

Survey and Collection of Mycotoxin Producing Fungi in Dried Fruits and Nuts Sample from Bihar

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Abstract

The point of this examination was directed to survey the mycotoxigenic parasitic affiliation and cooccurrence of aflatoxins and citrinin tainting in raisins, pistachio nut, pecan and almonds from Indo-gangetic plain territory of Bihar. 188 examples of raisins, pistachio nuts, walnuts and almonds were gathered from gangetic territory of Bihar state. Subjective and quantitative assessments of mycotoxins were led by Enzyme Linked Immuno Sorbant Assay (ELISA). 76.3% of raisins and 82% of pistachio tests were sullied with either aflatoxins or citrinin or both. 462 ppb of aflatoxins were identified from pistachio though just 174 ppb were distinguished in almonds tests. The aftereffects of this examination propose that raisins, pistachio, pecan and almonds are defenseless substrate for aflatoxigenic just as citrinin producing fungi and further aflatoxins and citrinin creations.

Keywords: *Mycotoxigenic Fungi, Aflatoxins, Citrinin, ELISA, Dried Fruits*

1. INTRODUCTION

Measurement of fungi each example was investigated for the aggregate sum of molds and yeasts and for the convergence of conceivably oxygenic species. The evaluation of fungi in the examples was performed by NMKL. From the start 40.0 grams of each example was said something a stomacher channel sack. Following 30 minutes of absorbing peptone water without salt, the example was squashed in a stomacher gadget for 2 minutes. Each example was weakened and the 10-1, 10-2, 10-3 and 10-4 weakening were spread on DG18 plates. To bring down the restriction of measurement, the 10-1 weakening was additionally examined by pupating 1 ml of test suspension straightforwardly in a petri dish after which roughly 30 ml of fluid DG18 was poured. Suggest DG18 as an overall substrate for nourishments of $a_w > 0.90$, and as a specific substrate for xerophilic fungi in nourishments of $a_w < 0.90$. The specific standards is the high convergence of sugars and the dichloran which represses the development of quickly developing fungi empowering measurement of province framing units (cfu).

Citrinin Contamination in Some Dried Fruits and Nuts

Probability of mycotoxin producing confined fungi Mycotoxin producing possibility of *A. flavus*, *A. ochraceus*, *A. paraticus*, *P. citrinum*, and *P. verrucosum* were inspected. The subjective and quantitative assessment of mycotoxins producing possibility of fungi were finished by the technique for aflatoxins producing probability of *Aspergillus* spp.; for testing ochratoxin An and citrinin producing probability of *A. ochraceus*, *P. citrinum* and *P. verrucosum*.

Mycoflora in Some Commercially Important Agricultural Commodities

Mycotoxin pollution of different groceries and horticultural items is a significant issue in the jungles and sub-jungles, where climatic conditions and farming and capacity rehearse are helpful for parasitic development and poison creation. Mycotoxins are contagious optional metabolites distinguished in numerous rural items screened for toxigenic molds. Mycotoxins have been accounted for to be cancer-causing, teratogenic, tremorogenic, hemorrhagic and dermatitic to a wide scope of living beings, and known to cause hepatic carcinoma in man. There are numerous such mixes yet a couple of them are routinely found in food and creature feedstuffs, for example, grains and seeds.

2. LITERATURE REVIEW

Luca Dellafiora (2019) Mycotoxins are low-sub-atomic weight harmful mixes synthesized by various kinds of molds having a place for the most part with the genera *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria*. They may enter the evolved way of life worldwide as an outcome of the capacity of mycotoxin-producing molds to contaminate a wide number of yields and food products. It has been accounted for that up to 25 % of world harvests might be debased with mycotoxins and over 4.5–5.0 billion individuals are believed to be persistently presented to these food foreign substances.

