Morphological and cultural characters of fungi isolated from copra and copra oil

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ABSTRACT : Copra samples were colllected from local farmers orchard at Dapoli, Ta. Dapoli, Dist. Ratnagiri (MS). From these collected samples, five fungus pathogens *viz.*, *Aspergillus niger, A. fumigates, A. oryzae*, *Chaetomium* spp. and *Lasiodiplodiatheo bromae* were isolated, which were used for cultural studies. These were cultured on five different culture media, Potato dextrose agar medium, Malt extract agar medium, Potato carrot agar medium, Sabouraud's dextrose agar medium and Czapek's yeast extract agar media. All five media used supported the growth of these five fungi in considerable amount.

Key Words : *Aspergillus niger, A. fumigates, A. oryzae*, *Chaetomium* spp., Czapek's yeast extract agar media, *Lasiodiplodiatheo bromae,* Malt extract agar medium, Potato dextrose agar medium, Potato carrot agar medium and Sabouraud's dextrose agar medium.

Coconut and its extracted oil from copra have served man as important foods for thousands of years. Since it contains mostly saturated fatty acids it is believed to be hypercholestrolemic in action. In India, coconut is although consumed in different forms such as tender nuts, raw kernel, copra, coconut oil and desiccated coconut. The principle product of the coconut is the dried endosperm, commonly called "Copra". It is used for the production of edible oil as well as preparation of some delicious traditional food items. The general standard of quality and the keeping properties of the world's copra are decreasing considerably. A variety of factors contribute to this, mainly fungi which cause deterioration of copra in field and during storage. Diseases of coconut palm are well documented but little is known about fungi associated in deterioration of copra. Hence, an attempt was made to study the morphological and cultural characters of fungi isolated from copra and copra oil.

Materials and Methods

Isolation of fungi associated with dried copra and oil extracted from copra

The copra samples showing symptoms of infection were collected form local farmers. The copra was sliced into small pieces and these pieces were then surface sterilized in 0.1 per cent mercuric chloride for 2 minutes. They were further washed thrice in sterilized distilled water to remove the traces of mercuric chloride and aseptically transferred to sterilized Petri plates already poured with sterilized potato dextrose agar (PDA) medium. Similarly, infected oil was taken with the help of pipette and single oil drop was placed at the center of Petri plates already poured with sterilized potato dextrose agar medium without spreading. A single bit of the fungus growing on PDA was aseptically transferred on PDA slants to obtain pure culture of the associated fungus. The slants of the pure culture were preserved in the refrigerator for future use.

Morphological and cultural characters of isolated fungi

The isolated fungi were cultured on five different culture media *viz.*, Potato dextrose agar medium, Malt extract agar medium, Potato carrot agar medium, Sabouraud's dextrose agar medium and Czapek'syeast extract agar media by inoculating 1 bit of the each fungal sample on each plate separately. These were repeated for all samples thrice. The inoculated media plates were incubated at 28±2°C. The colonies initiated on second day of inoculation. Cultural observations of the colonies were taken on fourth day after inoculation. Slide preparation and microscopic observations were carried out to study the morphological characters of the isolated fungi.

Results and Discussion

Identification of isolated fungi

On the basis of cultural and morphological characters, the isolated fungi were identified as *Aspergillus niger gr., A. fumigates gr., A. oryzae (*Ahlb) Cohn, *Chaetomium* spp*., and Lasiodiplodiatheo bromae*. The identifications were confirmed by Chief Mycologist, MACS- Agharkar Research Institute, (ARI) Pune.

Morphological and Cultural characters of isolated fungi

The morphological characters of the test fungus *viz.*,

Table-A : The morphological characters of the test fungus *viz.*, mycelium, conidiophores and conidia.

Sl.	Fungi	Morphological character			
		Mycelium	Conidiophore	Vesicle	Conidia
1.	*Aspergillus fumigates*	Septate, branched	Hyaline, long erect.	Ovate to flask shaped	Globose to spherical, 2-3µm
2.	*Chaetomium* spp.	Septate	-	Peritheciaglobose	Ascospore olive brown, lemon shaped, 10-12µm.
3.	*Lasiodiplodiatheobromae*	Sepate	-	Flask shaped pycnidia	Cylindrical – subovoid, double layered, hyaline, 7.2-2.8µm.
4.	*Aspergillusoryzae*	Septate	Hyline, Long erect	Globose	Globose-pyriform, ornamented, 3.68-4.40µm.
5.	*Aspergillusniger*	Branched, septate.	Long erect,	Globose to spherical	Globose, 3-5µm.

mycelium, conidiophores, conidia, were as given in Table-A.

