AFLATOXINS – SIX DECADES OF RESEARCH. 1. CERTAINTY OF AFLATOXINS OCCURRENCE AND PREVALENCE

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ABSTRACT
Recently and after 6 decades of research on aflatoxins and aflatoxicosis, since the first famous outbreak report of turkey X-disease in 1960, mentioning the etiological causing factor by the term "aflatoxin", a lot of literature and reports had been published. The available literature and reports contain bulk of knowledge with different levels of certainty. Some published data could be accepted as approved scientific evidence, while others could not be accepted at all, meanwhile the great bulk of work could represent assumptions and suggestions. Revising the available literature and data is very important to help and facilitate the job of researchers, decision makers and others who are interesting the issue of food safety. Hereby, some selected topics representing wide range of the factors affecting occurrence and prevalence of aflatoxins including, not limited to; causative organisms, seasons of occurrence, environmental factors affecting aflatoxins occurrence, geographical zones, methods of detection, socioeconomic considerations.


INTRODUCTION
Aflatoxins are approved as a group of natural occurring secondary metabolites produced by certain strains of the fungus Aspergillus flavus and/ or Aspergillus parasiticus when grown on most of the agriculture commodities and other substrates (Saad, 1991, Hell et al. 2002, Nel & Steyn, 2002 and Kpodo et al., 2006). The prevalence of aflatoxins is positively affected by certain biotic and abiotic factors, mainly to occur at high relative humidity (13-18%) and high temperature 25-37 C (Goldblat, 1969, Goldblat & Stollof, 1983 and WHO, 1979). So far, more than 20 aflatoxins are identified; only 6 are common in foods and feedstuffs, the
aflatoxins B1, B2, G1 and G2 are found in foods of plant origin, while aflatoxins M1 and M2 are occurred in foods of animal origin (Bahat & Vasanthi, 1991, Van Egmond, 1994 and El-Nezami et al., 1995). It’s well known that aflatoxin B1 is the parent compound and most potent form of aflatoxins, the molecule which is complying the authentic standard formula \(C_{17}H_{12}O_6\) with molecular weight equal 312.3 Daltons, referring in Sigma Aldrich catalog book carrying the No. A6636, (Sigma, 1997).

Although the fungal genera of Aspergilli, Penicillium and Fusarium had the capability to produce many mycotoxins, aflatoxins still receiving the great attention of scientific community and decision makers. The contradicting knowledge and conflicting data concerning aflatoxins occurrence and aflatoxicosis leads to urgent need to revise thoroughly the available literature published during the last 6 decades. This review is an attempt to revise some research studies and reports dealing with the prevalence and occurrence of aflatoxins, tailing by specific comments and arbitrary guiding by the well established scientific basis and accepted approaches. Also, this attempt hoping to differentiate between the solid and concreted facts, assumptions and speculations commonly handled when discussing the problem of occurrence and prevalence of aflatoxins.

1. **Title:** A survey of some species of *Aspergillus* and *Penicillium* for production of aflatoxins and kojic acid.

**Results and Findings**
A total of 165 fungus strains representing 14 species of Aspergillus and 7 species of Penicillium were screened for production of aflatoxins and kojic acid in a defined media. Aflatoxins were obtained only from 2 of the 14 species of Aspergillus and from none of the 7 species of Penicillium examined. Regarding kojic acid, it obtained from 9 and 4 species of both Aspergillus and Penicillium species, respectively.

**Comments and Arbitrary**
This very early study indicated two essential issues; 1) only Aspergillus species are capable to produce aflatoxins, while Penicillium species could not produce aflatoxins, 2) not all species, but only some species of Aspergillus had the capability to yield aflatoxins. In other words, Aspergillus- contamination is not a certain indicative for aflatoxins occurrence. Referring to this study, only 14% of the tested species of Aspergillus were able to produce
aflatoxins. Sure the obtained percentages of aflatoxins-producing strains were affecting by many etiological factors, but such factors are not included between the ultimates of the study. Also, the study did not report the qualitative and the quantitative properties of the obtained aflatoxins.