Punam Jeswal, Dhiraj Kumar (2017) Mycotoxins are poisonous optional metabolites principally delivered by the three contagious genera *Aspergillus*, *Penicillium* and *Fusarium*. In excess of 400 mycotoxins are known so far the main mycotoxins are aflatoxin, ochratoxin and citrinin. Aflatoxins are commonly delivered by *A. flavus* and *A. parasiticus*, which produce four distinct aflatoxins i.e AFB1, AFB2, AFG1 and AFG2 in farming ware.

Eskola, M.; Kos, G (2019) Although the most elevated levels of food defilement are all the more much of the time found in low-pay nations, mycotoxins really speak to a developing danger likewise because of atmosphere changes. The tainting of food and feed by mycotoxins brings about huge monetary misfortunes around the world, not just regarding food and feed waste, yet in addition as far as a weight on human wellbeing, creature profitability and global exchange.

Gotthardt, M.; Asam, S (2019) this situation is additionally convoluted by the conceivable presence of supposed "veiled mycotoxins". This term alludes to adjusted types of mycotoxins because of their metabolic changes in plants. Concealed mycotoxins have been accounted for to bounteously co-happen in tainted food and crude materials alongside their separate parent partners

Sanzani, S.M (2019) examined the co-event of 17 distinct mycotoxins in new, singed, dried or grounded sweet pepper items. Prominently, every one of them were debased by more than one mycotoxin all the while. In more detail, 6 out of 39 examples contained 2, 3 or 4 distinct mycotoxins, while the leftover examples were positive for various mycotoxins going from 5 to 16. The seared peppers indicated the least normal degree of defilement (with a normal mycotoxin tainting of 231 µg/kg), while the new pepper tests were the most polluted (27,280 µg/kg).

3. MATERIAL AND METHODS

Review and Sample collection A broad study of various locale of Bihar situated in Indo-Gangetic plain zones have been completed. Buxar, Bhojpur, Patna, Chapra and Bhagalpur regions were chosen for test collection. These regions have been chosen since they have thick populace. All out 188 examples (55 raisins, 50 pistachios, 40 pecan and 43 almonds) were gathered from various business sectors. 100 gm of each example were kept into the sterile cellophane then into clean earthy colored encompass and kept it at 4°C to capture any mycotoxins development.

Screening of Fungi

Tests were set on Potato Dextrose Agar (PDA) media and Standard blotting surface paper and brood at 28 °C for 5 – 7 days. Plates were inspected outwardly and by binocular stereomicroscope and contagious checks were recorded. Distinguishing proof of fungi was done by morphological qualities and followed the ordered plans for different genera.

4. INHERENT AND QUANTITATIVE ANALYSIS BY TLC AND ELISA

The Qualitative and quantitative location for cooccurrence of aflatoxin and citrinin in raisin, pistachio, pecan and almond tests were investigated by Thin Layer Chromatography (TLC) and protein connected immunosorbent measure (ELISA). Subjective discovery of tests was broke down by the techniques. Further sure examples were reconfirmed by ELISA and quantitative investigations of tests were broke down (Eslami et. al., 2015). AgraQuant absolute aflatoxin (COKAQ1000) was utilized for complete aflatoxins from ROMER LAB (ASTRIA) and RIDASCREEN FAST citrinin Assay (6302) for Citrinin. 20 gm of test were pounded and added 100 ml of 70% methanol mixed for 3 moment. The arrangements were sifted and supernatant was gathered. 4ml of concentrate was moved through cleanup segments.

The presence of aflatoxins and citrinin was recognized with explicit ELISA units (aflatoxins and citrinin pack) and the optical thickness was recorded by the ELISA peruser (MERK mios smaller than usual) utilizing a 450 nm channel with a differential channel of 630 nm. The base distinguishable measure of ELISA unit for aflatoxins was 4ppb and 15ppb for citrinin. Standard bend was set up with standard arrangement furnished with ELISA packs. The optical densities of the examples were contrasted with the optical thickness of principles and interpretative outcomes were resolved utilizing weakening element.

