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Incidences of fungal contaminations in dry fruits and nuts in Jabalpur (M.P.) India

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ABSTRACT
The present study is an attempt to show the fungal diversity associated with seven common dry fruits and nuts being sold in Jabalpur, Central India, for identification of fungi associated with seven dry fruits and nuts viz. almonds, raisins, dry figs, dry dates, almonds, cashew nuts and dry apricots. A sample survey of 84 samples of 7 dry fruits and nut samples (12 each) showed that all dried figs (100%), 91.6 % of almonds, approx 84% of cashew nuts, raisin and dry dates, 75% of walnut and 50% apricot sample carried live fungi. Loose samples had higher fungal contamination in rainy season (75%) followed by winter season. Dry dates was found to be infested by maximum number of fungal isolates among which 59.61% was recovered in rainy season. Among the fungal genera, Aspergillus species accounted for maximum 38.46 % of the mycoflora followed by Fusarium species (15.38 %) The results revealed that A. flavus and A. niger dominate the fungal strains with 19.2 % colonization rate. The study postulates that dry fruits and nuts in Central India carry potential fungi.
KEYWORDS: *Fungi, Fungal diversity, Dry fruits, Mycotoxin*

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INTRODUCTION

Due to high nutraceutical and nutriceutical values dry fruits and nuts are esteemed and highly priced world over [1, 2]. High nutrient content specially soluble carbohydrate, low water activity and moisture provide a conducible environment for multiplication an survival of a variety of fungi [1-3].Many of these fungi are get associated with dry fruits and nuts during storage, handling, drying and packaging processes known to synthesise toxic metabolites which are responsible for several human and animal health problems [4, 5].In addition, such fungi are also reduced the aesthetic as well as market values of the dry fruits and nuts.

Since, these food products are consumed directly, without any pretreatment and cooking, the toxicants, even in less concentration, can be very harmful [1]. Therefore, it is important to determine that these such commodities are not contaminated by toxigenic fungi.The present paper deals with the diversity of fungi associated with common dry fruits and nuts sold at Jabalpur, Madhya Pradesh (India).

MATERIALS AND METHODS

Sample collection

Packed and loose samples of cashew nuts, raisins, almonds, dried figs, walnuts, dry dates, and dried Apricots were collected from the different local shops of Jabalpur (MP)during June 2017 to May 2018 as per Agarwal and Hasija [6].

Isolation and identification of fungi:

Isolation of fungi from dried fruits and nuts were carried out by dilution and direct plating technique. One ml suspension prepared in sterilised distilled water / small piece of surface sterilised samples were placed on sterilized petri-plates containing Potato Dextrose Agar (PDA) medium supplemented with Streptomycin (250 mg L^{-1}) to inhibit the bacterial growth.[6]Petriplates were incubated at 28±1° C for 7 days. The fungi were identified using macro- and micro-morphological features using standard keys.

Fungal diversity analysis

The diversity of fungi was studied using the following statistical formulae:

a. Frequency: Percent frequency of each dry fruits sample contaminated with fungal species was calculated by using the given below [7]:

b. Isolation rate: Isolation rate (IR) was determined as the number of isolates obtained from individual sample divided by the total number of samples incubated.

c. Frequency (%) = $\frac{\text{No. of samples from which an organism was isolated}}{\text{Total number of samples tested}} \times 100$

d. Colonization frequency percentage: The colonization frequency percentage (% CF) was calculated according to Suryanarayanan *et al.*[8], using the formula-

$$\%CF = (N_{col}/N_t) \times 100$$

Where, N_{col} = number of samples colonized by each fungus and N_t = total number of samples studied

e. Relative Abundance: Relative Abundance was calculated as the percentage of the amount of a particular type of fungi isolated to the total isolates.

RESULTS

It is evident from the data presented in fig.1 that the association of fungi varied significantly with different dry fruits. Maximum associations was recorded in case of dried figs where cent percent samples were contaminated with fungi. It was followed by almonds(91.6 %), cashew nuts, raisin and dry dates(84%)walnut (75%) and apricot (50%).Similarly it was also recorded that loose samples had higher fungal contamination, as compared to packed samples. Maximum number of fungi were isolated from the samples collected during rainy season both in loose (75%) and packed (42.8%).It was followed by samples collected during winter season. Summer had comparatively less fungal contamination(Fig.2) .

