNA	
	The reverse	submerged	submerged	sequence	
	side was	hyphae, were up	hyphae, were up	comparisons rapidly	
	colorless.	to 1 to 2 mm in	to 1 to 2 mm in	identify pathogenic	
	Growth rate	length, colorless,	length, colorless,	molds). Journal of	
	was rapid (3	with walls	with walls	Clinical	
	days)	becoming	becoming	Microbiology 43:	
		abruptly thinner	abruptly thinner	3324- 3333).	
		at the base of the	at the base of the		
		vesicle. Vesicles	vesicle. Vesicles		
		were globose to	were globose to		
		subglobose, 15 to	subglobose, 25 to		
		32m in diameter	50m in diameter		
		with fairly thin	with fairly thin		
		walls which	walls which		
		frequently crush	frequently crush		
		in mounts, fertile	in mounts, fertile		
		over almost the	over almost the		
		entire surface.	Sentire surface.		
		Sterigmata, were	Sterigmata, were	Dr.	
		in one series in	in one series in		
		small heads and	small heads and	e Vi	
		in two series in	in two series in	S V.	
		large	large	S B	
		heads.Conidia	heads.Conidia	5 8	
		ranged from more	ranged from more	<u>a</u> 8	
		or less pyriform,	or less pyriform,		
		through sub-	through sub-	58	
		globose to	globose to	8	
		globose,	globose,	A	
		conspicuously	conspicuously	7	
		roughened from	roughened from		
		prominent	prominent		
		tubercles and bars	tubercles and bars		
		of orange-yellow	of orange-yellow		
		coloring matter	coloring matter		
		deposited	deposited		
		between the loose	between the loose		
		outer wall and the	outer wall and the		
		firm inner wall,	firm inner wall,		
		commonly	commonly		
		ranging from 5.0	ranging from 5.0		
		to 6.5mm in	to 6.5mm in		
		diameter,	diameter,		
		occasionally up to	occasionally up to		
		8mm. Sclerotia	8mm. Sclerotia		
		were purplish,	were purplish,		
		globose with apex	globose with apex		
		white.	white.		
SC 5	On SDA,	Conidiophores	Conidiophores		Aspergillus
	colonies had	were hyaline or	were hyaline or		niger
	rapid growth	pale-brown, erect,	pale-brown, erect,		

