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Influence of different culture media on growth of plant pathogenic fungi

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Abstract
Fungi from a large and heterogenous, eukaryotic group of living organisms characterized by their lack of photosynthetic pigment and their chitinous cellwall. Among these some are the pathogenic to the higher plants. The pathogenic fungi showed variation in the growth and development, when grown on various nutrient media. In the present investigation, the growth has been studied in various media viz. Potato Dextrose Agar (PDA); Czapek's Dox Agar (CZA); Corn Agar (CA); Nutrient Agar (NA); Sabouraud Agar (SA). The Potato Dextrose Agar medium was most commonly used in the laboratory for fungal isolation due to its good and balanced nutrient contents. The best growth fungal isolates of present investigation was recorded on Potato Dextrose Agar and Corn Agar media, the optimum growth on Czapek's Dox Agar media and minimum growth on Nutrient Agar media.

Keywords: plant pathogenic fungi, nutrient media, growth, agar, isolation, investigation

1. Introduction
Fungal kingdom contains more than 1.5 million species, but only around 1,00,000 has so far been described, with yeast, mold and mushroom being the most familiar (Hawksworth DL, 1991) [3]. A number of them are parasitic in order to complete their biological cycle, animals or plants, with around 15,000 of them causing disease in plants, the majority belonging to the Ascomycetes and Basidomyces (Reddy SM, 2001) [9].
In laboratory fungi are isolated on specific culture medium for cultivation, preservation, microscopical examination and biochemical and physiological characterization (Sharma G. and Pandey RR, 2010) [11].
The fungus showed variation in growth, when grow on various nutrient media. In the present study the five different culture media were used for their growth. The fungi showed the vegetative growth and colony morphology with pigmentation and sporulation depending up on the composition of specific culture medium, pH, temperature, light, humidity and surrounding atmospheric gas mixture (Northholt and Bullerman 1982; Kuhn and Ghannoum 2003; Kumara and Rawal 2008) [8, 5].
In the present study an attempt was made to evaluate six fungi isolated from different host for their growth on different culture media viz. *Rhizoctonia solani* (from Bajra), *Uromyces appendiculatus* (from Moong) *Cercospora beticola* and *Aspergillus fumigatus* (from Sugarbeet), *Alternaria alternata* (from Sissoo) and *Alternaria helianthi* (from Sunflower) were used in this study.

2. Materials and Methods
Infected plant materials were cut into small pieces and surface sterilized by using 0.1% Mercuric chloride ($HgCl_2$) solution for about 10 minutes. Sterilized materials were thoroughly washed with sterilized distilled water to get free from disinfecting solutions. Surface sterilized pieces were placed in moist chamber by using enrichment techniques. After 48 to 78 hrs they were transferred aseptically on the sterilized Potato Dextrose Agar (PDA) in petriplates. The petriplates were then incubated at 28 ±2°c temperature. Repeated sub culturing was practiced to obtain pure fungal culture.

2.1 Maintenance and Preservation of Cultures:
The isolated cultures were preserved on PDA slant at 4°c temperature. When needed they were grown on PDA at 28±2°c. The seven days old cultures were used for further experimentation.

2.2 Preparation of Media
The five different culture media were prepared by using their composition for the study of seven different isolates.
1. Potato Dextrose Agar (PDA) [Potato peeled 200g; Dextrose 20g; Agar 20g;Distilled water 1L].
2. Nutrient Agar (NA) [Pentose 5g; $NaCl_2$ 5g; Beef extract 1.5 g; Yeast extract 1.5 g; Agar 20g; Distilled water 1L].
3. Sabouraud Agar (SA) [Dextrose 40g; peptone 10g; Agar 20g; Distilled water 1L).
4. Czapek's Dox Agar (CZA) [NaNo3 3g; K2HPO4 1g; MgSo4 o.5g; KCL 0.5g; FeSO4 o.o1g; sucrose 30g; Agar 15g; Distilled water 1L].
5. Corn Agar (CA) [Corn grains 200g; Dextrose 20g; Agar 20g; Distilled water1L].

The pH of the test media was maintained at 5.5 being optimal for the growth and sporulation.