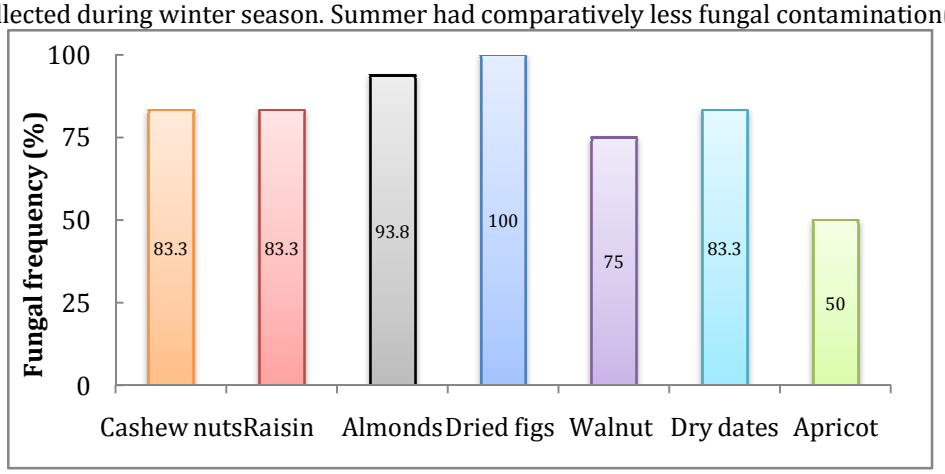

Fig 1: Frequency (%) of mycoflora contaminated dried fruits sample

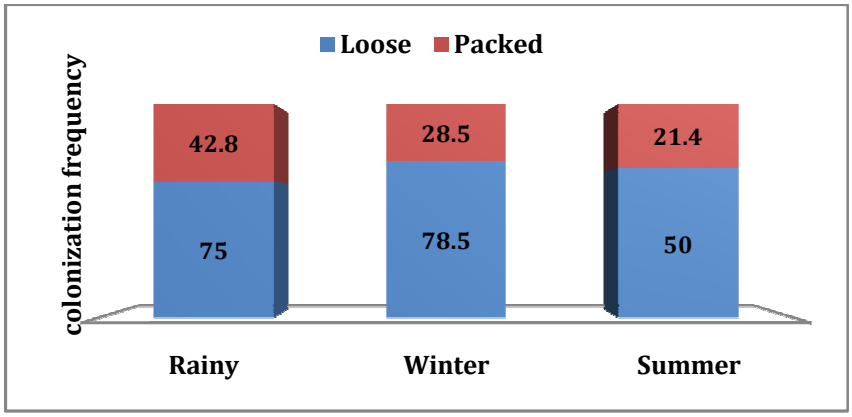

Fig 2: Mycoflora colonization frequency during different season

A total of 224 fungal isolates belonging to eight genera viz., *Aspergillus*, *Fusarium*, *Mucor, Alternaria, Cladosporium*, *Penicillium, Rhizopus* and *Trichoderma* represented by thirteen species were recovered(Fig. 3-7).It is evident from the data presented in Fig 3 dry dates samples yielded maximum number of fungal isolates (16.82 %) from the samples collected during rainy season(59.61%) which was followed by

winter (23.07%) and summer (17.30 %) season. Almond was the second most contaminated dry fruit with 15.53 % fungal isolates. Abundance of fungal isolates in remaining dry fruits were in order of raisin (11.32%) >dry figs (9.06 %)>cashew nuts (8.41 %)>walnut (7.76 %)>apricots (2.91 %).

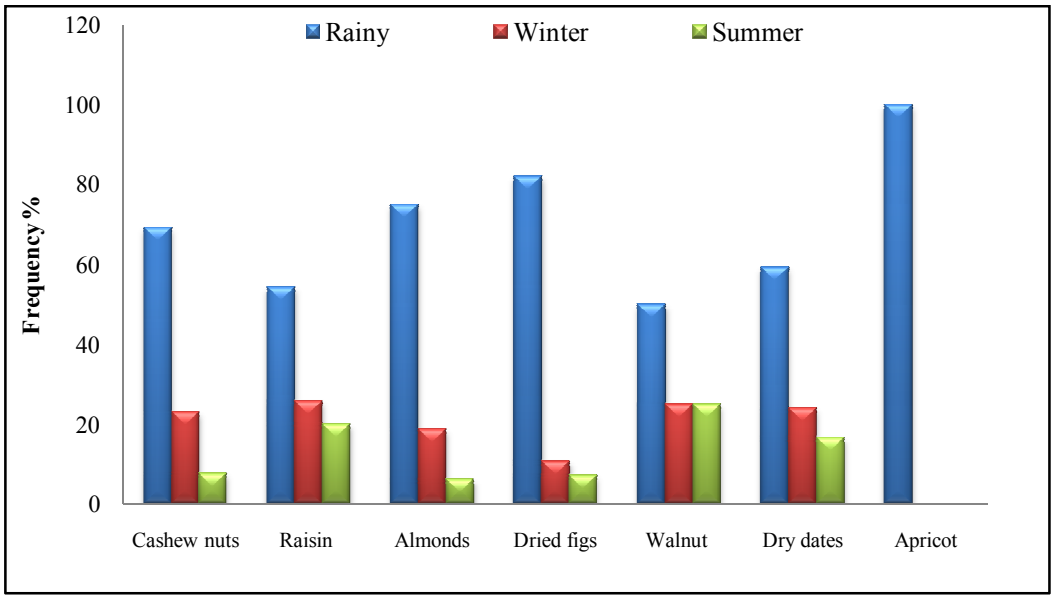

Fig 3: Frequency (%) of mycoflora in dry fruits during different season.

