OCCURRENCE OF MYCOFLORA ASSOCIATED WITH CASHEW NUTS (ANACARDIUM OCCIDENTALE L.)

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Abstract

Poor harvesting practices, improper storage, and less than optimal conditions during transport and marketing can also contribute to fungal growth. Fungal contamination of various agricultural commodities and foodstuffs (like dry fruits), is a major problem in the developing countries like India. Fungi play a significant role in deteriorating the aesthetic and nutritive value of stored food commodity. Therefore, the aim of this study was to evaluate the mycoflora associated with cashew nuts.

Samples of cashew nuts were collected from local shops of Amravati region during 2016-17. Samples were analyzed for the moisture contents and the presence of fungi by adopting direct plating and dilution plating methods. Altogether 14 fungal species were isolated from figs viz. Alternaria alternata, Aspergillus chevalieri, Aspergillus flavus, Aspergillus fumigatus, Aspergillus nidulans, Aspergillus niger, Aspergillus parasiticus, , Cladosporium herbarum, Cladosporium macrocarpum, , Fusarium solani, Mucor varians, Penicillium verrucilosum, Rhizopus stolonifer and Verticilli puniceum. Among all the fungi, genus Aspergillus was the most predominant isolate with 6 different species. Two species from Aspergillus, Section Flavi- A. flavus and A. parasiticus are known to produce the toxic and carcinogenic compounds aflatoxins (AFs) which are hazardous to animal and human health. Therefore, the occurrence of contamination with spoilage and toxigenic fungi on cashew nuts could be avoided or at least diminished if good agricultural (harvesting and handling), manufacturing (sorting and packaging) and proper storage practices will be applied.

Keywords: Cashew nuts, postharvest, ecological factors, mycoflora, afiatoxins(AFs).

Introduction

The cashew plant *(Anacardium occidentale L.)*, is a medium sized tree belonging to the family Anacardiaceae. The seeds are the source of cashew nuts and they are normally removed from the pericarp after the fruits are roasted. Worldwide, nuts are esteemed and highly priced food delicacy because of their pleasant taste and flavor in addition to their content of proteins and antioxidants. Fungi are found in different food commodities including cereals, nuts, spices, figs and dried fruits (Pitt and Hocking, 2009). They may contaminate foods by colonizing them at several stages of the food chain; pre-harvesting, processing, transportation and storage (Manonmani *et. al.*, 2005). The economic loss resulting from fungal and mycotoxin contamination of nuts is difficult to estimate. However, judging from the widespread occurrence of fungal and mycotoxin contamination and the large number of nuts affected, one can assume that such losses must be large. These losses constitute direct nut losses, human illness and reduced productivity and livestock losses from deaths and lower growth rates (El-Magraby and El-Maraghy, 1988). Some of the species, especially of *Aspergillus and Penicillium* associated with the nuts are known to have strains that produce toxic metabolites (Cole and Cox, 1981). Several environmental factors like humidity and temperature during storage influence the infestation by fungi and aflatoxin production (Drusch and Ragab, 2003).

Natural occurrence of fungal contamination of dry fruits and spices have been investigated in many parts of the world by different authors (Zohri and Abdel-Gawad, 1993; Ozay *et. al.*, 1995; Abdel-Sater and Saber, 1999; MacDonald *et. al.*, 1999; Bayman *et. al.*, 2002; Möller and Nyberg, 2003; Aksoy *et. al.*, 2007; Juan *et. al.*, 2007; Zinedine *et. al.*, 2007; Musaiger *et. al.*, 2008; Ozay and Özer, 2008; Bircan, 2009; Hedawoo and

Chakranarayan, 2011; Yilmaz and Aluc,2014; Adeniyi and Adedeji, 2015; Hedawoo *et.al*., 2017).

Cashew nut infection by toxigenic fungi has been reported in a number of studies and revealed a high risk due to contamination with mycotoxins (Mohammed, 2012). Moreover, fungi contaminated dry fruits cause considerable changes of all the biochemical contents (total Carbohydrates, Sugar, Proteins, Fat and dietary fibers) as well as affecting quality (Embaby *et. al.*,2012).