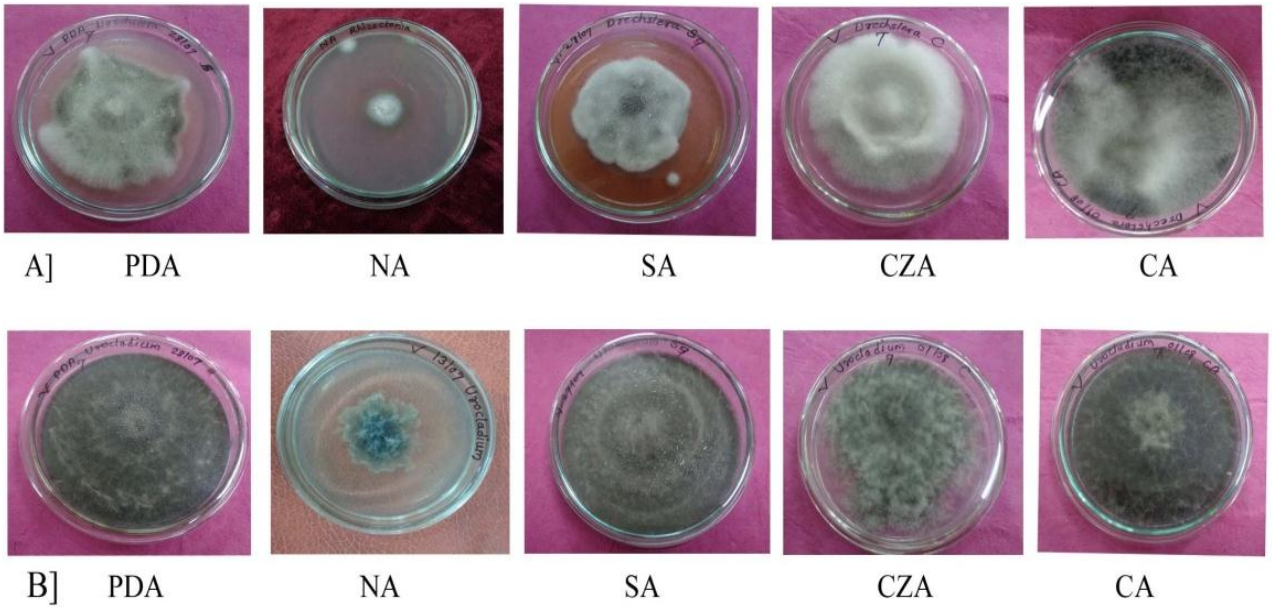
2.3 Preparation of Inoculums
The seven days old cultures were used by taking 5mm diameter disc for growing on the five different culture media for study its radial growth along with morphological characters in triplicates.
The petriplates were then incubated for seven days at 25±2°c in a BOD incubator and colony characters of each fungus was recorded, sporulation was assessed on glass slides by mounting a small portion of mycelia in Lacto-phenol-Cotton blue stain and observed under microscope.

3. Result and Discussion
All five culture media supported the growth of test fungi to

various degrees. Out of them, four fungi showed maximum mycelia growth on PDA after 7 days of incubation period (Table no.1), while *Rhizoctonia solani* (91.3±0.3) and *Alternaria alternata* (72.0±1.3) exhibited higher colony growth on CA. *Uromyces appendiculatus* (93.3±0.3) and *Aspergillus fumigatus* (68.6±0.6) showed maximum growth on PDA medium. *Alternaria helianthi* (47.0±0.7) exhibited higher growth on CZA, while *Cercospora beticola* (52.0±1.4) showed same growth on PDA, SA, and CZA. All six tested fungi exhibited minimum mycelia growth on NA, however majority of tested fungi exhibited maximum mycelial growth on PDA and minimum on NA.

