MYCOTOXINS IN GRAINS AND NUTS: I) PREVENTION OF THEIR FORMATION

TAHILLARDA VE KURUYEMİŞLERDE MİKOTOKSIN OLUŞUMUNUN ÖNLENMESİ, BULAŞMIŞ ÜRÜNÜ VE MİKOTOKSİNLERİ AYIRMA YÖNTEMLERİ

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SUMMARY

Mycotoxins are worldwide important problem in term of public health, agriculture, and economics. Turkiye, with its high potential in agriculture, produces large amount of grains and nuts (e.g., hazelnut), besides dried fruits (e.g., dried figs, raisins) which are susceptible to mycotoxin contamination. Insufficient and/or improper preharvest and postharvest technologies employed during cultivation, harvesting, drying, transportation and storage increase the risk involved for the Turkish products. Mycotoxins and their prevention methods are reviewed with special reference to grains and nuts besides detoxification and decontamination methods.

ÖZET

Sağlık, tarım ve ekonomi açısından mikotoksinler dünya çapında önemli bir sorundur. Türkiye tarımdaki büyük potansiyeline koşut olarak büyük miktarlarda tahıl, kuruyemiş (fındık), kuru meyve (kuru incir, kuru kayısı, kuru üzüm) üretmektedir. Üretim, hasat, kurutma, taşıma, ve depolama sırasındaki yetersiz ve yanlış uygulamalar adı geçen ürünlerde mikotoksin oluşumu riskini arttırmaktadır. Bu makalede tahıllarda ve kuruyemişlerde mikotoksin oluşumunun önlenmesi yöntemleri tartışılmaktadır.

MYCOTOXINS

Mycotoxins exhibit properties of acute, sub-acute, and chronic toxicities in animals and/or human with some also being carcinogenic, capable of causing mutations in susceptible organisms and teratogenic, capable of causing deformities in developing embryos. Moreover, they causes loss of viability of the seeds and reduces the quality and acceptability of all type of products, limit storability and decreases nutritional quality of the foods. Therefore, mycotoxins are world-wide important problem in term of public health, agriculture, and economics (Anonymous, 1987; Jones, 1995).

A poisoning caused by ingestion of a food or feed that contains a mycotoxin(s) is called a mycotoxicosis. Not all toxins produced by moulds cause human or animal diseases. The ones that are considered to be greatest potential risk to human health are given in Table 1 together with their effects in animals and the commodities in which they have been found (Bullerman, 1986).

Aflatoxins are produced by some strains of *Aspergillus flavus*, and most, if not all, strains of *A. parasiticus.* Both species grow on agricultural commodities and produce the

aflatoxins as secondary fungal metabolites for which there is no known function within the fundamental life processes of the organism. There are four main aflatoxins: B_1, B_2, G_1, G_2, and M toxins, M_1 and M_2 (Bullerman, 1986). Aflatoxin was first noted in the early 1960s and has been significant health concern and trade problem since that time (Anonymous, 1993). They are hepatotoxic and liver-carcinogenic (Anonymous, 1993) because the mycotoxins bind to nucleic acids (Jones, 1995). Effects of the toxins are dosage and length of the exposure dependent that may cause dead, chronic toxicity, or liver cancer in both human and animals. B_1 is the most toxic of the group (Bullerman, 1986). Toxicity also vary among species. Although aflatoxin B_1 is most liver-carcinogenic compound known for rat, mouse appears to be totally resistant to the carcinogenic effects. Due to different resistance to aflatoxin among experimental animals, it is difficult to make a meaningful estimation of human risk from aflatoxins. Moreover, diets that are low in protein, but rich in A and B vitamins are protective against aflatoxin B_1 induced carcinogenesis. Indoles from cabbage family vegetables, and antioxidants such as butylated hydroxytoluene inhibits aflatoxin-induced carcinogenesis. Sex and age are the factors that affects human response to the aflatoxins. Males and children are more susceptible than females and adults. Hepatitis B virus or other comparable intestinal pathogens may be required together with aflatoxin exposure for a significant increase in cancer risk. Therefore, for countries such as USA where indicidence of hepatitis B and comparable intestinal pathogens is fairly low, aflatoxins is not a significant hazard. This can also be traced from incidences of liver cancer that is also low in USA, and not increasing. However, it is not true for developing countries. In fact, aflatoxin-related liver cancer often occurs in African males 20-30 years old (Jones, 1995).

