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Battilani P.

in

Zakynthinos G. (ed.).
XIV GREMPA Meeting on Pistachios and Almonds

Zaragoza : CIHEAM / FAO / AUA / TEI Kalamatas / NAGREF
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 94

2010
pages 167-173

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P. Battilani

Institute of Entomology and Plant Pathology, Faculty of Agriculture,
Università Cattolica del Sacro Cuore, Via E. Parmense, 84, 29100 Piacenza (Italy)

Abstract. Nuts are among the crops that can be contaminated by aflatoxins (AFs), mycotoxins mainly produced by *A. flavus* and *A. parasiticus.* These fungi are well adapted to tropical and sub-tropical areas and tend to contaminate damaged or stressed crops. Both peanuts and pistachio nuts are well protected by their shells during the growing season, but damage caused by parasites, or early split in pistachio nuts, can result in contamination by Aspergilli in field. The amount of AFs in nuts can also increase considerably during harvest/post-harvest. As well as careful crop management, promising results in reducing AFs contamination have been achieved applying biological control in field. Sorting can effectively reduce AFs, but a high percentage of production is discarded, which is not always contaminated at unacceptable levels. Decontamination is difficult; it frequently leads to limited results or variations in the edibility or quality of nuts.

Keywords. Nuts – Aflatoxins – Crop management – Sorting – Decontamination.

Les mycotoxines chez les noix

Résumé. *Les fruits secs sont parmi les récoltes qui peuvent être contaminées par des aflatoxines (AFs), mycotoxines principalement produites par* A. flavus *et* A. parasiticus*. Ces champignons sont bien adaptés au milieu tropical et subtropical et affectent de préférence les récoltes endommagées ou soumises à stress. Les arachides ainsi que les pistaches sont bien protégées pendant la saison de croissance, tandis que les dommages provoqués par des parasites ou la déhiscence chez les pistaches peuvent produire la contamination par* Aspergilli*. La quantité d'AFs dans les fruits secs peut également augmenter de manière significative pendant et après la récolte. Des résultats prometteurs réduisant la contamination par AFs ont été obtenus par une gestion correcte de la culture, en appliquant un contrôle biologique aux champs. Sélectionner est intéressant pour réduire les AFs, mais un pourcentage élevé, à un niveau inacceptable, de production pas toujours contaminée est jeté. La décontamination, difficile, mène fréquemment à des résultats limités ou des variations de la comestibilité ou de la qualité.*

Mots-clés. *Fruits secs – Aflatoxines – Gestion de la culture – Sélection – Décontamination.*

I – Introduction

Mycotoxins are natural compounds, secondary metabolites produced by fungi which mostly belong to the *Aspergillus, Penicillium* and *Fusarium* species. They can have acute or chronic effects on humans and animals and they were recently defined as a major food safety concern (Kuiper-Goodman, 2004; Table 1). Current knowledge of their toxicity is still very limited, particularly regarding immunosuppressive activity and interaction between different toxins. Syndromes related to mycotoxins have generally been studied in animals where target organ and dangerous dosage are strictly related to the toxin and the species (Anonymous, 2003).

Mycotoxins have an important economic impact because of the direct loss of products which are unfit for sale and the indirect costs of mitigation strategies. It is difficult to estimate the financial cost because of the involvement of many factors, but direct annual costs in the US could be as high as 1.66 billion dollars and mitigation costs about 466 million dollars (Anonymous, 2003).

Nuts are among the crops that can be contaminated by mycotoxins. Those most studied are peanuts and pistachio nuts for aflatoxin (AFs) contamination, considered a major problem in the US (Bhatnagar *et al*., 2004), and also in Asia (Pitt and Hocking, 2004; Bonjar, 2004) and Africa (Bankole *et al*., 2006) while there are no reports available regarding Europe.

