Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

Research Papers

First report of *Aspergillus* species in green pistachio of Bronte

Wanissa MELLIKECHE[1], Giulia CASINI[2], Marilita GALLO[3], Anna Maria D'ONGHIA[3], Giancarlo COLELLI[1], Alessandra RICELLI[4,*]

[1] *Dipartimento di Scienze Agrarie, degli Alimenti e dell'Ambiente, Università di Foggia, Via Napoli 25, 71122 Foggia, Italy*
[2] *Enbiotech S.r.l., Via Quarto dei Mille, 6, 90129 Palermo, Italy*
[3] *Centre International de Hautes Etudes Agronomiques Méditerranéennes Bari (CIHEAM Bari), Via Ceglie 9, 70010 Valenzano, Bari, Italy*
[4] *Istituto di Biologia e Patologia Molecolari (IBPM-CNR) P.le A. Moro 5, 00185, Roma, Italy*
*Corresponding author. E-mail: alessandra.ricelli@cnr.it

Summary. *Aspergillus* contamination of pistachios causes significant product losses and potential presence of mycotoxins, particularly aflatoxin B1 (AFB1), and ochratoxin A (OTA). These toxins, which threaten human health, are strictly monitored by most nations. Italian pistachios produced in Bronte, Sicily, have high nutritional value and unique organoleptic properties, but the extent to which they contain these contaminants is unknown. *Aspergillus* spp. isolated from Bronte pistachios (cultivar Napoletana) were assessed for their ability to synthesize OTA or AFB1. *Aspergillus* occurrence in pistachio samples was measured at 1137 cfu g^{-1} for in shell pistachios and 770 cfu g^{-1} for kernels. The predominant isolated *Aspergillus* species was *A. niger* representing 74% of section *Nigri* (black isolates) and 47% of all *Aspergillus* isolates. Within section *Flavi*, *A. flavus* comprised 83% of green isolates. Only one black isolate (identified as *A. carbonarius*) had high OTA production, but all the *A. flavus* isolates had potential to produce AFG1 and AFB1, with AFB1 produced amount ranging from 0.1 to 8498 ng mL^{-1} of culture filtrate.

Keywords. *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus carbonarius*, *Aspergillus tamarii*, *Aspergillus tubingensis*, aflatoxins, ochratoxin A.

OPEN ACCESS

Citation: W. Mellikeche, G. Casini, M. Gallo, A.M. D'Onghia, G. Colelli, A. Ricelli (2024) First report of *Aspergillus* species in green pistachio of Bronte. *Phytopathologia Mediterranea* 63(1): 103-110. doi: 10.36253/phyto-14949

Accepted: March 5, 2024

Published: April 29, 2024

Copyright: ©2024 W. Mellikeche, G. Casini, M. Gallo, A.M. D'Onghia, G. Colelli, A. Ricelli. This is an open access, peer-reviewed article published by Firenze University Press (http://www.fupress.com/pm) and distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: Lluís Palou, Valencian Institute for Agricultural Research, Valencia, Spain.

ORCID:
WM: 0000-0001-6383-4094
GC: 0000-0001-7224-7158
AR: 0000-0002-1151-6120
GCo: 0000-0001-8619-3541
MG: 0000-0003-4981-485X
MD'O: 0000-0002-1817-4637

INTRODUCTION

Pistachio nut tree (*Pistachia vera* L.), native to the arid zones of Central and West Asia, is widely distributed across the Mediterranean region. Pistachios are one of the most highly valued nut commodities consumed raw, toasted, and salted, or as an ingredient in many foods including desserts, ice-cream, pastry, and some sausages (Arena *et al.*, 2007). Pistachios also have high nutritional value and contain phytochemical antioxidants (D'Evoli *et al.*, 2015; Sheikhi *et al.*, 2019). Pistachio trees are cultivated mainly in the United States of America, Iran, and Turkey and in the Medi-

Figure 1. Pistachio trees spontaneously growing in volcanic soil in Bronte, Sicily.

terranean countries of southern Europe and North Africa (Mandalari *et al*., 2022).

Pistachios were brought to Italy from Syria during the Roman era in the 9th Century AD (Marino and Marra, 2019). In Sicily, where agriculture was highly influenced by Arabs, pistachios became an integral part of the island's cuisine and culture. Pistachios produced in Bronte are very high-quality and particularly flavourful, due to the island's volcanic soils and climatic conditions. Pistachio trees in this area grow spontaneously on the volcanic soils (Figure 1), and are harvested once every 2 years, in September, and then dried in greenhouses. Despite their low production volume, "green pistachios of Bronte" cultivar Napoletana (also known as "Nostrale" or "Bianca") are the most expensive in the world, and are of economic importance in Italy (Wilson *et al*., 2018).