Consumption of aflatoxin contaminated product, or indirectly through the consumption of foods such as milk, eggs and liver from food-producing animals that have been fed aflatoxin-contaminated feed can bring the risk to humans through aflatoxin metabolites/reaction products including aflatoxin M_1, a metabolite of aflatoxin B_1, found in milk dairy cows exposed to aflatoxin contaminated feed (Anonymous, 1993).

Aflatoxins occur in the sub ppb (mg/kg) to ppm (mg/kg) range in peanut and peanut products, corn, and other grains, such as, wheat, sorghum, and millet besides dried fruit and tree nuts. The incidence of violative levels (total aflatoxins >20 ppb) of aflatoxins in consumer peanut products in the US for the time period 1980-84 and 1986 is low. In fact, over 95% of processed peanut products samples showed levels below 5 ppb. Corn also showed similar results from manufacturers (Anonymous, 1993). Moreover, no aflatoxin was detected in any of the domestic hazelnut samples, but 8% of 142 samples of hazelnut imported from Turkiye were positive for aflatoxins with average of 33 ppb, and a range of 2-100 ppb (Anonymous, 1979).

A. Flavus, A. tamarii, A. ochraceus, A. Terreus, A. wentii species produced aflatoxin on hazelnut. Concentration of aflatoxin stated to be highest between 1-4 month storage while decreasing during prolonged storage due to decomposition (Anonymous, 1978). The aflatoxin production by *A. parasiticus*, grown on ground raw hazelnut with 0.77 a_w is highest 20 ppm at 30^oC. It is about 5 ppb for unground raw kernels at the same conditions. Aflatoxin is much lower in the toasted unground hazelnuts compared to raw ones at the same conditions. When hazelnut a_w is 0.78-0.81, hazelnut becomes a good substrate for production of aflatoxin and ergosterol. Presence of potassium sorbate in the medium enhances aflatoxin production while it inhibits fungal growth (Sanchis et al., 1988).

Sterigmatocystin is produced by several species of *Aspergillus, Penicillium luteum, Bipolaris* species. It is thought to be a precursor of biosynthesis of aflatoxin since it resembles aflatoxin. It's acute toxicity is low but it is mainly classified as liver carcinogen, about one-tenth as potent as a carcinogen as aflatoxin B_1 (Bullerman, 1986). It is found heavily moulded wheat and green coffee beans (Jones, 1995).

Ochratoxins are group of related compounds that are produced by *Aspergillus ochraceus*, and related species, as well as *Penicillium viridicatum* and certain other *Penicillium* species (Bullerman, 1986). It is found in mouldy grain. Like zearalenone, high levels of the toxin cause infertility in both males and females . Ochratoxin A is the most potent mycotoxin in the group and is associated to kidney tumours in Balkans (Jones, 1995).

Citrinin is produced by several *Penicillium* species, as well as *Aspergillus* species. Toxicity of citrinin is lower than ochratoxin but have several synergistic activities. Like ochratoxin, it causes kidney damage in laboratory animals (Bullerman, 1986).

Patulin is produced by numerous *Penicillium* and *Aspergillus* species and *Byssochlamys nivea*. *Penicillum expansum* (Bullerman, 1986) that commonly presents in apple and apple juice products besides in mouldy feed, cherries, apricots, grapes, and peaches (Jones, 1995) . It is toxic to many biological systems, such as bacteria, mammalian cell cultures, higher plants and animals, but its role in causing animal and human disease is unclear (Bullerman, 1986).

Penicillic acid is produced by strains *A. ochraceus* and related species and several *Penicillium* species. Some strains of *A. ochraceus*is are capable of producing penicillic acid along with ochratoxin. It has been found in large quantities in high moisture corn stored at low temperature. Like patulin, it rapidly reacts with sulfhydry-containing compounds in foods to form non-toxic products. It has been shown carcinogenic to rats when injected subcutaneously and its potency is lower than aflatoxins like patulin (Bullerman, 1986).

