COMPARATIVE GROWTH PATTERN STUDIES OF *ASPERGILLUS* SPECIES ON VARIOUS SOLID AGAR MEDIA

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Abstract:

The aim of the present work is to study the comparative growth patterns of *Aspergillus* species grown on various solid media. In order to understand growth and physiological behaviour, all the species of *Aspergillus* were grown on the Potato Dextrose Agar, Capex's Dox Agar and Glucose Nitrate Agar solid media. This has helped to understand and classify the different group of *Aspergillus* occurring on different seeds. Regarding morphology of the colony it was found that *A. flavus* produced zonate growth on PDA and GNA and zonation was absent on CZA. While, *A. ornatus* shows zonate of growth on all the three media. Similarly all other species produced growth with zonation (smooth and uniform) irrespective of medium.

Key Words: *Aspergillus* species, Growth patterns, Plant seeds, Solid media

INTRODUCTION:

14 species of Aspergillus were isolated from different crops were grown on three commonly used solid media, GNA, PDA, CZA for comparative growth. 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). 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. filamentous fungi, where significant morphological and physiological variations exist (Meletiadis et al., 2001). Hence, the present study was undertaken to observe the influence of three different culture media on the mycelial growth, colony characters and sporulation patterns of fourteen dominant fungi isolated from stored plant seeds.

METHODS AND MATERIALS:

Isolation of *Aspergilli*

1) Collection of Seed Samples:

The methods described by Neergaard (1973) have been adopted for the collection of seed samples. Accordingly seed samples were collected from field, store houses and market places and from farmers. A composite sample was prepared by mixing the individual sample together, preserved in cloth bags at room temperature during the studies.

2) Detection of Seed Mycoflora:

The procedure for blotter test and agar plate methods was followed as described by International Seed Testing Association, ISTA (1966) De Tempe (1970), Neergaard (1973) and Agarwal (1976).

i) Blotter Test Method:

A pair of white blotter paper of 8.5 cm diameter was jointly soaked in sterile distilled water, placed in pre-sterilized corning Petriplates of 10 cm diameter. Ten seeds per plate were placed at equal distance on the

moist blotters. One hundred seeds were tested for each treatment. The plates were incubated at 25±2^{0}C under diurnal condition. On 7th day the seeds were examined under stereoscopic microscope for the preliminary determination of *Aspergillus.* Identification and further fungi occurred on seeds was made by preparing slides of the fungal growth and observing under compound microscope.

ii) Agar Plate Method:

In this method, pre-sterilized corning glass Petri-plates of 10 cm diameter were poured with 25 ml of autoclaved Potato Dextrose Agar (PDA) medium. On cooling the medium, 10 seeds per plate were equispaced aseptically; incubation condition and other details were same as described for the blotter test method.

In order to isolate only internal seed mycoflora, seeds were pre-sterilized with 0.1% solution of mercuric chloride for 1 minute. Subsequently, thoroughly washed twice with sterile distilled water and placed on agar plates. Seeds without any such pre-treatments were employed for the total seed mycoflora (control).

3) Composition of Media used for Growth Pattern of *Aspergillus* Sp.*:*

Three different growth media are used namely Glucose Nitrate Agar, Potato Dextrose Agar and Capex's Dox Agar (CZA.

i) Potato Dextrose Agar (PDA):

200g peeled potatoes were boiled until soft and passed through muslin cloth, 20 g of dextrose was added to it and final solution was made up to 1000 ml. In this 20 g of agar was added, pH of the medium was adjusted to 5.6.

ii) Capex's Dox Agar (CZA):

Sucrose 15 g, $NaNO_3$ 2 g, K_2HPO_4 0.1 g, M_gSO_4 0.5 g, Agar 20 g and distilled water 1000 ml, pH 5.6 was adjusted.

iii) Glucose Nitrate Agar (GNA):

Glucose 10 g, KNO_3 2.5 g, K_2HPO_4 0.1 g, M_gSO_4 0.5 g, Agar 20 g and distilled water 1000 ml, pH 5.6 was adjusted.

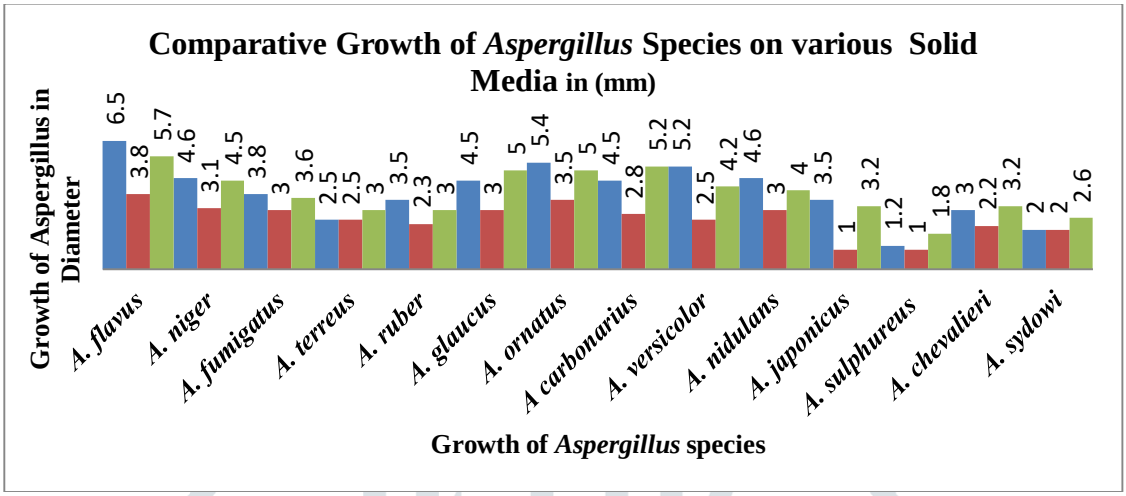
Results and Discussions:

