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# A study on presence of Aflatoxin in deried coconut

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The present investigation was carried out with a view to isolate the fungi associated with dried coconut collected from different places and to study their toxin producing ability. Nine samples of dried coconut of different grades were collected from different places of Ahamednagar district. Isolation of fungi from these samples yielded 8 types of different fungi, out of which three belonged to the genus *Aspergillus* and genus *Pencillium*, and one each of *Rhizoups* spp., *Alternaria* spp. and *Fusarium* spp. Results indicated that dried coconut samples were heavily infected with *Aspergillus* spp. (green and black pigment) than other fungi. 26 cultures obtained from the dried coconut samples were screened for their toxin producing ability.

Key words : Aflatoxin, Aspergillus, Dried coconut

## INTRODUCTION

Mycological contamination of agricultural commodities now being viewed with serious concern. In our country the presence of moulds in foods has been given importance mainly because of storage loss and from the sale point of view like color and taste. During the entire post harvest period of storage, food crops are highly vulnerable to mould attack. The fungal growth is usually accompanied by the simultaneous production of the toxic metabolites. Tropical conditions in India like high temperature and high moisture level during the monsoon season, unseasonable rains and inadequate pre-and –post-harvest practices that promote elaboration of toxins in foods. For many years, moulds have been known to produce toxic metabolites but their effects were largely ignored. The fungal toxins are chemical pollutants of biological origin. They can occur wherever fungi proliferate but present most serious hazards in foods and animal feeds. These chemicals are toxic and are found to be carcinogenic and hemorrhagic to human beings and animals.

Among the known mycotoxins, the most important from the direct hazard point of view to human health are aflatoxins. The relative concentrations of aflatoxin, however, very greatly depending on fungal strain, substrate and condition of growth. Aflatoxins cause two types of toxicity in human beings namely acute and chronic. In acute toxicity, deaths are caused in which encephalopathy and fatty degeneration of the viscera (EFDV) and fetal hepatitis are the incident of aflatoxin ingestion, where as in chronic toxicity aflatoxin caused primary liver cancer, Indian Childhood Cirrhosis (ICC), liver enlargement and other melodies.

All Fruits and fruit products produced in India are not consumed immediately after harvest. They are required to be stored for some time. In developing countries like India, it is not possible to store all fruits and fruit products under controlled conditions. Improper storage practices accompanied by high temperature and high moisture conditions are favorable for fungal invasion and elaboration of aflatoxins.

Therefore, it was felt to study the presence of aflatoxins in above-mentioned commodity *i.e.* dried coconut with the objective to isolate and identify different fungal contaminants with the post harvest spoilage of dried coconut.

## MATERIALS AND METHODS

To achieve the objective mentioned earlier, the following methodology was followed.

***Collection of samples:***

The samples of dried coconut were randomly collected from different places in the Ahamednagar district. The samples were collected from the market where common people purchased such food material. Each sample weighing about 100 g, was collected in the sterilized polyethylene bags.

The samples of the following grades were collected in triplicate.

– Low grade
– Medium grade
– Super grade

The physical characteristics of the samples collected form different places were observed critically with respect to fungus growth, insect damage, color appearance and general conditions. The samples collected form different places were as given below.

| | | |
|---|---|---|
| Rahuri | a) Copra | 3 |
| Jamkhed | a) Copra | 3 |
| Vambori : | a) Copra | 3 |
| | Total | 9x3 = 27 |

***Aflatoxin B1 standard:***

Aflatoxin standard required for the analysis was obtained from RAJKINSTITUTE VOOR DE VOLKSGEZONDHEID, Antonie Van Leeuwenhoak Lann, 9-postbus -3720. B.A. Bilthoven the Netherlands.

***Ultravoilet viewing cabinet:***

It was used for examination of the fluorescence produced by the aflatoxin standard, sample extract and toxin producing fungal strains.

***Standard culture:***

A standard culture of *Aspergillus flavus* (aflatoxin producing) was procured from the laboratory of the Department of Microbiology Central for Food Technology and Research Instittue, Mysore, and used for comparative study in BGY test.

***Estimation of mycoflora associated with dried coconut***

Microorganisms present in the samples of dry coconut were isolated by using tissue isolation method.

