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# Ochratoxigenic fungi and Ochratoxin A determination in dried grapes marketed in Tunisia

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## Abstract

**Purpose:** With the present work, we aimed to assess the occurrence of ochratoxigenic fungi and Ochratoxin A (OTA) in dried grapes from Tunisia.

**Methods:** Dried grapes samples ( $n = 90$ ) were investigated for the presence of ochratoxigenic fungi, which were further characterized at the species level through amplification of the internal transcribed spacer (ITS) region and polymerase chain reaction (PCR) product sequencing. Fungal isolates were tested for their ochratoxigenic potential by high-performance liquid chromatography with fluorescence detection (HPLC-FLD), as well as dried grapes samples after an immunoaffinity column (IAC) clean-up procedure.

**Results:** Black *Aspergilli* isolates were the dominant genre among the filamentous fungi found in dried grapes samples and were the only OTA-producing fungi encountered. *Aspergillus niger* aggregate were the most frequently found isolates reaching 70%, 80%, and 85% in dried grapes samples from regions of Kelibia, Sfax, and Rafraf, respectively, while covered 100% of the relevant mycobiota found in imported samples. *Aspergillus carbonarius* isolates were found only in Sfax's and Kelibia's samples, while uniseriate *Aspergilli* were found between 7 and 20% in dried grapes from Kelibia, Sfax, and the imported samples. The in vitro OTA production test showed that 88.9% of OTA-producing isolates belonged to *A. carbonarius* with OTA levels varying from 0.06 to 1.32  $\mu\text{g/g}$  of Czapek Yeast Agar (CYA). The remaining OTA-producing fungi (11.1 %) belonged to *A. niger* aggregate group having a maximum OTA potential of 2.88  $\mu\text{g/g}$  CYA, and no uniseriate *Aspergilli* isolate was able to produce OTA. All dried grapes samples were free of OTA presence.

**Conclusion:** According to the present study's findings, no OTA contamination was recorded in the investigated samples from Tunisian market. Nevertheless, the presence of strong OTA producers *A. carbonarius* in samples originated from the two out of three studied Tunisian regions, as well the high incidences of *Aspergillus niger* aggregate group with an attested potential for OTA production in all samples, necessitates further research on Tunisian dried grapes. Additionally, a continuous analysis of staple food of the Mediterranean diet is imperative to insure the best quality for the consumers and prevent potential health problems.

**Keywords:** Toxigenic fungi, *Aspergillus carbonarius*, *Aspergillus* spp., Molecular identification, Ochratoxin A, Dried grapes, HPLC-FLD

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## Introduction

*Vitis vinifera* L. (Vitaceae) is a species of *Vitis*, native to the Mediterranean region (Fernandes et al. 2013). Its production is widespread worldwide for fresh consumption and industrial processing of grapes. In 2014, world production of grapes exceeded 74 million tons (FAOSTAT 2014), of which more than 1.5 million tons correspond to dried grapes, having an increase of 10% compared with 2010 data (FAO-OIV 2016). Grape is a very sensitive fruit to various pathogens such as bacteria and molds. Indeed, the effects of fungal growth in grapes lead to numerous effects such as the production of metabolites that may affect the organoleptic properties of grapes and derived products, but also the production of toxic compounds namely Ochratoxin A (OTA) (Dachery et al. 2019), which may be produced in vineyards by several fungal species such as *Penicillium verrucosum* in temperate climates and *Aspergillus ochraceus* and *Aspergillus carbonarius* in tropical and warm ones (Amézqueta et al. 2012). Such fungi can contaminate crops prior to harvest and more commonly during storage (EFSA 2006) and lead therefore to the contamination of dried grapes, grape juices, and several types of wine (Welke 2019).

