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Research Article

IMPACT OF DIFFERENT CULTURE MEDIA ON THE GROWTH RATE OF FUNGI ISOLATED FROM DIFFERENT INFECTED PLANTS

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ABSTRACT

The present investigation was carried out to find out and study the effect of different culture media on the growth rate, colony character and sporulation of four different fungi grown on five different culture media viz., Potato Dextrose Agar (PDA), Sabouraud Dextrose Agar (SDA), Czapek'sDox Agar, Nutrient Agar (NA), and Corn Meal Agar at room temperature. The growth of fungi was greatly influenced by the type of media used. The fungi showed different growth rate, colony characteristics with different media. Amongst five different media used PDA exhibited excellent growth followed by Corn Meal Agar and SDA while slow and poor growth rate was observed on CZ and NA.

INTRODUCTION

A wide range of media are used for growing fungi. Media will affect colony characters such as morphology, colony colour, texture, mycelium growth whether particular structures are formed or not and may affect whether the fungus will even grow in culture. All fungi require several specific elements for growth and reproduction. The requirement for growth is generally easily met as compared to that for sporulation. Therefore it is important to try several types of media to identify a fungus in culture. Media generally contain a source of carbon, nitrogen and vitamins. Glucose (dextrose) is the most widely utilizable carbon source and hence is the most commonly used in growth media. Fructose and mannose are the next most commonly utilized sugars by fungi and are found in media from natural sources. Sucrose (table sugar) may be used in some media. Nitrogen sources include peptone, yeast extract, malt extract, aminoacids, ammonium and nitrate compounds.

Fungi have natural deficiencies for vitamins that are satisfied at micro molar (uM) tonano molar (nM) concentrations. Other nutrients such as glucose are often contaminated with vitamins sufficient to supply the growth requirements of fungi. Various types of media are available and used for isolation of different groups of fungi that affect the vegetative growth, colony character, sporulation, pigmentation depending upon P^H, temperature, light, water availability and surrounding atmospheric condition (Norholt and Bullerman, 1982; Kuhn and Ghannoum, 2003; Kumara and Rawal, 2008).

Fungal taxonomy have a wide scope and nowadays it is in great demand because of recent researches based on molecular study due to which the presently existing scenario of fungal systematic and the older classification system has been changed (Hibbet 2006). Of various different concepts used by mycologists to identify fungal species, the classic ones are: Morhology and reproductive stages. Physical and chemical factors greatly affect the diagnostic characters of fungi. Hence, it is necessary to use several different media while identifying a fungus in culture because mycelia growth and sporulation on artificial media are important biological characteristics (St. Germain&Summerbell, 1996).

With these perspectives, the present study was undertaken to observe the influence of five different culture media (natural and Synthetic) on the growth pattern of four different fungi isolated from infected plants which may be helpful for fungal taxonomic studies and laboratory evaluation.

MATERIAL AND METHODS

Four different infected plants were selected for this study. The infected plant parts (leaves) were collected from different fields through field surveys and brought to the laboratory and were washed firstly with tap water and then were submerged in alcohol for 1 min. The infected leaf samples were washed 4-5 times with sterile distilled water. Then the infected leaf parts bearing diseased spot were placed on sterile petriplates containing PDA medium supplemented with Streptomycin (100mg/l) and incubated at room temperature (27° C) for seven

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days. The fungal colonies that appeared on the infected leaf parts were isolated in fresh sterilized petriplates containing PDA and were identified. Four common fungi were selected for further study.

Each of these were inoculated by point method at the centre of the sterile petriplates (in triplicates) containing five different media, namely Potato Dextrose Agar (PDA); Sabouraud Dextrose Agar (SDA); Czapek'sDox Agar (CZ);Nutrient Agar(NA); Corn Meal Agar (CMA). The $P^H$of the test media was maintained at 5.5 being optimal for the growth and sporulation in a majority of fungi. The petridishes were incubated for 7 days at room temperature in BOD incubator. Growth pattern was observed for 7 days and sporulation was studied by mounting a slide of small portion of mycelia in Lactophenol Cotton blue stain and observed under microscope.

RESULT AND DISCUSSION

All the five culture media used supported the growth of test fungi to various extents. All the four fungal isolates showed maximum mycelial growth on PDA followed by SDA and CMA while poor growth was observed on NA and CZ. *Aspergillusflavus* was the fastest growing fungus followed by *Alternariaricini* and *Helminthosporium* but *Penicillium* was reported to be slow.

Growth of all the four fungi was almost of the same type on PDA and CMA but the colony characters of Penicillium showed considerable differences on both the media. Aspergillusflavus shown extensive and fast mycelial growth on PDA, SDA, CMA but waspoor and slow on CZ and much more slow on NA. Moreover, Aspergillusflavus was reported to be the excellent and fastest growing fungus on all the five media as compared to *Alternariaricini, Helminthosporium* sp. And *Penicillium* sp.

