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Studies on cultural and morphological characters of black mould of onion caused by *Aspergillus niger* (Van Tiegh.)

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Abstract
Cultural and morphological studies of black mould of onion caused by *Aspergillus niger* on different media revealed that there were variations in its colony characters, shape, growth and morphological characters. Richard's agar medium shown maximum mycelial growth (90 mm) and sporulation (+++++), Conn's agar medium shown mycelial growth (89.63) and sporulation (+++++) and Potato dextrose agar supported best mycelial growth (88.60) and sporulation (+++++) of *Aspergillus niger*. However, the large sized conidiophore were produced on the Richard's agar which was followed by others. However, small sized conidia were noticed on Tap water agar and Ashbys agar. The maximum average conidiophore length was recorded on Richard's agar while minimum setae width was observed on Ashby's agar.

Keywords: Morphological, cultural characters, *Aspergillus niger*

Introduction
Onion (*Allium cepa* L.) is one of the most important commercial vegetable crop grown in India. Popularly it is also known as 'Queen of kitchen.' It belongs to the family *Liliaceae*. Onion is an important underground vegetable bulb crop of tropical and sub-tropical countries (Thompson and Kelly, 1979) [8]. The onion originates from the region comprising of North West India, Afghanistan, the Soviet Republics of Tajik and Uzbek, and Western Tien Shan. According to Vavilov (1951) the primary centre of origin lies in Central Asia. The near east and Mediterranean is secondary centre of origin. The genus *Allium* is very large comprising of more than 500 spp. usually perennial bulbous plants. Out of these, *Allium cepa* is the major cultivated species grown all over the world. Among post-harvest diseases of onion, black mould rot caused by *Aspergillus niger* is the predominant one (Rajam 1982) [6]. Black mould rot is most destructive disease of onion in storage as well as in field conditions. Generally this disease occurs more in white onions as compared to red one because of less phenolic contents. It has been estimated that an average of 20–30% of crop yield is lost annually from the field. In the present investigation an attempt was made to study the morphological and cultural characters of *Aspergillus niger*.

Material and Method
Media
To find out the most suitable medium for cultural and morphological characters of test pathogen total ten media were studied.

Composition of Media
1. **Potato Dextrose Agar (PDA):** Peeled Potatoes (200 g), Agar (20 g), Dextrose (20 g), Distilled water (1000 ml).
2. **Conn's agar:** Potassium nitrate (2 g), Magnesium sulphate (1.20 g), Monopotassium phosphate (2.70 g), Maltos (7.20 g), Potato starch (10 g), Agar (15 g)
3. **Richard's agar:** Sucrose (50 g), Potassium dihydrogen phosphate (5 g), Potassium nitrate (10 g), Magnesium sulphate (2.5 g), Ferric chloride (0.02 g), Agar (20 g), Distilled water (1000 ml).
4. **Peptone glucose agar:** Glucose (5 g), Peptone (10 g), Agar (15 g), Distilled water (1000 ml)

5. **Ashby's agar:** Manitol (20 g), Dipotassium phosphate (0.2 g), Magnesium sulphate (0.2 g), Sodium chloride (0.2 g), Potassium sulphate (0.1g), Calcium carbonate (5 g), Agar (15 g), Distilled water (1000ml).
6. **Tap water agar:** Agar (15g), Tap water (1000 ml)
7. **Czapek's dox agar:** Sodium nitrate (3.0 g), Potassium dihydrogen phosphate (0.5 g), Magnesium sulphate (0.5 g), Potassium chloride (0.5 g), Ferrous sulphate (0.01g), Sucrose (20.0 g), Distilled water (1000ml)
8. **Oat meal agar:** Oat flakes (30 g), Agar (20 g), Distilled water (1000ml)
9. **Plain agar extract:** Dextrose (20 g), Agar (20 g), Distilled water (1000ml)
10. **Host extract agar:** Onion bulb (200 g), Agar (20 g), Distilled water (1000ml)

20 ml of each medium listed above was poured into 90 mm diameter Petri plate. After solidification, 5 mm disc of *Aspergillus niger* from actively growing culture were cut using a cork borer and a single disc was placed upside down at the centre of petri dish. Each set of experimental was replicated thrice and the plates were incubated at 27 ± 1 ºC. The measurement of colony diameter was taken when the maximum growth was attained in any of the media tested. The cultural characteristics *viz*., colony colour, colony texture and sporulation were also recorded 15 days of incubation. The morphological characteristics of *viz*., length of conidiophore, breadth of conidiophore, size of conidiophore, length of conidia and width of conidia were recorded by using binocular microscope, stage micrometer and compound microscope.