After several toxicological studies, OTA have shown various health implications to human and animals' health that make it to be considered as one of the most important mycotoxins with a worldwide concern. OTA was implicated in renal toxicity, mutagenicity, genotoxicity, teratogenicity, immunotoxicity, and possibly neurotoxicity (JECFA 2001; Pfohl-Leszkowicz and Manderville 2007). OTA has also been classified as a possible human carcinogen (group 2B) by the International Agency for Research on Cancer (IARC 1993). The Food and Agricultural Organization, the World Health Organization, and the Joint Expert Committee on Food Additives established a provisional OTA tolerable daily intake of 14 ng/kg body weight (JECFA 1995). Several countries have established regulations on OTA occurrence in food, and the European Community established maximum permitted OTA levels are 10 and 2 ng/g for dried vine fruits and for grape juices, respectively (European Commission 2006).

Another related to OTA emerging risk, but still under investigation by the scientific community, is the transformation of parent mycotoxin to several modified forms, defined as "modified mycotoxins" due to physical, chemical, or biological phenomena taking place on the field, during processing, and storage of products (Freire and Sant'Ana 2018; Welke 2019). Modified mycotoxins could either perform toxic effects when consumed or even converted again into the parent mycotoxin in the human digestive system (Berthiller et al. 2013).

The Tunisian population consumes high amounts of dried fruits directly, mixed to special salted or sweet dishes such as couscous (a semolina main typical dish

from Northern Africa) which are very appreciated by consumers and especially children. It is important to note that this consumption becomes very high in special celebrations such as Ramadan month and weddings.

OTA and its modified forms could potentially contaminate grapes and derived products like raisins all over the production cycle and especially at a post-harvest stage—in cases of processing under conditions promoting the growth of ochratoxigenic fungi (Freire and Sant'Ana 2018; Merlela et al. 2015; Palumbo et al. 2015).

Several studies have investigated the contribution of dried vine fruits (raisins, sultanas, and currants) to the total dietary exposure to OTA (Akdeniz et al. 2013; Hajok et al. 2019; Galvis-Sanchez et al. 2008; Lombaert et al. 2004; Miraglia and Brera 2002; Wei et al. 2018; Yurdakul et al. 2019). Numerous surveys have been carried out in Mediterranean countries on the occurrence of ochratoxigenic mycoflora in grapes from setting and/or veraison to harvest time (Abrunhosa et al. 2001; Battilani et al. 2003, 2004, 2006a, b; Bau et al. 2005; Bejaoui 2005; Belli et al. 2006; Covarelli et al. 2012; Lasram et al. 2007, 2013; Oueslati et al. 2010; Serra, et al. 2003; Stefanaki et al. 2003). However, very scarce data from surveys are available on the contamination with ochratoxigenic fungi and the OTA levels in dried grapes marketed in Tunisia up to now (Azaiez et al. 2015). The aim of this work was to determine the occurrence of ochratoxigenic fungi and OTA in dried grapes and assess the OTA contamination risk in this type of products.

## Material and methods

### Dried grapes sampling

Samples of dried grape ( $n = 90$ ) were purchased from local markets of three traditional grape-producing regions in Tunisia during the production period of August 2017. Thirty samples of local dried grapes were purchased from each one. The three regions were Sfax, Rafraf, and Kelibia located in the South, in the North, and in the North East of Tunisia, respectively, and are characterized by different climatic conditions. The most common traditional grape dehydration procedure relates pre-treatment and drying in the sun as follows: fresh grapes are washed with water and then soaked 15 s in 5% NaOH solution at 95 °C, drained and rinsed again with cool water, soaked in an emulsion of 7%  $K_2CO_3$  and 0.4% olive oil for 2–3 min, drained again, and distributed on drying racks in a single layer. Finally, sulfur fumigation (4 g/kg of grapes) was performed for 10 h before a direct sun drying process.

