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Full Length Research Paper

Influence of culture media on growth, colony character and sporulation of fungi isolated from decaying vegetable wastes

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The mycelial growth rate, colony character and sporulation pattern of ten fungal isolates, grown on three different culture media viz., Potato Dextrose Agar (PDA), Czapek's Dox + Yeast Extract Agar (CYA) and Lignocellulose Agar (LCA) were observed after seven days of incubation at 25±1°C. The colony diameter, culture characteristics (texture, surface and reverse colouration, zonation) and sporulation of selected test fungi were greatly influenced by the type of growth medium used. LCA exhibited comparatively higher mycelial growth in six test fungi, whereas all the ten isolates revealed heavy sporulation on this culture medium. *Penicillium* sp. and *Acremonium kiliense* exhibited maximum colony growth on PDA, while *Chaetomium funicola* and *Fusarium oxysporum* showed highest growth on CYA medium. These results will be useful for fungal taxonomic studies.

Key words: Mycelial growth, colony character, sporulation, culture media.

INTRODUCTION

Fungi grow on diverse habitats in nature and are cosmopolitan in distribution requiring several specific elements for growth and reproduction. In laboratory, these are isolated on specific culture medium for cultivation, preservation, microscopical examination and biochemical and physiological characterization. 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, water availability and surrounding atmospheric gas mixture (Northolt and Bullerman, 1982; Kuhn and Ghannoum, 2003; Kumara and Rawal, 2008). However, the requirements for fungal growth are generally less stringent than for the sporulation.

Nowadays, fungal taxonomy is in a state of rapid flux, because of the recent researches based on molecular approaches, that is DNA comparisons of selected strains either isolated locally or obtained from culture collection centre, which has changed the existing scenario of fungal systematic and often overturn the assumptions of the older classification systems (Hibbett, 2006). Different concepts have been used by the mycologists to characterize the fungal species, out of which morphological (phenetic or phenotypic) and reproductive stages are the classic approaches and baseline of fungal taxonomy and nomenclature that are still valid (Davis, 1995; Guarro et al., 1999; Diba et al., 2007; Zain et al., 2009). It seems evident that in near future, modern molecular techniques will allow most of the pathogenic and opportunistic fungi to be connected to their corresponding sexual stages and integrated into a more natural taxonomic scheme.

Physical and chemical factors have a pronounced effect on diagnostic characters of fungi. Hence, it is often necessary to use several media while attempting to identify a fungus in culture since mycelial growth and sporulation on artificial media are important biological characteristics (St-Germain and Summerbell, 1996). Furthermore, findings for one species are not readily extrapolated to others, particularly for filamentous fungi, where significant morphological and physiological

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variations exist (Meletiadis et al., 2001). With these perspectives, the present study was undertaken to observe the influence of three different culture media on the mycelial growth, colony characters and sporulation patterns of ten dominant fungi isolated from decaying vegetable wastes.

MATERIALS AND METHODS

Collection of vegetable waste

Different parts of vegetable wastes, that is, peel of fox nut (*Euryale ferox* Salisb. Nymphaceae), rice bean (*Phaseolus* sp.- Fabaceae), potato (*Solanum tuberosum* Linn.- Solanaceae), Squash (*Sechium edule* Sw.- Cucurbitaceae); petioles of cauliflower (*Brassica oleracea* Linn. var. *botrytis* -Brassicaceae), edible banana (*Musa paradisiaca* Linn.- Musaceae) and sheath of bamboo (*Bambusa arundinaceae* Willd.- Poaceae) were collected from various households in Imphal City, Manipur, India. After sorting out, each type of vegetable waste material was air-dried.

Study site

Decomposition studies were conducted in a experimental plot located at Department of Life Sciences, Manipur University, Imphal (24°45'259" N Latitude; 93°55'690" E Longitude) at an elevation of 768 msl (Etrex, 12 Channel, GPS). The climate of the area is monsoonic with distinct rainy, winter and summer seasons in a year. The mean minimum temperature during 10 months study period (November 2008 to September 2009) ranged between 5.6 to 20.9^{o}C and mean maximum varied between 23.1 to 31.6°C. The relative humidity varied from 47.1 to 78.9% while the total rainfall ranged from 0 to 352.8 mm. Surface soil (0 to 10 cm depth) temperature during the study period varied from 22 to 35°C whereas the soil moisture content ranged from 2.5 to 31.4%. The soil pH was acidic ranging from 4.5 to 5.2.