2. Title: Aspergillus flavus and Aspergillus parasiticus: Aflatoxigenic fungi of concern in foods and feeds.

**Results and Findings**

The species of Aspergillus flavus and the closely related subspecies parasiticus have long been recognized as major contaminants of organic and non-organic items. Aspergillus flavus strains flavus, Aspergillus flavus subspecies parasiticus and Aspergillus nomius share the ability to produce aflatoxins. Identification of the Aspergillus species group is mainly base on the color and macroscopic and microscopic characteristics of the isolated fungus. Aspergillus flavus growth and biosynthesis of aflatoxins depend upon many environmental conditions including; substrate, moisture, aeration, pH and competing micro flora. Aspergillus flavus growth and aflatoxins production are sometimes unavoidable. The detection of members of the Aspergillus flavus group of foods and feeds contaminants is generally conducted by using plate technique such as surface spread or direct plating. Mycological techniques to detect Aspergillus flavus are not an evident to indicate aflatoxins occurrence. Aflatoxins are generally analyzed by chemical methods, although immunochemical methods are becoming common analytical tools for aflatoxins.

**Comments and Arbitrary**

This study confirmed that only some Aspergillus flavus verities, not all, could be potentially capable to produce aflatoxins. The current data revealed that the sub-specie of Aspergillus nomius share the ability to produce aflatoxins. Most reports and survey studies emphasized that only the species of Aspergillus flavus and parasiticus had the potential capability to produce aflatoxins. It's worthy to differentiate between fungal- and aflatoxins contamination of certain substrates. The adopted methods in both cases are completely different to detect and determine the potent biological or chemical hazards. Aspergillus flavus species group in foods and feeds is generally detected by using plate techniques or direct plating, while aflatoxins are generally analyzed by chemical analytical methods. Also, it's well agreed that
Aspergillus flavus moldy contamination of certain foods or substrates could not considered as an indicative for aflatoxins occurrence in such foods and substrates.

3. Title: A survey of aflatoxins and Aflatoxigenic Aspergillus flavus in corn-based products from the Spanish markets.

Results and findings
The natural occurrence of aflatoxins B1, B2, G1 and G2, incidence of Aspergillus flavus and the capability to produce aflatoxins by Aspergillus flavus isolates were investigated in 100 corn-based samples from Spain destined for human and animal consumption. Data revealed that only one sample (1%) for animal consumption and none (0%) for human consumption were found to be contaminated with aflatoxins B1 and B2. The levels of contamination were 0.15 and 0.08 ug/ g sample of aflatoxin B1 and B, respectively. Of 43 isolates of Aspergillus flavus, only 4 (9.3%) were aflatoxins producers.

Comments and Arbitrary
This random study showed very low levels of aflatoxins occurrence (1%) in corn-based products marketed in Spain. Although corn had been widely identified as one of the most commodities exposed to Aspergillus flavus invasion and aflatoxins contamination, data revealed only 1% of corn-based feeds and none 0% of corn-based foods were contaminated with aflatoxin B's. The findings added that none of aflatoxins G1 and G2 were detected, meanwhile most of the previous studies reported that both aflatoxins B's and G's had been naturally occurred, simultaneously. This study focused on 3 main issues; 1) the negligible percentages (0-1%) of aflatoxins contamination, although corn and corn-based products are commonly described as sensitive foods and feeds for aflatoxins-contamination, 2) the 4 common aflatoxins of B1, B2, G1 and G2 are not necessarily to be occurred together, 3) out of 43 isolates of Aspergillus flavus, only 4 (9.3%) showed the capability to produce aflatoxins when tested in vitro and 4) quantitatively the obtained concentrations of aflatoxins B1 and B2 were low and tolerable ranging between 0.08 – 0.15 ug/ g sample.

4. Title: Aspergillus flavus infection and aflatoxin contamination of pre-harvest maize in Benin.
Results and Findings
This study was extended for 2 years to monitor Aspergillus infection and aflatoxins contamination of pre-harvest maize in the Asian country of Benin. Three Aspergillus species were isolated from different agro-ecological zones with Aspergillus flavus being the most prevalent. Data revealed that the country wide mean percentage of kernel infection was 20% in both 2 years of the study. Aflatoxins were extracted from maize in at least 30% of the field samples. Toxin concentrations exhibited distinct zonal variations with relatively high levels in the Guinea Savanna. There was a trend toward higher rate of aflatoxin accumulation per percentage of Aspergillus flavus infection from the south to the north. Also, damaging maize grains due to some insects' invasion have been shown to be significantly related to the incidence and level of aflatoxins contamination in pre-harvest maize.