### Mycobiota isolation and molecular identification of ochratoxigenic fungi

Dried grapes were randomly chosen from each sample and were surface-sanitized with 8% sodium hypochlorite,

followed by 10% alcoholic solution (Covarelli et al. 2012). Grapes were rinsed thoroughly with sterilized distilled water and dried aseptically on sterilized filter paper. Five grapes per sample were placed with triplicate repetitions on Petri dishes containing Malt Extract Agar medium (MEA) supplemented with chloramphenicol (100 mg/L). Plates were incubated at 25 °C for 7 days. Colonies of potential OTA-producing fungi (*Aspergillus* and *Penicillium* spp.) were classified by genera according to the appropriate keys for the identification of Pitt and Hocking (2009). Purified and single-spore isolates of *Aspergillus* and *Penicillium* spp. were inoculated on Czapek Yeast Agar medium (CYA) and incubated at 25 °C for 7 days in order to evaluate their OTA production in vitro. Black *Aspergilli* were identified at species level microscopically, based on the spores and conidial heads morphology using appropriate identification keys as described by Abarca et al. (2004). Molecular identification of fungi was done after DNA extraction from fresh mycelia as described by Lecellier and Silar (1994). DNA was purified with classical phenol-chloroform mixture, followed by ethanol precipitation. DNA pellet was resuspended in 100 µL of sterile water before further PCR and sequencing steps.

#### PCR and sequencing analysis

The amplification of the ITS region was performed using ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primers as described by Lasram et al. (2013). PCR products were directly sequenced in both directions with the ITS primers. The resulting DNA sequences were analyzed using CLUSTAL X 2.1. Isolates identification was determined based on the best score obtained by the comparison of the obtained DNA sequences with those available in the data of the National Center for Biotechnology and Information (NCBI) (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

#### OTA production

Pure and single-spore cultured strains were tested for their OTA production on CYA medium according to Bragulat et al. (2001). Briefly, plates were inoculated at three different points and incubated for 7 days at 25 °C. From each plate, three agar plugs ( $\varnothing = 6$  mm) were removed, placed into an amber vial, and extracted with 1 mL of analytical grade methanol for 1 h. Then, the extract was filtered through a membrane filter (MillexR SLHV 013NK; Millipore, USA) before chromatographic analysis.

#### OTA quantification in dried grapes samples

OTA content was quantified in the analyzed samples according to Stroka et al. (2000) with slight modifications. All chemicals used for the analysis were HPLC grade,

and briefly, 10 g of dried grapes were blended with 40 mL of methanol-water (80:20, v/v) for 3 min. Extracts were filtrated on Whatman filter paper No. 4. An aliquot of 10 mL was diluted with 60 mL of PBS buffer (pH 7.4). OTA clean-up was performed using an immunoaffinity column (IAC OchraStar™, Romer Labs Diagnostic GmbH, Austria). The column was conditioned with 10 mL of PBS at a flow rate of 5 mL/min. Then, the diluted filtrate was applied to the IAC column with a rate of 1–2 drops per second. The column was washed with 20 mL of water and then dried with gentle air stream. OTA was eluted using 2 mL of methanol into a glass amber vial. The eluent was evaporated to dryness under a gentle stream of nitrogen and reconstituted with 500 µL of the mobile phase before HPLC analysis.