Decomposition study

Decomposition studies were carried out following Nylon mesh bag technique (Bocock et al., 1960). Equal proportions of the seven vegetable waste types were mixed to make a lot of 5 g. A total of 50 Nylon mesh bags (10×15 cm; 1 mm) were filled with 5 g samples in each bag and were placed on soil surface of the experimental site. Five bags containing decomposing vegetable wastes were randomly recovered at monthly interval and brought to the laboratory in sterile polythene bags for fungal isolation. Surface soil was collected separately to assess the physico-chemical properties.

Isolation of fungi

Isolation of fungi was performed by surface sterilization method (Kinkel and Andrews, 1988). Twenty five bits (5×5 mm) of each decaying vegetable type were submerged in 70% ethanol for 1 min, then transferred into 15% H_2O_2 for 1 min and again kept in 70% ethanol for 1 min. Thereafter, the bits were serially washed in 10 changes of sterile distilled water, blotted dry, then placed in each of five Petri plates (5 bits/plate) containing PDA medium supplemented with Streptomycin (100 mg/l) and incubated at 25±1°C for 7 days. The fungal colonies that appeared on the vegetable bits after incubation period were isolated in fresh sterilized Petri plates containing PDA and were identified.

Ten common and dominant fungal isolates, namely*, Aspergillus candidus* Link*, Aspergillus niger* Tiegh, *Aspergillus sulphureus* (Fresen.) Wehmer*, Aspergillus versicolor* (Vuill.) Tirab., *Penicillium corylophilum* Dierckx*, Penicillium expansum* Link, *Penicillium* sp., *Acremonium kiliense* Grütz*, Chaetomium funicola* Cooke and *Fusarium oxysporum* Schltdl. were selected and 5 mm discs of each fungus obtained from pure cultures were transferred at the centre of sterile Petri dishes (in triplicates) containing three different growth media e.g. (i) Potato Dextrose Agar (PDA) [Potato (peeled) 200 g; Dextrose 20 g; Agar 20 g; Distilled H_2 O 1L] (ii) Czapek's Dox + Yeast extract agar (CYA) [Sucrose 30 g; $NaNO_3$ 2 g; K_2HPO_4 1 g; $MgSO_4$ + H_2O 0.5 g; KCl 0.5 g; $FeSO_4$ + $7H_2O$ 0.01 g ; Agar 15 g; Distilled H_2O 1 L] (Onion's et al., 1981) and (iii) Lignocellulose Agar (LCA) [Glucose 1 g; KH_2PO_4 1 g; $MgSO_4$.$7H_2O$ 0.2 g; KCl 0.2 g; $NaNO_3$ 2 g ;Yeast Extract 0.2 g; Agar 13 g; Distilled H_2O 1 L] (Miura and Kudo, 1970). The pH of the test media was maintained at 5.5 being optimal for the growth and sporulation in a majority of fungi. The Petri dishes were then incubated for 7 days at 25±1°C in BOD incubator and colony character of each fungus was recorded. Sporulation was assessed on glass slides by mounting a small portion of mycelia in Lactophenol-Cotton blue stain and observed under microscope.