The green pistachio of Bronte was officially registered as an Italian Protected Designation of Origin (PDO) product in 2010. This designation aims to promote and protect product authenticity and characteristics, to improve economic conditions for producers in the specified areas (within 'the municipalities' of Bronte, Adrano, and Biancavilla), and to provide consumers with information about product origins and production methods (Wilson *et al*., 2018; Marino and Marra, 2019).

The quality of pistachios does not depend only on their flavor and nutritional value, but also on the absence of mycotoxins. These are secondary metabolites produced by several widespread fungi, mainly *Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp. Pistachios have been associated with several types of mycotoxins including cyclopiazonic acid (Hua *et al*., 2012), and HT-2 toxin, fumonisins, ochratoxin A and aflatoxins, with this latter group being of the greatest interest on pistachios (Soares Mateus *et al*., 2021).

Aflatoxins are commonly found in pistachios, due to contaminations by green *Aspergillus* spp. (section *Flavi*), and particularly *A. flavus* and *A. parasiticus*. These species produce several aflatoxins including aflatoxin B1 (AFB1), AFB2, AFG1, and AFG2. While all of these compounds are hazardous due to their interference with the immune system, AFB1 is also characterized by the most potent mutagenic and carcinogenic activity. Therefore, it was confirmed as Group 1 agent by IARC (IARC, 2002).

In addition to aflatoxins, analyses of other varieties of pistachios found presence of ochratoxin A (OTA), which is linked to black *Aspergillus* (section *Nigri*), mainly *A. carbonarius* and *A. niger* (Fernane *et al*., 2010). OTA is a nephrotoxic, carcinogenic, teratogenic, immunotoxic and hepatotoxic mycotoxin. It is classified in Group 2B by IARC (IARC, 1993).

AFB1 and OTA are strictly regulated in many countries including those of the European Union. For pistachios intended for direct human consumption or use as food ingredients, the maximum acceptable levels are 8 μg kg^{-1} of AFB1 (with 10 μg of total aflatoxins), and 5 μg kg^{-1} of OTA (EU Reg EC 1881/2006, as amended by 165/2010).

Because of the high economic value of pistachio production and the potential for expansion of this industry in Italy, studies addressing the composition of pistachios (Tomaino *et al*., 2010) and the pathogens that could endanger pistachio production (Gusella *et al*., 2022) have increased. However, there are no reports on the presence of toxigenic *Aspergilli* in Bronte's pistachios. The present study aimed to determine the state of pistachio mycotoxin contamination, as knowledge to support the healthi-

ness of this valuable product. A survey of *Aspergillus* species on Bronte pistachios was conducted, and the potential capacity of isolated strains to produce AFB1 and OTA was evaluated.

MATERIAL AND METHODS

Sampling and isolation of fungi

Ten samples of dried pistachios (1 kg each) obtained from the area of Bronte (Catania province, Italy) were used in this study. To estimate fungal contamination levels (cfu g^{-1}) on these samples, isolations were performed by plating water spore suspensions. Fifty nuts from each sample were weighed and added to 50 mL of sterile water containing 0.1% Tween20 in a sterile Erlenmeyer flask, which was then shaken for 1 h on an orbital shaker (120 rpm), at room temperature. The resulting suspension was filtered and was used to prepare serial 10-fold dilutions.

One hundred µL of spore suspension was seeded onto three potato dextrose agar (PDA: Difco, Becton Dickinson) plates, which were incubated at 25°C and monitored daily for 7 d. Each resulting fungal colony was transferred to a new Petri dish containing PDA and was left to grow at 25°C. The total number of colonies grown on each original isolation plate was expressed as colony forming units per gram of matrix (cfu g^{-1}). For each sample, isolations were carried out from in shell pistachios (kernels and shells) and from kernels.

Identification of isolated Aspergillus *spp.*

Among the isolated fungi, *Aspergillus* isolates belonging to *Nigri* and *Flavi* sections were identified by their distinctive morphological structures, including colony characteristics and shape and size of conidia and conidiophores (Pitt and Hoking, 1997). Green isolates were tested using a LAMP assay (Mellikeche *et al.*, 2024) designed specifically for the detection of *A. flavus,* the greatest producer of AFB1. To detect *A. carbonarius*, the greatest producer of OTA, black isolates were grown on semi-selective malt extract agar, (MOA-B), containing 10 mg L^{-1} of Boscalid®, (Merck) (Samson *et al.*, 2007), on which only this species can sporulate. A LAMP assay for the detection of *A. carbonarius* (Enbiotech S.r.l.) was used to confirm identification of this fungus.

Remaining black and green isolates were divided into groups based on similarities of morphological characteristics on potato dextrose agar (PDA), czapek yeast agar (CYA), *Aspergillus* differentiation agar (ADA Difco, Becton Dickinson). Representative isolates from each group were subjected to DNA extraction (Carlucci *et al.*, 2013), and PCR assays using Calmodulin primers described by O'Donnell *et al.* (2000), followed by sequencing of the amplified regions to identify the species.