5. RESULTS AND DISCUSSIONS

In our investigation, predominance of mycoflora was related with raisins, pistachio nut, pecan and almonds tests in which some were toxigenic (Figure. 1). A sum of 6 distinctive parasitic genera have a place with 21 animal varieties were separated (Table 1). Fungi were recognized based on their way of life and morphological attributes, these were distinguished as.

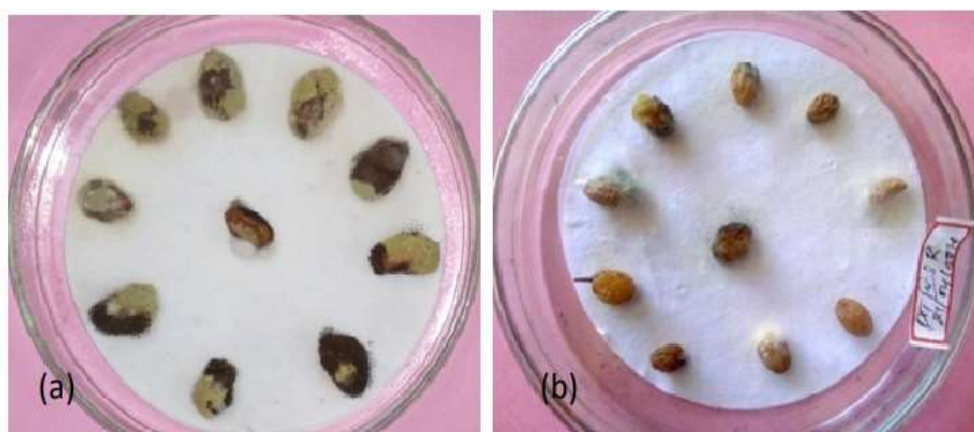


Figure 1.1: (a) Pistachio nut and (b) Raisins plated in Standard Blotter Paper indicating vivacious development of fungi

Aspergillus paraciticus, *A. oryzae*, *A. tamari*, *A. niger*, *A. Yemen*. Pistachio tests were likewise debased with *flavus*, *A. ochraceus*, *A. versicolour*, *A. disinfects*, *A. another aflatoxigenic*, *A. paraciticus* (3.1×10^4) *fungi. terrus*, *A. sydowi*, *Penicillium citrinum*, *P. islandicum*, *P. verrucosum*, *P. cyclopium*, *Fusarium oxysporum*, *F. moliniforme*, *Chaetamium globosum*, *Rhizopus nigricans*, *R. oryzae* and *Mucor hiemalis*. *Aspergillus flavus* and has revealed that pistachio nut of Spain had greatest aflatoxigenic parasitic tainting and aflatoxin defilement level up to $1134.5 \mu\text{g kg}^{-1}$. In present examination, Raisins, pecan and almonds *Aspergillus niger* was the most predominant present in totally dried fruits and nuts.

Pistachios are normally thick food and rich wellspring of proteins, unsaturated fats, dietary filaments, Vitamin B6, calcium, riboflavin, Vitamin An and K. The bits of pistachio nut are regularly eaten entire, either new or simmered and salted. It controls the pulse and Foods and Drugs Administration; USA additionally affirmed that the pistachio seeds bringing down the danger of coronary illness.

Table: 1.1 Mycofloral affiliation and it's include in various investigated dried fruits tests