RESULTS AND DISCUSSION

All three culture media supported the growth of test fungi to various degrees. Out of them, six fungi showed maximum mycelial growth on LCA after 7 days of incubation period (Table 1), while *Penicillium* sp. (32.0±2.6 mm) and (26.3±2.3 mm) exhibited higher colony growth on PDA. *Chaetomuim funicola* (58.3±1.2 mm) and *Fusarium oxysporum* (85.7±0.3 mm) showed maximum growth on CYA medium. High growth rate of *F. oxysporum* (85 mm) in Czapek's Dox agar, after incubation period, has also been observed by Farooq et al. (2005). Difference in surface and reverse colouration of fungal colonies was distinct on three growth media as observed in case of *F. oxysporum* (magenta pink in PDA, white in CYA and hyaline in LCA) and *Aspergillus versicolor* (white to orange with green spores at centre and bright orange on reverse side in PDA, white to cream with reddish exudates on surface and reddish colour on reverse in CYA and colourless on both sides in LCA). Moreover, the surface and reverse colony colour in six and nine tested "fungi" respectively were hyaline and/or colourless on LCA, except that of *Acremonium kiliense* where both sides colour were bright orange.

In the present study, zonations observed in fungal colonies were found to be influenced by the culture media used. In PDA, almost all tested fungi were characterized with distinct radial furrows on the reverse with exception of concentric dark and light reddish colouration in *F.* oxysporum. In CYA, radial furrows were less prominent in *Aspergillus candidus*, *Aspergillus niger* and *Penicillium expansum* whereas, concentric and/or circular zonations were observed in *Penicillium corylophilum* and *Penicillium* sp. On the other hand, none of the fungal colonies exhibited radial furrows in LCA. However, majority of the tested fungi exhibited concentric zonations in LCA except *A. versicolor*, *Penicillium* sp. and

Table 1. Mycelia growth, colony characters and sporulation pattern of fungal isolates on three culture media.

Fungi	Media type	*Colony diam (mm)	Colony character			Zonation	Sporulation
			Texture	Surface colour	Reverse colour		
A. candidus	PDA	23.3±0.3	Velvety thick	Creamish white	Slightly creamish	Radially furrowed on the reverse	Moderate
	CYA	31.3±2.2	Velvety thick	Creamish with brownish sporulating area	Yellow brown	Radially furrowed	Poor
	LCA	41.3±0.9	Fine	White with black-brown spores	Colourless	With concentric zones	Heavy
A. niger	PDA	65.0±2.3	Velvety	White with typical black spores	Yellow	Heavily furrowed on the reverse	Heavy
	CYA	78.0±3.6	Powdery	White with black spores	Yellow	With single concentric ring at periphery and radial furrow at the centre.	Moderate
	LCA	83.3±1.2	Powdery	Hyaline with black spores	Colourless	With light concentric zones	Heavy
A. sulphureus	PDA	17.3±1.2	Velvety	Dirty white with yellow spores at the centre	Orange to chocolate colour	Slightly radially furrowed	Moderate
	CYA	19.3±0.3	Powdery	Sulphur yellow with less spores at the centre	Brownish	None	Poor
	LCA	21.7±1.7	Fine	Hyaline with yellow spores.	Colourless	Concentric zones	Heavy
A.. versicolor	PDA	26.3±0.3	Floccose	White to orange-cream with green spores at the centre	Bright orange	Heavily wrinkled on reverse	Moderate
	CYA	19.3±0.3	Velvety	White to creamish with reddish exudates	Reddish	None	Poor
	LCA	28.0±1.5	Fine	Hyaline with white to dark green spores at the centre	Colourless	None	Heavy
Penicillium corylophilum	PDA	10.7±2.3	Velvety	Dark green	Colourless to Creamish	With shallow centre and radially furrowed raised margin	Moderate
	CYA	33.7±1.2	Velvety	Grey green with sterile Margin	Brown	With concentric zones and slightly raised margin	Moderate
	LCA	46.7±3.1	Fine	White with green spores	Colourless	With concentric zones	Heavy

Table 1 Contd.