Production of AFB1 and OTA

Fungal cultures were incubated in czapek yeast broth (CDY), which is conductive to biosynthesis of aflatoxins and ochratoxin A by Aspergilli (Visagie *et al.*, 2014; Frisvad *et al.*, 2019). The cultures were grown at 24°C in the dark in static conditions, and were filtered through Whatman no. 4 filter paper, using a a vacuum pump system. Resulting filtrates were frozen at -25°C until they were used for mycotoxin analyses.

Production of OTA and AFB1 by the black and green *Aspergillus* species isolated from pistachio nuts was first qualitatively evaluated using High-Performance Thin-Layer Chromatography (HPTLC, Merck). Two mL of each culture filtrate were acidified by 0.2 mL of formic acid and then extracted using 2 mL of ethyl acetate. Each extraction was repeated twice, and the extracts were collected and concentrated under a nitrogen stream. The concentrated extracts were reconstituted with ethyl acetate to 1 mL, and then 5 µL were plated on HPTLC plates together with known amounts of the pure AFB1 and OTA as references. The eluent phase used was a mixture of toluene, ethyl acetate and formic acid (6:3:1, v/v/v).

For quantitative analyses, culture filtrates were purified using immunoaffinity columns (AflaOchra® Immuno Affinity columns, VICAM), with 20 mL of each culture filtrate used for each purification. After passage through the column, washings were each carried out with 20 mL of distilled water. Water was then eliminated from the columns by applying light pressure, and mycotoxins potentially present were eluted from the column with 1.5 mL methanol, as prescribed in the manufacturer's instructions. Each sample was then evaporated under nitrogen flow at room temperature. The dry sample was then reconstituted in 200 µL of a mixture of acetonitrile, water, acetic acid (99:99:2 v/v/v).

Mycotoxin analyses were carried out using the procedure of Solfrizzo *et al.* (1998). A stock solution (1.0 mg mL^{-1}) of OTA (Sigma) was prepared in toluene plus acetic acid (99:1, v/v). OTA calibration standard solutions for HPLC determination were prepared by dissolving appropriate amounts of stock solution in acetonitrile-water-acetic acid (99:99:2, v/v/v) to obtain final concentrations of 1 ng mL^{-1}. For AFB1, the stock solution (Sig-

ma) was prepared at 1.0 mg mL^{-1} in methanol. Calibration standard solutions for HPLC determinations were prepared by further dissolving appropriate amounts of stock solution in methanol to obtain final concentrations of 1 ng mL^{-1}. Each dried sample was resuspended in 200 µL of an acetonitrile-methanol mixture (1:1, v/v), and was analyzed using an HPLC (1260 Infinity Agilent) equipped with a diode array detector and a C18 column (Poroshell 120; 50 × 4.6 mm, 2.7 µm, Agilent). The mobile phase consisted of a mixture of acetonitrile-water-acetic acid (99:99:2, v/v/v) at a flow rate of 1 mL min^{-1}. The chromatographic runs were carried out in isocratic mode, whereby an aliquot of 10 µL, taken from 200 µL of each dissolved extract, was injected into the HPLC system. AFB1 and OTA in the samples were detected and quantified by comparing the retention times and the absorbance spectra of the authentic standards.

Each mycotoxin was quantified by measuring peak areas and comparing them with the relevant calibration curves. The Limits of Quantification (LOQ) were 2 ng for OTA and 2.5 ng for AFB1. Detection and quantification of AFB1 were carried out at 363 λ and for OTA at 333 λ, which are the relative maxima for these molecules.

RESULTS AND DISCUSSION

Isolation and evaluation of Aspergillus *occurrence in pistachios*

Most isolated fungi were identified as *Aspergillus*, comprising 95.8% of the isolates from in shell pistachios and 94.5% of the isolates from pistachio kernels. Morphological analyses of the other isolated fungi indicated they belong to the genera *Penicillium* and *Alternaria* (Figure 2). Average *Aspergillus* occurrence in kernels was 770 cfu g^{-1}. A total of 133 isolates were obtained from kernels, with the majority (78%) belonging to section *Nigri*, while only 18% were classified as section *Flavi*. Greater occurrence was recorded from in shell pistachio nuts averaging 1137 cfu g^{-1}. A total of 141 fungal isolates were obtained, with 56% of these classified as section *Nigri* and 43% as section *Flavi*.