Zearalenone is primarily produced by *F. graminearum (roseum).* It has been found in mouldy hay, high moisture corn infected before harvest and pelleted feed rations. Its human toxicity has not been reported (Bullerman, 1986).

Trichothecenes are family of closely related compounds produced by several *Fusarium* species. *Fusarium* species produce more than 20 naturally occurring compounds including T-2 toxin, diacetoxyscirpenal, deoxynivalenol, HT-2 toxin, DON vomiton. Don has been implicated in mouldy corn toxicosis of swine that ultimately cause death. T-2 toxin is quite toxic to rats. Large doses ultimately resulted in death. It also causes severe dermal responses in rabbits, rats, and other animals, including human when applied to skin. However it is not thought to be carcinogenic. Although it is very toxic, its natural contamination in foods and feeds appears to be low. Unlike T-2, deoxynivalenol contamination in commodities is significant in certain years. It may have teratogenic potential besides low-acute toxicity (Bullerman, 1986).

Other mycotoxins such as mycophenolic acid, cyclopiazonic acid, b-nitropropionic acids have been reported, and believed to be antibiotic substances of low toxicity. Many of these mycotoxins have been associated with cheeses produced in Europe. Tremorgens has also been associated with cheese (Bullerman, 1986).

PREVENTION OF CONTAMINATION OF GRAINS AND NUTS BY MYCOTOXIN

Mycotoxin contamination of grain crops may start with filamentous fungi contamination in the field, increase during harvesting and drying operations, and continue accumulating during storage due to improper postharvest treatment of the commodity. Contamination is not usually associated with one stage described above, but one stage may be the most important depending on the crop, mycotoxin and environment (Wilson and Abramson, 1992). The factors effecting mycotoxin formation are given in Table 2. Hesseltine (1976; cited by Wilson and Abramson,, 1992) separated mycotoxigenic fungi by habitat: those growing in the living plant, those growing in stored plant material, and those growing in decayed material (Table 3). Turkish Hazelnut microflora separated as those present at harvest on the hazelnut and those growing in the stored plant is given in Table 4 (Anonymous, 1978). When the damage is extensive the whole crop may have to be destroyed or sold at lower grade (Anonymous, 1977). Pre-harvest sanitation and post-harvest safe handling are essential to reduce moulding of the crops and subsequent mycotoxin formation.

Preharvest Field Management

Aflatoxin formation during the preharvest stages of the peanut can increase due to environmental conditions such as drought or excess rain, insect infestation. The most significant contamination of peanuts usually occurs prior the harvest during periods of late-season drought stress as peanuts are maturating which can only be controlled by proper irrigation that is unavailable to majority of the peanut producers. Thai et al., (1990) reported that aflatoxin formation for peanuts under drought stress is dependent on the current level of aflatoxin and daily mean soil temperature. An alternative approach is biological control of contamination through biological competition by addition of a highly competitive, nontoxigenic strain of *A. parasiticus* (biocompetitive agent) to soil which can dominate the soil microflora and prevent the build-up of native, aflatoxin producing stains of *A. flavus/ A. parasiticus*. So that peanuts subjected to late-season drought stress is predominantly invaded by the biocompetitive agent that can not produce aflatoxin. The method has great potential, but practical demonstration of biological control is needed. The method have also advantage of producing chemical free produce (Dorner et al., 1992). Brown et al., (1991) stated that nontoxigenic strains of *A. flavus* reduced 80-95% preharvest contamination of corn when applied either simultaneously with or one day prior to toxigenic strain in field plot experiments. It is also stated that biocompetitive strains prevent postharvest infection and subsequent contamination of corn by toxigenic strains. Paster et al., (1992) stated that *A. niger* is capable of producing metabolites at even early stages of its growth, inhibiting on the growth of *A. ochraceus* and *A. Flavus* as well as aflatoxin formation. Investigation is being carried out to find out the inaction between *A. niger* and mycotoxigenic fungi *in vitro* and on grains.