#### HPLC determination

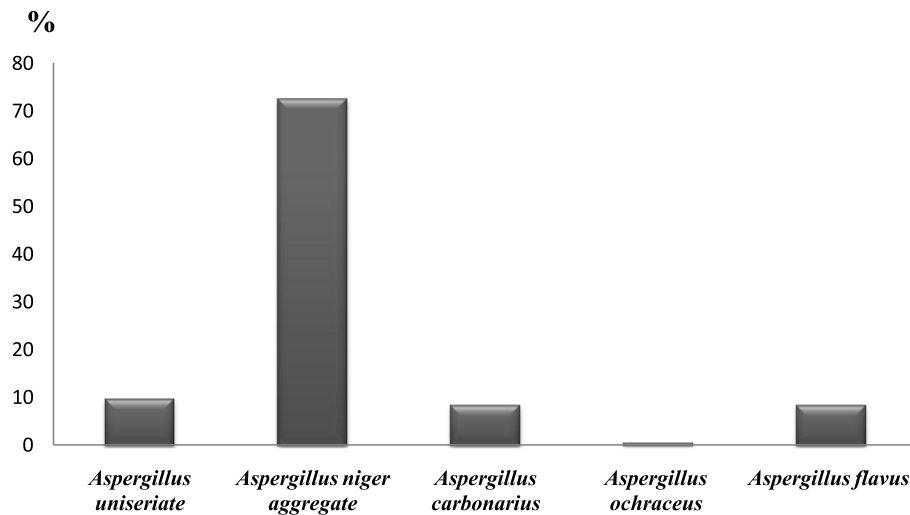
HPLC analysis was performed using a Smartline liquid chromatography system (Knauer, Germany) equipped with an online vacuum degasser (Smartline 5000), an auto-sampler (Smartline 3900), a quaternary pump (Smartline 1000), and Shimadzu RF-10AXL fluorescence detector (IET, USA). The analytical column was a Euro-spher RP C18 (5 µm, ODS2, 4.6 × 150 mm, Milford, MA, USA) with a guard column packed with the same phase. OTA detection was carried out using 330 nm and 460 nm as fluorescence excitation and emission wavelengths, respectively. ClarityChrom chromatography station software (Knauer, Germany) was used to control the system and the signal process. Quantification of OTA was performed by measuring its peak area with the help of a calibration curve calculated from standard solutions. The mobile phase was a mixture of acetonitrile to water to acetic acid, (57:41:2, v/v/v). The OTA retention time was ca. 4 min. Detection and quantification limits (LOD and LOQ) for the analysis of ochratoxigenic potential of isolated species were 0.02 and 0.04 µg OTA/g of CYA medium with a signal-to-noise ratio of 3:1, while for dried grapes samples were 0.03 µg/L and 0.05 µg/L, with a signal-to-noise ratio of 10:1. The OTA recovery was  $89 \pm 4\%$  (mean  $\pm$  SD,  $n = 3$ ).

#### Statistical analysis

The distribution of the ochratoxigenic isolates found was statistically analyzed with SAS software (version 8.02, SAS Institute, Inc., Cary, NC, USA) to assess the effect of the sample origin by analysis of variance followed by LSMEAN. Statistical significance was judged at both  $p < 0.05$  and  $p < 0.0001$ .

#### Results

The occurrence of the identified fungi from dried grapes samples is presented in Fig. 1 and showed the presence of *Aspergillus niger* aggregate (73%), *Aspergillus carbonarius*



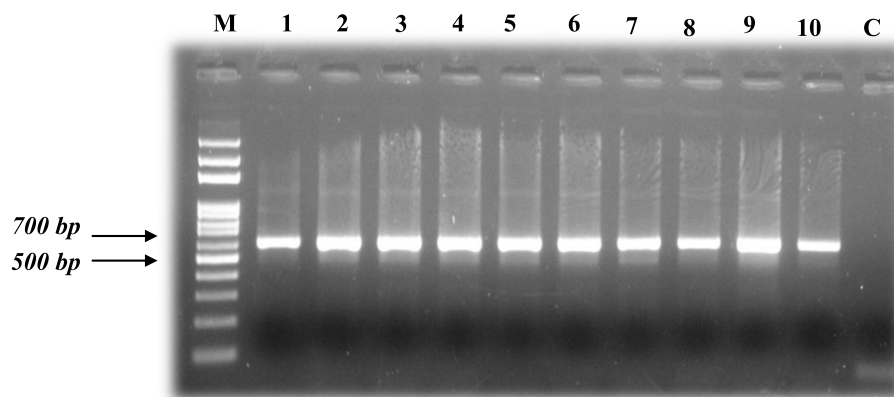
**Fig. 1.** Occurrence of *Aspergillus* species isolated from dried grapes samples marketed in Tunisia (n=90)

