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MOULDS ASSOCIATION WITH MEDICINALLY IMPORTANT SEEDS OF PUMPKIN (*Cucurbita maxima* L.) AND THEIR HEALTH HAZARDS

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ABSTRACT

Pumpkin seeds are considered as nutritional power house packed with high protein, healthy polyunsaturated fats including omega-3 & 6 fatty acids, Mg, Zn, Cu, Fe and Vitamin E. A small daily serving can boost immunity, protect heart, balance blood sugar, prostate and urinary health, hormonal balance and ease menopause symptoms. Due to its high nutritive value, pumpkin seeds are used by human beings for better health. As such they are stored by manufacturers as well as traders and during storage they can be contaminated with toxic fungi. In view of this fact, 20 samples were randomly collected from the market of Agra district and analysed for association of moulds by Agar seed plating technique. In all 18 fungi were recorded which include 5 species of *Aspergillus*, 2 species each of *Alternaria*, *Curvularia*, *Penicillium*, *Fusarium* and 1 species each of *Helminthosporium*, *Macrophomina*, *Rhizoctonia*, *Rhizopus* and *Verticillium*. Out of these, *Aspergillus flavus* was found to be most frequent and dominant mould followed by *Aspergillus ochraceus*, *Alternaria alternata*, *Fusarium solani* and *Penicillium citrinum*. It is needless to say that all these fungi are known to produce highly toxic mycotoxins which can cause health hazards in human beings. On the basis of our study it can be suggested that pumpkin seeds should be dried/roasted before use and should be properly stored so as to avoid fungal contamination. Out of various toxins produced by the above mentioned fungi the aflatoxin B_1 is considered as carcinogenic, mutagenic, teratogenic and cytotoxic and regarded as cause of liver cancer (hepatocarcinoma).

***Key words*:** *Cucurbita maxima*, Pumpkin seeds, *Aspergillus;* Mycotoxins, Health hazards.

Introduction

Pumpkin (*Cucurbita maxima* L.), a member of the family Cucurbitaceae and is widely cultivated in India for its edible fruit and seeds. Pumpkin seeds have gained increasing attention as a functional food because of their high protein, lipid, mineral and vitamin content. Their nutritional composition contributes to their dietary value and has supported their growing consumption in domestic and commercial markets. (Glew *et al*., 2006; Montesano *et al*., 2018).

The nutritional richness of pumpkin seed may however be accompanied by susceptibility to fungal colonisation during harvesting, handling, transportation and storage. The relatively high oil content and the presence of residual moisture together with prolonged storage under unsuitable conditions, can provide favourable conditions for storage associated moulds. Genera such as *Aspergillus*, *Penicillium*, *Alternaria* and *Fusarium* are frequently associated with stored agricultural commodities and include species known for their ability to produce mycotoxins. (Bhat & Reddy, 2017)

The composition of seed associated fungi may vary considerably with geographical location, climatic conditions and post-harvest practices. Previous observations on pumpkin seed have reported different dominant fungal groups under different storage and climatic conditions, emphasizing the need for region-specific assessment of seed-borne mycoflora. (Bhat &

Reddy, 2017; Weidenbörner, 2001)

Despite the increasing consumption of pumpkin seed in India, information on the fungal flora associated with pumpkin seeds marketed for human consumption in the Agra region remains limited. The present investigation was therefore undertaken to isolate and identify fungi associated with market samples of pumpkin seeds using the agar seed-plating method, to screen and characterize the recovered fungal colonies and to determine the predominant taxa. The health significance of the dominant fungal groups was subsequently considered in relation to their documented mycotoxin producing potential.

Materials and Methods

Study Material and Sample Collection: Twenty seed samples of pumpkin (*Cucurbita maxima* L.) were collected at random from different retail and wholesale outlets in the Agra district of Uttar Pradesh. The samples represented seed lots available for direct human consumption. Each sample was transferred into a separate sterile polythene bag, appropriately labelled, sealed and transported to the laboratory of the Department of Botany, Agra College, Agra. Samples were maintained under ambient laboratory conditions and processed, generally within 48 h of collection.