	PDA	28.3±1.8	Velvety	Dark green with clear exudates and distinct sterile white margin	Yellow	Radially furrowed	Heavy
P. expansum	CYA	42.3±0.3	Velvety	Greenish- brown ,with clear exudates and sterile white margin	Dirty white to Creamish	Slightly radially furrowed	Moderate
	LCA	48.3±2.3	Light velvety	Hyaline with green spores	Colourless	With concentric zonations	Heavy
	PDA	32.0±2.6	Powdery	Olivaceous green with sterile white margin	Orange to red, wrinkled	Radially furrowed	Heavy
Penicillium sp.	CYA	22.0±0.6	Floccose	Gray green	Orange	With concentric zonations	Moderate
	LCA	27.7±2.1	Fine	Hyaline with green spores and sterile margin	Colourless	None	Heavy
	PDA	26.3±2.3	Fine Floccose	Peach coloured	Slightly yellowish	Radially furrowed	Poor
Acremonium kiliense	CYA	19.0±0.6	Fine Floccose	White to pale yellow	Colourless to light orange	None	Moderate
	LCA	21.7±0.7	Fine Floccose	Bright orange	Bright orange	Light zonation at the margin	Heavy
	PDA	36.7±3.6	Velvety	Light Yellow	Light yellow	With venation like radial furrows	Nil
Chaetomium funicola	CYA	58.3±1.2	Velvety	Golden brown to yellow	Dark brown	With a distinct marginal zone	Moderate (few perithecia)
	LCA	41.3±0.7	Fine	Light greenish yellow	Colourless	With distinct white margin	Heavy (numerous perithecia)
	PDA	52.0±3.1	Floccose	Magenta pink	Magenta-red turning violet	With concentric zones of dark and light reddish colouration	Poor
Fusarium oxysporum	CYA	85.7±0.3	Floccose	White	Colourless	None	Moderate
	LCA	79.7±0.3	Light cottony	Hyaline	Colourless	None	Heavy

*Data represents mean of three replicates ± SE.