Segregation of *A. flavus* contaminated peanuts at the field level by close observation of peanuts prior to digging also reduces the risk involved. So that reveal of areas of plant stress such as isolated pockets of severe drought stress that are often the area of *A. flavus* infection and insect infestation. In irrigated fields, the areas missed by irrigation system may contain high concentrations of insect damaged or *A. flavus* infected peanuts. Harvests from such areas must be handled and processed separately to remove the aflatoxin contaminated kernels, and

should be diverted to non-food use or decontaminated by removal of all mouldy kernels. Like peanut, careful examination and separate harvesting of mouldy heads prevent the mouldy grains from mixing with other grains (Anonymous, 1979).

Crops such as sorghum, corn, rough-rice and beans are liable to internal and external pre-harvest attack by insects and moulds in the field. The oviposition by storage pests or mechanical injury by insects and other arthropods are largely responsible for fungal infection. The prevention of oviposition by the insects on the kernels and pods may be achieved by applications of repellents or insecticidal compositions. Pre-harvest disinfection and prophylactic application of chemicals such as captan, thiram, zined, propionic acid, and acetic acids have been found to reduce incidence of fungal contamination of grains. Spraying operations carried out during milky and post-milky stages may prevent internal infection. Combinations of pesticides of low mammalian toxicity such as malathion and captan at 0.3 % concentration applied with low-volume nozzle have given prophylactic effects on the treated panicles of rough-rice, corn and legumes. It is necessary to watch out for development of resistance to pesticides (Anonymous, 1993). Moreover, benlate, Bordeaux mixture, orthophenyl phenate, aureofungin can be applied to grains that are to be long-stored at moisture content less than 16% (d.b.) and temperatures less than 15^{o}C. They inhibit fungi and prevents enzymatic deterioration of the grain (Anonymous, 1993).

In addition, insect and fungal damage can be reduced by adaptation of appropriate cultural practices, such as use of resistant varieties, crop rotation, proper, uniform and adequate irrigation. Sowing at the recommended spacing for the specific crop species and/or varieties to avoid overcrowding of plants is also important in the prevention. Application of calcium to peanuts in the from of gypsum during cultivation reduced aflatoxin contamination in limited field trials. It also improved quality and yield of the peanut besides germination property can be a potential way of cost-effective method to reduce aflatoxin production (Reding et al., 1993).

Certain varieties of seeds have been recognised among peanut, sorghum to have potential for inhibiting aflatoxin production though not necessarily support the fungal growth. The variety must also be equally resistant to other types of mycotoxins and must ascertain that nutritive value of the crop is maintained. In such a study, Brown et al., (1993) stated that MAS:gk and MAS:pw,nf shows a significant postharvest resistance to aflatoxin contamination by *A. flavus* but no significant inter-population variation for this resistance. They also suggested that the resistance in the two population is due to metabolic activities of living corn embryo.

Wild grasses, debris, crop residues are reservoirs for fungal inoculum while weeds compete with the crop during growing. Therefore they must be destroyed. Alternating host plants is also helpful (Anonymous, 1979).

Harvesting the crops in dry weathers conditions at full maturity completely reduces risk involved. Sowing time should not coincide with periods in which mould infections are likely.

Careful handling, minimising mechanical damage during cultivation, harvesting, processing is also important to ensure mycotoxin free products (Anonymous, 1979).

Drying

Drying is essential step in the preservation of grains against fungal attack. It is especially important when harvesting is done in very humid weather. Drying must start immediately after harvesting of the crops, particularly if harvested at high moisture levels, and must be as rapidly as possible to safe moisture level before placing the crop in storage. It is 13% (d.b.) or less for prolonged storage of cereal grains, 8% (d.b.) for peanut, 6 % (d.b.) for hazelnuts, 10% (d.b.) for cottonseed, 10% (d.b.) for soybean and below 11% (d.b.) for sorghum.. Prior the drying remove foreign particles and contaminants that generally carry a higher load of mould spores than does the crop itself besides the pod that are damaged (Anonymous, 1979).