Aspergillus species are the most prominent postharvest contaminants in pistachios. However, Bronte pistachios exhibited greater predominance of *Aspergillus* over other molds, compared to pistachios assessed in other countries. Fernane *et al.* (2010) analyzed pistachios from the Spanish market, and showed a more diverse mycoflora including *Fusarium* and *Penicillium*, with *Penicillium* surpassing prevalence of *Aspergillus*. Although section

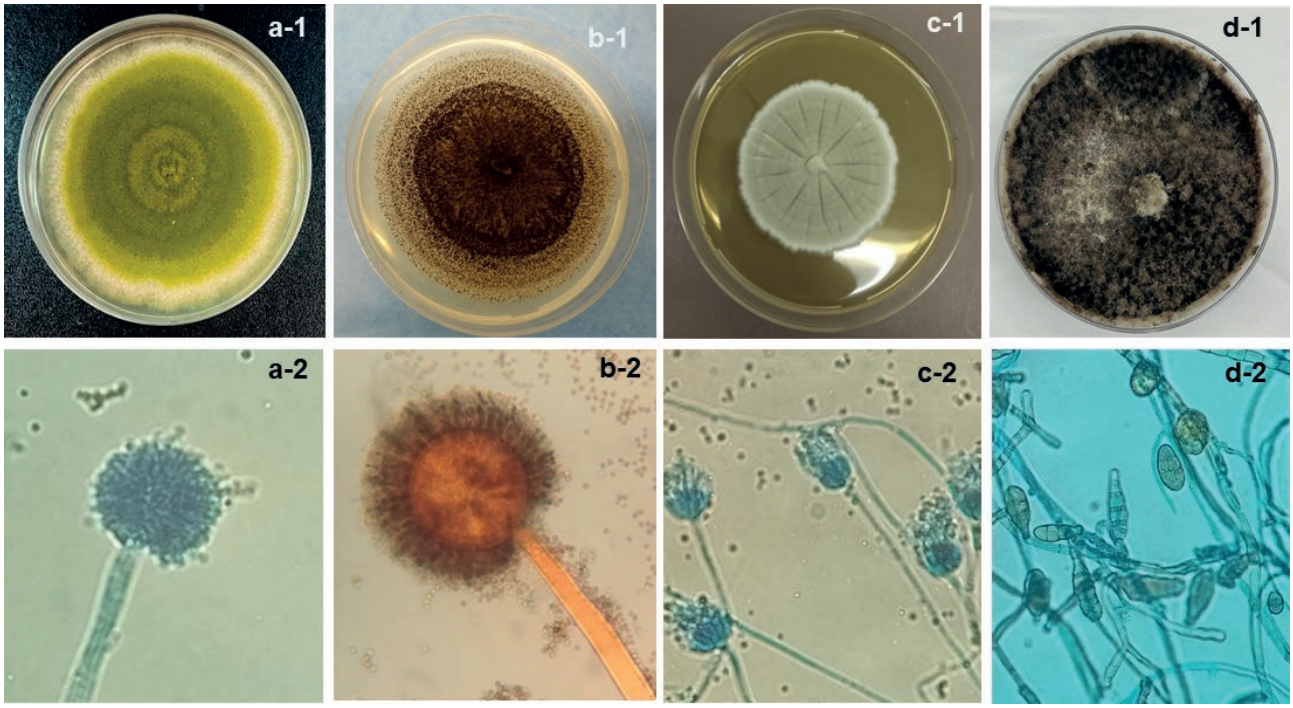

Figure 2. Fungal genera isolated from pistachio samples: a) *Aspergillus* section *Flavi* 1 colony on PDA and 2 conidiophore; b) *Aspergillus* section *Nigri* 1 colony and 2 conidiophore; c) *Penicillium* 1 colony and 2 conidiophore; d) *Alternaria* 1 colony and 2 conidiophore.

Nigri is often the most isolated among *Aspergillus* from pistachios (Doster and Michailides, 1994), greater attention is directed towards section *Flavi*. This is due to the favourable substrate provided by pistachios for the production of Aflatoxins by section *Flavi* species.

The presence of Aflatoxins is often associated with marketing and exportation issues, due to strict regulations. An example is the European ban of Iranian pistachios in 1994, due to their high aflatoxin contents (Bui-Klimke *et al.*, 2014). In Iran, Moghadam *et al.* (2020) showed green *Aspergilli* contamination in pistachio kernels ranged from 1.6×10^3 to 1.6×10^4 cfu g^{-1}. This contamination is much greater than recorded in the present study on Bronte pistachio kernels, where the average contamination was 1.3×10^2 cfu g^{-1}, and on in shell pistachios (4.9×10^2 cfu g^{-1}).

The low fungal contamination in Bronte pistachios could be due to several factors that can affect fungal growth and mycotoxin accumulation in the complex host plant-fungus-environment interactions, e.g., climatic conditions, soil type, agricultural practices, crop variety and characteristics (Kaminiaris *et al.*, 2020). The green pistachios of Bronte grow spontaneously on the volcanic soil. Therefore, agricultural practices, which often allow the introduction and spread of fungi, are practically absent. The cultivar Napoletana is possibly less prone to fungal contamination than other varieties, especially in the susceptible period of the maturity (Panahi and Khezri, 2011). However, these aspects should be verified by testing this cultivar in other Italian pistachio's growing areas.

