

Original Research Article

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## Morphological and Cultural Characters of Fungi Isolated from Copra and Copra Oil

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### A B S T R A C T

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Copra samples were collected 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 *Lasiodiplodia theobromae* 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.

### Introduction

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 hypercholesterolemic 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 from 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's yeast 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  $28\pm 2^{\circ}\text{C}$ . 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 *Lasiodiplodia theobromae*. The identifications were confirmed by Chief Mycologist, MACS-Agharkar Research Institute, (ARI) Pune.

### Morphological and Cultural characters of isolated fungi

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  $\mu\text{m}$  in diameter. *Chaetomium spp.* produced septate mycelium with globose perithecia. The ascospores were lemon shaped, olive brown, 10-12  $\mu\text{m}$  in diameter.

*Lasiodiplodia theobromae* produced septate mycelium with flask shaped pycnidia. The conidia were sub ovoid, double layered on maturity, hyaline and 7.2-2.8  $\mu\text{m}$  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 $\times$ 8-10  $\mu\text{m}$  in *A. oryzae* and 3-5  $\mu\text{m}$  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 *Lasiodiplodia theobromae* 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 fumigatus*. The best media for *A. fumigatus* were PDA, CYEA and PCA followed by SDA and MEA. The colony on all five media was 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.

*Lasiodiplodia theobromae* 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. 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 30-20mm. 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 fumigatus* were best on PDA, CYEA and PCA followed by SDA and MEA. The colony on all five media was 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 myceliums were well developed with long erect conidiophores. The vesicle was ovate to flask shaped with globosely conidia measuring 2-3  $\mu\text{m}$ .

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 globose perithecia. The ascospores were lemon shaped, olive brown, 10-12  $\mu\text{m}$  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 $\times$ 8-10  $\mu\text{m}$  in *A. oryzae* and 3-5  $\mu\text{m}$  in *A. niger*.

**Table.1** The morphological characters of the test fungus viz., mycelium, conidiophores, conidia, were as under

No	Fungi	Morphological character			
		Mycelium	Conidiophore	Vesicle	Conidia
1.	<i>Aspergillus fumigates</i>	Septate, branched	Hyaline, long erect.	Ovate to flask shaped	Globose to spherical, 2-3µm.
2.	<i>Chaetomium spp.</i>	Septate	-	Peritheciaglobose	Ascospore olive brown, lemon shaped, 10-12µm.
3.	<i>Lasiodiplodia theobromae</i>	Separate	-	Flask shaped pycnidia	Cylindrical – subovoid, double layered, hyaline, 7.2-2.8µm.
4.	<i>Aspergillus oryzae</i>	Septate	Hyline, Long erect	Globose	Globose-pyriform, ornamented, 3.68-4.40µm.
5.	<i>Aspergillus niger</i>	Branched, septate.	Long erect,	Globose to spherical	Globose, 3-5µm.

**Table.2** 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	<i>Aspergillus fumigates</i>	22.22	Whitish Blue	Flat growth	Radiating irregular margin
		<i>Chaetomium spp.</i>	20.67	Greenish centre and white margin	Flat growth raised at centre	Regular margin
		<i>Lasiodiplodia theobromae</i>	99.67	Slightly white Colony with light green centre	Flat with slightly raised at centre	Filamentous radiating margin
		<i>Aspergillus oryzae</i>	39.29	Dark green centre and white margin	Slightly raised centre and flat margin	Regular margin
		<i>Aspergillus niger</i>	33.44	Black centre with white margin	Slow growing, flat growth	Regular margin
2.	Czapek's yeast extract agar medium	<i>Aspergillus fumigates</i>	22.17	Whitish blue colony	Flat growth	Slightly irregular margin
		<i>Chaetomium spp.</i>	20.00	White color colony	Slow growing, raised at centre	Regular margin
		<i>Lasiodiplodia theobromae</i>	72.41	Grey centre	Sparse growth	Regular margin
		<i>Aspergillus oryzae</i>	33.44	White margin with green centre	Colony raised at centre	Regular margin