colonies were compact with yellow basil felt covered by a dense layer of black conidial heads (up to 3 mb by 15 to 20 µm in diameter), with powdery with pow		rate. However,	simple, with foot	simple, with foot		
fat and compact with ycllow basal felt covered by a dense layer of black conidial heads (up in diameter) split into over 4 texture. The colour on the com pale-brown, globose vesicles and phialdes on pale-brown, globose vesicles <br< th=""><th></th><th>colonies were</th><th>cells basally,</th><th>cells basally,</th><th></th><th></th></br<>		colonies were	cells basally,	cells basally,		
compact with yellow basal felt covered by a dense layer of black conidial heads (up to 3 mm by 15 to 20 µm in diameter), split into over 4 loose conidial columns with over 4 fragments apically empared at apex. Conidial incubated at 30 "C for 5 days.apex forming globose vesicles, bearing conidial heads (up to 3 heads (up to 3) multi diameter), split into over 4 columns with oorer 4 fragments apically empared at apex. Conidial discritate phialdosporous, brown, black in mass globose vesicles, and phialides acutely tapered at apex. Conidia vere grayish sepia to black, fuffy with abundant erevres side was paleapex forming total composed of catenulate conidial (over 15 conidia/ composed of catenulate conidial discritate phialdosporous, brown, black in mass globose vesicles, and phialides acutely tapered at agers. Conidia vere simple or compound. offer aggregated, aggregated, simple, "C for 5 days.apex forming total total tapes. Conidia vere simple, sometimes brown, black in mass globose cosiclate, frequently servers, black to black, Conidiophores were hyaline, simple, "C for 5 days.peridiaspores splitalosporous, brown, black in mass globose cosiclate, frequently servers, diagregated, simple, sometimes sometimes cells were hyaline, sometimes cells were hyaline, sometimes cells were hyaline, sometimes cells were hyaline, sometimes cells were hyaline, sometimes cells were hyaline, simple, conidiogenous cells were hyaline, simple, conidiogenous cells were hyaline, simple, conidiogenous cells were hyaline, simple, conidiogenous cells were hyaline, simple, conidiogenous c		flat and	inflated at the	inflated at the		
yellow baal felt covered by a dense layer of black conidial heads (up to 3 mm by 15 to 20 µm in diameter), split into over 4 loose conidial columns with over 4 fragments apically ecomposed of colonies were incubated at 30 "C for 5 days.globose vesicles, apically composed of cotaminate onidia (over 15 conidia/ chain borne on uniscriate aphialides and phialides acutely tapered at apically apically composed of cotaminate conidia (over 15 conidia/ chain borne on uniscriate aphialides) on pale-brown, globose vesicles, and phialides brown, black in mass globose and minutely colonies were grayish sepito to mose gray to black, fuffy with abundant the reverse side was faccus black to High Colonies were grayish sepito to mouse grey to black to High Colonies were grayish sepito to mouse gray to black to Black. Colonies were incubated at 30 "C for 5 days.globose vesicles, agregated, agregated, agregated, arising from the pyendial were sometimes sometimes sometimes sometimes sometimes sometimes sometimes sometimes sometimes sometimes sometimes sometimes sometimes sometimes sometimes sometimes sometimes sometimes sometimes sometimes sometimes sometimes sometimes sometimes sometimes sometimes sometimes sometimes sometimes sometimes sometimes sometimes <b< th=""><th></th><th>compact with</th><th>apex forming</th><th>apex forming</th><th></th><th></th></b<>		compact with	apex forming	apex forming		
SC 6     On SDA, colonies were grayish sepit to back     Dearing conidial heads (up to 3 mm by 15 to 20 µm in diameter), split into over 4 loose conidial columns with columns with reverse side was pale yellow.     Dearing conidial heads (up to 3 mm by 15 to 20 µm in diameter), split into over 4 split into over 4 split into over 4 columns with columns with columns with columns with reverse side was pale yellow.     Dearing conidial heads (up to 3 mm by 15 to 20 µm in diameter), split into over 4 split into over 4 split into over 4 columns with columns with diameter) are phialosporous, brown, black in muss globose and minutely was fuscous black to black, Colonies were incubated at 30 °C for 5 days.     Pycnidia were simple, coll direct was paterial more layers of coll swere hyaline, simple, coll direct was byabyriform to blobbystiform to blobbysti		yellow basal	globose vesicles,	globose vesicles,		
a dense layer of black conidial heads with powdery texture. The colour on the reverse side was pale yellow.       heads (up to 3 mm by 15 to 20 µm in diameter), split into over 4 lose conidial columns with over 4 fragments apically composed of colonies were incubated at 30 "C for 5 days.       heads (up to 3 mm by 15 to 20 µm in diameter), split into over 4 lose conidial columns with over 4 fragments apically composed of colonies were incubated at 30 "C for 5 days.       heads (up to 3 mm by 15 to 20 µm in diameter), split into over 4 lose conidial columns with over 4 fragments apically composed of condibies on pale-brown, globose vesicles and phialides acutely tapered at apex. Conidial (3.55 µm in diameter) are phialosporous, brown, black in mass globose and minutely compound, often agregated, ostiolate, to black, Colonies were grayish sepia to black to black. Colonies were grayish sepia to black to black. Colonies were grayish sepia to black to black, fuffy with abundant acrial mycelia; the reverse side vas fuscous black to black. Colonies were grayish sepia to black to black, Colonies were hyaline, simple, collidical cavity. Condidophores black to black collidial cavity. Condidophores septate, rarely branched subboyriform to holobbatic collis were hyaline, simple, collidical, subpyriform to blobbypriform to blobbypriform to blobbypriform to blobbypriform to blobbypriform to blobbypriform to blobbypriform to blobbypriform to blobbatic at collidical cavity. Condicidagenous cells were hyaline, simple, collidical to collidingenous cells were hyaline, simple, collidical cavity. Condidiagenous cells were hyaline, simple, subbobypriform to blobbatical to collidingenous cells were hyaline, simple, collidical to collidingenous ce		felt covered by	bearing conidial	bearing conidial		