Materials and Methods:-

a) Sample collection:-

Ten samples of Cashew nuts were purchased from local markets of Amravati region. The collected samples were put in paper bags and brought into laboratory for isolation of fungi.

b) Moisture content:-

The moisture content of Cashew nuts was determined using the International Organization for Standardization (ISO) method (Hamid and Lopez,2000).

c) Mycological analysis:-

i) Direct plating method- Direct plating is considered to be the more effective technique for mycological examinations of particulate foods. The Cashew nuts pieces were surface disinfected with 2% Sodium hypochlorite solution for 2 min. then rinsed with sterile distilled water. Seven pieces were placed in each petri plates containing PDA medium. The plates were incubated at 27^0 C for 7 days (Pitt and Hocking, 1999).

ii) Dilution plating method- The dilution plating method is the most commonly used technique for the examination of food and feedstuff (Jarvis *et. al*., 1985). According to International Commission on Microbiological Specifications for fruits (ANON, 1989), sample suspension were prepared by adding 40gm of sample in 200ml sterile distilled water for 2 – 4 hours. Then shake well using a mechanical shaker for 20-30 minutes. Serial dilutions were prepared from 10^{-1} to 10^{-5}ml under aseptic condition, fungal spores sediment more quickly, so it is important to draw aliquots for dilution or plating as soon as possible (Beuchat,1992). One ml of appropriate dilution was transferred into petri plates contains PDA medium by sterile pipette, for each sample three replicates used, then plates were incubated at 27^oC for 7days (Akerstrand, 1995).

d) Identification of fungi:-

All the fungi were identified on the basis of their colony morphology and spore characteristics (Rajankar *et.al*., 2007). All species identifications were according to the keys, manuals and descriptions provided by Raper and Thom (1949); Raper and Fennel (1965); Gilman (2001); Subramanian (1971); Nagmani *et.al*., (2006)

e) Percent occurrence:-

For calculating the percent occurrence, following formula was used -

$$\% \text{ occurrence of fungus} = \frac{\text{No. of colonies of a particular fungal species in all plates}}{\text{Total no. of colonies of all the fungal colonies in all the plates}} X100$$

Results and Discussion

The experiments were carried out during winter season in the months of Dec. 2016, Jan.2017 and Feb.2017. In that period the minimum temperature was recorded as- 8^0C, 10^0C & 14^0C and maximum temperature was 32^0C, 35^0C & 38^0C in the respective months. Also the average humidity percentage was recorded as- 56%, 54% and 42% respectively. (**Table-1**). It was noticed that, the growth of fungi is directly proportional to optimum temperature. As the temperature increases fungal growth also increases. Similarly, growth of fungi is also directly proportional to humidity. As humidity increases, fungal growth also increases as supported by Pal (2015).

In the beginning of experiment, moisture content of the cashew nuts was measured as 7.33 % (**Table-1**). This range showed appropriate moisture content of the nut samples which allow the growth of xerophilic fungi. Incidence of fungi depends on the number of factors including temperature, moisture and storage time (Chelack *et.al.*, 1991). Our results agree with Beatriz *et. al*., (2006) which states that, high sugar concentration and low water activity in dried fruits assist the development of xerophillic fungi like *Aspergilli* and *Penicilli* especially *A. niger* (Toma and Rajab, 2014).

For mycological analysis, cashew nuts were plated aseptically in direct plating or indirect plating (Serial dilution plating). In direct plating technique, total 8 fungi were noticed viz *Alternaria alternata, Aspergillus chevalieri, A. flavus, A. fumigatus, A. niger, Cladosporium herbarum* , *Mucor varians* , and *Rhizopus stolonifer*. Fourteen fungal species representing eight genera were isolated by serial

dilution technique. Their percent occurrence (contamination) is presented in **Table-2**.

Most of the recovered fungi were previously reported from cashew nuts in many parts of the world (Cole and Cox, 1981; Chelack *et.al.* 1991; Mohammed, 2012; Yilmaz and Aluc, 2014; Adeniyi and Adediji, 2015).