(10%), *Aspergillus japonicus* (10%), *Aspergillus flavus* (8%), and *Aspergillus ochraceus* (1%). *Aspergillus niger* aggregate were the most frequently found isolates reaching 70%, 80%, and 85% in dried grapes samples from the regions of Kelibia, Sfax, and Rafraf, respectively, while covered 100% of the relevant mycobiota found in imported dried grapes samples. *Aspergillus carbonarius* isolates were found only in Sfax's and Kelibia's samples, while the uniseriate Aspergilli were found between 7 and 20% in dried grapes from Kelibia, Sfax, and the imported samples. Furthermore, *Aspergillus niger* distribution in the Sfax and Kelibia regions was significantly higher than in the imported and Rafraf samples. Similarly, *Aspergillus japonicus* distribution was significantly higher in the Sfax and Kélibia regions. However, both *Aspergillus carbonarius* and *Aspergillus ochraceus* distributions were not affected significantly by the sample origin (Table 1). Identification procedure also revealed the presence of the genera *Penicillium*, *Alternaria*,

*Botrytis*, and *Rhizopus*. Among the potential OTA-producing fungi, only black Aspergilli represented by *Aspergillus* section Nigri group and *Aspergillus ochraceus* were isolated from the analyzed samples, while no isolates of *Penicillium verrucosum* were identified.

PCR amplification and DNA sequencing were used to confirm the macroscopic and microscopic identification of the observed genera. The electrophoresis profile (Fig. 2) and DNA sequencing resulted in eight strains of *Aspergillus carbonarius*, one strain of *Aspergillus niger*, and one strain of *Aspergillus ochraceus*, with high scores reaching 100% and permitting the confirmation of microscopic identification made previously.

A total of 139 black Aspergilli isolates were tested for their OTA production on CYA. Chromatograms of an OTA-positive isolate and OTA standard are presented in Fig. 3. The OTA quantification revealed that only nine isolates were able to produce OTA. Eight out of



**Fig. 2.** PCR amplification of ochratoxigenic isolates in dried grapes samples. Lanes 1 to 6, 8 and 9: *A. carbonarius*; Lane 7: *Aspergillus niger*; Lane 10: *Aspergillus ochraceus*; M: 1 Kb DNA Ladder; C: negative control

**Table 1** Effect of the sample origin on the mean distribution of the different ochratoxigenic species

Isolate	Region				<i>p</i> > F	SEM
	Sfax	Kelibia	Rafraf	Imported		
<i>Aspergillus niger</i>	3.533 <sup>a</sup>	2.2667 <sup>a</sup>	0.8667 <sup>b</sup>	0.6667 <sup>b</sup>	**	0.457
<i>Aspergillus carbonarius</i>	0.4667	0.4	0	0	NS	0.159
<i>Aspergillus japonicus</i>	0.2667 <sup>a</sup>	0.6667 <sup>a</sup>	0 <sup>b</sup>	0.0666 <sup>b</sup>	***	0.1768
<i>Aspergillus ochraceus</i>	0	0	0	0.06667	NS	0.0333

NS not significant

\*Different letters in superscript represent statistically significant differences between regions

\*\**p* < 0.0001, \*\*\**p* < 0.05

thirteen identified *A. carbonarius* isolates (88.88%) and only one from 111 *A. niger* aggregate isolates (11.11%) were confirmed for OTA production (Table 2). No OTA production was detected for the uniseriate *Aspergillus japonicus* and the *Aspergillus ochraceus* isolates. The OTA production by *Aspergillus carbonarius* isolates varied between 0.09 and 1.32 µg/g CYA. However, the OTA production by the *Aspergillus niger* isolate was higher and reached 2.88 µg/g CYA.

Despite the isolation of highly OTA-producing strains, all the analyzed dried grapes samples have not shown quantifiable OTA concentrations after their analysis with IAC for clean-up and HPLC-FLD for quantification.