Identification of isolated Aspergillus *spp.*

A total number of 75 green isolates were analyzed with the LAMP assay specific for *A. flavus*. Among these, 48 isolates were positive, while the remaining isolates were identified as *A. tamarii* through PCR amplification using calmodulin primers and sequencing of resulting products.

Black isolates were grown on MEA-B on which only one isolate, UPB36, sporulated and was identified as *A. carbonarius* (Figure 3a). This was further confirmed by LAMP analysis, which gave a positive result only for isolate UPB36 (Figure 3b). PCR amplifications identified the remaining isolates as *A. tubingensis* and *A. niger* which was the predominant species (74% of section *Nigri* and 47% of all sections).

The most commonly occurring *Aspergillus* species were *A. niger* and *A. flavus* belonging, respectively, to the sections *Nigri* and *Flavi* (Perrone *et al.*, 2007). On pistachios, the distribution of Aspergilli probably differs depending on the growing region and the cultivar. For instance, Khodavaisy *et al.* (2012), examined pistachios from Samandaj, Iran, and found high incidence of *A. flavus* (56.2%) followed by *A. niger* (12.5%) among all detected fungal contaminants. Moghadam *et al.* (2020) also confirmed the dominance of *Aspergillus* section *Flavi* in Iranian pistachios, with its distribution varying depending on the cultivar. However, Rahimi *et al.* (2007), assessed the contamination of pistachios from Kerman, Rafsanjan, and Isfahan, recorded greatest occurrence of *A. niger* (49%) followed by *A. flavus*

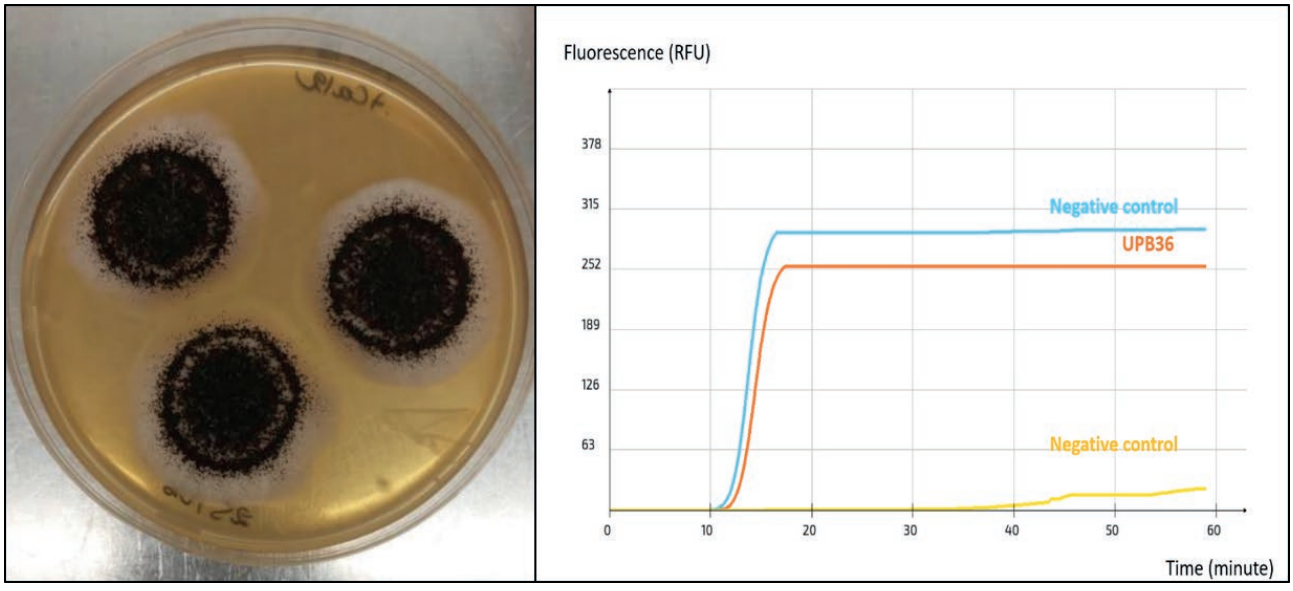

Figure 3. *Aspergillus carbonarius* UPB36 a) colonies on MEA-Boscalid. b) Positive signal with LAMP set ACS02

(33%). The present study also recorded high diversity in aspergilli associated with pistachios, with 11 species identified. This is not the case for Italian pistachios where we have detected dominance of *A. niger* (74%), and low diversity amongst aspergilli with only five species detected. The situation of Italian Bronte pistachios is more comparable to the Californian pistachios on which *A. niger* has been frequently reported as the most common species (Doster and Michailides, 1994; Bayman *et al.*, 2002).