Two species were isolated with high frequency namely- *Aspergillus chevalieri,* (43.60%) and *Aspergillus niger* (38.83%); followed by *Aspergillus flavus* (5.08%), *Aspergillus fumigatus(3.14%), Rhizopus stolonifer* (2.64%) *Cladosporium macrocarpum* (2.34%) and *Aspergillus parasiticus* (2.04%). While *Mucor varians* showed the least frequency i.e. (0.57%). Our results are in line with the reports of Saadulllah and Abdullah (2015); Adeniyi and Adediji, (2015).

Aspergillus was represented by 6 species and showed the widest diversity among all isolated fungi *viz. A. chevalieri , A. flavus, A. fumigatus, A. niger, A. parasiticus* and *A. nidulans* followed by *Cladosporium* (2) species and single species of *Penicillium, Alternaria, Fusarium, Rhizopus, Mucor and Verticillium* . These species were found common to soil, different agricultural and food commodities in India (Srivastava *et. al*., 2014).

A.flavus (5.08%) and *A. parasiticus* (2.04%) and to less extent some species in the genus *Fusarium* (1.20%) are the most important species contaminating cashew nuts because of their potential to produced mycotoxins (Heperkan *et. al*., 2012) which pose a potential hazard to consumer's health.

Due to the contamination of aflatoxins, the cashew nut is considered as a high risk commodity. The problem of aflatoxin contamination is worldwide; but in India the poor harvesting practices, high temperature, high moisture levels and post harvest practices are conductive for fungal growth proliferation and aflatoxin contamination (Reddy *et. al*., 2011).

TABLE- 1 :- Optimum temperature, atmospheric humidity and moisture %

Month	Temperature ^{0}C		Average humidity (%)	Moisture content in Cashew nuts (%)
	Min.	Max.		
December- 2016	8	32	56	7.33
January- 2017	10	35	54	
February- 2017	14	38	42	

(**Source:-** Weather report in Amravati, India *https://www.timeanddate.com*)

TABLE- 2 :- Isolated mycoflora & their percent occurrence on Cashew nuts

Sr. No.	Fungi Isolated	Direct plating method	Dilution plating method	% Occurrence
1.	*Alternaria alternata*	+	+	1.29
2.	*Aspergillus chevalieri*	+	+	43.60
3.	*Aspergillus flavus*	+	+	5.08
4.	*Aspergillus fumigatus*	+	+	3.14
5.	*Aspergillus nidulans*	-	+	1.14
6.	*Aspergillus niger*	+	+	38.83
7.	*Aspergillus parasiticus*	-	+	2.04
8.	*Cladosporium herbarum*	+	+	1.94

9.	*Cladosporium macrocarpum*	-	+	2.34
10.	*Fusarium solani*	-	+	1.20
11.	*Penicillium verrucilosum*	-	+	1.29
12..	*Mucor varians*	+	+	0.57
13.	*Rhizopus stolonifer*	+	+	2.64
14.	*Verticillium puniceum*	-	+	0.88

(+) Fungus present, (-) Fungus absent

Conclusion

The present study revealed that cashew nuts are highly contaminated with several mycotoxigenic fungi such as *A. flavus, A. parasiticus* and others. Therefore, strict hygiene mycological measureds should be done during harvest, storage and drying to minimize contamination with such fungi. Therefore, the authorities should take the lead in the efforts to establish mandatory regulations in cashew nut 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. Development of efficient pre- and post-harvest hygienic practices must be considered as components to be integrated into cashew nut production processing.