Table 1. List of acute and chronic food related risks (Kuiper-Goodman, 2004)

ACUTE risks		CHRONIC risks
	HIGH	
Microbiological		**Mycotoxins**
Phycotoxins		Anthropogenic contaminants
Some phytotoxins		Some phytotoxins
Mycotoxins		Unbalanced diet
Anthropogenic contaminants		Phycotoxins
Pesticide residues		Food additives
Food additives		Pesticide residues
		Microbiological
	LOW	

Regarding other toxins, the only signalled presence of ochratoxin A in nuts regards pistachio nuts in a survey managed by the British Food Standard Agency (2002); therefore, this toxin is not considered a concern.

Pistachio and peanuts are important crops at world level and problems relating to mycotoxins are a growing health and economic concern. Global pistachio production is around 600 hundred thousand metric tons (2002 data). The top ten producers are Iran (53%), USA (24%), Syria (9%), Turkey (7%), China (5%), Greece (2%) and Afghanistan, Italy, Uzbekistan and Tunisia, each with less than 1% (www.fas.usda.gov/htp/hort_circular/2004). China, with an annual production of over 15 million tons (2003 data) is the world's biggest peanut producer (42%), followed by India (20%), Nigeria (7%), USA (5), Sudan (3%) and Senegal (2%) (www.lana.uga.edu/peanuts/knowledgebase).

Pistachio, a tree nut, and peanuts, an annual crop, vary greatly for plant behaviour and size, nut production and growing areas, but they both have interaction with *A.* Section *Flavi* and concerns relating to aflatoxins can be approached in the same way. All stages of nut production in field and management at harvest and post-harvest are important and can contribute to the final contamination level. A profound knowledge of "all the rings in the chain", intended as all the steps in nuts production and management till to consumption, is fundamental in order to produce healthy nuts.

II – Aflatoxins producing fungi in nuts

1. Aflatoxins

Aflatoxins (AFs) are a group of mycotoxins, secondary metabolites produced by members of *Aspergillus* Section *Flavi*, mainly *A. flavus* and *A. parasiticus* (Kurtzman *et al.*, 1987) in several crops. Four toxins, AFB_1, AFB_2, AFG_1 and AFG_2 can be detected in plant products, but AFs can also contaminate animal products. In fact, another known AF is AFM_1, produced during AFB_1 metabolism in animals and excreted in milk; it represents almost all AFs detectable in cattle milk (Galvano *et al.*, 1996).

Aflatoxin B_1 is the most dangerous toxin, for its acute and chronic effects, and consequently it is the most studied; it was classified by the International Agency for Research on Cancer (IARC) in class 1, the only natural compound, because of its demonstrated carcinogenicity to humans (Castegnaro and Wild, 1995). Aflatoxins are potent epatotoxins and their carcinogenicity has forced governments and regulatory agencies to set very low tolerance levels in food (Van Egmond, 2002).

Most countries have aflatoxin regulations for various products, including nuts. Current European legislation regarding AFs in nuts is incorporated in EU Regulation 1881/2006 (EC, 2006a) and is summarised in Table 2. Sampling methods and analysis for the official control of mycotoxin levels in foodstuffs is detailed in EU Regulation 401/2006 (EC, 2006b) where a sample of 30 kg is considered necessary for nuts, and the required analytical procedures are also defined.

Table 2. Maximum level of aflatoxins admitted in nuts, in µg kg^{-1}, according to EU regulation 1881/2006. The maximum levels refer to the edible part of groundnuts and nuts

Foodstuff	Aflatoxin B_1	Sum of B_1, B_2, G_1 and G_2
Nuts to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs	5	10
Groundnuts and nuts and processed products thereof, intended for direct human consumption or use as an ingredient in foodstuffs	2	4

2. *Aspergillus* Section *Flavi*

It is generally considered that *A. flavus* produces only AFB_1 and AFB_2, while *A. parasiticus* produces all the four principal AFs (AFB_1, AFB_2, AFG_1 and AFG_2) (Diener *et al.*, 1987; D'Mello and McDonald, 1997), but Gabal *et al.* (1994) reported *A. flavus* strains producing also AFG_1 and AFG_2. The percentage of strains able to produce AFs in a fungal population varies in different areas and probably years.