Name of Fungi	Fungal count (cfu/g)			
	Raisin	Pistachio nut	Walnut	Almond
<i>Aspergillus parasiticus</i>	2.1×10^4	3.1×10^4	-	1.2×10^2
<i>Aspergillus oryzae</i>	-	1.2×10	-	-
<i>Aspergillus tamar</i>	2.4×10	-	1.4×10	-
<i>Aspergillus niger</i>	5.4×10^3	7.2×10^4	4.6×10^3	6.4×10^3
<i>Aspergillus flavus</i>	6.4×10^3	8.5×10^4	2.8×10^3	3.5×10^2
<i>Aspergillus ochraceus</i>	4.8×10^2	5.1×10^3	6.1×10	2.2×10
<i>Aspergillus versicolor</i>	1.2×10	-	2.1×10^2	-
<i>Aspergillus fumigatus</i>	1.4×10^2	4.1×10^2	-	1.1×10
<i>Aspergillus terreus</i>	-	-	-	1.7×10
<i>Aspergillus sydowi</i>	-	1.5×10	-	1.4×10^2
<i>Penicillium citrinum</i>	4.8×10^2	3.4×10^3	2.6×10^3	2.1×10
<i>Penicillium islandicum</i>	0.4×10	-	-	1.2×10^2
<i>Penicillium verrucosum</i>	3.2×10^3	5.1×10^2	-	2.6×10^2
<i>Penicillium purpurogenum</i>	-	-	-	1.2×10
<i>Penicillium cyclopium</i>	1.4×10	-	-	2.8×10
<i>Fusarium oxysporum</i>	4.6×10^3	3.2×10^3	4.1×10^2	4.1×10^3
<i>Fusarium moniliforme</i>	3.8×10^2	4.6×10^2	-	3.4×10^2
<i>Chaetomium globosum</i>	-	-	1.1×10	0.6×10
<i>Rhizopus nigricans</i>	4.1×10^2	2.3×10^2	2.4×10^3	3.5×10^2
<i>Rhizopus oryzae</i>	-	3.1×10^3	-	-
<i>Mucor hiemalis</i>	4.8×10^3	6.4×10^3	-	5.2×10^3

A. flavus samples were likewise tainted with mycotoxigenic fungi yet lower than pistachio tests. Pollution was most elevated in Pistachio nut (8.5×10^4) were revealed that *Aspergillus* and *Penicillium* genera were followed by raisin (6.4×10^3). the most pervasive in raisins tests and tainted detaches A. flavus and A. niger from the raisins tests in with mycotoxins. Citrinin producing P. citrinum was profoundly separated from raisins (4.8×10^2 cfu/g) and least in almonds (2.2×10 cfu/g). Another citrinin producing fungi, P. verrucosum was likewise detached from all substrates aside from pecan tests.



Figure 1.2: (a) Almond (b) Pistachio nut and (c) Raisin sample contaminated with *Aspergillus flavus* and *Aspergillus niger*

Table 1.2: Percent contamination of aflatoxins and citrinin in different samples

Samples	No. of sample analyzed/Positive samples	% contamination	Aflatoxins /(%)	Citrinin/(%)	Aflatoxins + Citrinin/(%)
Raisin	55/42	76.3	35 /(64)	2 (3.6)	5/ (9)
Pistachio nut	50/41	82.0	34 /(68)	0	7/ (14)
Walnut	40/29	72.5	21(52.5)	4 (10)	4 (10)
Almond	43/30	69.7	28/ (65)	0	2/ (4.6)

Co – event of aflatoxins and citrinin

Aflatoxins and citrinin both were available in raisins, pistachio, pecan and almond tests. 82 % of pistachio aflatoxin polluted and 9 % was co-debased with aflatoxin and citrinin both (Figure. 3). By and large citrinin is available in nature as co-impurity with different mycotoxins nuts example were sullied with either aflatoxins or however 2 examples of raisin were debased with just citrinin or both where as just 76.3 % of raisins, 72.5 % of citrinin. Almond tests were least tainted and just pecan and 69.7 % of almond tests were polluted.

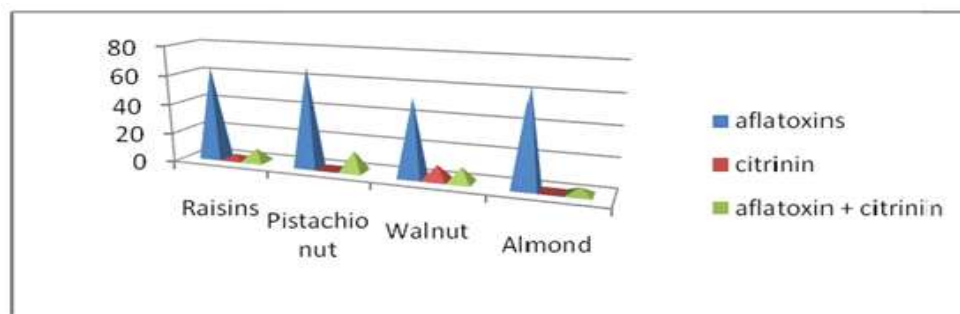


Figure 1.3: Aflatoxins and citrinin and co-occurrence in dried fruits samples and maximum contamination of aflatoxins in pistachio nut samples.