Results and Discussion

Effect of different media in supporting the cultural growth of pathogen

To find out the best medium, ten different solid media were selected to study their effects on the cultural growth and sporulation of *A. niger*. The results obtained are presented in Table 1. Significantly highest cultural growth was recorded in Richard's agar (90 mm) the second best media was reported Conn's agar medium (89.63 mm) and both are at par with each other. In case of, Potato dextrose agar and Oat meal agar the cultural growth were (88.60 mm) and (86.73 mm), respectively. Followed by Peptone glucose agar and Host extract agar (84.67 mm and 82.56 mm). Significantly lowest cultural growth was recorded in Ashby's agar and Tap water agar medium (16.67 mm) and (24.33 mm), respectively. Richard's agar performed better than other media. The difference in performance is probably due to the fact that it contains sucrose and minerals that have been identified as vital for *A. niger* growth.

Similar result in case of *A. niger* was also noted by Pathak (1993) [5]. He observed maximum growth of *A. niger* on Richard's solution followed by PDA medium. Shashikala and Krishnamurthy (2005) also observed excellent mycelial growth of *A. flavus* on Czapek's dox agar medium. Further Cabrera *et al.*, (2005) noted highest mycelial growth of *A. niger* on Czapek's yeast extract medium after seven days of inoculation. Narsimha *et al.*, (2006) [4] reported that Czapek's dox medium (410 mg) supported maximum mycelial growth of *Aspergillus niger* after two weeks of incubation. Amrita and Richa (2014) [1] also reported that *Aspergillus niger* showed the highest growth in Richards's broth followed by Potato dextrose agar and Yeast extract agar.

Colony Colour

The colours of the test fungal colonies were observed. The observations on colony pigmentation were taken on 8th day after incubation. The data from table revealed that the cultures were initially whitish in colour, later on it became dark black in colour. On potato dextrose agar, it produced brownish black in centre, while on Conn's agar colonies were first light yellow colour then black. On Richard's agar colonies were found brown to black in centre. On Peptone glucose agar medium the test fungal colonies were black in colour. On Tap water agar, it produced colonies which were thick black colour in centre. On Ashby's agar, it produced brown to yellow green coloured colonies of test fungus. On Czapek's dox agar, it exhibit white mycelial mat at periphery and centre brown in colour. On Oat meal agar, it produced colony which was initially white then dark brown to black, while Plain agar extract, it showed colonies which were grayish black in centre and Host extract agar, it produced white mycelial mat at periphery and brown in a centre.

The present result are supported by the earlier observations of Pathak (1993) [5] where he noted the luxuriant brown to black or carbon black, grayish black growth on Richard's agar. Growth on synthetic media was sub merged and sparse. Disalvo (2000) [2] also reported that culturally *Aspergillus species* require 1-3 weeks for growth. The colonies begin as a white dense mycelium which later assume a variety of colours according to the species based on the colour of their conidia.

Sporulation Characteristics

All the ten culture media tested exhibited a wide range of sporulation of the test pathogen.

Table 1: Growth characteristics of *A. niger* on different solid media

Sr. No.	Medium	Colony* Diameter (mm)	Sporulation**	Growth characters
1.	Potato Dextrose Agar	88.60	+++++	Colony compact, circular, thick, white mycelial mat at periphery, brownish black in center.
2.	Conn's Agar	89.63	+++++	Colonies are first light yellow coloured then black and luxuriant growth.
3.	Richard Agar	90.00	+++++	Colony compact, circular, thick, brown to black in center.
4.	Peptone Glucose Agar	84.67	++++	Texture dense velvety, mycelium entirely covered by black spore.
5.	Tap Water Agar	16.67	+	Colony compact, thick black colour in center.
6.	Ashbys Agar	11.33	+	Colonies are brown to yellow green coloured.
7.	Czapek's Dox Agar	74.76	+++	Colony loose, circular, thin, white mycelial mat at periphery, center brown.
8.	Oat Meal Agar	86.73	++++	Colony first white then dark brown to black. Exudates absent, reverse cream-coloured to light brown.
9.	Plain Agar Extract	24.33	++	Colony loose, circular, thick, grayish black in center.
10.	Host Extract Agar	82.56	+++	Colony loose, not circular, thin, white mycelial mat at periphery, brown in a center.
	SE±	0.56		
	C.D. @ 0.01	1.56		

* Average of three replications.

** + = Trace; ++ = Poor; +++ = Fair; ++++ = Good; +++++ = Abundant.

However, excellent sporulations (+++++) were induced on Richard's agar, Conn's agar and Potato dextrose agar whereas good sporulations (++++) were recorded on Peptone glucose agar and Oat meal agar. While, fair sporulations (+++) was recorded on Czapek's dox agar and Host extract agar. Poor sporulations (++) were observed on Plain agar extract media and Trace sporulations (+) observed on Tap water agar and Ashby's agar medium.

The observations of cultural studies of present investigations resembled with earlier reports of Xu *et al.,* (1984) [10]; Maheshwari *et al.,* (1999) [3] and Saha *et al.,* (2008) [7]. Several workers stated PDA to be the best medium for mycelial growth.

Morphological characteristics of *Aspergillus niger* on different media

Results depicted in Table. 2 that *Aspergillus niger* exhibited a wide range of variability in respect of length of conidia, width of conidia, length of conidiophore, breadth of conidiophore and size of conidiophore.

