Indian J. Plant Pathol. 1984, 2(1) : 46—49

Occurrence of Fungi in Marketed Betel Nut

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ABSTRACT

Betel nut samples collected from grocer's shop, betel and betel nut shops and betel nut vendors, were analysed for their microfungal population using the blotter and agar-plate methods. Moisture content of nut samples was also determined. Of a total of 33 species belonging to various genera isolated, species frequently encountered were of *Aspergillus*, *Mucor* and *Penicillium*. Many of these fungi are known mycotoxin producers. Seasonal occurrence of fungi was evident which had direct relationship with the moisture content of the nuts.

In India, areca palm (*Areca catechu* L.) according to Nayar (14) occurs over a land of 1,74,300 hectare and is consumed for several religious and social ceremonies in various forms. It is one of the most important and common masticatories used by the rural and urban folks.

Except for a preliminary report by Misra and Misra (11), surprisingly little work has been done on the fungi and their seasonal occurrence in betel nuts (9, 13, 16). It, therefore, appeared profitable to undertake systematic and extensive collection of betel nuts from various sources and examine them for the presence of microfungi. This paper embodies results of the above study.

MATERIALS AND METHODS

Samples of betel nut were collected each month from betel nut shops, grocer's shops and also from vendors. The collections were made from various districts of U. P., particularly from eastern ones. Samples were stored aseptically in plastic bottles. Moisture content of each sample was estimated by drying method and the fungal isolations were done by the blotter and agar plate methods (5, 12) using potato-dextrose and Czapek-Dox agar. Plates were incubated at 27±1°C and examined every 24 hours for the fungal growth. Colonies which appeared were isolated and maintained on agar slants for further study.

RESULTS AND DISCUSSION

Moisture content data are given in Table 1. It was found to be the highest in July and lowest was in May. In other months too, it was appreciably high due to the open and poor storage conditions prevailing in shops.

Fungi isolated in different months are given in Table 2. Thirty three fungal species belonging to twelve genera were isolated. As regard the frequency of occurrence of different fungal forms, fungi were more frequently obtained during the months when moisture content was relatively high i.e. July to March. In April, May and June, the moisture content was not that sufficient to support greater number of fungi. Besides, the higher air temperature in these months also appears to limit the

Accepted for Publication April 26, 1984

TABLE 2. *Frequency percentage of fungi in different months.**

Fungus/Month	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Alternaria tenuis	16	16	16	—	—	—	33	50	50	66	33	16
Aspergillus flavipes	16	—	—	—	—	—	50	50	66	—	—	—
A. flavus	80	80	100	100	50	50	100	100	100	100	100	100
A. fumigatus	16	16	16	100	100	100	100	100	100	50	33	16
A. japonicus	16	16	16	—	—	—	—	—	33	33	50	50
A. mellens	16	16	—	—	—	—	—	—	33	50	50	50
A. nidulans	33	33	—	—	—	—	—	—	—	33	50	50
A. niger	16	16	33	—	33	33	50	50	66	66	80	80
A. niger var. *phoenicis*	16	33	33	16	16	33	50	50	33	33	16	16
A. ochraceus	50	33	33	16	50	50	66	80	80	50	16	16
A. sydowi	16	33	33	50	50	50	66	80	33	—	—	—
A. tamarii	33	33	—	—	—	—	—	—	50	50	33	16
A. terreus	16	16	—	33	33	33	50	50	50	50	—	—
A. ustus	—	—	—	—	—	—	—	—	50	—	—	—
Chaetomium globosum	33	33	33	—	—	—	—	—	50	—	—	16
Cladosporium cladosporioides	33	33	—	—	—	—	—	—	33	50	16	16
Curvularia lunata	33	33	—	—	—	—	50	50	33	33	—	—
C. prasadii	16	16	—	—	—	—	50	50	16	16	—	16
Fusarium sp.	33	33	—	—	—	—	—	50	33	50	—	16
Mucor hiemalis	—	—	—	—	—	—	100	100	50	50	—	—
M. racemosus	—	—	—	—	—	—	100	100	33	33	—	—
Paecilomyces varioti	—	—	—	33	—	—	50	50	—	33	—	—
Penicillium citrinum	33	16	—	—	—	—	—	—	33	50	50	50
P. frequentans	33	33	—	—	—	—	—	—	33	33	50	50
P. funiculosum	33	33	—	—	—	—	—	—	50	50	66	50
P. lividum	16	16	—	—	—	—	—	—	50	66	90	80
P. purpurogenum	33	16	—	—	—	—	—	—	66	50	66	50
P. variable	50	—	—	—	—	—	—	—	—	66	33	50
Rhizopus arrhizus	—	—	—	—	—	—	100	100	50	16	—	—
R. oryzae	—	—	—	—	—	—	100	100	16	16	—	—
Scopulariopsis brevicaulis	—	—	—	—	—	—	16	16	33	33	—	—
Talaromyces stipitatus	66	50	—	—	—	—	—	—	50	66	100	80
T. wortmanii	50	33	—	—	—	—	—	—	66	66	100	100

*Percent occurrence = $\frac{\text{Samples of occurrence}}{\text{Total number of samples}} \times 100$

TABLE 1. *Moisture content of betel nuts in different months.*

Month	Moisture (%)*
January	9.0
February	9.5
March	8.5
April	6.5
May	2.0
June	8.7
July	11.0
August	10.0
September	8.5
October	8.5
November	8.7
December	8.5

*Calculated using the formula :

$$\text{Moisture percentage} = \frac{\text{Initial weight-Final weight after drying}}{\text{Initial weight}} \times 100$$

fungal flora, excepting for the dominant Aspergilli like *A. flavus, A. fumigatus* and *A. niger* which are known to withstand and grow even under higher temperature regimes. The genus *Aspergillus* which dominates the fungal scene of the nut was isolated throughout the year representing thirteen species followed by *Penicillium*, being represented by eight species including two ascosporic species of *Talaromyces*. Species of *Curvularia, Mucor* and *Rhizopus* were also recovered. Mucorales were most abundant during raining months i.e. July to September. Some fungi like *Gladosporium cladosporioides* appeared more frequently during winter months. This reflects that the fungi infesting nuts have seasonal periodicity governed by factors like moisture and air temperature besides poor storage conditions.

Lalitha Kumari *et al.* (10) reported that areca nuts have antimicrobial properties. Inspite of having such a property, the nuts support apparently quite a good number of fungi. Boyland (4) has reviewed the carcinogenic effect of alkaloids of tobacco and areca nut. Ashby *et al.* (1) studied betel nut, arecaidine and oral cancer relationship. Recently, Shivapurkar *et al.* (15) have studied the effect of aqueous polyphenolic fraction of betal nut in Swiss strain mice, and reported that polyphenol fraction of betel nut induced 100% tumour in treated mice; 80% of these being the fibrocarcomas at the site of injection. During the course of this study, *Aspergillus flavus, A. ochraceus* and a few species of *Penicillium*, known for their mycotoxin producing capabilities and causing maladies in human beings and animals (2, 3, 6,

7, 8), were isolated. The fact that the association of toxicogenic fungi may aggravate already existing carcinogenic potential of areca nut can not be ignored in the light of above findings.

ACKNOWLEDGEMENTS

The authors are thankful to the Head of Department of Botany for providing all necessary facilities and to the Principal, Sri R. C. Dikshit for all encouragements. Financial assistance received from Indian Council of Medical Research, New Delhi is also gratefully acknowledged.

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