Table 2 shows that out of 82 % of contamination in pistachio nut, 68 % were contaminated with only aflatoxins and 14 % with aflatoxins and citrinin both but none of the sample was only citrinin contaminated. 76% of raisins Co – occurrence of aflatoxins and citrinin samples were contaminated I which 64 % was only.

Table 1.3: Quantitative values of mycotoxins in different dried fruits

Samples	Mycotoxins (ppb)	
	Total aflatoxins (Mean ± SE)	Citrinin (Mean ± SE)
Raisin	248 ± 24.5	225 ± 35.2
Pistachio nut	462 ± 16.2	115 ± 28.5
Walnut	210 ± 24.0	130 ± 24.6
Almond	174 ± 21.8	95 ± 25.4

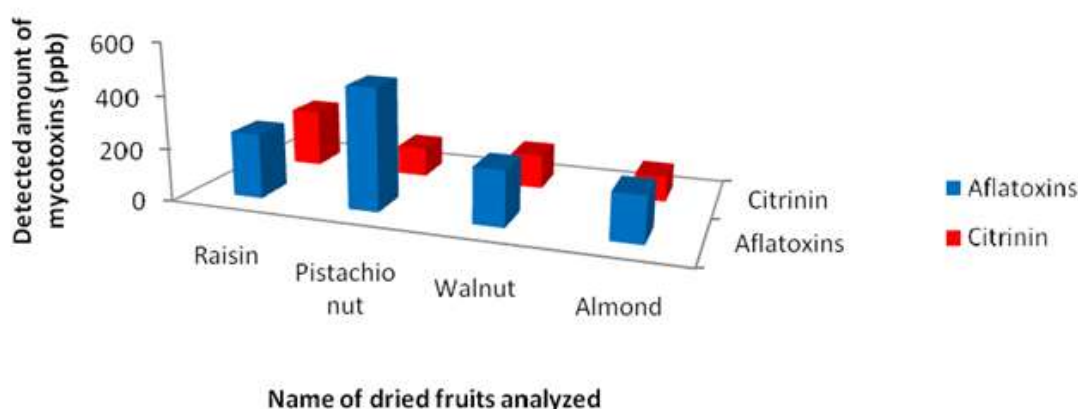


Figure: 1.4 Showing aflatoxins and citrinin level a lot higher than EU limit (4ppb for aflatoxins and 15 ppb for citrinin)

6. NORMAL OCCURRENCE OF MYCOTOXINS AND RISK ASSESSMENT

Aflatoxins are cancer-causing in nature and there are numerous reports with respect to cancer-causing nature by aflatoxin. Citrinin is nephrotoxic and by and large present as co-foreign substance with different mycotoxins. Both of the mycotoxins are unsafe for the soundness of buyers. The greatest constraint of 4 ppb for absolute aflatoxins and 15 citrinin in dried fruits and nuts. In our investigation, pistachio nut has most extreme aflatoxins defilement (462 ppb) trailed by raisins and Walnut (Table 3). Additionally distinguished the aflatoxins level up to 1134.5 $\mu\text{g kg}$ pistachio nut from Spain. Citrinin was additionally not many of the dried fruits tests. The recognized degree of citrinin was most noteworthy in raisins (225 ppb) which was a lot higher than reasonable breaking point. While was least tainted example either by aflatoxins or citrinin or both (Figure 4).