Significant fungal diversity was recorded in the samples of dry fruits and nuts.Of the isolated fungal species, Ascomycota (85%) was most dominant group represented by six genera included elavenspecies when compared to Zygomycota (15%) represented by two each genera and species. Amongst the fungi recovered , *Aspergillus* species accounted for maximum (38.46 %)of the mycoflora followed by *Fusarium* species contributed for 15.38 % whereas restof species independently formed 7.69% of the fungal population (Fig 4). Colonization rate of fungal species is shown in Fig 5which revealed that *A. flavus* and *A. niger* were the most dominat fungal isolates with 19.2 % colonization rate, followed by *P. chrysogenum* (17.86 %), *R. oryzae* (9.82 %) and *M. racemosus* (8.93%). Rest of the isolates ranked as *Fusarium oxysporium* accounts>*F. equiseti*>*A. alterneta*> *A. parasiticus*> *A. fumigatus, C. herbarum* and *richoderma sp*.

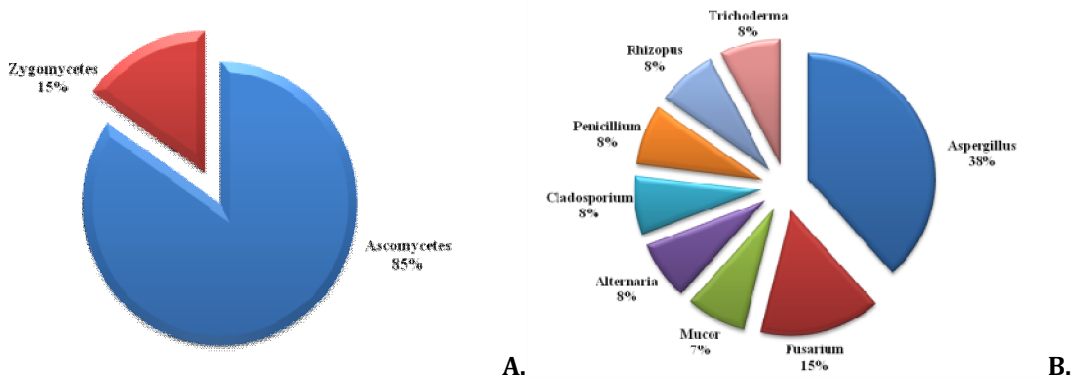

Fig 4: Fungal isolation rate of different fungi from dry fruits samples collected from Jabalpur (A) as per the fungal class and (B) fungal genera

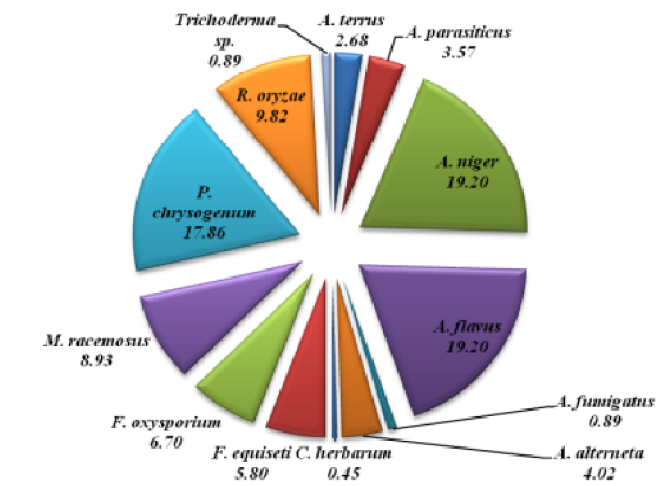

Fig 5: Colonization frequency percentage of fungal species recovered from the samples of dried fruits

Occurrence of individual fungal species varied significantly during different season(Fig 6). *A. flavus* was most dominated during rainy , *A. niger* in winter while *P. chrysogenum* showed maximum appearance during summer season. *Trichoderma sp*. contamination was observed only during rainy season. There was no appearance of *Trichodermasp,A. fumigatus* and *F. equiseti* in the samples collected during summer season .

