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Diversity of fungal contamination in peanut products locally available in Peshawar Region, Pakistan

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Abstract

Peanut (*Arachis hypogaea*) used as a major ingredient in varieties of products including; bakery products and poultry feed. Peanut is a valuable economic crop in Pakistan. Currently, there is no fungal contamination information available of the groundnut products in Peshawar region, Pakistan. Therefore, the current study is to highlight the fungal contamination associated with peanut products (Kernels, Cakes, and Oils) in City, Cantt and University areas of Peshawar, Pakistan. Among the products, peanut kernels samples were found most contaminated (3.0 x 10^4 CFU/g) in Cantt area, peanut cakes samples were found most contaminated (5.2 x 10^4 CFU/g) in City area, and peanut oils samples were found most contaminated (1.5 x 10^3 CFU/g) in Cantt area. *Aspergillus flavus* (29.2%) was found the most frequently isolated fungal species, followed by, *A. niger* (21.1%), *A. fumigatus* (16.5%) and *Penicillium notatum* (1.6%), respectively. It were observed, that peanut products obtained from Peshawar city and Cantt areas could be unsafe for consumption as food or feed ingredients due to contamination by fungi, which is an indicative factor for the presence of Aflatoxins, Ochratoxin and other types of mycotoxins. Therefore, implications of inadequate drying of peanut, in storage conditions and at the time of processing this should be consider in first priority.

Key words: Peanut, Fungi, Quality Assessment, Contamination, Peshawar

Introduction

Fungus infects peanuts products during production, processing and in all stages of peanuts products supply chain (Murphy *et al.* 2006). In damp condition certain levels of mould infection can occur in peanut in the field including; at the time of harvest, insect infestation, impropre post-harvest handling, delayed harvesting, transporting, drying, Storage and différent processing techniques. Due to mould grwoth, crop yields reduction and poor live stock productivity has been reported (Reddy *et al.* 2013; Tiffany, 2013). Production of Peanut and trade are faces various challenges due to mycotoxin producing fungus. Due to these fungal contamination lose of quality and Trade value of the agricultural commodites has been reported (Mutegi *et al.* 2007; Soler

et al. 2010). Mycotoxins are natural toxins produced by fungus belonging to genus Aspergillus. These are the secondary metabolites, which may cause high levels of acute to chronic teratogenic and mutagenic effects due to contamination of food. These can create hurdles to international trade for commodities, which cannot maintain regulatory limits of commodities from market and typically highly resistant with high regulations of feeds and foods. Neurotoxicity, birth defects, immune suppression, skin irritation, and death are the symptoms of mycotoxicoses (Chang *et al.,* 2013).

Consumption of mouldy groundnut causes the death of some children in Ibadan. Furthermore, the use of mycology as weapons, cannot be ignored in the current state of world concerns with bio-terrorism and biosafety in relation to food or water supplies. These fungi are also getting credibility as source of health problems. Indeed, in mycology taxonomic research the unambiguous identification of fungus remains the most critical area because of some what confused state of the systematic and importance of mycology (Salau *et al.,* 2017).

The purpose of this study was to evaluate fungal contamination in peanut products collected from three main areas of Peshawar region. This will help to determine the level of fungal contamination in peanut products available in local market assessing the quality of the products, which may causes health risks due to consumption of these products.

Materials & methods

Collection of Samples

One hundred and fifty (n=150) peanut products samples were purchased from different stores of City, Cantt and University areas of Peshawar, Pakistan. Fifty samples (50), each included peanut kernels, peanut cakes, and peanut oils were collected from different selling shades, stores, and centres, respectively. Approximately, 200g representative each samples, were collected in small polyethylene packs according to sampling method reported by Whitaker (Whitaker, 2003).

