

“Black Aspergilli” in vineyards of conventional and organic farming: Investigating the population structure and mycotoxigenic capacity of *Aspergillus* species section *Nigri*.

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Aim:

Investigation of the population structure and determination of the *in vitro* mycotoxigenic capacity of black *Aspergilli* species collected from conventional and organic vineyards.

Introduction:

Grapevines considered to be one of most important crops worldwide as well as in Greece. Grape's Black rot disease caused by several mycotoxigenic species of Black *Aspergilli* section *Nigri*, is one of the most important pre- and post-harvest diseases of grapevines, while contaminated grape products and derivatives with *Aspergillus* mycotoxins (OTA, FB₂) may have an important impact on consumers health.

Materials & Methods:

- Asymptomatic grape berries were collected for 2 consecutive years (2018 & 2019) from organic and conventional vineyards located in different regions all over Greece.
- Fungal isolation was performed in petri dishes amended with a semi-selective medium DRBAC (Dichloran Rose Bengal Agar Chloramphenicol)
- In total, 300 isolates of *Aspergillus* spp. were collected and identified by amplicon sequencing of 3 reference genes (*ITS*, *β-tubulin*, *calmodulin*).
- The *in vitro* production of mycotoxins was evaluated in YESA (Yeast extract Sucrose Agar) growth medium, after cultures incubation for 7 and 15 days.
- Overall, *in vitro* mycotoxin capacity of 110 *Aspergillus* isolates originating from organic (n=55) and conventional (n=55) vineyards, was tested.
- The liquid chromatographic analysis was performed on a ExionLC AC system consisting of an in-line degasser, two LC pumps, an autosampler and a column oven associated with a X500R QTOF mass spectrometer (AB Sciex, Framingham, MA, USA).

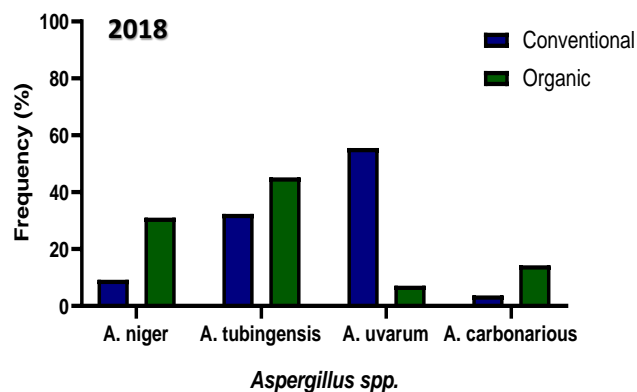


Figure 2. Frequency of *Aspergillus* species on grape berries originating from conventional and organic vineyards (2018 sampling).

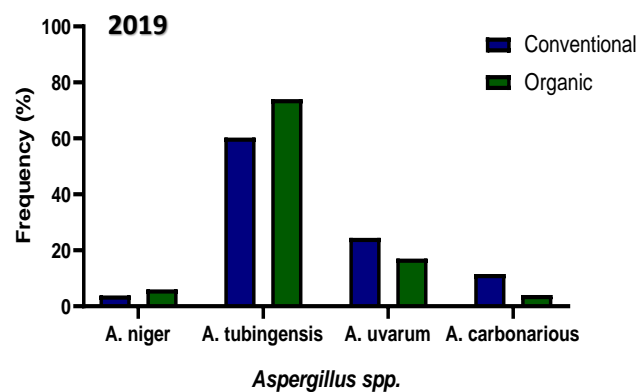


Figure 3. Frequency of *Aspergillus* species on grape berries originating from conventional and organic vineyards (2019 sampling).

Table 1. Minimum and maximum *in vitro* production of OTA and FB₂ from *Aspergillus* species.