Comments and arbitrary
This study concluded that both the geographic pattern and the exposure of maize to insects' invasion have been significantly related to the incidence and level of aflatoxins contamination in pre-harvest maize. It's worthy to differentiate between Aspergillus and aflatoxins contamination. It's accepted to observe some fungal plant diseases in the field including the molds of Aspergillus flavus but most, if not all, those field species were identified as "field fungi" according the classification of Kristensen (1957) which are commonly not capable to yield aflatoxins. Also, the adopted method to determine the concentrations of aflatoxins depended upon the bio-technique of inhibition zone which is not accurate or specific. Thus, it's accepted to report that both geographical pattern and insect invasion are related to fungal contamination of pre-harvest maize. But the occurrences of aflatoxins during the pre-harvest stage still represent doubtful, at least because the Aspergillus flavus species potentially capable to produce aflatoxins were classified as "storage fungi" commonly occurred and activated during post-harvest stage.

5. Title: Aflatoxin contamination food products in Thailand.

Results and Findings
This report is an overview concerning 34 years of research on aflatoxin contamination in various foods of Thailand. The accumulated data based on confident accepted statistical number of samples (n = 3.206 samples) concerning the variables of; type of food, seasons and
geographical areas. Data showed that 1.248 samples representing (38.9%) were highly contaminated with aflatoxins. Over half of the contaminated samples were peanuts, milk and poultry. Analyzed data indicated that there was significant relation between the type of food and seasonal influence, but not geographical influence. Detection methods of aflatoxins based on fundamental techniques have been also reviewed.

**Comments and Arbitrary**

The findings of this report could represent valued accumulated data due to the extended period of data collection (34 years) and the large number of investigated food samples. The numbers of collected samples of each food type were not reported, so the conclusions stating that over one half of the positive samples were related to peanuts, milk and poultry is not satisfied enough. Since the first discovery of aflatoxins in 1960, aflatoxicosis was related to peanut products, more suspicious and less satisfaction are oriented towards peanut products as common aflatoxins contaminated foods and feeds. Concerning the contaminated samples of milk and poultry, sure both are representing the indirect route of exposure to aflatoxins. In other words the livestock of lactating animal or poultry should previously ingest aflatoxins-contaminated rations before the toxin is accumulating in carcasses or milk. Thus, the direct occurrence of aflatoxins on certain substrates should be discussed far away the indirect accumulation of aflatoxins of edible animal tissues.

6. Title: Interaction between certain moulds and aflatoxin B1 produce Aspergillus flavus NRRL-3251.


**Results and Findings**

To evaluate biotic interaction between some mould species and the active producer of aflatoxin B1 Aspergillus flavus NRRL-3251 in vitro, 25 mould strains of 5 species were studied. Data confirmed antagonistic interaction between all tested strains. The most competent strains are belonging to Alternaria species and Cladosporium species capable to decrease 100% of aflatoxin B1 production comparing with detected concentrations in a single culture of Aspergillus flavus NRRL-3251. The inhibition was decreased to 50-70% in mixed culture with Mucor species. Data revealed that the effect is not only related to mould specie difference, but also is within the same specie. Four of 5 non-aflatoxin producing strains interfered with aflatoxin production in mixed cultures and completely reduced the productivity of aflatoxin B1 by 100%. Also, a decrease in toxin production was observed in
dual cultivation with Aspergillus niger strains, such decrease reached 100% in 3 tested strains.

Comments and Arbitrary

It's well agreed that the occurrence of aflatoxins is a result of very complicated process involving the availability of 3 main reactants; 1) the fungus potentially capable to produce aflatoxins, 2) the substrate and 3) the proper environmental conditions which are subdivided to many factors. The findings of this study confirmed that antagonistic interactions between all tested strains either between or within the specie of Aspergillus flavus were observed. The tested mould strains representing both in- and out-doors environment besides the storage conditions of post-harvest maize. Data confirmed that aflatoxin B1 production was decreased sharply in all co-cultured media. Both Alternaria and Cladosporium sp. of co-cultured media had the capability to decrease aflatoxin B1 production up to 100% comparing with a single culture of Aspergillus flavus NRRL-3251. In mixed cultures with Mucor sp. aflatoxin B1 dropped sharply and the inhibition zones were decreased by 50-70%. Data revealed very interesting observation when reporting that 4 of 5 aflatoxins non-producing strains of Aspergillus flavus were interfered with aflatoxin production in mixed cultures and reduced aflatoxin B1 productivity by 100%. When applying those logic and interesting findings on what's happen in nature, undoubtedly all substrates, foods and feeds are exposed to be contaminated with all the available environmental fungi and the expected results of such contamination will be the resultants of the biotic reactants. Also, it could be concluded that in most and common cases of fungal contamination, the opportunity of Aspergillus producing aflatoxins strains to yield significant amounts of aflatoxins is very rare due to the great challenge of environmental conditions and biotic interactions.