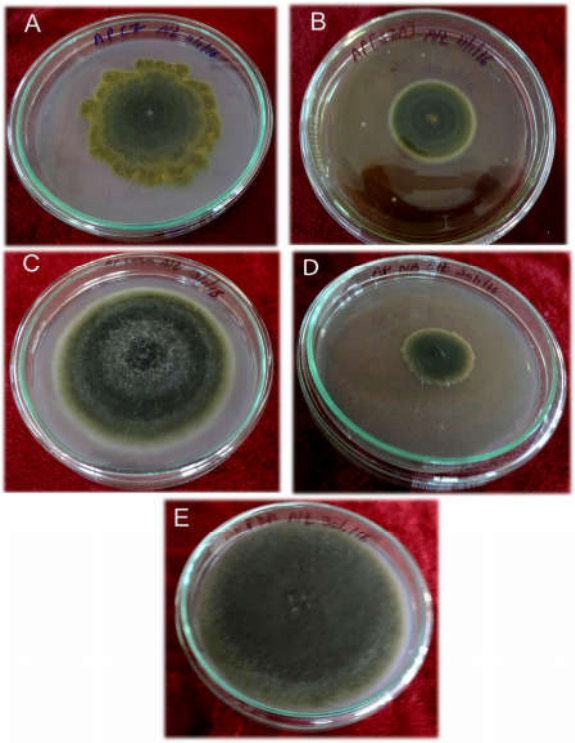

Fig. 1. Growth of *Alternaria* on all the five media. A.*Alternaria ricini* on CZ, B. *Alternaria ricini* on SDA, C. *Alternaria ricini* on CMA, D. *Alternaria ricini* on NA, D. *Alternaria ricini* on PDA.

Growth of all the four fungi was almost of the same type on PDA and CMA but the colony characters of *Penicillium* showed considerable differences on both the media. *Aspergillusflavus* shown extensive and fast mycelial growth on PDA, SDA, CMA but was poor and slow on CZ and much more slow on NA. Moreover, Aspergillusflavus was reported to be the excellent and fastest growing fungus on all the five media as compared to *Alternariaricini*, *Helminthosporium* sp. and *Penicillium* sp.

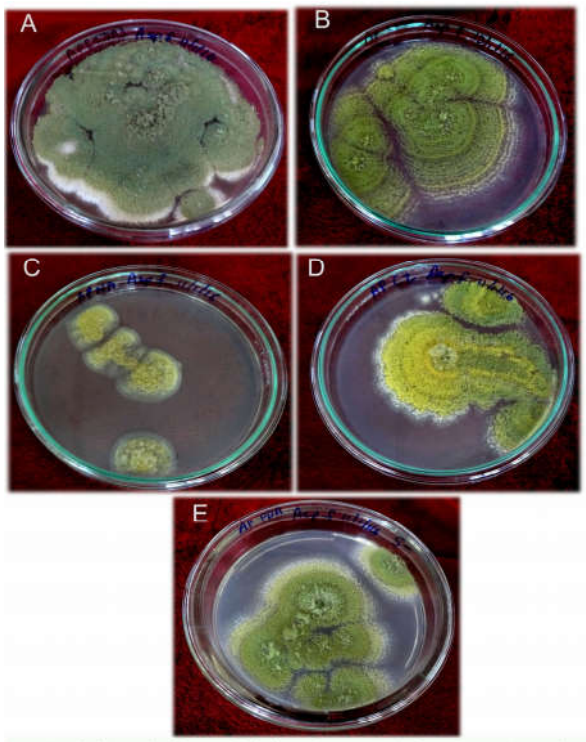

Fig. 2. Growth of *Aspergillus flavus* on all the five media. A. *Aspergillus flavus* on SDA, B. *Aspergillus flavus* on CMA, C. *Aspergillus flavus* on NA, D. *Aspergillus flavus* on CZ, E. *Aspergillus flavus* on PDA.

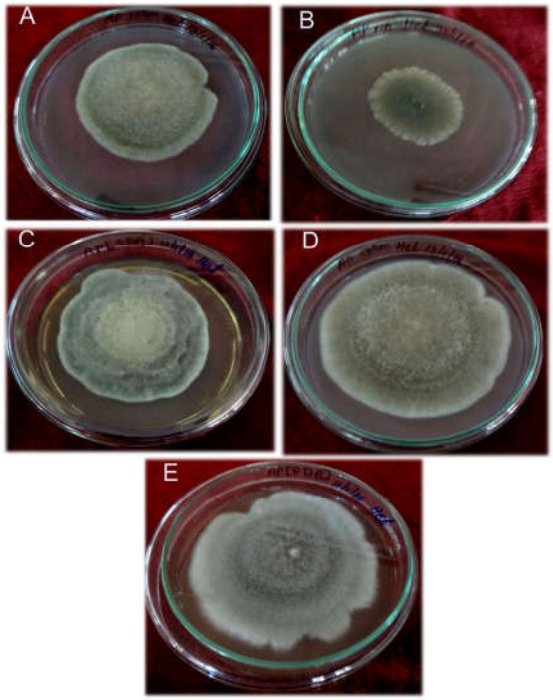

Fig. 3. Growth of *Helminthosporium* on all the five media. A. *Helminthosporium* on CZ, B. *Helminthosporium* on NA, C. *Helminthosporium* on SDA, D. *Helminthosporium* on CMA, E. *Helminthosporium* on PDA.