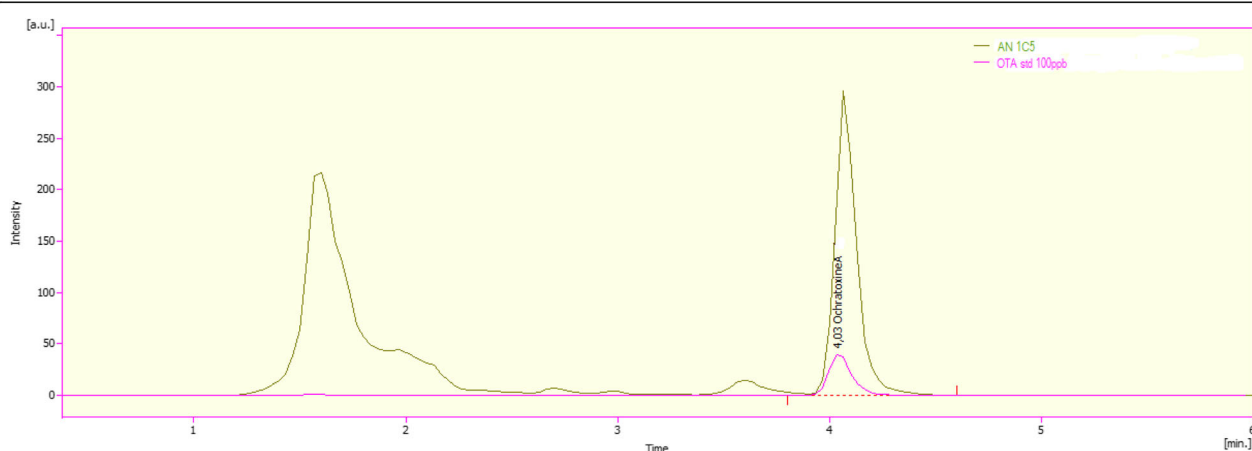
## Discussion

In warmer climate countries, such as Tunisia, *Alternaria*, *Cladosporium*, *Botrytis*, and *Rhizopus* spp. are the most abundant fungi at the beginning of grapes ripening, while the genres *Aspergillus* and *Penicillium* are the most common fungi contaminating grapes during the late stages of ripening, harvest, and grapes' solar drying, with the predominance of *Aspergillus* species which are capable of mycotoxin production like OTA (Abarca et al. 2003; Belli et al. 2005; García-Cela et al. 2015; Oliveri and Catara 2011; Valero et al. 2005). During this post-harvest treatment, grapes are dried until their sugar

level is extremely high, but at the same time, this process can produce adverse effects in the fruit mycoflora like an increase of fungal development and OTA production (Bau et al. 2005; Merlela et al., 2015; Palumbo et al. 2015; Pardo et al. 2005).

Several studies conducted in southern European countries had shown that other ochratoxigenic spp. like *Aspergillus ochraceus* was detected at very low incidences while *Penicillium verrucosum* was not isolated from grapes grown in warm climates (Pitt et al. 2000; Covarelli et al. 2012). Therefore, the fungal population's diversity depended on several factors such as the grapes variety, maturity stage, climatic conditions, and agricultural practices, but OTA in warm climates was linked only with black Aspergilli (Battilani et al. 2004; Cabanés and Bragulat 2018; Esteban et al. 2004; Lopez de Cerain et al. 2002; Mitchell et al. 2004; Oliveri and Catara, 2011; Serratos et al. 2010).

As it is shown in Fig. 1, black Aspergilli were the most abundant mycobiota in our study and relevant studies have shown these isolates are very resistant to sunlight exposure and to hot and dry environments (Akdeniz et al. 2013; Serra et al. 2003). Indeed, grapes are subjected to a drying process until they acquire the desired sugar content, and *a<sub>w</sub>* decrease quietly from 0.95 to 0.75. Within these conditions, only a few microorganisms