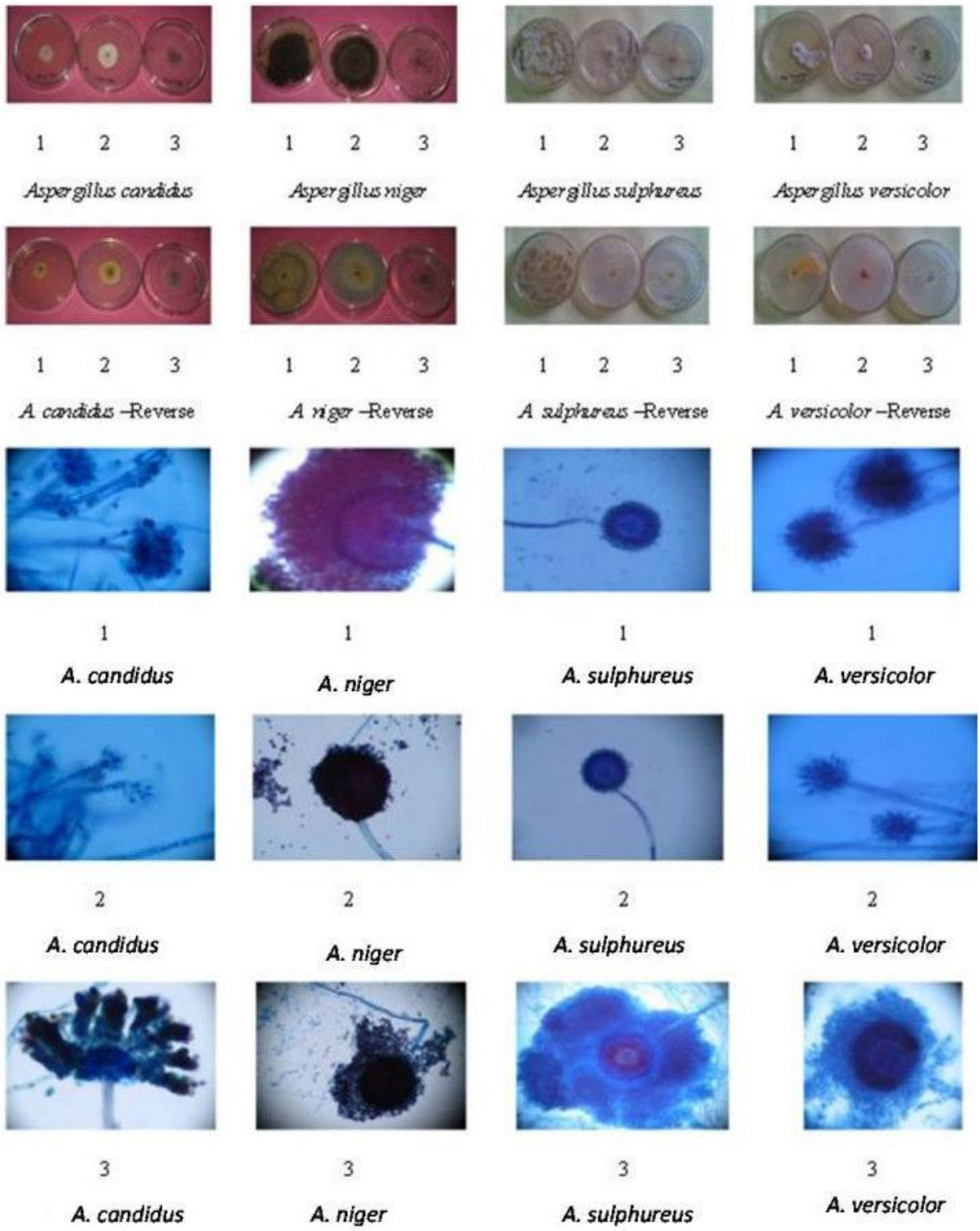

Figure 1. Colony growth, colour (surface and reverse) and sporulation pattern in test fungi on different culture media (1-on PDA, 2-on CYA, 3-on LCA).

F. oxysporum.

Our findings revealed marked differences in the sporulation patterns of all tested fungi on three culture media used. *C. funicola* could not sporulate in PDA, while *A. niger, P. expansum* and *Penicillium* sp. revealed heavy conidial production after seven days of incubation period. In LCA, all the tested fungi exhibited heavy sporulation (Table 1, Figures 1, 2 and 3), whereas comparatively