7. Title: Aspergillus flavus, the major producer of aflatoxin.

Results and Findings

Aspergillus flavus is an opportunistic pathogen of crops. It's important because it produces aflatoxins as secondary metabolites in the seeds of a number of crops both before and after harvest. In the field, aflatoxins are associated with drought-stressed oilseed crops including maize, peanut, cottonseed and tree nuts. Under the proper conditions, the fungus will grow and produce aflatoxins in almost any stored crop seed. In storage, aflatoxins could be
controlled by maintaining available moisture at levels below that which will support growth of Aspergillus flavus.

**Comments and Arbitrary**

The author emphasized on the importance of the mould Aspergillus flavus due to its capability to produce the secondary metabolites of aflatoxins in both stages of pre- and post-harvest of a number of crops. The author stated that under the right conditions, the fungus will grow and produce aflatoxins in almost any stored crop seeds. The linkage between fungal growth and aflatoxins production is not absolute issue, at least because of the great isolate numbers of Aspergillus flavus which most of them are not capable to yield aflatoxins. Contradicting to many of the available studies, this study referred to the association between aflatoxins occurrence and certain category of foods "oilseed crops". Also, the study reported that aflatoxins occurrence is obtained during both the stages of pre- and post-harvest, in contrary with approved findings reported by many studies confirming that aflatoxins are commonly occurred during post-harvest stage and depending upon the storage conditions.

8. Title: Production of aflatoxins by Aspergillus flavus and Aspergillus niger strains isolated from seeds of pulses.


**Results and Findings**

Fourteen Aspergillus flavus and 3 Aspergillus niger strains were isolated from seeds of the pulses of lupine, mung- bean, faba bean and lentil. The aflatoxin production of isolates was tested by 2 different bioassay methods measuring the inhibitory effect on okra seeds germination and bacterial growth. Toxic effects had different degrees including delay of seed germination, yellowish plant and inhibition of Bacillus subtilis growth. Aflatoxins B1 and B2 were found in all fungal filtrates at concentrations ranging between 38-496 ug/Liter. As well, aflatoxins G1 and G2 were also produced by some isolates. Autoclaving of fungal filtrates, freezing or microwaving has no effect on bacterial growth inhibition, providing the evidence of the stability of aflatoxins.

**Comments and Arbitrary**

This study showed that all tested 14 Aspergillus flavus and 3 Aspergillus niger had fungal filtrates with aflatoxins B1 and B2 at wide level of concentrations ranged between 38 and 496 ug/ Liter. Also data revealed that aflatoxins G1 and G2 were produced by some isolates. It's
accepted to report that Aspergillus flavus strains are capable to produce aflatoxins, even the percentage of toxin-producing strains reached 100% of the tested strains. On the other hand, the findings that all the 3 tested Aspergillus niger strains had the capability to produce aflatoxins are contradicting the most of the available and reported data accumulated through six decades of research. The author reported wide range of the concentrations of the occurred aflatoxins B1 and B2 between 38 to 496 ug/liter. The study did not refer to the levels of the concentrations of aflatoxins G1 and G2. The author added that the aflatoxins produced by the tested strains were tested by 2 bioassay techniques, measuring the inhibitory effect on okra seed germination and Bacillus subtilis growth. To accept such statement an accurate analytical chemical assay should be applied to confirm that the inhibitory effects are due to aflatoxins in particular.