**Fig. 3.** HPLC chromatograms of one ochratoxigenic isolate (1C5) and OTA standard (100 ppb)



**Table 2** OTA potential after 7 days incubation at 25 °C on CYA of dried grapes' isolates

Isolates	OTA (µg/g CYA)
<i>Aspergillus carbonarius</i> 1M3	0.098
<i>Aspergillus carbonarius</i> 1M4	0.060
<i>Aspergillus carbonarius</i> 1E8	1.326
<i>Aspergillus carbonarius</i> 1E3	0.509
<i>Aspergillus carbonarius</i> 1O3	0.136
<i>Aspergillus carbonarius</i> 1L1	0.472
<i>Aspergillus niger</i> 1C5	2.887
<i>Aspergillus carbonarius</i> 2L6	0.167
<i>Aspergillus carbonarius</i> 2I2	0.454

such as black *Aspergilli* are still able to develop (Hocking et al. 2007). Even if in the case of solar dehydration, the direct sunrays reduce the viability of *A. carbonarius* spores (Leong et al. 2006); in general, black *Aspergilli* are less susceptible than other species to the germicidal UV rays and the strong sunlight heating due to their thick, heavily melanized walled pigmented spores (García-Cela et al. 2016). In comparison with the other species, the dried grape substrate provides a competitive advantage for black *Aspergilli* (Pitt and Hocking 2009).

Moreover, it is noticed that in Tunisia, a caustic soaking with  $K_2CO_3$  is implemented as a bleaching solution to accelerate the drying process by making the grape skin weaker (Covarelli et al. 2012; Simate and Ahrné 2005). This procedure may enhance the fungal development especially the OTA-producing isolates. In addition, the use of  $SO_2$  as an antioxidant to fresh grapes can also be the origin of the variability in the level of contamination since it is showing an antifungal activity (Dilip 2003). These pretreatments aim to control fungal growth and their potential OTA production.

Regarding the fact that OTA production by the *Aspergillus niger* isolate was higher of *A. carbonarius* isolates, culture substrate used for the fungal growth may play an important role in the enhancement of OTA production. In fact, it was reported by Esteban et al. (2004) that mean OTA concentration produced by the *A. niger* aggregate and *A. carbonarius* isolates on YES and CYA medium was different.

Lasram et al. (2007) reported that *Aspergillus carbonarius* was the stronger OTA-producing fungi isolated from Tunisian grapes with a production level reaching 10 µg/g CYA, while several studies reported also that *A. carbonarius* was the most important source of OTA in dried grapes (Covarelli et al. 2012; Somma et al. 2012; Merlera et al. 2015).

It may be concluded that the occurrence of OTA producers in some dried grapes samples may occur regardless of the grapes varieties or the resveratrol content

which may decrease the OTA concentration (De Rossi et al. 2012). However, environmental conditions (mainly temperature and Rh) in the vineyards offer the most important stimulations to the fungal contamination and their mycotoxin production (Oueslati et al. 2010; Zhang et al. 2014). In addition, in a Tunisian survey on table and wine grapes, Lasram and co-workers (2012) found out that most contaminated grapes, presenting also considerable levels of OTA (up to 5.85 µg/L), were those originating from Rafraf region which is characterized by a humid climate in contrast with those from the Regueb region, characterized by an arid climate, where furthermore *A. carbonarius* was rarely isolated. Also, mycobiota interactions may be a cause for the OTA production, as for example, the presence of *Eurotium amstelodami* can help *Aspergillus niger* to colonize grapes, through its enzymatic activity which offers to *Aspergillus* isolates nutrients release and entrance ability to grapes (Valero et al. 2007), while filamentous fungi of grapevine may trigger either stimulation or decrease in OTA production by *A. carbonarius* depending to the species (Kogkaki et al. 2015). In contrast, other interactions, as for example with yeasts or bacteria, may cause an inhibition or a decrease in OTA production (Kapetanakou et al. 2012).