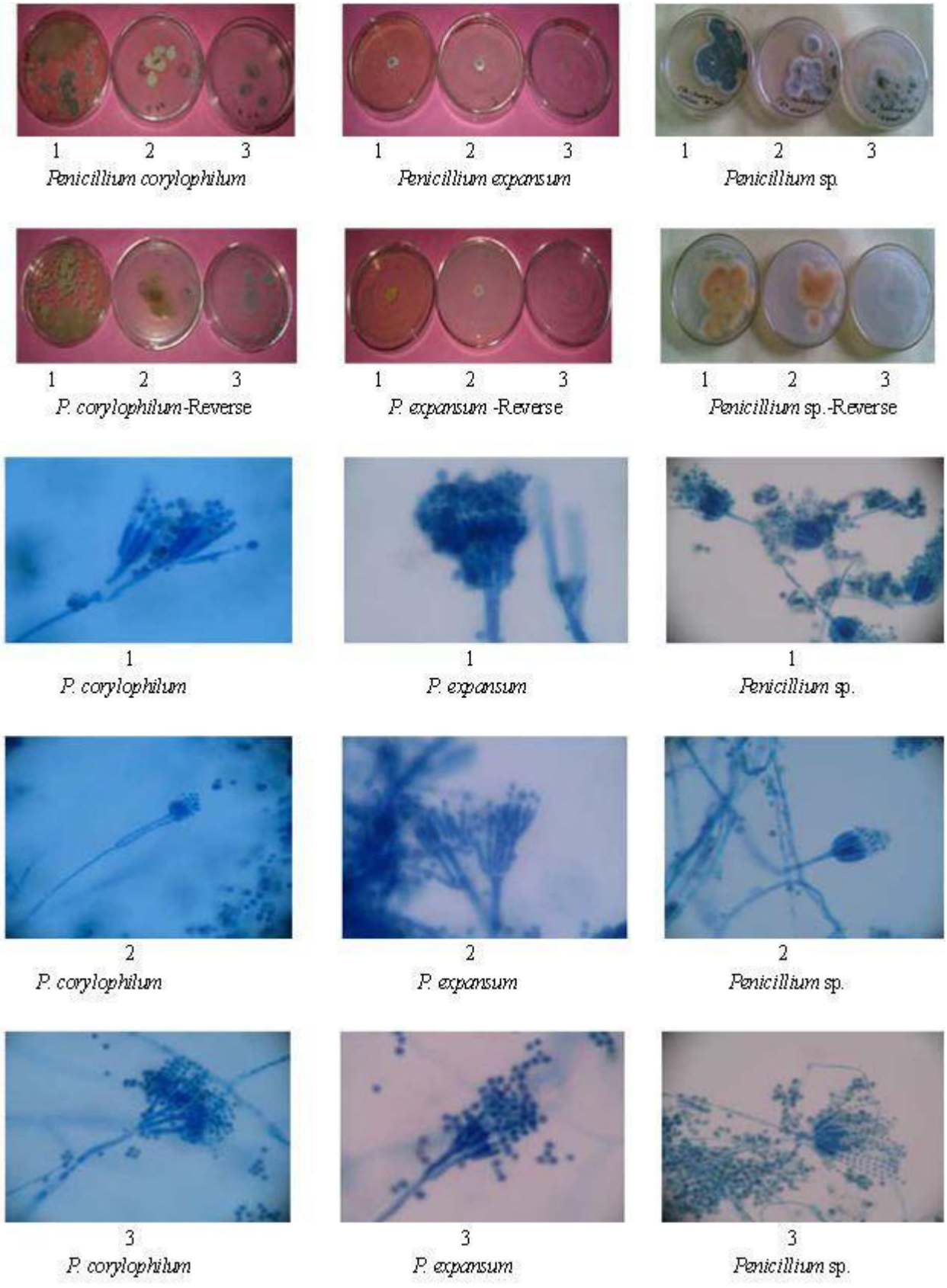

Figure 2. Colony growth, colour (surface and reverse) and spore mass in test fungi on different culture media (1- PDA, 2- CYA, 3- LCA).