Mycotoxin production

For the green *Aspergillus* isolates, TLC analyses showed that all *A. flavus* isolates produced AFB1 and AFG1, while *A. tamarii* isolates did not produce these mycotoxins. Therefore, HPLC analyses were carried out for all *A. flavus* isolates to confirm and quantify their AFB1 production. Some isolates of *A. tamarii* were also analyzed with HPLC, which confirmed the TLC results. HPLC analyses confirmed that all *A. flavus* isolates produced AFB1 at quantities ranging from 0.1 to 8497.8 ng mL^{-1} of culture filtrate (Table 1).

The only *A. carbonarius* isolate that produced OTA was UPB36, at an amount of 34.2 ng mL^{-1} (Table 2). For *A. niger*, TLC analyses showed that five isolates were potential OTA producers. These isolates were further analyzed with HPLC, and the ability to produce OTA was confirmed, at relatively low levels, for three of them (Table 2).

Aspergillus flavus is the species most commonly associated with aflatoxin production in pistachios. Previous studies have linked *A. flavus* strains with high potential to produce aflatoxins (Hua *et al.*, 2012; Marín *et al.,* 2012). The present study showed that in Bronte pistachios the majority of *A. flavus* isolates have high potential to produce AFB1. Previous studies have detected the presence of OTA and OTA-producing Aspergilli in pistachio samples (Fernane *et al.*, 2010; Singh *et al.*, 2023). In the present study, one *A. carbonarius* isolate produced OTA at 34.2 ng mL^{-1}, and only five *A. niger* isolates were potential OTA producers. Despite their low production, these isolates cannot be dismissed as threats to human health, due to their possession of genes for OTA production, and because this production could be triggered by the occurrence of appropriate growth conditions.

Table 1. AFB1 production in culture by green *Aspergillus* isolates.

Isolate	*Aspergillus* species	Amount (ng mL^{-1}) of AFB1
UP60	*A. flavus*	576.6
UP66	*A. flavus*	414,6
UP67	*A. flavus*	178.7
UP68	*A. flavus*	604.6
UP74	*A. flavus*	253.3
UP85	*A. flavus*	5624.8
UP86	*A. flavus*	246.6
UP87	*A. flavus*	154.0
UP88	*A. flavus*	2223.1
UP89	*A. flavus*	691.3
SP5	*A. flavus*	8497.8
SP6	*A. flavus*	6350.0
Sp7	*A. flavus*	4424.9
SP9	*A. flavus*	4247.6
SP11	*A. flavus*	4612.9
SP12	*A. flavus*	3509.7
SP22	*A. flavus*	879.9
SP27	*A. flavus*	978.6
SP30	*A. flavus*	1147.2
SP31	*A. flavus*	3927.6
SP34	*A. flavus*	1513.2
SP38	*A. flavus*	234.6
SP39	*A. flavus*	50.00
SP40	*A. flavus*	1983.8
SP42	*A. flavus*	683.9
SP44	*A. flavus*	66.0
SP64	*A. flavus*	2651.7
SP91	*A. flavus*	368.0
SP92	*A. flavus*	0.1
SP93	*A. flavus*	0.1
SP95	*A. flavus*	0.1
SP96	*A. flavus*	0.1
SP97	*A. flavus*	0.1
SP99	*A. flavus*	0.1
SP100	*A. flavus*	0.1
SP102	*A. flavus*	0.1
SP104	*A. flavus*	0.1
UP82	*A. flavus*	170.7
SP107	*A. flavus*	77.3
SP108	*A. flavus*	89.3
SP109	*A. flavus*	152.7
SP110	*A. flavus*	214.0
SP119	*A. flavus*	0.1
SP128	*A. flavus*	0.1
SP12(2)	*A. flavus*	6387.4
UP48	*A. flavus*	59.6
UP50	*A. flavus*	63.5
SP53	*A. flavus*	43.5
SP17	*A.tamarii*	0.0
SP80	*A.tamarii*	0.0
SP20	*A.tamarii*	0.0
SP25	*A.tamarii*	0.0

CONCLUSION

This is the first study of *Aspergillus* spp. contamination in green pistachios that have been produced on the

Table 2. OTA production by black *Aspergillus* isolates.

Isolate	Species	Amount (ng/mL) of OTA
UPB16	*A. niger*	0.6
UPB31	*A. niger*	1.0
UPB33	*A. niger*	0.2
UPB36	*A. carbonarius*	34.2
UP51	*A. niger*	0.0
UP55	*A. niger*	0.0

volcanic soils in Bronte, Sicily. This study estimated that *Aspergillus* occurrence in these pistachios is less than for pistachios produced in other countries. This could be related to the type of plantation, soils, climatic conditions, and/or the pistachio variety.