References

1. **Abdel-Sater MA, and Saber SM (1999).** Mycoflora and mycotoxins of some Egyptian dried fruits. *Bulletin of the Faculty of Science of Assiut University D. 28: 92–107*. (Chemical Abstracts 133, 3874 p. 2000).
2. **Adeniyi DO and Adedeji AR (2015).** Evalutation of fungal flora and mycotoxin potential associated with postharvest handlings of Cashew Nut Scholars Research Library 30- 33.
3. **Akerstrand K (1995).** Mould and yeast Determination in foods. *UDC.Nordic Committee on Food Analysis.*582.23-633.1.
4. **Aksoy U, Eltem R, Meyvaci KB, Altindisli A, and Karabat S (2007).** Five-year survey of ochratoxin A in processed sultanas from Turkey. *Food Additives and Contaminants*. 24: 292–296.
5. **Alghalibi SM and Shater AM (2004).** Mycoflora and Mycotoxin Contamination of some Dried Fruits in Yemen Republic. Ass. *University Bulletin for Environmental Researches.* 7(2):19-27.
6. **Anon (1989).** Council for Agricultural Science and Technology. *Mycotoxinsq: Economic and Health Risks. Task Force Report No. 116.*
7. **Baitriz TI, Taniwaki MH, Vicente E and Menezes HC (2006).** Fungi producing ochratoxin in dried fruits. *Advances in Experimental Medicin and Biology*. 571:181-188.
8. **Bayman P, Baker JI, Doster MA, Michaildes TJ, and Mahoney NF (2002).** Ochratoxin production by *Aspergillus ochraceus* group and *Aspergillus alliaceus*. *Appl.Environ. Microbiol*. 68: 2326-2329.
9. **Beuchat LR (1992).**Enumeration of fungi in grain flours and meals as influenced by settling time in diluent and by the recovery medium. *Journal of Food Protectionection*. 55: 899–901.
10. **Bircan C (2009).**Incidence of ochratoxin A in dried fruits and co-occurrence with aflatoxins in dried figs. *Food Chemical Toxicology*. 47: 1996–2000.
11. **Chelack WS, Borsa J, Marquardt R and Frohlich AA (1991).** Applied and Environmental Microbiology. 57:2492.2496.
12. **Cole RJ and Cox RH(1981).** Handbook of toxic fungal metabolites. Academic press, New York.
13. **Doster MA, Michailides TJ,and Morgan DP (1996).** Aspergillus species and Mycotoxinsin figs from Calofornia Orchards. *Plant Dis.*, 80:484-489.
14. **Drusch and Ragab (2003)**. Mycotoxins in fruits, fruit juices and dried fruit. *Journal of Food Protection*, Vol.66, No.8,pp. 1514-1527, ISSN 0362-028X.
15. **El-Magraby OM and El- Maraghy SS(1988).** *Mycopathologia* 104:19-24.
16. **Embaby EM, Hagagg LF and Abdel-Galil MM (2012)**.Decay of some fresh and dry fruit quality contaminated by some mold fungi. *J.Appl.Sci.Res.*8:3083-3091.