The mycelium of *A. fumigates* were well developed, branched and septate. The conidiophores were hyline, long erect with flask shaped vesicle. The conidia were globose about 2-3 µm in diameter. *Chaetomium* spp. produced septate mycelium with globoseperithecia. The ascospores were lemon shaped, olive brown, 10-12 µm in diameter.

Lasiodiplodiatheo bromae produced septate mycelium with flask shaped pycnidia. The conidia were subovoid, double layered on maturity, hyaline and 7.2-2.8µm in diameter. *Aspergillus oryzae* and *A. niger*both produced septate, branched mycelium with long erect conidiophores. The vesicle in *A. oryzae* was globose, in *A. niger* it was spherical to globose. The conidia in both species were globose, about 5-6×8-10 µm in *A. oryzae*and 3-5 µm in *A. niger*.

Cultural characters of isolated fungi

Growth, colony characters and sporulation of *Aspergillus niger gr., A. fumigates gr., A. oryzae (*Ahlb)Cohn, *Chaetomium* spp., and *Lasiodiplodiatheo bromae* were studied *in vitro* using different culture media *viz,*. Potato dextrose agar medium (PDA), Malt extract agar medium (MEA), Potato carrot agar medium (PCA), Sabouraud's dextrose agar medium (SDA) and Czapek's yeast extract agar medium (CYEA). The data on mean colony diameter, colony characteristics and sporulation of above fungi are presented in Table-1.

All five media supported the growth of *Aspergillus fumigates.* The best media for *A. fumigates* were PDA, CYEA and PCA followed by SDA and MEA. The colony on all five media were very slow growing with whitish blue color, flat growth and irregular margins. The diameter of colony was about 20mm on PDA, CYEA and PCA, whereas it was about 15mm on SDA and MEA.

The best medium for growth of *Chaetomium* spp. was PCA followed by PDA, CYEA, SDA and MEA. The colony on PCA was slow growing about 20mm in diameter with slightly green raised centre and white margin. On PDA it showed greenish center and whitish margin about 18mm diameter. The colony growth on other media was also slow with whitish margins and diameter about 15-18mm.

Lasiodiplodiatheo bromae showed best growth on PDA and PCA followed by SDA, MEA and CYEA. The colony on PDA was slightly raised at centre with dark sporulation and filamentous radiating margins with diameter about 99.67 mm. The colony on PCA showed submerged growth towards margin and grayish centre because of sporulation at the centre. The colony showed slightly cream color with flat sparse growth on SDA, blackish irregular margin with few aerial hyphae on MEA and irregular thin margin with grey centre on CYEA.

Table-1: Cultural characters of isolated fungi on different media.

Tr. No.	Culture media	Fungal Isolate	Radial mycelial growth (mm)*	Colony characters Color	Types of growth	Margin
1.	Potato dextrose agar medium	*Aspergillus fumigates*	22.22	Whitish Blue	Flat growth	Radiating irregular margin
		Chaetomium spp.	20.67	Greenish centre and whitemargin	Flat growth raised at centre	Regular margin
		Lasiodiplodia theobromae	99.67	Slightly white Colony with light green centre	Flat with slightly raised at centre	Filamentous radiating margin
		Aspergillus oryzae	39.29	Dark green centre and white margin	Slightly raised centre and flat margin	Regular margin
		Aspergillus niger	33.44	Black centre with white margin	Slow growing, flat growth	Regular margin
2.	Czapek's yeast extract agar medium	*Aspergillus fumigates*	22.17	Whitish blue colony	Flat growth	Slightly irregular margin
		Chaetomium spp.	20.00	White color colony	Slow growing, raised at centre	Regular margin
		Lasiodiplodia theobromae	72.41	Grey centre	Sparse growth	Regular margin
		Aspergillus oryzae	33.44	White margin with green centre	Colony raised at centre	Regular margin
		Aspergillus niger	33.00	Black centre with white margin	Flat growth	Regular margin
3.	Sabour-aud's dextrose agar medium	*Aspergillus fumigates*	16.72	Whitish blue colony	Slow growing, Flat growth	Regular margin
		Chaetomium spp.	16.67	White colony	Raised growth at centre	Regular margin
		Lasiodiplodia theobromae	90.29	Slightly cream color colony	Flat sparse growth	Regular margin
		Aspergillus oryzae	33.44	Green centre and white margin	Raised at centre with flat margin	Regular margin
		Aspergillus niger	38.92	Black centre, white margin	Sparse flat growth	Regular margin
4.	Malt extract agar medium	*Aspergillus fumigates*	17.17	Whitish blue colony	Slow growing, Flat growth	Regular margin
		Chaetomium spp.	16.59	White color colony	Dense raised growth	Regular margin