Sun drying by spreading on a paved floor with intermitted stirring is usually the most commonly used method especially in developing countries. In cloudy and rainy weather, it is not sufficient to dry the commodity to safe moisture level in a reasonable time. The occurrence of re-wetting of the crop during or after drying processes due to improper (use of plactic sheets) and inadequate protection from rain during sun-drying or due to water vapour condensation at night, etc. are also possible. They result in prolongation of drying process. Prolonged sun-drying of a crop, in conditions of high humidity, leads to contamination of the crop with moulds. At collection centres that are easily accessible by road and which are not too far from the fields, large-scale artificial drying may be considered, is practical.

Storage

Bulk storage of food grains has become a necessity in many parts of the world. Well ventilated warehousing facilities are essential if grains and other food crops are to be adequately stored to prevent formation of layers or pockets of high moisture content while airtight warehouse allows fumigation when required. Storage structures must be dry and do not permit the entry of water either by leakage or seepage of ground water. Flooring of the warehouse must also be rodent and bird proof. Stacks of bagged grain on dunnage such as polyethylene sheet or wooden pallets under the stacks can help to avoid upward movement of ground water. In non-rodent proof warehouses, stacks should be protected by rodent harbourage removal, rodent stoppage techniques, rodenticidal baits and tracking powders (Hoseney, 1994).

Crops to be stored must be whenever possible of a high quality: free from moulds, insects and off odours and have been dried to safe moisture level for that particular crop. During storage, insects can be controlled by use of fumigation, radiation and good sanitary practices, while mould growth is best controlled with safe moisture level storage. It is important to prevent insect infestation because they crawl on the grain and deposit fungi that results in increase of the moisture levels in pockets of grain where fungal growth then invariable occurs. Treatment of the gunny bags, cloth or other textile bags for infection and infestation by either fumigation or insect-proofing with approved pesticides such as malathion, lindane is also helpful. The level of the active ingredients on the treated containers should not exceed 0.5 g/m^2 (Hoseney, 1994).

Methyl bromide or mixtures of ethylene dibromide and methyl bromide are effective for large-scale fumigation when applied under tropical temperature conditions. Fumigants act very quickly as disinfectant, but any leakage or purging makes the grains susceptible to reinfestation by insects. Pesticides can also be used. Its amount is dependent on grain

temperature and intragranular air both of which affects decomposition rate (Singh et al., 1993). Rat burrows must be fumigated in a safe manner with a suitable and approved fumigant such as phospine, hydrogen cyanide and a combination of 60:40 (w/w) ethylene bromide. Cross infestation of different lots of produce in the warehouse can be prevented by suitable prophylactic insecticide treatment.

Constant temperature and relative humidity is important since moisture migration and condensation resulting from thermal gradients within stored grain masses can cause an accumulation of moisture in certain areas where mould growth could occur. Most fungi require at least 0.7 a_w, and spoilage increases greatly above 0.8 a_w. So safe storage a_w is below 0.65. Water vapour moves along temperature gradients and increases the a_w of the cooler grain. When it is high enough to allow rapid growth of micro-organisms, their respiration releases heat and water of metabolism. If the heat and water are not dissipated, both the temperature and water activity rise, leading to growth of potentially mycotoxigenic fungi. Aeration could prevent moisture re-distribution and cool the crop but it is unsafe for moist climate. If RH and temperature of the air are higher than the conditions within the warehouse, ventilation or aeration may increase the moisture content and temperature of grain. In such conditions ballooning technique is used. It prevents the grain from moisture penetration and absorption by the crop during the humid season. Low-temperature storage whenever possible must be preferred, as mycotoxin contamination is correlated directly with temperature except for some species of *Fusarium* that can produce mycotoxins at low temperature, and storage under nitrogen inhibits their growth (Hoseney, 1994). At temperatures around 10^{o}C, growth of fungi is very slow. However, with time, fungi will grow and produce toxins at low stored temperatures if the water content is adequate. Most fungi associated with stored fungi grow best between 10 and 30^{o}C (mesophiles), some psychrophiles are able to grow as -5^{o}C, and thermophiles may grow up to 60^{o}C. *Aspergillus, Penicillium,* and *Fusarim* species are the most prevalent of the mycotoxigenic fungi in the stored product (Wilson and Abramson, 1992).