		<i>Aspergillus niger</i>	33.00	Black centre with white margin	Flat growth	Regular margin
3.	Sabouraud's dextrose agar medium	<i>Aspergillus fumigates</i>	16.72	Whitish blue colony	Slow growing, Flat growth	Regular margin
		<i>Chaetomium spp.</i>	16.67	White colony	Raised growth at centre	Regular margin
		<i>Lasiodiplodia theobromae</i>	90.29	Slightly cream color colony	Flat sparse growth	Regular margin
		<i>Aspergillus oryzae</i>	33.44	Green centre and white margin	Raised at centre with flat margin	Regular margin
		<i>Aspergillus niger</i>	38.92	Black centre, white margin	Sparse flat growth	Regular margin
4.	Malt extract agar medium	<i>Aspergillus fumigates</i>	17.17	Whitish blue colony	Slow growing, Flat growth	Regular margin
		<i>Chaetomium spp.</i>	16.59	White color colony	Dense raised growth	Regular margin
		<i>Lasiodiplodia theobromae</i>	88.63	Slightly white colony	Sparse flat growth	Regular margin
		<i>Aspergillus oryzae</i>	33.61	Light green centre,white margin	Flat growth	Regular margin
		<i>Aspergillus niger</i>	27.85	Black centre, white margin	Sparse flat growth	Regular margin
5.	Potato carrot agar medium	<i>Aspergillus fumigates</i>	22.41	Slightly blue colony	Slow growing, Flat growth	Regular margin
		<i>Chaetomium spp.</i>	22.41	White colony	Dense raised growth at centre	Regular margin
		<i>Lasiodiplodia theobromae</i>	99.67	Light white color colony	Flat sparse growth	Regular margin
		<i>Aspergillus oryzae</i>	22.41	Green centre and white margin	Flat growth	Regular margin
		<i>Aspergillus niger</i>	22.24	Black centre, white margin	Flat growth at Margin, Slightly Raisedcentre	Regular margin

These results are in line with the findings of Gautam A. K. and Bhaduria R. (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.

*Lasiodiplodia theobromae* 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. *Lasiodiplodia theobromae* produced septate mycelium with flask shaped pycnidia. The conidia were subovoid, double layered on maturity, hyaline and 7.2-2.8 $\mu$ m in diameter.

## References

- Ali Sadiq R. M.; Ahmed J. Fradi and Alaa M. Al-Aaraji, 2016. Comparison between different cultural medium on the growth of five *Aspergillus* species. *World Journal of Pharmaceutical Research* Vol 8(5), ISSN 2277– 7105.
- Chaudhuri Tushnima, Birendranath Panja and Jayanta Saha, 2017. Cultural and morphological characteristics of *Lasiodiplodia theobromae* of *Dianella* in various carbon and nitrogen containing media. *Journal of Pharmacognosy and Phytochemistry*, Vol. 6(6), 160-164.
- Gautam A. K. and Bhaduria R., 2010. Characterization of *Aspergillus* species associated with commercially stored triphala powder. *Afr. J. Biotechnol.*, Vol. 11(104), pp. 16814-16823.
- Gupta Meera; Kumari Manisha and Ruby Grover, 2012. Effect of Various Media types on the rate of Growth of *Aspergillus niger*. *Indian Journal of Fundamental and Applied Life Sciences* Vol. 2 (2), ISSN: 2231-6345, pp.141-144.
- Munirah M.S.; Azmi A.R.; Yong S.Y.C. and NurAinIzzati M.Z., 2017. Characterization of *Lasiodiplodia theobromae* and *L. pseudotheobromae* causing fruit rot on pre-harvest mango in Malaysia. *Plant Pathology & Quarantine*, Vol. 7(2): 202–213.
- Nyongesa Beatrice Wabusya; Sheila Okoth and Vincent Ayugi, 2015. Identification key for *Aspergillus* species isolated from Maize and soil of Nandi County, Kenya. *Advances in Microbiology*, 5, 205-229.
- Prokhorov V.P. and M. A. Linnik, 2011. Morphological, cultural, and biodestructive peculiarities of *Chaetomium* species. Moscow Univ. Biol.Sci.Bull., 66:95.
- Rodrigues R.; C. Soares, Z. Kozakiewicz, R.R.M. Paterson, N. Lima, and A. Venancio, 2007. Identification and characterization of *Aspergillus flavus* and aflatoxins. *Communicating Current Research and Educational Topics and Trends in Applied Microbiology*, 527-534.
- Soumya K., L. Swathi, G.L. Sreelatha and T. Sharmila, 2014. Light influences Pigment, Biomass and Morphology in *Chaetomium cupreum* - SS02 - A Photoresponse Study. *Int. J. Curr. Microbiol. App. Sci.*, Vol. 3(4): 53-64.
- Thom Charles and Margaret B. Church, 1921. *Aspergillus flavus*, *A. oryzae*, and Associated Species. *American Journal of Botany*, Vol. 8, No. 2, pp. 103-126.

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