Aspergillus Section *Flavi* are plant pathogens, but living tissue is only a minor substrate for these soil-borne fungi, which are saprophytic during most of their life cycle. Aspergilli can grow at temperatures from 12° to 48℃ and water pot ential as low as -35 MPa; these organisms are semitermophilic and semixerophitic, well adapted to tropical and sub-tropical areas. The two major factors that influence their fungal population are soil temperature and moisture. They are very competitive with high temperature and low water activity and may become the dominant fungal species in host crops (Payne, 1998). These conditions also favour insect activity and often compromise the defence system of the host plant.

Aspergillus Section *Flavi* are not very aggressive and they tend to contaminate damaged or stressed crops, although they sometimes invade seeds directly. *Aspergillus flavus* is the most aggressive species and it dominates on all the commodities, probably supported by its ability to produce pectinase and cutinase, a relevant aid in host penetration (revised in Payne, 1998).

3. The infection cycle on the host plant

The inoculum of aspergilli comes from crop debris and soil; its level seems very important and may be a limiting factor in certain years and locations. Spores are air dispersed and easily reach all plant organs; nevertheless, the incidence of *A. flavus* and *A. parasiticus* in seeds is normally low (Doster and Michailides, 1995) and in pistachios *A. niger* was found as the dominant fungal species isolated (Doster and Michailides, 1994).

Both peanuts and pistachio nuts are well protected during the growing season against Aspergilli because their seeds are enclosed in a shell. Further, pistachio nuts have a cuticle and a hull that cover the shell for extra protection.

Drought stress and temperature are the two main factors which influence aflatoxin contamination in peanuts, particulary pod stress (Sanders *et al*., 1993), and the most critical growing period takes place during the last 3 to 6 weeks of the growing season (Cole *et al*., 1995). In pistachio nuts a significant correlation between temperature and incidence of *A. flavus* was also found, with the fungus more common in orchards situated in hot areas. Lower aflatoxin contamination was detected in irrigated pistachios where a lower incidence of early split was also observed (Michailides, 1996).

Parasitic damage (Sommer *et al*., 1986) or early split, intended as the hull split before harvest in pistachio nuts (Doster and Michailides, 1995; Mehrnejad and Panahi, 2006), can elicit infection by Aspergilli in field, and consequently contamination by AFs. Navel Orange worm larvae (*Amyelois transitella*) is cited as the main insect involved in tree nut damage in the US and lesser cornstalk borer (*Elasmopalpus lignosellus*) for peanuts, with an important role also for spore dissemination (Lynch and Wilson, 1991).

The harvest/post-harvest period can significantly increase the level of AF contamination if incorrectly managed. Harvesting must be completed immediately after physiological maturity and drying managed quickly because this period, with ripe fruits and water available for fungal activity, is crucial for AF production (Mehrnejad and Panahi, 2006). Studies on commercial pistachio nuts in Iran have indicated that mid to late September is the best harvest period (Panahi *et al*., 2005).

Harvest time has been more extensively studied in peanuts, where earlier-than-normal harvest is suggested when the risk of AF contamination is high; two different predictive systems of AF contamination at harvest are available, developed in the US (Parmar *et al*., 1997) and Australia (Wright *et al*., 2005), but they are considered by farmers only when penalties are imposed for AF contamination, which happens in Australia (Dorner, 2008).

The parameters of the drying process, mainly air temperature and air velocity, have been shown to influence the qualitative characteristics of nuts, without changes in AF content (Kashaninejad *et al*., 2005) and a specific study has been undertaken on the role of drying conditions on the characteristics of pistachio nuts (Kashaninejad *et al*., 2007). Safe moisture obtained through a proper drying process, with water activity at or below 0.83, must be maintained also during storage, until nut processing or consumption, to avoid fungal activity (Dorner, 2008).

III – Prevention of aflatoxin accumulation in nuts

Prevention of AF contamination is vital, while decontamination is difficult and frequently lowers the quality or quantity of the commercial product.