Farm Storage The produce must be stored in structures or containers that are moisture proof and amenable to fumigation treatment at the farm level. If produce is already infected in the field, fumigate and dry it before storage. The produce must periodically be inspected and fumigated with suitable fumigants to control infestation. The immature, discolored and broken kernels, weed seeds, stones must be removed before storage and drying. Maximum foreign matter and total refractors should not exceed 4 % on the farm level (Anonymous, 1979).

Transportation Practices

International shipments of aflatoxin susceptible products are usually in bulk such as ship or truck. Peanuts are shipped usually in bags either containerised or break bulk, i.e., stored in bags in the hold of the ship. The final purpose of the product (edible or human food stuffs, or animal feeds) is important in the choice of method of shipment since conditions of the product during shipment could cause an increase in aflatoxin levels in the commodity. Corn is shipped primarily as bulk and the end use are not necessarily known. If the product were exposed to added moisture such as condensation, leakage and holding temperatures were optimal for mould growth (*A. Flavus* or *A. Parasiticus*), aflatoxin levels could increase. Therefore, the extent and conditions under which mycotoxin develop in commodities during

transport must be evaluated and necessary measures must be applied. Tarpaulins, ballooning, or airtight containers are appropriate methods and suitable to prevent moisture absorbance during shipment. Use of packaging materials or containers that do not allow easy entry of insects, and are insect- and rodent-repellent, made by chemical treatment are recommended (Anonymous, 1993).

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Table 1:
The effects of mycotoxins and commodities in which contamination has been found (Bullerman, 1986)

Mycotoxin	Animals effected	Pathological effects	Commodities found contaminated
Aflatoxins B_1, B_2, G_1, G_2, M_1, M_2	Birds Ducking, turkey poult, pheasant chick, mature chickens Mamals Young pigs, pregnant sows, dogs, calves, mature cattle, sheep, cat, monkey, humans Fish Laboratory animasl	Hepatotoxin Liver damage Hemorrhage Intestinal tract Kidney Bile duct Hyperplasia Carcinogen Liver tumors	Peanuts, corn, wheat, rice, cottonseed, copra, nuts, various foods, milk, eggs, cheese
Sterigmatocysin	Mouse Rat	Carcinogen	Green coffe, moldy, wheat, dutch, cheeses
Ochratoxin A	Swine, dogs, ducklings, chickens, rats Humans	Nephotoxin Tubular necrosis of kidney Mild liver damage Enterisis Porcine nephropathy Teratogenic	Cereal grains: wheat, barley, oats, corn Dry beans
Citrinin	Swine, dogs, laboratory animals	Nephrotoxin Porcine nephropathy Acute kidney damage Swelling of kidney Tubular necrosis of kidney	Cereal grains: wheat, barley, rice, corn

Table 1:
Contiuned

Mycotoxin	Animals effected	Pathological effects	Commodities found contaminated
Patulin	Birds Chickens, chicken embryo, quail Mammals cat, cattle, mouse, rabbit, rat Others Brine shrimp, quppies, zebra, fish larvae	Edema Brain Lungs Hemorrhage Lungs Capillary damage Liver Spleen Kidney Paralysis of motor nerves Conculsions Carcinogen Antibiotic	Moldy feed, rottod apples, apple juice, wheat straw residue
Penillic Acid	Mouse, rat, chicken embyro, quail, brine shrimp	Liver damage Fattry liver cell necrosis Kidney damage Digitalis-like action on heart Dilates blood vessels Antidiuretic Edema in rabbit skin Carcinogenic Antibiotic	Stored corn, cereal grains, dried beans, moldy tobacco
Zearalenone (F-2)	Swine, dairy cattle, chicken, turkey, lamp, rat, mouse, quinea pig	Estrogenic effects Swelling and edema of vulva Prolapse of vagina Enlargement of uterus Atrophy of ovaries Atrophy of testicles Enlargement of mamary blands Abortion	Corn, moly hay, pelleted commercial feed