		Lasiodiplodia theobromae	88.63	Slightly white colony	Sparse flat growth	Regular margin
		Aspergillus oryzae	33.61	Light green centre, white margin	Flat growth	Regular margin
		Aspergillus niger	27.85	Black centre, white margin	Sparse flat growth	Regular margin
5.	Potato carrot agar medium	*Aspergillus fumigates*	22.41	Slightly blue colony	Slow growing, Flat growth	Regular margin
		Chaetomium spp.	22.41	White colony	Dense raised growth at centre	Regular margin
		Lasiodiplodia theobromae	99.67	Light white color colony	Flat sparse growth	Regular margin
		Aspergillus oryzae	22.41	Green centre and white margin	Flat growth	Regular margin
		Aspergillus niger	22.24	Black centre, white margin	Flat growth at Margin, Slightly Raised centre	Regular margin

The growth of *A. oryzae*was best on PDA. The colony on PDA was dark green at centre with white regular margin about 35mm in diameter. On other media the fungus produced colony with light green centre with flat white margin and diameter about 30-20 mm.

The colony of *A. niger* was showing sparse flat growth and black centre with white margin on all media. The diameter of the colony was about 38.92mm on SDA, 27.85mm on MEA, 22.24mm on PCA, 33.44mm on PDA and 33mm on CYEA.

Growth of *Aspergillus fumigates* were best on PDA, CYEA and PCA followed by SDA and MEA. The colony on all five media were slow growing. The diameter of colony was ranging from 16.72 to 22.41mm. It produced whitish blue colored colony on nearly all media. The mycelium were well developed with long erect conidiophores. The vesicle was ovate to flask shaped with globose conidia measuring 2-3 µm.

The best medium for growth of *Chaetomium* spp. was PCA followed by PDA, CYEA, SDA and MEA. The colony diameter were ranging from 22.67 mm on PDA up to 16.59 mm on MEA. It had produced slightly white colony with greenish centre. *Chaetomium* spp. produced septate mycelium with globoseperithecia. The ascospores were lemon shaped, olive brown, 10-12 µm in diameter. These observations are in conformity with the studies of Prokhorov and Linnik (2011), Soumya *et al.* (2014).

The growth of *A. oryzae* was best on PDA. The colony on PDA was dark green at centre with white regular margin about 39.29 mm in diameter. Whereas, the colony of *A. niger* was showing sparse flat growth and black centre with white margin on all media. The diameter of the colony of *A. oryzae* was ranging from 21-39 mm. *A. niger* colony diameter were ranging from 35 mm to 20 mm. *Aspergillus oryzae* and *A. niger*both produced septate, branched mycelium with long erect conidiophores. The vesicle in *A. oryzae* was globose, in *A. niger* it was spherical to globose. The conidia in both species were globose, about 5-6×8-10 µm in *A. oryzae* and 3-5 µm in *A. niger*. These results are in line with the findings of Gautam and Bhadauria (2012) and Gupta *et al.* (2012).

Ali *et al.* (2016) studied the growth rate of five *Aspergillus* species on five culture media to determine the best media. The results of tests done on five species of *Aspergillus*, showed that the best media was SDA, PDA, Czapek, MEA and CMA respectively.

Lasiodiplodiatheo bromae showed best growth on PDA and PCA followed by SDA, MEA and CYEA. The colony on PDA was slightly raised at centre with dark sporulation and filamentous radiating margins with diameter about 99.67 mm. The colony on PCA showed submerged growth towards margin and grayish centre because of sporulation at the centre. *Lasiodiplodiatheo bromae* produced septate mycelium with flask shaped

pycnidia. The conidia were subovoid, double layered on maturity, hyaline and 7.2-2.8µm in diameter.

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