***Tissue isolation method:***

Potato Dextrose agar (PDA) medium was used for the isolation of mycoflora associated with the above dry fruit. The PDA medium was prepared in bulk and autoclaved at 15 pounds pressure for 15 minutes. After cooling, a thin layer of medium was poured in sterilized glass Petridishes under aspetic conditions. After solidification of the medium the Petridishes were used for the isolation of the fungus. Three or four pieces previously sterilized in $HgCl_2$ (1:1000) for about 1 to $1^{1/2}$ minutes of each of the sample were taken from the collected samples and were equidistantly placed in the previously prepared agar plates. The plates were labeled and incubated at room temperature ($27^0$C + $2^0$C) for 48 to 72 hours. Each plate was examined for fungus growth and the types of fungi grown were observed.

Observations of different fungi grown in plates were recorded by examining the fungal cultures under microscope and identified up to generic level by consulting manual of Illustrated genera of imperfect Fungi by Barnett and Hunter (1965). The pigmentation formed by these fungi was also recorded.

***Maintenance of pure cultures:***

Pure cultures of the isolated organisms were maintained on PDA slants and stored in a refrigerator for further study.

***Toxin producing ability:***

Aflatoxin producing ability of the isolated fungi from dried coconut samples was judged by the screening test as described by Hara *et al.* (1979).

***Screening test:***

A rapid screening test as described by Hara *et al.* (1979) was followed to detect the ability of cultures to produce aflatoxins. Czepek's dox agar solution was prepared, autoclaved and poured into the sterilized glass Petriplates, after solidification of the medium the plates were inoculated with the mycellial bits of fungi under study separately. Standard culture of *Aspergillus flavus* (aflatoxin producing) was also inoculated in one plate for comparative study. The plates were incubated $30^0$ + $1^0$ C for fungal growth.

After obtaining the luxuriant growth of fungus of the test strains after 7 days, the plates were examined upside down under ultraviolet viewing cabinet for 365 nm illuminations for the presence or absence of blue fluorescence. In the case of positive strains the toxin, investigative mycelium diffuses through agar and produces a clear blue fluorescence against dark back ground and in the case of negative strains typically blue or green fluorescence were never observed.

***Estimation of aflatoxin from samples:***

The samples, from which the fungal strains showed a positive fluorescence test, were taken up for the estimation of toxin. Methodology for aflatoxin estimation was followed as described by A.O.A.C.(1980).

***Extraction of Toxins from samples:***

The samples were ground to fine powder in Wiley ball mill. Fifty grams of the above powder was weighed in five hundred ml Erlenmeyer flask. To this 25 ml distilled water, 25 gm of elite (filter aid) and 250 ml of chloroform

**Table 1 : Mycoflora associated with copra studied for aflatoxin contamination**

| Sr. No. | Place of collection | Grade of sample | Fungi identified | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | *Aspergillus* spp. black pigment | *Aspergillus* spp. green pigment | *Aspergillus* spp. yellow pigment | *Pencillium* spp. Blue Pigment | *Penicillium* spp. dark green pigment | *Rhizopus* spp | *Alternaria* spp | *Fusarium* spp. |
| 1. | Jamkhed | Low | P | P | - | P | P | P | - | P |
| 2. | Jamkhed | Medium | P | - | P | - | - | - | P | - |
| 3. | Jamkhed | Super | - | - | - | - | - | - | - | - |
| 4. | Rahuri | Low | P | P | - | - | P | - | - | - |
| 5. | Rahuri | Medium | P | P | - | P | - | P | - | - |
| 6. | Rahuri | Super | P | P | - | - | - | - | - | - |
| 7. | Vambori | Low | P | P | - | P | - | - | P | - |
| 8. | Vambori | Medium | P | - | P | P | - | P | - | - |
| 9. | Vambori | Super | - | - | - | - | - | - | - | - |