Isolation of Seed Associated Fungi by Agar Seed Plating Method

The seed borne mycoflora was isolated by the agar seed plating technique following the standard procedure described by the International Seed Testing Association (ISTA, 1966). Before plating, the seeds were surface sterilised with 1% sodium hypochlorite solution for 2 min and subsequently rinsed three times with sterile distilled water to remove residual disinfectant. The seeds were blotted dry on sterile filter paper. (International Seed Testing Association, 1966).

For each sample, five treated seeds were aseptically placed on each PDA plate with five replicate plates maintained for each sample. Potato Dextrose Agar (PDA) amended with chloramphenicol was used as the isolation medium to restrict bacterial interference. The inoculated plates were incubated at 28 ± 2 °C for 5–7 days in B.O.D incubator. Emerging fungal colonies were examined periodically and recorded on the basis of their growth from individual seeds.

Screening, Purification and Maintenance of Fungal Isolates

All morphologically distinct fungal colonies developing on the seed plating plates were considered for preliminary screening. Colonies were screened on the basis of visible differences in colony colour, texture, margin, growth pattern, pigmentation and sporulation. Representative colonies were aseptically transferred to fresh PDA plates to obtain pure cultures. The purified isolates were maintained on PDA for subsequent morphological and microscopic examination.

Morphological and Microscopic Identification

The purified fungal isolates were identified to genus and where possible, species level using a combination of colony morphology and microscopic characteristics colony (colour, texture, growth rate, pigmentation and sporulation). Microscopic preparations were made using lactophenol cotton blue, and diagnostic structures including conidiophores, conidia, conidial arrangement and other relevant morphological features were examined. Identification was carried out with reference to standard mycological taxonomic literature.

Frequency and Relative Occurrence of Fungi

The occurrence of each fungal taxon was assessed from the seed plate observations obtained during isolation. The frequency was used to compare the distribution of recovered fungi among the examined seed samples, and the taxa occurring most consistently were considered predominant or dominant within the study material.

Results

A total of 20 pumpkin seed samples were examined by the agar seed plating method. Fungal growth was recovered from the plated seed material and morphologically distinct colonies were purified and examined microscopically. The recovered mycoflora comprised of 18 fungal species belonging to 10 genera. *Aspergillus* was the most diverse genus represented by five species, whereas *Alternaria*, *Curvularia*, *Penicillium* and *Fusarium* were each represented by two species. *Helminthosporium*, *Macrophomina*, *Rhizoctonia*, *Rhizopus* and *Verticillium* were represented by one species each.

Preliminary screening of the developing colonies showed clear variation in colony morphology, pigmentation, texture and sporulation. These cultural differences, together with microscopic characters observed in lactophenol cotton blue preparations, permitted separation and identification of the principal fungal taxa.

The genus-wise distribution of the identified fungi is presented in Table 1.

Among the identified fungi, *Aspergillus flavus* was the most frequent and dominant species, followed by *Aspergillus ochraceus*, *Alternaria alternata*,

Table 1: Genus-wise distribution of fungal species isolated from pumpkin seed samples.

Genus	No. of species	Species recorded	Remarks
Aspergillus	5	*A. flavus*, *A. ochraceus*, *A. niger*, *A. fumigatus*, *A. terreus*	Dominant genus; *A. flavus* most frequent overall
Alternaria	2	*A. alternata*, *A. tenuissima*	*A. alternata* among the five most frequent species
Curvularia	2	*C. lunata*, *C. pallescens*	Moderate frequency
Penicillium	2	*P. citrinum*, *P. chrysogenum*	*P. citrinum* among the five most frequent species
Fusarium	2	*F. solani*, *F. oxysporum*	*F. solani* among the five most frequent species
Helminthosporium	1	*Helminthosporium* sp.	Low frequency
Macrophomina	1	*M. phaseolina*	Low frequency
Rhizoctonia	1	*Rhizoctonia* sp.	Low frequency
Rhizopus	1	*R. stolonifer*	Low frequency
Verticillium	1	*Verticillium* sp.	Low frequency

Penicillium citrinum. The remaining taxa occurred comparatively less frequently in the examined seed samples. The recovery of *Aspergillus* species from the market samples is particularly relevant because several members of this genus are established producers of mycotoxins under favourable storage conditions.