Mycotoxins are secondary metabolites which are produced independently from mycelium growth and sporulation. However, examination of *Aspergillus* spp. occurring on pistachios and their ability to produce mycotoxins can indicate their potential production under favorable conditions. Although the contamination of Bronte pistachios with green *Aspergillus* spp. is low, these contaminants showed high levels of production of AFB1.

These results indicate that investigations dealing with mycotoxigenic fungi developing on pistachio nuts grown in Italy should be expanded. In particular, considering areas with different envirnmental, climatic, and management conditions. It is also important that agricultural and post-harvest practices should be improved to mitigate human health risks related to mycotoxin contamination. This will help to preserve the organoleptic and nutritional qualities of these nut products.

LITERATURE CITED

Arena E., Campisi S., Fallico B., Maccarone E., 2007. Distribution of fatty acids and phytosterols as a criterion to discriminate geographic origin of pistachio seeds. *Food Chemistry* 104: 403-408. https://doi.org/10.1016/j.foodchem.2006.09.029

Bayman P., Baker J.L., Mahoney N.E., 2002. *Aspergillus* on tree nuts: incidence and associations. *Mycopathologia* 155: 161–169. https://doi.org/10.1023/a:1020419226146

Bui-Klimke T.R., Guclu H., Kensler T.W., Yuan J.M., Wu F., 2014. Aflatoxin regulations and global pistachio trade: Insights from social network analysis. *PLoS ONE* 9(3): e92149. https://doi.org/10.1371/journal.pone.0092149

Carlucci A., Raimondo M.L., Cibelli F., Phillips A.J.L., Lops F., 2013. *Pleurostomophora richardsiae*, *Neofusicoccum parvum* and *Phaeoacremonium aleophilum* associated with a decline of olives in Southern Italy. *Phytopathologia Mediterranea* 52(3): 517–527. https://doi.org/10.14601/Phytopathol_Mediterr-13526

D'Evoli L., Lucarini M., Gabrielli P., Aguzzi A., Lombardi-Boccia G., 2015. Nutritional value of Italian pistachios from Bronte (*Pistacia vera* L.), their nutrients, bioactive compounds and antioxidant activity. *Food and Nutrition Sciences* 6(14): 1267. https://doi.org/10.4236/fns.2015.614132

Doster M.A., Michailides T.J., 1994. Aspergillus molds and aflatoxins in pistachio nuts in California postharvest pathology and mycotoxins. *Phytopathology* 84: 583–590. 10.1094/Phyto-84-583

Fernane F., Cano-Sancho G., Sanchis V., Marín S., Ramos A.J., 2010. Aflatoxins and ochratoxin A in pistachios sampled in Spain: occurrence and presence of mycotoxigenic fungi. *Food Additives Contaminants Part B Surveill*. 3(3): 185–192. https://doi.org/10.1080/19440049.2010.497257.

Frisvad J.C., Hubka V., Ezekiel C.N., Hong S.B., Novakova A., and Houbraken J. 2019. Taxonomy of *Aspergillus* section *Flavi* and their production of aflatoxins, ochratoxins and other mycotoxins. *Studies in Mycology* 93: 1–63.

Gusella G., Vitale A., Polizzi G., 2022. Potential role of biocontrol agents for sustainable management of fungal pathogens causing canker and fruit rot of pistachio in Italy. *Pathogens* 11: 829. https://doi.org/10.3390/pathogens11080829

Hua S.S., McAlpin C.E., Chang P.K., Sarreal S.B., 2012. Characterization of aflatoxigenic and non-aflatoxigenic *Aspergillus flavus* isolates from pistachio. *Mycotoxin Research*. 28(1): 67–75. https://doi.org/10.1007/s12550-011-0117-4

IARC 1993. Working Group on the evaluation of carcinogenic risks to humans. Monograph Volume 56: *Some Naturally Occurring Substance: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins*. Lyon, France: IARC Press, World Health Organization.

IARC 2002. Working Group on the evaluation of carcinogenic risks to humans. Monograph Volume 82: *Some Traditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene*. Lyon, France: IARC Press, World Health Organization.

Kaminiaris M.D., Camardo Leggieri M., Tsitsigiannis D.I., Battilani P., 2020. AFLA-PISTACHIO: Development of a mechanistic model to predict the aflatoxin contamination of pistachio nuts. *Toxins* 12(7): 445. https://doi.org/10.3390/toxins12070445

Khodavaisy S., Maleki A., Hossainzade B., Rezai S., Ahmadi, F., and Ghahramani E., 2012. Occurrence of

fungal contamination in pistachio and peanut samples from retail shops in Sanandaj province, Iran. *African Journal of Microbiology Research* 6: 6781–6784. https://doi.org/10.5897/AJMR12.722

Mandalari G., Barreca D., Gervasi T., Roussell M.A., Klein B., Feeney M.J., Carughi A., 2022. Pistachio nuts (*Pistacia vera* L.): Production, Nutrients, Bioactives and Novel Health Effects. *Plants* 11(1): 18. https://doi.org/10.3390/plants11010018