Fungal culture and isolation

Agar dilution method was used for the isolation of fungi (Pittet, 1998, Salau *et al.,* 2017), 10 gram each sample of peanut kernel and cake powder were thoroughly mixed with peptone buffer (90 ml) and then serial dilutions (10^{-1} - 10^{-4}) were made. Fungal species isolation were done on Potato Dextrose Agar (PDA). The medium in sterile Petri dish were taken and each samples solution (0.1 ml) were spread onto PDA media in plates. The plates were incubated for 5 to 7 days at 25°C. Purification of fungal isolates were performed through, sub-culturing on Sabouraud Dextrose Agar (Oxide, UK), and incubated at 25°C for 5 to 7 days. Identification of fungi was performed by microscopic observation and culture appearance such as reverse colour, colonies colours, hyphae arrangement, texture, nature of spores and conidia spores (Singh *et al.,* 1993). The total fungal counts for each plates were expressed as colony forming units per gram of sample (cfu/g). Identification of each specie or genus was expressed in percentage (%) of the total isolated fungi. The total colonies of fungi were enumerated and results were reported in mean and average fungal counts (Pitt and Hocking, 1997; Dachoupakan *et al.,* 2009).

Identification of Mold

Identification of fungal Genera and the enumeration of fungal specie including *Aspergillus a*nd *Penicillium* were performed by observing both morphology of the colonies and microscopic characteristics on PDA and SDA medium (Klich, 2002; Pitt and Hocking, 1997).

Data Analysis

One-way ANOVA statistics was done for comparison of means of Total Fungal Count among peanut products and fungal species overall percentage (%). The means were separated for significance test by Duncan's Multiple Range Test (*P>0.05*).

Results

In this study, the entire peanut products sample showed different levels of fungal contamination. In these samples, two hundred and fifty (250) isolates of fungus belonging to eight (8) identified species including; *Fusarium*, *Rhizopus*, *Mucor*, *Penicillium*, *A. niger*, *A. flavus, A. fumigates* and *A. parasiticum*, respectively. In peanut kernel samples, maximum fungal count (3.0×10^4 CFU/g) observed from Cantt area, whereas, minimum fungal count (1.9×10^4 CFU/g) from University area. Higher fungal loads (5.2 x 104 CFU/g) were observed in peanut cake samples of City area, as compared to others areas. In peanut oil samples fungal load was higher in Cantt area (1.5×10^3 CFU/g) in comparison with others areas.

Aspergillus species were detected in all samples, whereas, species of *Fusarium* were found in all samples except in peanut oil sample. *Penicillium* species were present in only peanut cake samples from all areas except in City, while *Rhizopus* species occurred only in peanut kernel and cakes samples from all areas of Peshawar. *A. paraciticum* was present in peanut kernel samples. Overall the highest (29.2%) levels of *A. flavus* was detected being higher significant difference (*P<0.05*) as compared to other species of fungus. *Penicillium* species were found lowest (1.6%) having significant difference (*P<0.05*) as compared to *Rhizopus* and *A. paraciticum* (Table 1).

Table 1: Distribution of fungal contamination in different Peanut products of three areas at Peshawar region, Pakistan

Products	Areas of Peshawar	*TFC (cfu/g)	Percentage (%) of fungal species occurring in peanut products samples							
			Aspergillus flavus	*Aspergillus niger*	*Aspergillus fumigates*	*Fusarium solani*	*Aspergillus parasiticum*	*Penicillium notatum*	*Rhizopus stolonifer*	*Mucor racemosus*
Peanut Kernel	City	2.8×10^4	22.0	15.2	15.3	12.7	10.1	-	14.5	12.0
	University	1.9×10^4	18.6	18.6	13.7	5.2	12.2	-	3.9	8.8
	Cantt	3.0×10^4	26.8	17.8	15.8	19.0	6.8	-	17.0	15.6
	Mean	2.6×10^4								
Peanut cakes	City	5.2×10^4	35.6	27.8	22.8	6.1	-	-	5.7	2.9
	University	1.7×10^4	30.2	13.9	16.9	11.2	-	8.1	13.8	5.1
	Cantt	4.2×10^4	33.9	26.0	13.5	8.8	-	5.2	11.5	-
	Mean	3.7×10^4								
Peanut oil	City	1.4×10^3	41.0	25.5	34.2	-	-	-	-	-
	University	1.3×10^3	28.7	34.1	-	-	-	-	-	39.0
	Cantt	1.5×10^3	36.6	28.9	15.9	-	-	-	-	15.9
	Mean	1.4×10^3								
Overall (%)			29.2	20.1	16.1	8.0	5.3	1.6	9.5	10.2