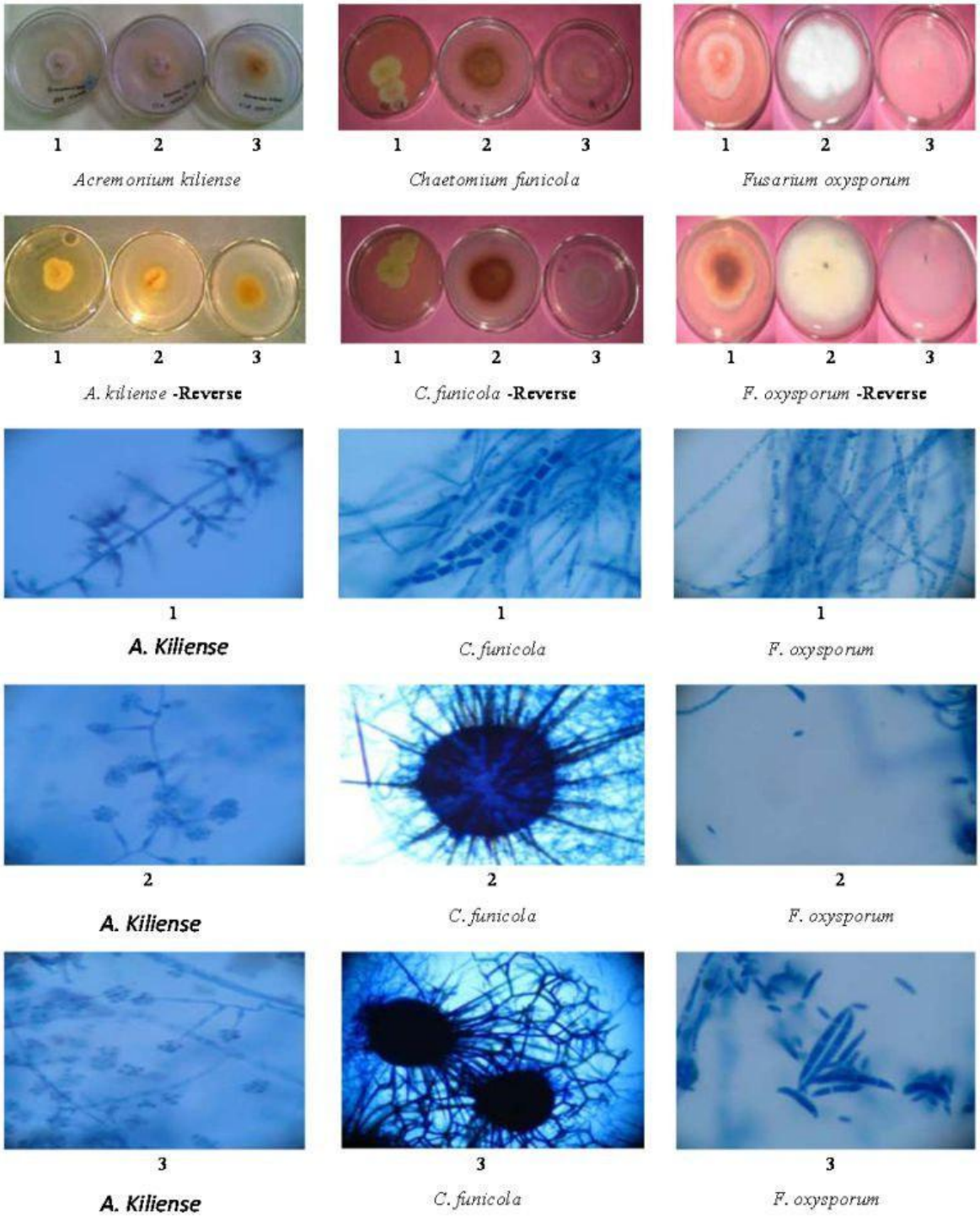

Figure 3. Colony growth, colour (surface and reverse) and spore mass in test fungi on three culture media (1- PDA; 2- CYA; 3- LCA).

poor or moderate fungal sporulation was observed in CYA. Okunowo et al. (2010) also observed least sporulation and minimum mycelia growth of *Myrothecium roridum* on Czapek' s Dox agar which may be due to the presence of chloride ion in the test medium. The mycelia growth of the organism on different nitrogen sources was found to be highest on sodium glutamate containing medium and lowest on ammonium chloride containing medium. However, in LCA despite the presence of chloride ion, as in case of CYA, mycelial

growth and sporulation were high in the present study. Several workers have recognized the importance of reproductive structures for inoculums production and studies have been conducted on the effects of various media components along with important physiological parameters that lead to maximum sporulation (Kim et al., 2005; Saxena et al., 2001; Saha et al., 2008). Type of culture media and their chemical compositions significantly affected the mycelia growth rate and conidial production of *Phoma exigua* (Zhae and Simon, 2006).

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 workers stated PDA to be the best media for mycelial growth (Xu et al., 1984; Maheshwari et al., 1999; Saha et al., 2008). Most fungi thrive on PDA, but this can be too rich in nutrients, thus encouraging the mycelial growth with ultimate loss of sporulation (UKNCC, 1998). In the present study, *C. funicola* showed heavy perithecia formation in LCA. Osono and Takeda (1999) stated that LCA because of its low glucose content suppresses the overgrowth of fast growing species and induces sporulation, hence this medium is useful for fungal identification. The fungal systematics is still based mainly on morphological criteria as observable characteristics. Hence, fungi are recognized and identified basically by their phenotypes (Zain et al., 2009). Moreover, the variations in colour of spores, especially among *Aspergillus* and *Penicillium* species, are one of the main criteria used widely for their identification and taxonomic placement (St-Germain and Summerbell, 1996) which seems to be mainly attributed to the constituents of a medium.

Conclusion

Our findings revealed that culture media differentially influenced the growth, colony character and sporulation of the test fungi. Out of three test media employed in the present study, LCA was found to be most suitable for heavy sporulation while PDA reproduced most visible colony morphology. It is concluded that instead of using any single culture medium a combination of two or more media will be more appropriate for routine cultural and morphological characterization of fungi to observe different colony features.

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