17. **Gilman JC (2001).** A manual of soil fungi. *Biotech. Book Publi. Delhi*, pp. 392.
18. **Hamid A and Lopez AS (2000)**. Quality and weight changes in cocoa beans stored under two warehouses conditions in East Malaysia. *The planter*. 76:619-637.
19. **Hedawoo GB and Chakranarayan RG (2011).** Isolation of fungal species from the seeds of some Indian spices. *Biosci. Biotech. Res. Comm*. Vol. 4, No. 2: 208-210.
20. **Hedawoo GB, Bijwe HV and Maggirwar RC (2017).** Occurrence of Mycobiota Associated with *Ficus carica L. Inte. Jou. Of Res. In Bio. Agri. And Tech* Special Issue (2)vol. 5. 985 -989.
21. **Imanaka BT, Castle de Menezes H, Vicente Leite RSF and Taniwaki MH (2007).** Aflatoxigenic fungi and aflatoxins occurrence in sultans and dried figs commercialized in Brazil. *Food Control.* 18:454-457.
22. **Jarvis B, Seiler DA, Ould AJ, and Williams AP (1985).**Observations on the enumeration of moulds in food and feeding stuffs. *Journal of Applied Bacteriology*. 55: 325-336.
23. **Javanmard M**. **(2010).** Occurrence of Mould counts and *Aspergillu*s species in Iranian dried figs at different stages of production and processing. *J. Agri. Sci. Tech.* Vol. 12:331-338.
24. **Juan C, Zinedine A, Molto JC, Idrissi L, and Manes J (2007).** Aflatoxins levels in dried fruits and nuts from Rabat-Sale´ area, Morocco. *Food Control.* 1:849–853.
25. **MacDonald S, Wilson P, Barnes K, Damant A, Massey R, Mortby E, and Shepherd MJ, (1999).** Ochratoxin A in dried vine fruit: method development and survey. *Food Additives and Contaminants*. 16: 253–260.
26. **Mohammed SA (2012).** *J. Am. Sci.* 8(12): 525-534.
27. **Manonmani HK, Anand S, Chandrashekhar A and Rati ER (2005).** Detection of aflatoxigenic fungi in selected food commodities by PCR. Process Biochemistry. 40:2859- 2864.
28. **Moller TEand Nyberg M(2003).** Ochratoxin A in raisins and currants: basic extraction procedure used in two small marketing surveys of the occurrence and control of the heterogeneity of the toxins in samples. *Food Addit Contam*.Vol.20:1072-1076
29. **Musaiger AO, Al-Jedah JH and D'souza R (2008).**Occurrence of contaminants in foods consumed in Bahrain. *Food Control.* 19: 854–861.
30. **Nagmani A, Kunwar, IK and Manoharachary, C (2006).** Handbook of soil fungi. I.K. *International Pvt. Ltd. N. Delhi*, pp.477.
31. **Ozay G and Ozer H (2008).**Mycotoxin Problems in nuts and dried fruits from the Mediterranean basin. In: *Mycotoxins: detection methods, management, public health and Agricultural Trade* (J.F. Leslie, R. Bandyopadhyay, A. Visconti, ed.), CAB International, Oxfordshire, UK, 133–138.
32. **Ozay G., Aran, M and Pala, M. (1995).** Influence of harvesting and drying technique on mycoflora and mycotoxins of figs. *Nahrung.* 39:156-165.
33. **Pal P(2015)** Effect of temperature and humidity on seed mycoflora of charoli and almond, India *International Research Journal of Environmental Seiences* Vol. 4(9),10-15.
34. **Pitt JI and Hocking AD (1999).** Fungi and Food Spoilage. *Aspen Publisher, Inc., Gaitherburg, Maryland.*
35. **Pitt JI and Hocking AD (2009).** Fungi and food spoilage, 3rd edition. *Springer, New York, USA* . 540 PP.
36. **Rajankar PN, Tambekar DH and Wate SR (2007).** Study of phosphate solubilization efficiencies of fungi and bacteria isolated from saline belt of purna river basin. *Research Journal of Agriculture and Biological Science.* 3(6):701-703.
37. **Raper KB and Thom C, (1949).** A manual of *Penicillia*, The Williams and Wilkins Co., Baltimore.
38. **Raper KB and Fennel DI (1965).** The genus *Aspergillus*, The Williams and Wilkins Co., Baltimore.
39. **Samson RA, Noonim, P., Meijer, M. Houbraken, J., Frisvad, JC and Varga, J. (2007)**. Diagnostic tools to identify black *Aspergilli. Stud. Mycol.*59:129-145.
40. **Senyuva HZ, Gilbert J, Saneson RA, Ozcan S, Ozturkoglu S and Onal D (2008).** Occurrence of fungi and their mycotoxins in individual Turkish dried figs. *World Mycotoxin Journal.* 1:79-86.
41. **Srivastava M**., **Pande S, Srivastava, L and Srivastava, C. (2014).** Fungal infestation in some dry fruits during storage in different seasons. *International J. of Multidisciplinary and Current research, Jan/Feb* 2014.
42. **Subramanian, CV (1971).** Hyphomycetes:An account of Indian Species except Cercosporae.ICAR Publi.N Delhi.pp.930.
43. **Toma FM and Rajab ALNN (2014).** Isolation and Identification of Fungi from Dried Fruits and study of Quantitative

Estimation of Aflatoxin. *Zanco Journal of Pure and Applied Sciences*. Vol. 26, No. 4:49-60.

44. **Tournas VH, Niazi, NSand Kohn, JS (2015).** Fungal presence in selected tree nuts and dried fruits. *Microbiology Insights.* 2015:8.
45. **Yilmaz I and Aluc M (2014).** Determination of Aflatoxin levels in Cashews on Turkish markets. Foodbalt 321-323.
46. **Zinedine A, Soriano JM, Juan C, Mojemmi B, Molto JC and Bouklouze A (2007).** Incidence of ochratoxin A in rice and dried fruits from Rabat-Sale´ area, Morocco. *Food Additives and Contaminants*. 24:285–291.
47. **Zohri AA and Abdel-Gawad KM (1993).** Survey of microflora and mycotoxins of some dried fruits in Egypt. *J. Basic Microbiology* 4: 279-288.