TFC (cfu/g) = Total fungal count in colony forming units per gram, Overall (%) = Mean with different letters in the same row are statistically different ($P < 0.05$) according to Duncan's test

Discussion

In this study, the fungal population recovered from peanut products have great public health hazards due to presence of notable toxins producing species whereas some others were saprophytes. Food items were being deteriorated during their adaptation and survival in their environment. It has been reported that species of *Aspergillus, Rhizopus* and *Penicillium* recovered from peanut products samples corroborate to similar fungi as contaminants of peanut cake alongside other fungi which could not be identify or not recovered during the study (Adebesin *et al.,* 2001).

The fungi along with *Fusarium* species were reported as major contamination fungi in storage of peanut (Gachomo *et al.,* 2004; Jimoh *et al.,* 2008). Therefore, the occurrence of contamination in these products might be originated from the raw peanut, which has been utilized in processing of peanut cake and may be due to exposure of these marketed products to spores of fungus presence in air after production of products. The fungal count were found higher in samples of markets located in Cantt area than City and University areas in the region, so the earlier contamination may be a minor contributor as compared to exposure of products in market due to fungal spores in air.

Many researchers reported their findings about presence of *Penicillium* species, *Fusarium* species, *Rhizopus*, *A. flavus* and *A. niger* in peanut cake samples and further concluded that majority of fungal species produces toxigenic isolates may pose a toxicological threat to the consumers (Akano and Atanda, 1990; Jimoh *et al.,* 2008, Makun *et al.,* 2010). A metabolite rhizonin-A is known to produce by *Rhizopus* and during metabolism of this fungus may produce aflatoxins, ochratoxins, fumonisins, trichothecenes, citrinin and patulin (Wilson *et al.,* 1984). It was previously reported, that consumption of aflatoxins contaminated foods causes deaths in Ibadan, Oyo state, Nigeria (Jimoh *et al.,* 2008). Range of factors such as processing, implicit factors, intrinsic nutritional factors and extrinsic may cause seed spoilage due to presence of fungi in stored peanut represents a complex ecosystem. Factors in combination among them or alone affect the composition of the fungal population due to changes throughout the storage period. In all peanut products samples the presence of fungal population of *A. flavus* species was significantly higher as compared to other isolates, without any change in processing method, supported the previous findings about contamination of peanut due to presence of *Aspergillus* species (Soler *et al.,* 2010; Sultan and Magan, 2010; Nyirahakizimana *et*

al., 2013). In this study, the presence of three species of *Aspergillus* agrees with the findings of Salau *et al.,* 2017.

Conclusion

This study showed high incidence of fungal contamination (65.4%) by species of *A. niger, A. flavus* and *A. fumigates* in peanut products of three areas in Peshawar region. The findings in this research suggested that peanut products could be contaminated with high levels fungi (*A. flavus* and *A. niger*), which is an indication factor for the real and confirmed presence of Aflatoxin, ochratoxin A and other mycotoxins suggesting several products of peanut unsafe for human consumption and also as animal feeds ingredients used in feed formulation. The contamination level of peanut products by fungus is closely related to the use of fungicide, organic manuring and un-removal of peanut kernel (sorting) during different agricultural practices such as before harvest, poor handling, storage and processing methods.

Recommendations

Based on these findings in this study, it was recommended that there is a need to create awareness among farmers, processors, sellers and consumers to develop strong and suitable protocols for proper handling of peanut products from production to storage sites and to ensure safety of the products for both humans and animals consumption. The implications of inadequate drying of peanut, in storage conditions and at the time of processing this should be consider in first priority.

References

Adebesin AA, Saromi OT, Amusa NA, Fagade SO. (2001). Microbiological quality of some Groundnut products hawked in Bauchi, a Nigerian City. Journal of Food Technology in Africa. 6(2), 53-55.

Akano DA, Atanda OO. (1990). The present Level of Aflatoxin in Nigeria Groundnut Cake ('Kulikuli').Letters in Applied Microbiology. 10, 187-18.

Balogun BO, Surajudeen AA. (2010). Fungal and Aflatoxin contamination of some human food commodities in Nigeria. African Journal of Food Science. 4(4), 127–135.