black conidial heads with powdery texture. The colour on the reverse side was pale yellow. Colonies were incubated at 30 "C for 5 days.mm by 15 to 20 µm in diameter), split into over 4 ragments apically composed of catenulate conidia (over 15 conidia/ (over 15 conidia/ (over 15 conidia/ icotan) borne on uniseriate and biseriate phialides a untely tapered at agregated, storwn, black in mass globose and minutely cechinulate.mm by 15 to 20 µm in diameter), spit into over 4 ragments apically composed of catenulate conidia (over 15 conidia/ icotan) borne of uniseriate and biseriate phialides acutely tapered at agregated, storwn, black in mass globose and minutely cechinulatemm by 15 to 20 µm in diameter), spitalosprous, phialosprous, phialosprous, phialosprous, spitalosprous, storwn, black in mass globose and minutely cechinulatemm by 15 to 20 µm in diameter), spitalosprous, phialosprous, spitalosprous, spitalosprous, spitalosprous, spitalosprous, storwn, black in mass globose and minutely cechinulatemm by 15 to 20 µm in diameter), spitalosprous, spitalosprous, spitalosprous, spitalosprous, spitalosprous, spitalosprous, spitalosprous, spitalosprous, spitalosprous, spitalosprous, spitalosprous, spitalosprous, spitalosprous, spitalosprous, spitalosprous, spitalosprous, spitalosprous, spitalosprous, spitalosprous, spitalosprous, spitalosprous, spitalosprous, spitalosprous, spitalosprous, spitalosprous, spitalosprous, spitalosprous, spitalosprous, spitalosprous, spitalosprous, spitalosprous, spitalosprous, spitalosprous, spitalosprous, spitalosprous, spitalosp		a dense layer of	heads (up to 3	heads (up to 3		
sconidial heads with powdery texture. The colour on the colour on the reverse side was pale yellow.       µm in diameter), split into over 4 split into over 4 columns with over 4 fragments apically apically wellow.       µm in diameter), split into over 4 columns with over 4 fragments apically composed of catculate conidia (over 15 conidia/ chain) borne on uniseriate and biseriate phialides on pale-brown, globose vesicles and phialides acutely tapered at apex. Conidia (3:55 µm in diameter) are phialosporous, brown, black in mass globose and minutely echinulate       A blast search using the GenBank sequence database, showed that the sequence obtained from L. Heoromae is perfect state acutely tapered at agregated, ostiolate, up to somple, conidial, colonies were grayish sepia to mouse grey to black fulfy with abundant acrial mycelia; the reverse side some were grayish sepia to mouse grey to black to black. Colonies were grayish sepia to mouse grey to black fulfy with abundant acrial mycelia; the reverse side colonies were grayish sepia to mouse grey to black to black Colonies were grayish sepia to mouse grey to black to black fulfy with abundant acrial mycelia; the reverse side colonies were grayish sepia to mouse grey to black to black to black Colonies were septate, rarely sometices septate, rarely sometices cells were hyaline, simple, cells were hyaline, simple, subobypyriform to subobypyriform to subobypyriform to holoblatis and cells were hyaline, simple, suback to bl		black	mm by $15$ to $20$	mm by $15$ to 20		
with powdery texture. The colour on the vas pale yellow.'split into over 4 loose conidial columns with over 4 fragments apically composed of catenulate conidia (over 15 conidia/ chain) borne on uniseriate and biseriate philaldes on pale-brown, globose vesicles and philaildes and philaildes acutely tapered at apex. Conidia (3.5-5 µm in diameter) are philaosporous, brown, black in mass globose and minutely echinulateisplit into over 4 losse vesicles and philaildes acutely tapered at apex. Conidia (3.5-5 µm in diameter) are philaosporous, brown, black in mass globose and minutely echinulateA blast search using the GenBank sequence database, showd that the sequence database, showd that the sequence database, showed that the sequence database, sometimes sometimes sometimes septate, rarely branched incubated at 30 eclis lining the pyenidia cavity. Condiogenous cells were hyaline, simple, colonigenous cells wire hyaline, simple, colonigenous cells were hyaline, simple, colonigenous cells were hyaline, simple, colonigenous cells were hyaline, simple, colidiogenous cells were hyaline, simple, colidiogenous cells were hyaline, simple, colidiogenous cells were hyaline, simple, colidiogenous cells were hyaline, simple, colidiogenous cells were hyaline, simple, colidiogenous cells were hyaline, simple,<		conidial heads	um in diameter).	um in diameter).		
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annelidic. Conidia	subobpyriform to	descriptions of	
were initially	holoblastic and	Diplodia africana	
unicellular,	annelidic. Conidia	and <i>L. plurivora</i> sp.	
hyaline,	were initially	nov. 99 (5): 664-	
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subovoid to	hyaline,	was found to be a	
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oblong,thick-	subovoid to	theobromae by	
walled, base	ellipsoid-	combined ITS/Ef1	
truncate; mature	oblong,thick-	gene analysis but	
conidia one-	walled, base	was differentiated	
septate, cinnamon	truncate; mature	morphologically by	
to fawn,	conidia one-	the conidia size of	
longitudinally	septate, cinnamon	26-33 x 14.5- 17	
striate, 10-15 x 1-	to fawn,	μm, whereas	
5µm. Paraphyses	longitudinally	those of L.	
were hyaline,	striate, 20-30 x	theobromae were	
cylindrical	10-15µm.	shorter, about 24-28	
sometimes septate	Paraphyses were	X	
up to 20 µm.	hyaline,	12- 15 µm.	
	cylindrical	,	
	sometimes septate		
B nd II	up to 50 µm.		
		NA	



Fig 5: Micrograph of Aspergillus sp. using our Improved Method



Fig 6: Micrograph of Aspergillus sp. using our Improved Method



Fig 3: Micrograph of Aspergillus flavus using the Traditional Method



Fig 4: Micrograph of Aspergillus flavus using our Improved Method



Micrograph of Mucor circinelloides.



Fig 1: Micrograph of *Fusarium solani* using the Traditional Method



Fig 2: Micrograph of Fusarium solani using our Improved Method



Micrograph of Aspergillus tamarii



Micrograph of Aspergillus niger



Micrograph of Lasiodiplodia theobromae

## DISCUSSION

The traditional teasing method does not allow for a holistic microscopic view of fungal isolates under the microscope, thereby making the study of the microscopic structures of fungi such as spore shape, spore heads and other special structures such as rhizoids, stolons, foot cells and hold fasts almost impossible. Other types of slide culture techniques are laborious, time-consuming, costly and almost impracticable to reproduce and as such the development of this simple, economical and efficient slide culture method.

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#### **COMPETING INTERESTS**

Authors have declared that no competing interests [ exist.

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