Aspergillus species	Concentration (µg/gr)	
	OTA	FB ₂
<i>A. niger</i>	0.01 to 0.3	20 to 60
<i>A. carbonarius</i>	0.1 to 20	nd
<i>A. tubingensis</i>	nd	nd
<i>A. uvarum</i>	nd	nd

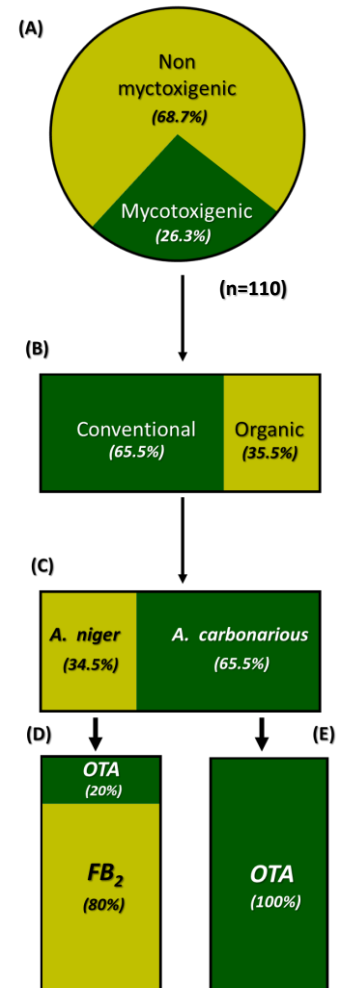


Figure 4. Panel of pie charts and bars that demonstrate: (A) Frequency of mycotoxigenic isolates within the tested population (n=110). (B) Frequency of mycotoxigenic strains originating from conventional and organic vineyards in Greece. (C) Frequency of mycotoxigenic strains within the *A. niger* and *A. carbonarius* subpopulations. (D & E) Frequency of OTA and FB₂ producing isolates of *A. niger* and OTA-producing isolates of *A. carbonarius*.

Key Results:

- A. tubingensis*, *A. uvarum*, *A. carbonarius* and *A. niger* were identified as the casual agents of Black rot disease in Greece.
- A. uvarum* and *A. tubingensis* considered to be the predominant species for 2018 and 2019, respectively.
- For both sampling years, higher frequencies of *A. tubingensis* and *A. uvarum* were found in the organic and in the conventional grapevines, respectively.
- In total, 26.3% of the selected isolates were capable to produce mycotoxins *in vitro*.
- The majority of the mycotoxigenic isolates originating from conventional (65.5%) vineyards and only 35.5% derived from organic grapevines.
- Among all tested *Aspergillus* isolates, *A. niger* and *A. carbonarius* were identified as the main OTA and FB₂ producers.
- Most of *A. niger* isolates produced only FB₂, while only 20% produced both mycotoxins.
- A. carbonarius* strains produced only OTA.

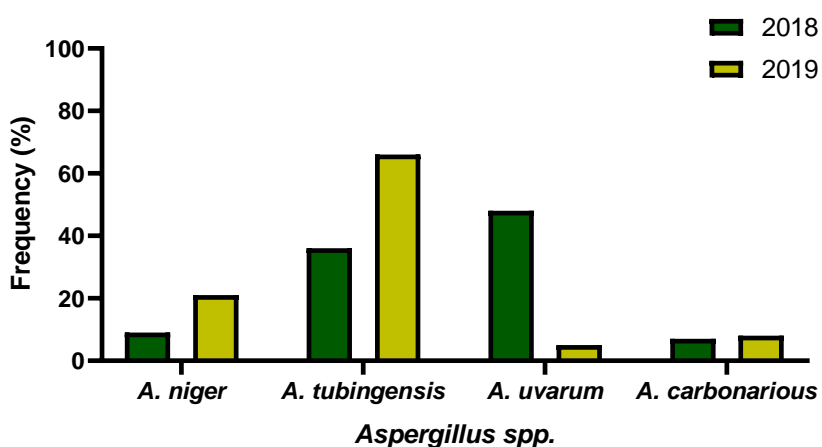


Figure 1. Frequency of *Aspergillus* species collected from asymptomatic grape berries during 2018 (dark green columns) and 2019 (light green columns)