Conidial dimensions

From the data presented in Table 2, conidial size of *Aspergillus niger* was ranged from 17.40 µm to 27.42 µm. However, large size conidia were produced on the Richard's agar (25.53-27.42×3.06-4.84µm). It was followed by Conn's agar (25.38-26.24×3.90-4.36µm) and Potato dextrose agar (24.08-25.80×3.40-4.85µm), Oat meal agar (23.71-25.62×3.75-4.01µm), Peptone glucose agar (21.64-24.60×3.20-4.01µm), Host extract agar (20.50-23.00×3.70-5.60µm), Czapek's dox agar (20.70-22.29×3.80-5.76 µm) and Plain agar extract (19.13-21.61×3.32-5.31µm). Comparatively, small conidial length were noticed on Ashby's agar (17.40-20.23×3.81-5.02 µm) and Tap water agar (18.13-21.51×3.90-4.25µm) of *Aspergillus niger*.

Table 2: Morphological characteristics of *A. niger* on different solid media

Sr. No.	Media	Conidial dimensions		Dimensions of conidiophore		
		Length (µm)*	Width (µm)*	Length (µm)*	Breadth (µm)*	Size (µm)*
1	Potato dextrose agar	24.08-25.80µm	3.40-4.85µm	1005-1230µm	14.40-15.60 µm	4.00-5.50 µm
2	Conn's agar	25.38-26.24µm	3.90-4.36µm	1246-1425µm	15.00-16.00 µm	4.20-5.50 µm
3	Richard's agar	25.53-27.42µm	3.06-4.84µm	1309-1561µm	15.06-16.20 µm	3.75-5.60 µm
4	Peptone glucose agar	21.64-24.60µm	3.20-4.01µm	988-1120 µm	14.10-15.10 µm	3.05-4.50 µm
5	Tap water agar	18.13-21.51µm	3.90-4.25µm	790-995µm	11.70-14.56 µm	2.11-3.06 µm
6	Ashby's agar	17.40-20.23µm	3.81-5.02µm	855-957µm	12.65-14.02 µm	2.70-3.01µm
7	Czapek's dox agar	20.70-22.29µm	3.80-5.76µm	868-1070µm	11.40-15.00 µm	2.50-3.25µm
8	Oat meal agar	23.71-25.62µm	3.75-4.01µm	1002-1168µm	13.25-15.11µm	3.60-4.50 µm
9	Plain agar extract	19.13-21.61µm	3.32-5.31µm	910-1069µm	12.73-14.68 µm	2.10-3.15µm
10	Host extract agar	20.50-23.00µm	3.70-5.60µm	927-1119µm	13.90-15.01µm	2.43-3.70µm

*: Average of three replications

Dimensions of conidiophores

Conidiophore length

From the data presented in Table 2, conidiophore length of *Aspergillus niger* was ranged from 855 µm to 1561 µm. However, large length conidiophore were produced on the Richard's agar (1309-1561µm), followed by Conn's agar (1246-1425µm) and Potato dextrose agar (1005-1230 µm), Oat meal agar (1002-1168 µm), Peptone glucose agar (988-1120 µm), Host extract agar (927-1119 µm), Czapek's dox agar (868-1070 µm) and Plain agar extract (910-1069µm). Comparatively small conidiophore length were noticed on Ashby's agar (855-0957µm) and Tap water agar (970-995µm) of *Aspergillus niger.*

Conidiophore breadth

From the data presented in Table 2, conidiophore breadth of *Aspergillus niger* was ranged from 11.40 µm to 16.20 µm. However, large breadth of conidiophore were produced on the Richard's agar (15.06-16.20µm) followed by Conn's agar (15.00-16.00µm) and Potato dextrose agar (14.40-15.60µm), Oat meal agar (13.25-15.11µm), Peptone glucose agar (14.10-15.10µm), Host extract agar (13.90-15.01µm), Czapek's dox agar (11.40-15.00µm) and Plain agar extract (12.73-14.68µm). Comparatively small conidiophore breadth of *Aspergillus niger* were noticed on Ashby's agar (12.65-14.02µm) and Tap water agar (11.70-14.56 µm).

Conidiophore size

From the data presented in Table 2, conidiophore size of *Aspergillus niger* was ranged from 2.10 µm to 5.60 µm. However, large conidiophore size were produced on the Richard's agar (3.75-5.60µm). It was followed by Conn's agar (4.20-5.50µm) and Potato dextrose agar (4.00-5.50µm), Oat meal agar (3.60-4.50µm), Peptone glucose agar (3.05-4.50µm), Host extract agar (2.43-3.70µm), Czapek's dox agar (2.50-3.25µm) and Plain agar extract (2.10-3.15µm). Comparatively small conidiophore size of *Aspergillus niger* were noticed on Ashby's agar (2.70-3.01µm) and Tap water agar (2.11-3.06µm).

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