Chang AS, Sreedharan A, Schneider KR. (2013). Peanut and peanut products: a food safety perspective. Food Cont. 32, 296–303.

Dachoupakan C, Ratomahenina, Martinez R, Guiraud JP, Baccou JC, Schorr GS. (2009). Study of the phenotypic and genotypic biodiversity of potentially ochratoxigenic black aspergilli isolated from grapes. International Journal of Food Microbiology. 132, 14-23.

Gachomo EW, Mutitu EW, Kotchoni OS. (2004). Diversity of fungal species associated with peanuts in storage and the levels of aflatoxins in infected samples. Internal Journal of Agriculture and Biology. 6(6), 955-959.

Jimoh KO, Kolapo AL. (2008). Mycoflora and Aflatoxin production in market samples of some selected Nigerian foodstuffs. Research Journal of Microbiology. 3, 169-174.

Klich MA. (2002). Introduction; economic and medical importance of Aspergillus. In Identification of common Aspergillus species, 1-16 p.

Makun HA, Anjorin ST, Moronfoye B, Adejo FO, Afolabi OA, Fagbayibo G, Murphy PA, Hendrich S, Landgren C, Bryant CM. (2006). Food mycotoxins, an update. Journal of Food Science. 71(5), 51-65.

Mutegi CK, Hendrisks SL, Jones RB, Okello JJ, Ngugi HK. (2007). Role of collective action and handling practices on aflatoxin contamination of groundnuts: Evidence from Kenya. African Crop Science Conference Proceeding. 8, 1779-1782.

Nyirahakizimana H, Mwamburi L, Wakhisi J, Mutegi CK, Christie ME, Wagacha JM. (2013). Occurrence of Aspergillus spp. and Aflatoxin contamination in raw and roasted Groundnuts from formal and informal markets in Eldoret and Kericho towns, Kenya. Advances in Microbiology. 3, 333-342.

Pitt J, Hocking A. (1997). Fungi and Spoilage, 2nd Ed. London, N.Y. Blackie Academy and Professional.

Pittet A. (1998). Natural occurrence of mycotoxins in foods and feeds: an updated review. Revue Medicine Veterinary. 149, 479-492.

Reddy CR, Reddy AR, Waliyar F, Alur AS, Gowda CL, Reddy BV, Rao PP. (2013). Mycotoxins in sorghum and pearl millet: effect on human and animal health and their management. CFC/FAO. 1.

Salau IA, Shehu K, Muhammad S, Umar RA. (2017). Determination of aflatoxin level in groundnut oils marketed in Sokoto State, Nigeria. International Research Journal of Biochemistry and Biotechnology. 3(1), 051-054.

Salau IA, Shehu K, Farouq AA. (2017). Molecular characterization of mycotoxin producing fungi contaminating groundnut products in Sokoto Sate, Nigeria. J. Of Computation in Biosciences and Engineering. 3(4), 1-4.

Singh K, Frisvad JC, Thrane U, Mathu SB. (1991). An illustrated manual on identification of some seed borne Aspergilli, Fusaria, Penicillia and their mycotoxins. Heller up, Denmark: Danish Government, Institute of seed pathology for developing countries. 133.

Soler CMT, Olatinwo R, Diarra B, Waliyar F, Traore S. (2010). Groundnut contamination by Aspergillus flavus and Aflatoxin B1 in granaries of villages and markets of Mali, West Africa. Journal of Food Agriculture and Environment. 8, 195-203.

Sultan Y, Magan N. (2010). Mycotoxigenic fungi in peanuts from different geographic regions of Egypt. Mycotoxin Research. 26, 133-140.

Tiffany I. (2013). The implication of aflatoxin contamination for local food safety in Senegal. www.hungercenter.wpengine.netdna-cdn.com.

Whitaker TB. (2003). Standardisation of mycotoxin sampling procedure an urgent necessity. Food Contaminants. 14, 233-237.

Wilson T, Rabie CJ, Fincham JE, Steyn PS, Schipper MA. (1984). Toxicity of rhizonin A, isolated from *Rhizopus microspores*, in laboratory animals. Food Chemistry Toxicology. 22(4), 275-281.