9. Title: Mycoflora and occurrence of aflatoxin in dried vegetables in Benin, Mali and Togo, West Africa.

Results and Findings
Fungal contamination of dried vegetables including okra, hot chili, tomato, melon seeds, onion and baobab leaves collected from Benin, Togo and Mali were evaluated after plating on selective media. Data exhibited the isolation of 561 fungal strains. The species of Aspergilli were dominant on all marketed dried vegetables irrespective of the studied country. Chemical analysis using high performance liquid chromatography (HPLC) showed that only okra and hot chili were naturally contaminated with aflatoxins B1 and B2 at concentrations ranging between 0.6 to 3.2 ug/kg. This is the first time that mycotoxigenic fungi and resultant toxins were found on dried vegetables of African countries. The previous reports had highlighted the risk of mycotoxins exposure from staple crops of Africa.

Comments and Arbitrary
This study focused on the dried vegetables commonly marketed in some African countries. Evaluation of fungal contamination revealed 561 fungal isolates, most of Aspergillus species. The proper conditions for Aspergillus growth mandate at least 13% moisture content and water availability (aw) of 0.85-0.9 which is not commonly available in most of dried vegetables. The isolated Aspergillus species were capable to produce aflatoxins B1 and B2 only on 2 out of 6 examined dried vegetables. Although the findings of this study are the first time representing those mycotoxigenic fungi which could contaminate some dried vegetables,
the low yielded concentrations of aflatoxins B1 and B2 could reflect the improper conditions dealing with the moisture content and water availability of the studied substrates.

10. Title: Aflatoxin B1 producing potential of Aspergillus flavus strains isolated from stored rice grains.

Results and Findings
Eighty five strains of Aspergillus flavus were isolated from the discolored rice grains and tested for their aflatoxin B1 producing potential on different media. The isolated strains collected from different locations of India. Among 85, 43 strains were identified as aflatoxin B1 producers. The different used media include yeast extract sucrose (YES), Czapek's agar, potato dextrose agar (PDA) and Aspergillus flavus and parasiticus agar AFPA). The capability of the 43 aflatoxins producing strains are affected by cultivating media, being 100% on YES, 65% on Czapek's agar and 53% on PDA. Within the 43 aflatoxins producing strains, only 5 isolates showed the capability to grow on all tested agar media and rice cultivars, as well they could produce significant concentrations of aflatoxins. Moreover one strain of the 5 isolates capable to grow on both synthetic and natural media identified as DRAF-009 resulting high concentrations of aflatoxin B1 reaching 415 ug/ g. on the rice cultivars.

Comments and Arbitrary
The study confirmed some logic basis including; 1) the mould Aspergillus flavus is commonly found in the storages of the grains, 2) some but not all Aspergillus flavus strains had the potential capability to produce aflatoxins, 3) the potential capability of the aflatoxins producing strains are commonly dependent on the fungal genome, substrate and the environmental factors, and 4) the fungal growth and aflatoxins production are qualitatively and quantitatively influenced by the chemical composition and the nutritive value of the substrate. One more interesting finding is the very high concentration of aflatoxin B1 produced by a certain strain of Aspergillus flavus grown on the natural media of milled rice cultivars reaching 386 – 415 ug/ g. grain, comparing with 0.4 – 4.0 ug/ g agar, when grown on the most proper nutrient synthetic agar media.

11. Title: Association between aflatoxin and Aflatoxigenic fungi in Brazil nut.
Results and Findings

In order to evaluate Brazil nut safeties, 120 samples of different stages of the productive chain were analyzed for; moisture content, Aflatoxigenic fungi and aflatoxins (LOQ = 1.95 ug/ kg total aflatoxins) using thin layer chromatography (TLC). Data revealed that among 120 samples, only 4 from the receiving area and 5 from retail presented aflatoxins above the LOQ, but the amounts of aflatoxins were below the LOQ after the samples were dried in the plant. The positive samples were above the limit of total aflatoxins permitted by the European Union (4.0 ug/ kg) and Brazil standards (30 ug/ kg). The moisture content mean was 22-4% in the receiving area which is higher than that in the other stages of sampling. All the Aspergillus flavus strains were Aflatoxigenic and there was statistic association between the presences of aflatoxins and Aspergillus flavus strains.