Good agricultural practices are definitely the fundamental prevention strategies, with particular attention to correct manuring and irrigation; water supply is considered a useful preventive action in peanuts (Dorner *et al.,* 1989).

As well as care in crop management, an approach taken with pistachio crops involved the use of gibberellic acid; it effectively reduces early hull split and positive results were obtained in terms of AF reduction (Pakkish and Rahemi, 2005).

Genetic resistance to *A.* Section *Flavi* has not yet been considered in plant breeding, but differences in AF contamination in varieties has been observed both in pistachios (Moghaddam *et al*., 2006; Hokmabadi *et al*., 2007) and peanuts (Xue *et al*., 2005); an interesting screening has also been undertaken with almonds (Dicenta *et al.*, 2003).

Pest and aspergilli control are not common practices in nuts, but promising results in reducing AF contamination have been found applying biological controls in field. Several microrganisms have been studied (Hua, 2004; Palumbo *et al*., 2006), but the best results related to the distribution of *A. flavus* strains not able to produce AFs, good competitors of positive strains (Dorner, 2005). Commercial products are available in the US and good results have been reported on cotton and maize and a commercial product is also available for peanuts (Dorner and Lamb, 2006).

IV – Techniques for post-harvest aflatoxin management

Some techniques have been developed to manage AF contamination that has already occurred in field, during harvest, transport or storage. A good example of a rationale approach in peanuts has been reported by Dorner (2008).

The first step of correct management is lot segregation, which means that lots with visible moulds, confirming the presence of *A. flavus* or *A. parasiticus*, or with AF contamination above the legal limit must be stored separately and not used for edible purposes. After lot segregation, screening to separate high-aflatoxin-risk components, identified as loose shelled kernels and small, immature pods, can be effective and a 35% reduction in AF content can be achieved. Shelled kernels vary in density and size; consequently, they can be segregated based on gravity tables or slotted and round-hole screened. This is also brings good results and reductions as high as 83% and 43% in AF contamination were obtained using these two methods. Electronic colour sorting, before or after blanching (removal of seed coats) is also effective, based on linking discoloured kernels and AF contamination; this approach can reduce mean AF concentration up to 91%.

These approaches are very successful for reducing AF contamination, but there is a drawback: the altered kernels are linked to AF contamination, but they are not necessarily contaminated. It means that yield losses due to different sorting approaches are not always justified by AF reduction (reviewed in Dorner, 2008).

The relationship between small, discoloured or defective nuts in general and AF content has also been studied in pistachios (Takahashi *et al*., 2001; Ghadarijani and Javanshah, 2006) and an automated detection of defects by machine vision been proposed, with interesting results in terms of kernel safety (Pearson *et al*., 2001), and more recently a neural network based on bright greenish yellow fluorescent excitation (Karami and Mirabolfathy, 2006).

Decontamination is a further research line being followed. Heat treatments have shown that AF degradation is time and temperature dependent and effective treatments result in an inedible product (Hassa-Yazdanpanah *et al*., 2005). Ethanol (Versilovskis and Mikelsone, 2006) and ozone (Akbas and Ozdemir, 2006) applications have also been considered; the most promising results were obtained with pure ethanol, applied in laboratory conditions, which reduced AFs by between 44 and 99% depending on the initial contamination level, giving an edible product.

V – Conclusions

Nut growing areas have very similar meteorological conditions for *A.* Section *Flavi* growth and AF production. Due to the global importance of nuts, particularly peanuts and pistachios, and concern about the effects of AFs on humans and animals, a rationale management of these crops is vital.

Current knowledge on this issue is incomplete, but many critical points are clear and effective management procedures have been indicated. Based on the information available on these crops, but also on other crops with mycotoxin problems, prevention is the correct approach, while many operations aimed at reducing contamination levels often have limited effects and are expensive.

A "chain approach" should be followed to minimise nut contamination with AFs, with all operators informed and involved. This is always the best approach, especially in this case because all the stages of nut production and storage/processing play a role in determining the final level of contamination.

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