Discussion

The present investigation demonstrates that pumpkin seeds marketed in the Agra region can harbour a diverse assemblage of seed associated fungi. The recovery of 18 species belonging to 10 genera indicates that the seed surface and associated tissues may support a mixed fungal community. The predominance of *Aspergillus*, particularly *A. flavus*, is consistent with the importance of *Aspergillus* as a storage associated mould in warm agricultural environments.

The observed predominance differs from reports from temperate storage environments in which *Penicillium* and other storage fungi have been prominent. Such variation may reflect differences in temperature, humidity, storage duration, handling practices and local post-harvest conditions. The present findings therefore support the need for region specific surveillance of fungi associated with edible seed commodities (Weidenbörner, 2001; Bilgrami, 1984).

The health relevance of the recovered fungi is primarily associated with the known toxigenic potential of several dominant taxa (Magnussen & Parsi, 2013; Wild & Gong, 2010; Bui-Klimke & Wu, 2015; Flajs & Peraica, 2009; Escrivá *et al*., 2017). *Aspergillus flavus* is well recognised for its association with aflatoxin production, including aflatoxin B_1, a potent hepatocarcinogen. *Aspergillus ochraceus* has been associated with ochratoxin A and citrinin, while *Alternaria alternata* and *Fusarium* species are also associated in the literature with biologically active secondary metabolites and mycotoxins. *Penicillium citrinum* is a recognised source of citrinin.

Importantly, the present study established fungal association and taxonomic occurrence; it did not quantify aflatoxin or other mycotoxins in the seed samples. Accordingly, the results indicate a potential mycotoxin hazard based on the identity of the recovered fungi and their documented toxigenic capacity, rather than demonstrating the actual concentration of toxins in the investigated seed lots. Direct confirmation would require subsequent toxin specific analytical testing.

Taken together, the fungal profile observed in the present study warrants attention because the commodity is intended for human consumption. Good post-harvest

Table 2: Principal mycotoxins and reported toxicological effects associated with the dominant fungal isolates.

Fungal species	Principal mycotoxin(s)	Reported toxicological effects
Aspergillus flavus	Aflatoxin B_1, B_2, G_1, G_2	Hepatotoxic, carcinogenic, mutagenic, teratogenic and immunosuppressive
Aspergillus ochraceus	Ochratoxin A, Citrinin	Nephrotoxic and hepatotoxic effects reported
Alternaria alternata	Alternariol, Alternariol monomethyl ether, Tenuazonic acid	Mutagenic and cytotoxic effects reported
Fusarium solani	Fumonisins, trichothecenes (species-dependent)	Hepatotoxic and nephrotoxic potential reported
Penicillium citrinum	Citrinin	Nephrotoxic, hepatotoxic, embryotoxic and genotoxic effects reported

practices, including adequate drying, hygienic handling and storage under low-moisture conditions, may help restrict further fungal development. The findings also provide a basis for subsequent studies involving direct estimation of aflatoxins and other relevant mycotoxins using validated analytical methods such as thin-layer chromatography or high-performance liquid chromatography. (Bhat & Reddy, 2017; Alemayehu *et al*., 2023)

Conclusion

The present study establishes the occurrence of a diverse seed-associated mycoflora in pumpkin seeds marketed in the Agra region. Eighteen fungal species representing ten genera were recovered by the agar seed plating method with *Aspergillus flavus* identified as the most frequent and dominant species, followed by *Aspergillus ochraceus*, *Alternaria alternata*, *Penicillium citrinum*. The occurrence of these fungi is of food-safety significance because several are recognised as potent producers of mycotoxins. Since toxin concentrations were not directly quantified in the present investigation, the findings should be interpreted as evidence of potential mycotoxin risk rather than confirmed toxin contamination. Further analytical studies are recommended to quantify aflatoxins and other relevant mycotoxins in the corresponding seed lots.

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