Comments and Arbitrary

The importance of evaluating Brazil nuts is due to not only its high nutritive value as an important trade commodity but also to its bad history since the prevalence of turkey X-disease in 1960 as a causative etiological agent. Data showed that the moisture content mean was 22.4% in the receiving areas and higher than that in the other stages of sampling. All the Aspergillus flavus strains were Aflatoxigenic. Among the 120 analyzed samples, only 4 from the receiving areas and 5 from the retail presented aflatoxins above the LOQ (1.95 ug/ kg total aflatoxins) using TLC methods. The authors added that the amounts of produced aflatoxins were below the LOQ after the samples were dried in the plant. The study concluded that Brazil nut samples with moisture content less than 22.4% showed less aflatoxins even they were contaminated with Aflatoxigenic Aspergillus flavus strains.


Results and Findings

Aspergillus species are major causes of pre- and post- harvest degradation of corn grains. This study aimed to identify morphologically Aspergillus species isolated from stored corn grains in certain province "Kermanshah" of western Iran during 7 years (2006-2013) growing seasons. One hundred samples of mostly diseased corn grains for human and animal consumption representing the different geographic regions of the province were collected and examined. One hundred Aspergillus isolates were collected and identified into 6 species; Aspergillus niger (40%), Aspergillus flavus (27%), Aspergillus ochraceus (15%), Aspergillus
fumigates (10%), Aspergillus japonicus (5%) and Aspergillus sclerotorium (3%). Also, aflatoxin B1 was determined in the samples by enzyme-linked immune sorbent assay (ELISA). Data revealed that natural occurrence of aflatoxin B1 detected in 66 samples (66%). The detected concentrations of aflatoxin B1 had a very wide range (0.046 – 10.776 ug/kg) and the highest levels were detected in samples from certain geographic zones "Bisetoon and Sarpolzehab".

**Comments and Arbitrary**
Because maize is one of the most important primary food crops all-over the continents of the world and because of aflatoxins contamination of stored grains had long been a major problem in different countries, the problem of corn aflatoxins contamination had got the attention of many researchers. This study was extended for 7 years of corn growing seasons. Data showed that corn grains could contaminate with 6 different species of Aspergilli and Aspergillus flavus which are identified as aflatoxins producing species representing 27% of the total isolates of Aspergilli. Qualitative assay to differentiate the naturally occurred aflatoxins exhibited only aflatoxin B1 using ELISA technique. Quantitative data showed wide range of aflatoxin B1 concentrations reached its maximum at 10.776 ug/kg in samples of certain geographic areas. Undoubtedly, such geographic areas facilitate the most proper environmental conditions to enhance and promote fungal growth and aflatoxins production.

13. Title: A survey of aflatoxin producing Aspergillus species from peanut field soils in four geographical zones of China.

**Results and Findings**
Peanut pods are easily infected by aflatoxin producing Aspergillus species from field soil. To assess the aflatoxin producing Aspergillus species in different peanut field soils, 344 aflatoxins producing Aspergillus strains were isolated from 600 soil samples representing 4 geographical zones in China. Data revealed that 324/344 (94.2%) of the isolated strains were Aspergillus flavus and 20/344 (5.8%) were belonging to Aspergillus parasiticus. The Yangtze River Zone had the highest population density of Aspergillus species and positive rate of aflatoxin production in isolated strains (1039.3 cfu/g). The potential risk of aflatoxin B1 contamination in peanuts can increase with increasing population density and a positive rate of aflatoxin producing Aspergilli in field soils suggesting that reducing Aflatoxigenic Aspergillus species from field soils could prevent aflatoxin B1 contamination in peanuts.
Comments and Arbitrary
This study focused on the relation between aflatoxins occurrence and both qualitative and quantitative characteristics of Aspergilli found in filed soils used for peanuts cultivation. Data obtained from the 4 different geographical zones of China showed that the highest population density of Aspergillus species were 1039.3 cfu/ g soil of the Yangtze River zone (high humid zone), meanwhile the lowest corresponding population density was only 2.4 cfu/ g soil in less humid zones. The authors concluded that the potential risk of aflatoxin B1 contamination in peanuts can increased by increasing population density and a positive rate of aflatoxin producing Aspergillus species in field soils, and vice versa.