Table 1:
Continued

Mycotoxin	Animals effected	Pathological effects	Commodities found contaminated
Trichothecenes T-2 Toxin, diacetoxyscirpenol, neosolaniol, diacetylnivanenol, deoxynivalenol, HT-2 toxin, fusarenon X	Swine, cattle, chicken, turkey, horse, rat, mouse, dog, cat, humans	Digestive disorders, Emisis, diarrhea, refusal to eat Hemorrhaging stomach, heart, intestines, lungs, bladder, kidney Edema Oral Lessions Dermatitis Blood disorders (Leucopenial)	Corn, wheat, commercial cattle feed, mixed feeds

Table 2:
The factors effecting mycotoxin formation (Wilson and Abramson et al., 1992)

Factor	In Field	At Harvest and Drying	In Storage
Physical			
Moisture	+	+	+
Rapidy of drying	-	+	+
Rewetting	-	+	+
Relative humidity	+	+	+
Net evaporation	+	+	-
Temperature	+	+	+
Mechanical damage	+	+	+
Blending of grain	-	+	+
Hot spots	-	-	+
Time	+	+	+
Chemical			
CO_2	-	-	+
O_2	-	-	+
Nature of substrate	+	-	+
Mineral nutrition	+	-	+
Chemical treatment	-	-	+
Biological			
Plant stress	+	-	+
Invertebrate vectors	+	-	+
Plant vertical differences	+	-	+
Fungal strain differences	+	-	+
Spore load	+	+	+
Microbiological ecosystem	+	-	+
Insect damage	+	+	+
Damage by plant diease	+	-	+

Table 3:
Fungi that produce mycotoxins classified by habitat (Wilson and Abramson et al.,1992)

Fungi Growing on Living Plant	
Aspergillus flavus	*Fusarium moniliforme*
Claviceps purpurea	*Helminthosporium biseptatum*
Fusarium gramiearum (*Gibberella zeae*)	*Rhizoctonia leguminicola*
	Sclerotina sclerotiorum
Fungi Growing on Stored Plant Material	
Aspergillus flavus	*Penicillium islandicum*
A. chevallieri	*P. citreoviride*
A. clavatus	*P. citrinum*
A. fumigatus	*P. expansum*
A. ochraceus	*P. palitans*
A. parasitius	*P. puberulum*
A. rubrum	*P. roqueforti*
A. versicolor	*P. rubrum*
Chaetomium globosum	*P. rugulosum*
Fusarium graminearum	*P. urticae*
F. moniliforme	*P. verrucosum* var *cyclopium*
F. nivale	*P. verrucosum* var *verrucosum*
F. tricinctum	
Fungi Growing in Decaying Plant Material	
Alternaria lonqipes	*Myrothecium verrucaria*
Chaetomium globosum	*Pericania minutissima*
Cladosporium sp.	*Pithomyces chartarum*
Dendrodochium toxicum	*Stachybotrys atra*
Fusarium graminearum	*Trichoderma viride*
F. sporotrichoides	*Trichorhecium roseum*

Table 4:
Fungi that present on hazelnut classified by habitat (Data gathered from Anonymous 1978).

Fungi	Present on Hazelnut		Growing on Stored Hazelnut	
	Shell at Harvest	Dry-Shell Before storage	2-Months	6-Months
Penicillium sp.	+	+	+	+
Cephalosporium sp.	+	+	+	+
Cladosporium sp.	+	+	+	+
Verticillium sp.	+	+	+	+
Acremonium sp.	+	+	+	+
Alternaria sp.	+	+		+
Chaetanium sp.	+			
Mucor sp.	+			
Rhizopus sp.		+	+	+
Trichothecium Roseum		+		+
Oopora sp.			+	
Gromella sp.		+		
*Phoma*sp.				+
Pestilazza sp.				+
Trichoderma sp.				
Aspergillus sp.	+			