14 species of Aspergillus were isolated from different crops were grown on three commonly used solid media, GNA, PDA, CZA for comparative growth. The observations were made on the 7th day of incubation at room temperature. Results are given in table no -01

Table 1: Comparative Growth of *Aspergillus* Species on various Solid Agar Media

Sr. No.	*Aspergillus* sp.	Growth Diameter (mm)		
		PDA	CZA	GNA
1	*A. flavus*	6.5	3.8	5.7
2	*A. niger*	4.6	3.1	4.5
3	*A. fumigatus*	3.8	3.0	3.6
4	*A. terreus*	2.5	2.5	3.0
5	*A. ruber*	3.5	2.3	3.0
6	*A. glaucus*	4.5	3.0	5.0
7	*A. ornatus*	5.4	3.5	5.0
8	*A carbonarius*	4.5	2.8	5.2
9	*A. versicolor*	5.2	2.5	4.2
10	*A. nidulans*	4.6	3.0	4.0

11	*A. japonicus*	3.5	1.0	3.2
12	*A. sulphureus*	1.2	1.0	1.8
13	A. *chevalieri*	3.0	2.2	3.2
14	*A. sydowi*	2.0	2.0	2.6



Sr. No.	*Aspergillus* sp.	Growth Patterns of *Aspergillus* Sp.		
		PDA	CZA	GNA
1	*A. flavus*	Zonate	Smooth	Zonate
2	*A. niger*	Zonate	Zonate	Smooth
3	*A. fumigatus*	Smooth	Smooth	Smooth
4	*A. terreus*	Smooth	Smooth	Smooth
5	*A. ruber*	Smooth	Smooth	Smooth
6	*A. glaucus*	Smooth	Smooth	Smooth
7	*A. ornatus*	Zonate	Zonate	Zonate
8	*A carbonarius*	Smooth	Smooth	Smooth
9	*A. versicolor*	Smooth	Smooth	Smooth
10	*A. nidulans*	Smooth	Smooth	Smooth
11	*A. japonicus*	Smooth	Smooth	Smooth
12	*A. sulphureus*	Smooth	Smooth	Smooth
13	A. *chevalieri*	Smooth	Smooth	Smooth
14	*A. sydowi*	Smooth	Smooth	Smooth

Fig No-1 *A. niger*

Fig No-2 *A. ornatus*

Fig No-3 *A. ruber*

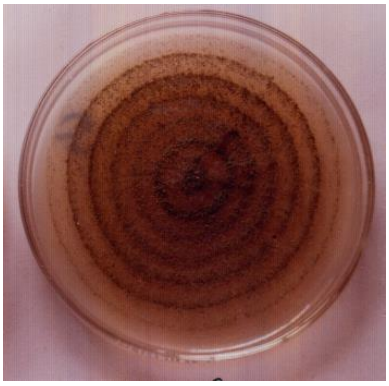


Fig No-4 *A. flavus*

Fig No-5 *A. glaucus*

It is clear from the data summarised in **(Table No, 1).** The species of *Aspergillus* vary in their growth on different media growth on PDA was found to be maximum in *A. flavus* followed by *A. ornatus*, *A. versicolor*, *A. niger* and *A. glaucus*, while it was very scanty in case of *A. sydowi* and *A. terreus*.

Growth of *Aspergillus* sp. on CZA was found to be uniformly less than on PDA. While growth of all the species on GNA was with more or less similar to that on PDA. However, *A. fumigatus*, *A. sulphureus* and *A. sydowi* showed improvement in growth on GNA as compared to PDA. Regarding morphology of the colony it was found that *A. flavus* produced zonate growth on PDA and GNA and zonation was absent on CZA. While, *A. ornatus* shows zonate of growth on all the three media. Similarly all other species produced growth with zonation (smooth and uniform) irrespective of medium (Figure no. 1,2,3,4,5)

Growth on different media growth on PDA was found to be maximum in *A. flavus* followed by *A. ornatus*, *A. versicolor*, *A. niger* and *A. glaucus*, while it was very scanty in case of *A. sydowi* and *A. terreus*.

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