Table 1: Mycelial growth, colony characters and sporulation pattern of fungal isolates on five culture media

Fungi	Media type	Colony dia. in mm	Colony character				Sporulation
			Texture	Surface color	Reverse color	Growth	
R. solani	PDA	72.3±0.4	Thick cottony	Blackish white	Black	Normal	Moderate
	NA	40.0±0.2	Thin velvety	White	Blackish white	Very slow	Poor
	SA	63.3±0.3	Cottony	White	Black	Normal	Poor
	CZA	82.6±o.5	Fine cottony	White	Pale black	Normal	Moderate
	CA	91.3±0.3	Cottony	Whitish black	Black	Fast	Moderate
U.appendiculatus	PDA	93.3±0.3	Fine cottony	Blackish	Bluish black	Very fast	Heavy
	NA	46.0±0.2	Cottony	Blackish white	Black	Very slow	Poor
	SA	90.0±0.3	Fine cottony	Black	Black	Fast	Heavy
	CZA	89.0±0.4	Fine cottony	Whitish black	Black	Normal	Moderate
	CA	90.0±0.3	Cottony	Black	Bluish black	Fast	Heavy
C. beticola	PDA	52.0±1.4	Cottony	White	Dark black	Normal	Moderate
	NA	32.6±0.5	Cottony	White	Pale black	Very slow	Poor
	SA	53.3±0.6	Cottony	White	Dark black	Normal	Moderate
	CZA	52.0±1.2	Velvety in circle	Grey	Black	Normal	Poor
	CA	51.0±1.3	Velvety cottony	Blackish white	Black	Normal	Poor
A. fumigatus	PDA	68.6±0.6	Floccose	Yellowish green	Pale yellow	Fast	Heavy
	NA	50.0±1.2	Powdery	Yellowish white	Yellowish	Slow	Poor
	SA	64.7±0.5	Floccose	Yellowish grey	Yellow	Normal	Moderate
	CZA	52.3±0.3	Powdery velvety	Yellowish white	Pinkish white	Slow	Poor
	CA	64.3±0.3	Powdery	Grey	Greenish	Normal	Poor
A. alternata	PDA	63.6±0.6	Velvety	Blackish	Black	Normal	Heavy
	NA	28.0±1.2	Powdery	Brown	Blackish brown	Very slow	Poor
	SA	39.3±0.3	Velvety	Black	Black	Slow	Moderate
	CZA	47.0±0.9	Velvety	whitish yellow	Purple black	Normal	Heavy
	CA	72.0±1.3	Fine cottony	Whitish black	Brownish black	Fast	Moderate
A. helianthi	PDA	40.0±0.2	Fine velvety	Blackish brown	Black	Normal	Heavy
	NA	27.0±1.2	Fine powdery	Brown	Brown	Very slow	Poor
	SA	37.3±0.3	Velvety	Whitish black	Black	Slow	Moderate
	CZA	47.0±0.7	Fine velvety	Whitish black	Purple black	Fast	Heavy
	CA	43.3±0.3	Powdery	Brownish black	Brownish black	Normal	Moderate