7. CONCLUSION

Based on the current examination, it very well might be reasoned that raisins, pistachio, pecan and almond zone are powerless substrate for contagious development a mycotoxins creations. Each of the 4 sorts of were tainted with just aflatoxins or citrinin or both. Pistachio was the most well-known as nuts and is of profoundly healthy benefits, had mycotoxin fixation. Almond and the measure of aflatoxins and other however it was adequately high to instigate poisonousness. Aflatoxins are cancer-causing though citrinin is nephrotoxic and presence of these two mycotoxins in dried fruits and nuts are perilous and straightforwardly worry with the strength of customers. Thus, it is imperative to mind in preparing, dealing with, transportation and capacity. 273 Citrinin (Mean \pm SE) Showing aflatoxins and citrinin level a lot higher than EU limit (4ppb for aflatoxins and 15 ppb for citrinin) most noteworthy in raisins (225 ppb) which was a lot of breaking point.

8. REFERENCES

1. Luca Dellafiora (2019)"Co-Occurrence and Combinatory Effects of *Alternaria* Mycotoxins and Other Xenobiotics of Food Origin: Current Scenario and Future Perspectives"Received: 6 September 2019 / Revised: 24 October 2019 / Accepted: 31 October 2019 / Published: 3 November 2019
2. Eskola, M.; Kos, G.; Elliott, C.T.; Hajšlová, J.; Mayar, S.; Eskola, M.; Kos, G.; Elliott, C.T.; Hajšlová, J.; Mayar, S.; et al. Worldwide contamination of food-crops with mycotoxins : Validity of the widely cited 'FAO estimate' of 25%. *Crit. Rev. Food Sci. Nutr.* 2019, 1–17.
3. Gotthardt, M.; Asam, S.; Gunkel, K.; Moghaddam, A.F.; Baumann, E.; Kietz, R.; Rychlik, M. Quantitation of Six *Alternaria* Toxins in Infant Foods Applying Stable Isotope Labeled Standards. *Front. Microbiol.* 2019, 10, 109.
4. Sanzani, S.M.; Gallone, T.; Garganese, F.; Caruso, A.G.; Amenduni, M.; Ippolito, A. Contamination of fresh and dried tomato by *Alternaria* toxins in southern Italy *Food Addit Contam Part A* 2019, 36, 789–799.
5. Punam Jeswal, Dhiraj Kumar(2017) "Evaluation of Mycotoxigenic Fungi and Aflatoxins, Ochratoxin A & Citrinin Contamination in some Dried Fruits and Nuts from Bihar" p-ISSN 2454-4582, e-ISSN 2454-7808, Volume 3, Issue 1; January-June, 2017 pp. 48-51
6. Toma FM, Rajab ALN "Isolation and Identification of Fungi from Dried Fruits and Study of Quantitative Estimation of Aflatoxin. *Zanco J. of Pure and Applied Sciences*, 26(4) (2014) : 49-60. 33.
7. Saadullah AAM, Abdullah SK (2015) Mycobiota and Incidence of Toxigenic Fungi in Dried Fruits from Duhok Markets, North Iraq. *Egypt. Acad. J. Bio log. Sci.*, 7(1): 61-68. 35.
8. Shaker RJ, Thalij KM, Bdooy AS (2013) Isolation and Identification of some molds that produce mycotoxins in some foods in Iraqi market. *The J of Tikrit University of Agricultural Sciences*, 2013,13(1).(in Arabic
9. Dellafiora, L.; Aichinger, G.; Geib, E.; Sánchez-Barrionuevo, L.; Brock, M.; Cánovas, D.; Dall'Asta, C.; Marko, D. Hybrid in silico/in vitro target fishing to assign function to "orphan" compounds of food origin—The case of the fungal metabolite atromentin. *Food Chem.* 2019, 270, 61–69.
10. Kollarova, J.; Cenk, E.; Schmutz, C.; Marko, D. The mycotoxin alternariol suppresses lipopolysaccharide-induced inflammation in THP-1 derived macrophages targeting the NF- κ B signalling pathway. *Arch. Toxicol.* 2018, 92, 3347–3358.