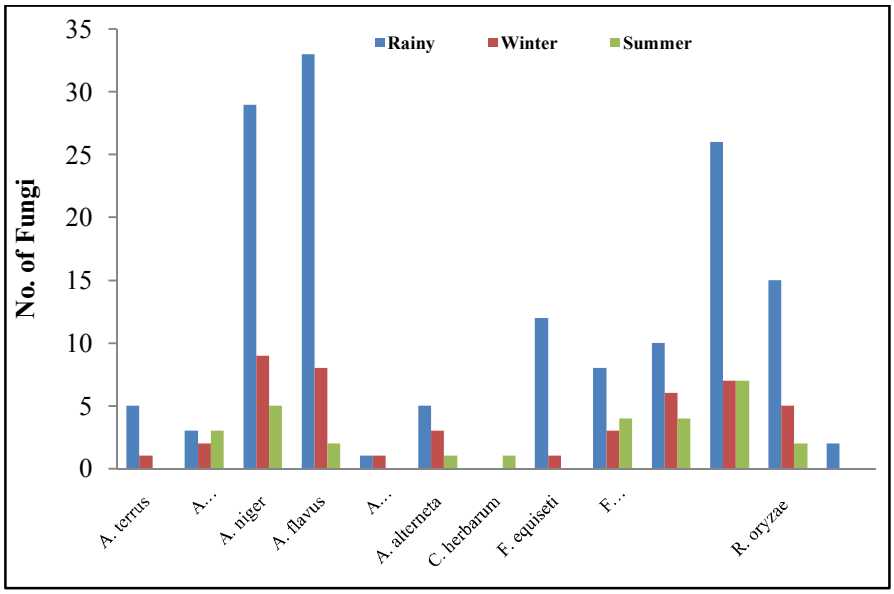

Fig 6: Occurrence of mycoflorafrom dry fruits and nuts during different seasons.

Relative abundance of mycoflora in dry fruits sample was depicted in Fig7. Out of 13 types of fungal species, maximum diversity was observed in dry dates samples (76.92 %). It was followed by raisin, almonds, Walnut(69.23%),cashew nuts(61.54%),dried figs(53.85%) and Apricot (38.46 %).

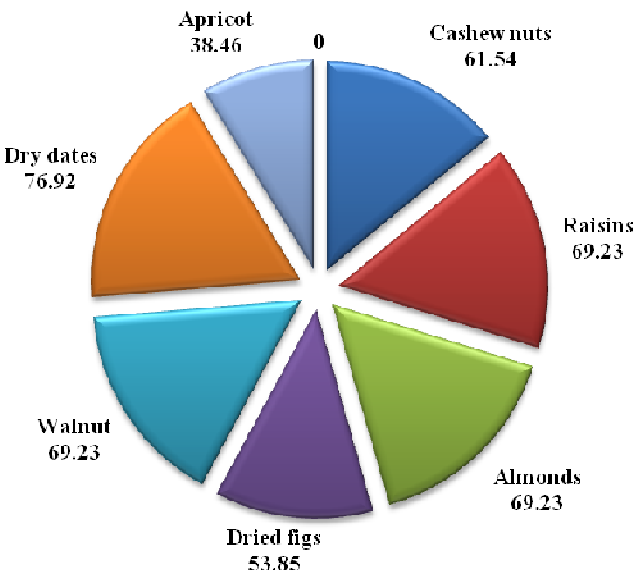

Fig 7: Relative abundance (%) of mycoflora in contaminated dried fruits sample

DISCUSSION

In spite of wide use of dry fruits and nuts as nutrecious foods, there are few publications on their contamination with moulds compared with numerous publications on the contamination of grains and oil seeds. In India, there is a practice to sell dry fruits in loose as well as packed conditions. The loose samples often absorb moisture and promote fungal growth. Further, the Food Safety and Standards Authority of India (FSSAI) has food safety norms only for nuts and dry figs among the dry fruits, and that too only, for the presence of total aflatoxins. The present study was carried out to identifying the diversity of fungal flora associated with different types of dry fruits and nuts shold in Jabalpur market. Significant diversity and dominance of *Aspergillus* specises in dry fruits and nuts have also reported by Alghalibi and Shater [9], Romero *et al.*[10] and Wei *et al* [11]; Amirmijani *et al.*[12] while working with moulds associated with medicinal plants, tea and other botanicals stated that simple association of fungi might not be correlated with the presence of mycotoxins. Therefore, the present findings are important and may be consider as milestone for the safety analysis of the dry fruits and nuts.

CONCLUSIONS

The present study revealed that dried fruits and nuts are highly contaminated with several fungi such as *A. flavus, A. niger*etc. which may release toxic metabolites during storage. The economic loss resulting from fungal contamination of nuts is difficult to estimate. In view of the fungal contamination detected from the market samples, an ardent need for proper storage, drying to minimize contamination with such fungi. Therefore, the authorities should take the lead in the efforts to establish mandatory regulations in dry fruits and nuts farming, processing and storage to decrease contamination risk to toxigenic fungi. These would lead to enhanced food safety, enhanced international trade efforts and improved public health.

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