Fig. 4. Growth of *Penicillium* on all the five media. A. *Penicillium* on SDA, B. *Penicillium* on CZ, C. *Penicillium* on NA, D. *Penicillium* on PDA, E. *Penicillium* on CMA.

to be white with grey spores at the centre on PDA, grey with white spores at the centre on SDA, dull white on CMA, whitish green shade on CZ and brown on NA.

The present study shows mark differences in the sporulation pattern of the four fungi on all the five culture media used. *Aspergillusflavus* and *Helminthosporium* showed heavy sporulation on SDA, moderate on PDA and CMA while poor on CZ.*Penicillium* heavily sporulated on PDA and CZ and moderately on SDA and CMA. *Alternariaricini* revealed heavy sporulation on PDA and CMA, moderate on SDA and poor on CZ. The sporulation was poor in NA of all these four fungi.

Okunowo *et al*. (2010) observed least sporulation and minimum mycelia growth of *Myrotheciumroridum* on CZapek's Dox Agar which may be due to the presence of chloride ion in the test medium. Several workers have realized the importance of reproductive structures for inoculums productionand 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 colonial growth and sporulation of Phomaexigua (Zhae and Simon, 2006). Several workers stated PDA to be the best media for mycelia growth (Xu *et al*., 1984; Maheshwari *et al*., 1999; Saha *et al* 2008).

Table No. 1 Patterns of fungal growth observed on different culture media

Fungi	Media type	Colony diameter (cm)	Colony Characters			Sporulation
			Texture	Surface Color	Reverse Color	
Aspergillusflavus	PDA	9.0	Fine	Green	Light yellow	Moderate
	SDA	9.0	Fine	Green	Light yellow	Heavy
	CMA	9.0	Fine	Green with white spores	Light yellow	Moderate
	CZ	7.3	Fine	Green with yellow at centre	Light Cream	Poor
	NA	6.2	Fine	Yellow	Colorless	Poor
Penicilliumsp	PDA	3.0	Velvety	Dark green	Black	Heavy
	SDA	3.1	Velvety	Greenish grey	Black	Moderate
	CMA	3.5	Velvety	Dark green	Black	Heavy
	CZ	1.6	Thick velvety	Light green	Black	Moderate
	NA	1.7	Thick velvety	Dark green	Black	Poor
Alternariaricini	PDA	7.5	Velvety	Dark green	Black	Heavy
	SDA	5.7	Fine	Dark green	Black	Moderate
	CMA	7.5	Fine	Dark green	Black	Heavy
	CZ	4.0	Velvety	yellow with dark green at centre	Dark green	Poor
	NA	2.5	Fine	Dark grey	Black	Poor
Helminthosporiumsp	PDA	6.1	Velvety	Whitish grey	Dark brown	Moderate
	SDA	4.3	Thick velvety	Greyish white	Dark brown	Heavy
	CMA	5.5	Velvety	Dull white	Dark Brown	Moderate
	CZ	3.9	Fine	Whitish green	Colorless	Poor
	NA	3.0	Fine	Brown	Dark Brown	Poor

In the present study difference in the surface and reverse color of the fungal colonies was found to be distinct on the five culture media. as observed in *Aspergillusflavus* pale green with whitish surrounding on PDA, SDA and CMA while pale green with yellow spores on CZ and Creamish on NA on surface view, whereas, on reverse side the color was observed to be creamish. In *Alternariaricini*, the surface color was appeared to be dark green on PDA, SDA and CMA while dull yellow with dark green at the centre on CZ and black on NA. In *Penicillium* the surface color was viewed to be dark green on PDA, CMA and NA and light green on CZ and greenish grey on SDA while on each of the five media the color on the reverse side was black. In *Helminthosporium* sp., the surface color was observed

Most fungi thrive on PDA, but this can be too rich in nutrients, thus encouraging the mycelia growth with ultimate loss of sporulation (UKNCC, 1998).

CONCLUSION

The present study concludes that although each of the media supported the growth of each of the selected four fungi, PDA has proved to be the best followed by CMA and SDA. These media can be successfully used for the mass production of the major fungi.

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