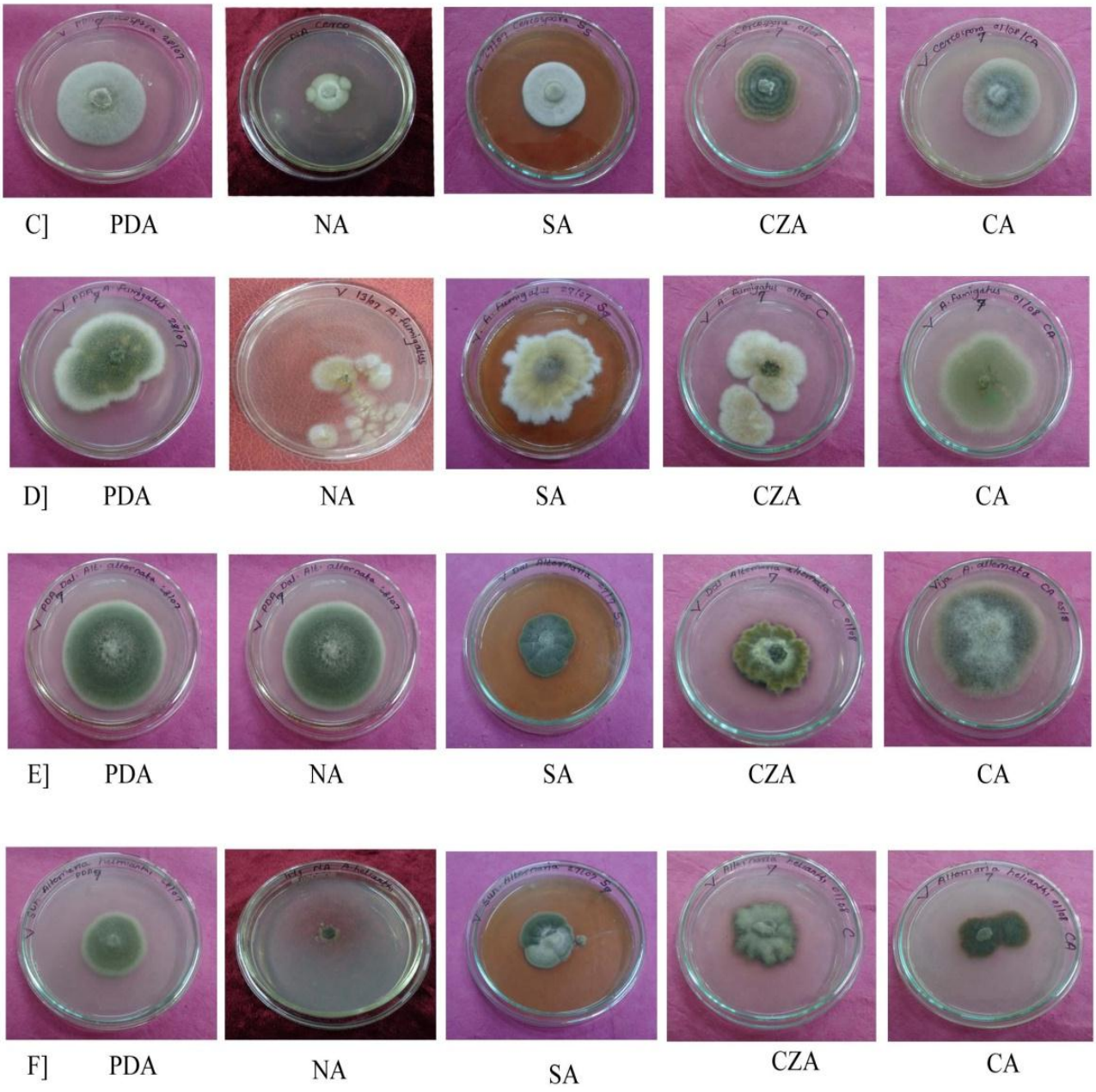


Fig 1: Colony growth and color : A) *Rhizoctonia solani*, B) *Uromyces appendiculalus*, C) *Cercospora beticola* D) *Aspergillus fumigatus*, E) *Alternaria alternata*, F) *Alternaria helianthi*

In the present study, the difference in surface and reverse coloration of fungal colonies was distinct on three growth media as observed in case of *Aspergillus fumigatus* (yellowish green in PDA, yellowish white in NA and CZA, yellowish grey in SA, grey in CA) and *Alternaria alternata* (blackish in PDA, brown in NA, black in SA, whitish yellow in CZA, and whitish black in CA). The difference in texture was observed in case of *Aspergillus fumigatus* (floccose in PDA and SA, powdery in NA and CA, powdery velvety in CZA) and *Alternaria helianthi* (fine velvety in PDA and CZA, fine powdery in NA, velvety in SA and powdery in CA).

In case of sporulation, our findings revealed that five culture media showed differences in sporulation pattern. The all six fungi exhibited heavy and moderate sporulation in PDA and the poor was observed in NA. Type of culture media and their chemical composition significantly affected the mycelia growth rate and conidial production of different fungi isolated from decaying vegetable wastes. (G. Sharma and R.R Pandey 2010) [11].

PDA is one of the most commonly used culture media because of its formulation and ability to support mycelia growth of wide range of fungi. Several workers started PDA to be the best media for mycelia growth (Maheshwari *et al.*, Saha *et al.*, 2008). Hence the PDA media is best for the growth and sporulation of fungi as compare to other media.

4. Conclusion

Our findings revealed that culture media differentially influenced the growth, sporulation and colony characters of the test fungi. It is concluded that out of five test media employed in the present study, PDA was found to be most suitable for heavy sporulation and higher mycelia growth, while SA and CA was also effective for better growth and sporulation of fungi as compare to CZA and NA. 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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