General discussion and conclusion
It's worthy to mention that the risk analysis process of the hazards of aflatoxin(s) is a general standard process with the 3 main sub-processes involving; 1) risk assessment, 2) risk management and 3) risk communication (FAO/WHO, 2006). Risk assessment as an essential step and the corner stone of the process offers the essential information and knowledge guiding food safety authorities and decision makers. The sub-process of risk assessment scientifically based on the identification of certainties of defined hazard and severity of the adverse effects threaten man, animal and environment (EC, 2000). Consequently, risk management is completely dependent upon the information and knowledge addressed by risk assessment. However, the 4 main components of risk assessment of any hazard should concern the hazard identification and characterization, exposure assessment and risk characterization of such hazard (Shephard, 2008). So, the contradicting data and information obtained from some studies lacking the basis of the scientific approach should be thoroughly reevaluated to avoid misleading and misunderstanding when dealing with the problem of aflatoxins contamination. It's very important to distinguish between the hazards of aflatoxins causing adverse health effects "severity" and the risk which represents the "probability" of the occurrence of such adverse effects. Thus, the authorized organizations of food safety are anxious to receive accurate and precise data on both severity and probability of aflatoxins occurrence and prevalence. As mentioned above and as a result of revising some topics dealing with the problem of aflatoxins occurrence tailing with comments and arbitrary, this is an attempt to determine the level of certainty of the obtained findings and results of each study.
As a conclusion concerning the problem of aflatoxins occurrence and prevalence, more than one level of certainty could be distinguished after revising the abovementioned results and findings. At least 3 levels of certainty are distinguished when describing or diagnosing the relation between the fungi, substrate and the environmental conditions affecting aflatoxins occurrence and prevalence. The highest level of certainty confirming the evidence of the following statements; 1) the secondary metabolites of aflatoxins are naturally produced by some, species of Aspergillus flavus and/or Aspergillus parasiticus, 2) not all the species of Aspergillus flavus or Aspergillus parasiticus had the potential capability to yield aflatoxins, 3) the percentages of the aflatoxins-producing strains varied widely as shown previously ranging between 0% to 80% based on many biotic and abiotic variables, 4) aflatoxins are occurred mainly during post-harvest stage, 5) the most common environmental factors affecting the occurrence of aflatoxins are, the moisture content, water availability and relative humidity, 6) the potential capability of aflatoxins-producing strains of Aspergillus flavus and Aspergillus parasiticus is influenced by many factors, so the concept of changing from negative aflatoxin-producers to positive ones and/or vice versa is completely accepted concept, 7) the stability of the occurred aflatoxins is varied widely based on the qualitative and quantitative characteristics of the occurred molecules, 8) fungal invasion and aflatoxins occurrence contaminated the natural substrates and agriculture commodities is influenced by the physical status, so the damaged and broken seeds, grains and kernels showed the highest level of contamination, 9) he naturally occurring aflatoxins of B1, B2, G1 & G2 could be only obtained from contaminated foods and feeds of plant origin, while aflatoxins M1 and M2 had the chance to be found in foods of animal origin, 10) to calculate the potency of aflatoxins, addition of the determined concentrations of aflatoxins B1, B2, G1 & G2 leads to wrong decision.

Another level of less certainty dealing with aflatoxins occurrence and prevalence referring to the following statements as suspected ones; 1) aflatoxins could occurred during pre-harvest stage of some commodities, 2) the occurred aflatoxins could maintain its stability for a long time, even they exposed to autoclaving or other thermal treatments, 3) the opportunity of aflatoxins occurrence is influenced by the chemical composition of the substrate especially their fat content, 4) Aflatoxins could be detected in dried crops, foods and feeds containing less than 13% moisture content with a_w less than 0.85.
The third level of certainty is concerning the knowledge and not approved data, lacking the scientific evidence which considered being just suggestions, speculations i.e.; 1) Fungal geneses and species rather than Aspergillus flavus and Aspergillus parasiticus had the capability to yield aflatoxins, 2) the naturally occurring aflatoxins had the same effects or could go through the same metabolic pathways on man, animal and the environment, and 3) the 4 aflatoxins of B1, B2, G1 & G2 are commonly and always occurred simultaneously together, and very rare to occur individually.

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The author should like to thank all authors mentioned in this article, although some of them had different opinions and sights about the issue of aflatoxins occurrence. Sure, all of us are welling and keen to differentiate between the solid and concreted facts dealing with the problem of aflatoxins and aflatoxicosis and the other speculations, suggestions and opinions. Again the efforts of the mentioned authors and others whom are searching and working on the same issue are highly appreciated aiming to offer the scientific evidence to all interested parties including decision makers.

REFERENCES


