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X-ray imaging for fungal necrotic spot detection in pistachio nuts

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Abstract

The potential of X-ray imaging for detecting pistachios with kernel necrotic (KN) spots was the main objective of this study. X-ray images of whole pistachios were compared with colour pictures of the same nuts taken after the kernel was opened by splitting it in two. The necrotic spots appear in the X-ray images as darker gray areas of almost round shape. Also, pistachios with KN spots contained about 60 times more aflatoxin than healthy ones in an aflatoxin contaminated sample. Elimination of such nuts will reduce aflatoxin in contaminated batches and improve the quality in batches free of aflatoxin.

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1. Introduction

Mycotoxin contamination of agricultural commodities is considered a serious food safety issue worldwide [1]. Aflatoxins are the most prominent groups of mycotoxins. They are naturally produced by fungal molds, mainly *Aspergillus flavus* and *Aspergillus parasiticus*, as secondary metabolites [2]. Many aflatoxins exhibit acute and chronic toxicity. The International Agency for Research on Cancer has posted aflatoxin B1 as a group I carcinogen, primarily affecting liver. Due to the significant health risks associated with the presence of aflatoxins in food, strict regulations govern the import and export sales of various food products to minimize the mycotoxins risk for human consumption and animal feed. According to the Annual report for 2008 of the European Rapid Alert System for Food & Feed (RASFF), mycotoxins were the hazardous category with the highest number of notifications within EU in the year

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2008 and in previous years as well. In the last years, there were many cases of aflatoxin detection above the EU limits in pistachio nuts. In all such cases, the nuts are not released for human or animal consumption and therefore a serious economic loss results due to the direct loss of product and the indirect cost of mitigation strategies.

The most studied nuts for aflatoxin contamination are peanuts and pistachios [3], considered a major problem in the US [4], in Asia [5], [6] and Africa [7], while there are very limited reports available regarding Europe. A previous study concerning aflatoxin contamination of pistachios produced in Greece was conducted in all production steps, from field to storage [8]. Aflatoxin was detected in all stages, from pre-mature nuts to stored dried nuts. It was detected in concentrations above the legal limits at the stage of maturity on the trees and onward. In consistence with other studies for pistachios, higher levels of aflatoxin were detected in orchards with heavy insect infestations indicating a positive correlation between them. At post-harvest, aflatoxin contamination varied among samples from different orchards depending on the aflatoxin quantity at pre-harvest stage, the drying method and the storage conditions. It was also observed that pistachio nuts which looked healthy on the outside but with a necrotic spot in the kernel (Figure 1), harvested at the stage of maturity, had higher probability for aflatoxin contamination at concentrations above legal limits, which increased dramatically (up to 600ppb total aflatoxins) after drying and during storage [8]. Consequently, it was decided to further study the correlation between aflatoxin contamination and kernel necrotic spots.

Pistachio nuts with kernel necrosis symptoms are considered to be infected by the stigmatomycosis disease. Stigmatomycosis of pistachio has been reported in Iran (1967), Russia -referring to central Asian countries- (1972), Greece (1979) and USA (1990) [9]. According to Michailides et al. [10], kernel necrotic symptoms differ from typical symptoms of stigmatomycosis, which is characterized by the wet, smelly, rancid, slimy appearance of the kernel. Actually, the term stigmatomycosis is the general name for a disease that occurs in nuts which appear healthy on the outside but inside are deteriorated by fungi which have been implanted by insects of the class Hemiptera [9]. Some native pests of Hemiptera class are visible in pistachio orchards from early May till late August. All these bugs use piercing-sucking mouthparts to feed directly on the nut. In California, more than 10 different species of hemipterans have been reported as pests which, after feeding on pistachio nuts, cause epicarp lesion on the developing fruit early in the season and kernel necrosis later in the season, after feeding directly on the kernel of the nuts [12]. As studied by Daane et al [13], small bugs damage nuts' pericarp (hull) early in the season and most damaged nuts are dropped from the cluster. On the other hand, large bugs have larger, stronger mouthparts and may continue to damage kernels until harvest time. The most critical period for potential kernel damage occurs in midseason, when fruit load is set, shell hardening is not complete and large bugs' mouthparts can easily penetrate hardening shells. The damaged nut remains in the cluster and the feeding punctures result either to epicarp lesion staining the outer shell or to necrotic spots on the kernels contaminated by fungi [13]. The subsequent feeding by large bugs presents potential "hidden" damage, since kernel necrosis may occur without the external sign (epicarp lesion) of insect feeding. Late-season feeding can also lead to kernel necrosis when the insect's mouthparts penetrate the shell near the "Achilles heel" of the pistachio, the region near the peduncle (nut's stem) [11].

Stigmatomycosis is caused by the pathogen *Nematospora coryli* (Saccharomycetales, Ascomycete) [9]. The pathogen seems to hibernate in the proboscis of the adult bugs and enters by the feeding punctures. According to other reports [10] this disease is also caused by *Aureobasidium pullulans*. In recent years, the problem of stigmatomycosis is serious in Greece due to the reduction of insecticide applications in pistachio orchards. Moreover, our previous studies concluded that nuts which look healthy on the outside



Fig. 1: Pistachio nut with brown necrotic spot in the kernel

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