Marín S., Ramos A.J., Sanchis V., 2012. Modelling Aspergillus flavus growth and aflatoxins production in pistachio nuts. *Food Microbiology* 32(2): 378–388, https://doi.org/10.1016/j.fm.2012.07.018

Marino G., Marra F.P., 2019. Horticultural management of Italian pistachio orchard systems: current limitations and future prospective. *Italus Hortus* 26 (3): 14–26. https://doi.org/10.26353/j.itahort/2019.2.1426

Mellikeche W., Ricelli A., Casini G., Gallo M., Colelli G., D'Onghia A.M. 2024. Development of Loop-Mediated Isothermal Amplification (LAMP) Assays for the Rapid Detection of Toxigenic Aspergillus flavus and A. carbonarius in Nuts. *International Journal of Molecular Sciences* 25(7): 3809. https://doi.org/10.3390/ijms25073809

Moghadam M.M., Rezaee S., Mohammadi A.H., Zamanizadeh H.R., Moradi M., 2020. A Survey on contamination of Iranian pistachio cultivars to *Aspergillus* section Flavi and Aflatoxin. *Journal of Nuts* 11(1): 13–22. https://doi.org/10.22034/jon.2020.1882572.1072

O'Donnell K., Kirenberg H.I., Cigelnik E., 2000. A multigene phylogeny of the Gibberella fujkuroi species complex detection of additional phylogenetically distinct species. *Mycoscience* 41: 61–78. https://doi.org/10.1007/BF02464387

Panahi B., Khezri M., 2011. Effect of harvesting time on nut quality of pistachio (*Pistacia vera* L.) cultivars. *Scientia Horticulturae* 129: 730–734. https://doi.org/10.1016/j.scienta.2011.05.029

Perrone G., Susca A., Cozzi G., Ehrlich K., Varga J., and Samson R.A., 2007**.** Biodiversity of *Aspergillus* species in some important agricultural products**.** *Studies in Mycology* 59(1): 53–66 https://doi.org/10.3114/sim.2007.59.07

Pitt, J.I., Hocking, A.D. 1997. Methods for isolation, enumeration and identification. In: *Fungi and Food Spoilage*. Springer, Boston, MA, USA. https://doi.org/10.1007/978-1-4615-6391-4_4

Rahimi P., Sharifnabi B., Bahar M., 2007. Detection of Aflatoxin in Aspergillus Species Isolated from Pistachio in Iran. *Journal of Phytopathology* 156(1): 15–20. https://10.1111/j.1439-0434.2007.01312.x

Samson R., Noonim P., Meijer M., Houbraken J., Frisvad J., Varga, J., 2007. Diagnostic tools to identify black aspergilli. *Studies in Mycology* 59: 129–145. https://doi.org/10.3114/sim.2007.59.13

Sheikhi A., Mirdehghan S.H., Ferguson L., 2019. Extending storage potential of de-hulled fresh pistachios in passive-modified atmosphere. *Journal of the Science of Food and Agriculture* 99(7): 3426–3433. https://doi.org/10.1002/jsfa.9560

Singh P., Jaime R., Puckett R.D. Lake J., Papangelis A., and Michailides T., 2023. Ochratoxin A contamination of California pistachios and identification of causal agents. *Plant Disease* https://doi.org/10.1094/PDIS-06-23-1233-RE

Soares Mateus A.R., Barros S., Pena A., Sanches Silva A. 2021. Mycotoxins in Pistachios (*Pistacia vera* L.): Methods for Determination, Occurrence, Decontamination. *Toxins* 13(10): 682. https://doi.org/10.3390/toxins13100682

Solfrizzo M., Avantaggiato G., Visconti A., 1998. Use of various clean-up procedures for the analysis of ochratoxin A in cereals. *Journal of Chromatography* 815: 67–73. https://doi.org/10.1016/s0021-9673(98)00271-4

Tomaino A., Martorana M., Arcoraci T., Monteleone D., Giovinazzo C., Saija A., 2010. Antioxidant activity and phenolic profile of pistachio (*Pistacia vera* L., variety Bronte) seeds and skins. *Biochimie* 92(9): 1115–1122. https://doi.org/10.1016/j.biochi.2010.03.027

Visagie C.M., Varga J., Houbraken J., Meijer M., Kocsub S., and Samson R.A. 2014. Ochratoxin production and taxonomy of the yellow aspergilli (Aspergillus section Circumdati). *Studies in Mycology* 78: 1–61. http://dx.doi.org/10.1016/j.simyco.2014.07.001

Wilson J.S., Petino G., Knudsen D.C., 2018. Geographic context of the green pistachio of Bronte, a protected designation of origin product. *Journal of Maps* 14(2): 144–150. https://doi.org/10.1080/17445647.2018.1438318