Note :- P = denotes presence of fungus growth
= denotes absence of fungus growth

**Table 2 : Screening of fungal cultures isolated from dried coconut for aflatoxin producing potentials**

| Sr. No | Places of collection | Grade of the sample | Condition of the sample | Isolated | Toxin production | |
|---|---|---|---|---|---|---|
| | | | | | Fluorescence | Toxin present |
| 1. | Jamkhed | Low | Color is Brownish black, fair in appearance, infected | *Aspergillus* spp. Black pigment | Absent | Absent |
| | | | | *Aspergillus* spp. Green pigment | Blue | Aflatoxin |
| | | | | *Penicillium* spp. Dark green pigment | Absent | Absent |
| | | | | *Penicillium* spp. Blue pigment | Absent | Absent |
| | | | | *Rhizopus* spp. | Absent | Absent |
| | | | | *Fusarium* spp. | Absent | Absent |
| 2. | Jamkhed | Medium | Color is Light Brown Fairly good, free from insect damage | *Aspergillus* spp. Black pigment | Absent | Absent |
| | | | | *Aspergillus* spp. Yellow | Absent | Absent |
| | | | | Pigment *Alternaria* spp. | Absent | Absent |
| 3. | Jamkhed | Super | Color is white good, free from insect damage | No growth of fungus was observed | Absent | Absent |
| 4. | Rahuri | Low | Fair in appearance, Color is Yellowish Black, infected | *Aspergillus* spp. Black pigment | Absent | Absent |
| | | | | *Aspergillus* spp. Green pigment | Absent | Absent |
| | | | | *Penicillium* spp. Dark green pigment | Absent | Absent |
| 5. | Rahuri | Medium | Fairly good in appearance color is Brown, free from insect damage, | *Aspergillus* spp. Black pigment | Absent | Absent |
| | | | | *Aspergillus* spp. Green pigment | Blue | Aflatoxin |
| | | | | *Penicillium* spp Blue pigment | Blue | Aflatoxin |
| | | | | *Rhizopus* spp | Absent | Absent |
| 6. | Rahuri | Super | Color is white good, free from insect damage | *Aspergillus* spp. Black pigment | Absent | Absent |
| | | | | *Aspergillus* spp. Green pigment | Absent | Absent |
| 7. | Vambori | Low | Color is Yellowish Fair, infected | *Aspergillus* spp. Black pigment | Absent | Absent |
| | | | | *Aspergillus* spp. Green pigment | Blue | Aflatoxin |
| | | | | *Penicillium* spp. Blue pigment | Absent | Absent |
| | | | | *Alternaria* spp. | Absent | Absent |
| 8. | Vambori | Medium | Good in appearance light brown in color, free from insect damage | *Aspergillus* spp. Black pigment | Absent | Absent |
| | | | | *Aspergillus* spp. Yellow pigment | Absent | Absent |
| | | | | *Penicillium* spp. Blue pigment | Absent | Absent |
| | | | | *Rhizopus* spp. | Absent | Absent |
| 9. | Vambori | Super | Good in appearance white in color, free from insect damage | No growth of fungus was observed | Absent | Absent |

was added. The flasks were stopper tightly and covered with aluminum foil and were kept on wrist action shaker for 30 minutes. After shaking the extract was filtered through What man filter paper No. 1.

## RESULTS AND DISCUSSION

The result in respect of presence of aflatoxins associated with dried coconut are presented in Table 1.

Total nine samples of dried coconut were studied for fungal contamination. It was found that out of nine, seven samples were contaminated with different fungi, where as two samples representing super grades collected from Jamkhed and Vombori were found free from fungal contamination. The fungi var. *Aspegillus* spp. (Black Green and Yellow Pigment). *Penecillium* spp. (Blue and Dark Green Pigment) *Rhizopus* spp, *Alternaria* spp., *Fusarium* spp. Were found associated with the samples of dried coconut. The fungi namely *Aspergillus* spp. (Black and Green pigment) were found predominantly in the sample representing low and medium grade collected from three places where as one sample representing super grade collected from Rahuri also showed the contamination of *Aspergillus* spp (Black and Green Pigment).

***Toxin producing ability:***

26 isolate obtained from 9 samples of dried coconut were screened as per the method described Hara *et al.* (1979) and are presented in Table 2. The cultures were screened by exposing them to ultra violate light (365nm) and were observed for the presence or absence of blue or green florescence. It was found that out of 26, only 4 cultures, representing from *Aspegillu*s spp. (Green and Black pigment). *Penicillium* spp. (Blue pigment) shown positive for aflatoxin production. The fluorescences produced by these test cultures were exactly similar to those produced by the standard culture of *A. flavus* (aflatoxin producer).

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