The absence of quantifiable concentrations of OTA in the tested dried grapes may demonstrate that the toxin-producing fungi may exist in a sample but no Ochratoxin A is synthesized within as it depends on several environmental factors, mainly temperature, rainfall, and humidity. Nevertheless, correlation between the incidence of OTA-producing strains and OTA contamination is frequently reported in relevant studies (Somma et al. 2012; Lasram et al. 2012). Regarding reports on dried grapes, Zinedine et al. (2007) detected OTA levels in samples from Morocco varying between 0.05 and 4.95 ng/g; Zhang et al. (2014) reported a high number of contaminated dried grapes from the Chinese market, with almost 60% of tested samples being positive, and OTA range between 0.07 and 12.83 µg/kg. Palumbo et al. (2015) found that 54% of conventionally cultured and 34% of organic cultivation raisins from the US markets were contaminated with OTA in ranges between 0.34 and 15.34 ng/g. In another recent study of Yurdakul et al. (2019) on Turkish dried grapes, none of the samples out of 17 tested presented detectable OTA concentration, although in previous surveys, for Turkey raisins, small incidence (8%) and mean concentration (1.15 µg/Kg) were reported (Akdeniz et al. 2013) and Sultanas were found highly contaminated (Aksoy et al. 2007; Meyvacı et al. 2005).

Drying process and pretreatments are also important factors influencing the OTA contamination of grapes (Somma et al. 2012; Valero et al. 2008; Zhang et al. 2014) but also its modified forms (Freire et al. 2018a). For example, Rafraf is a Tunisian region that is known

for using a soaking solution containing CaO, ash, and *Pistacia lentiscus* branches, which has an astringent oleoresin having therapeutic role, within fumigation processes (Harbi Ben Slimen 2005). Chemical pretreatments are also used to help drying procedure such as  $K_2CO_3$  solution. These pretreatments may cause the OTA decrease or inhibition of its production by the contaminating fungi (Valero et al. 2005). In fact, grapes composition will be modified quickly resulting in reduced nutrients not only for *Aspergillus* spp., but also for antagonistic mycoflora (Kogkaki et al. 2015), which may use the previously produced OTA as a source of carbon and energy for its metabolism, but also may transform the toxic OTA into modified mycotoxins (Bragulat et al. 2017; Freire et al. 2018b).

## Conclusion

We currently identified the potential ochratoxigenic fungi and studied the OTA contamination in dried grapes samples marketed in Tunisia. *Aspergillus* is the most frequent fungi genre contaminating the studied samples. *Aspergillus* section *Nigri* group represented by *Aspergillus niger* aggregate, *Aspergillus carbonarius*, and *Aspergillus japonicus* was frequently found. Among all isolates, only 6.47% were OTA producers and the only *Aspergillus ochraceus* strain found was not ochratoxigenic. All the analyzed samples were free of OTA presence, although they included OTA-producing strains within their mycoflora.

Nevertheless, if we account also the fact that a part of the produced OTA could undergo a transformation to its modified forms, normally remaining undetected during the testing for parent mycotoxin (Freire and Sant'Ana 2018), and acknowledging that their potential production could be triggered in dried grapes either by chemical treatments (soaking solution), as well from biological (antagonist microorganisms) or even environmental (traditional dehydration, origin, and type of grape) conditions, a more rigorous investigation is necessary to ensure to the level of safety needed (Freire et al. 2017, 2018a, 2018b, 2019).

As there is not enough information on OTA occurrence in dried grapes from Tunisia, further investigations should be carried out to further evaluate the impact of climatic conditions of the production regions, the effect of different chemical treatments, and the drying process of grapes on the ochratoxigenic fungi contamination and their OTA production potential. In addition, in order to guarantee food product safety, inspections should be legislated not only for those mycotoxins linked with a certain product but also for other or modified mycotoxins (Freire and Sant'Ana, 2018; Welke et al. 2019). It also remains important to conduct more toxicological studies to elucidate not only OTA but also its modified form intake from consumption of dried grape products and to ensure consumer safety.

## Authors' contributions

SC and HBI designed the study, AB-A and WR-B designed the molecular biology work, SO and WR-B have conducted the laboratory work and drafted the article, PN helped collating the experimental data and assessed the presented data, all authors helped editing the research paper. The author(s) read and approved the final manuscript.

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## Ethics approval and consent to participate

N/A

## Consent for publication

Informed consent was obtained from all individual participants included in the study.

## Competing interests

The authors declare that they have no conflict of interest.

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