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**Fungal flora from Tunisian olives carposphere:
diversity and volatile organic compounds**

Hend Bejaoui ¹; Guido Flamini ²; Paula Rodrigues ³; Nourhène Boudhrioua Mihoubi ⁴

ABSTRACT

Plant phyllosphere is an enormous environment on Earth densely colonized by microorganisms. Those microbiomes play essential roles in processes related to plant development and pathogen defense. They release different metabolites working as interspecies messages in the environment. Volatile organic compounds are among those released messages. Among the worldwide phyllosphere, oliveyards are widely spread in the Mediterranean basin. Like all foods, olives and oil quality begin in the field. Fungal microbiomes through their interactions with olives trees could have an impact on oil quality. In this work we were interested on fungal microbiomes from olives carposphere. Fifteen Tunisian fields from four climatic regions were studied. Fungi were isolated and identified to species with microscopic and molecular techniques. Their aromatic profiles were analysed by solid-phase micro-extraction (SPME) coupled to gas chromatography and mass spectrometry. Results showed that genus *Penicillium* (*P. polonicum*, *P. crustosum* and *P. expansum*) was predominant. The major volatiles identified were: Styrene, 1-octen-3-ol, 3-octanol, 3-octanone, 1,8-cineole. Styrene is a volatile hydrocarbon reported as 80 times more toxic than the volatile phase of toluene. The following three are oxylipins. They play essential roles in fungal morphogenesis and pathogenesis and are reported as metabolites with musty and earthy characteristics able to induce mycotoxin production. The last one is a terpene with a eucalyptus herbal camphor odor note. Could those volatiles be found on olives and olive oils? Could they impact chemical and sensory olive oils qualities? Could they induce mycotoxin production in olive oils? All those questions still to be answered...

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Keywords: Oliveyards, fungal microbiomes, Penicillium, oxylipins

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Fungal flora from Tunisian olives carposphere: diversity and volatile organic compounds

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Introduction: Plant phyllosphere is an enormous environment on Earth that is estimated to reach an area of 6.4×10^8 km² and is densely colonized by microorganisms. Those microbiomes play essential roles in processes related to plant development and pathogen defense. They are associated through networks and communicate by releasing different type of metabolites, working as chemical vocabulary and formulating interspecies messages in the environment. Volatile organic compounds are among those communicating languages. Among the worldwide phyllosphere, oliveyards are widely spread in the Mediterranean basin. Tunisia is the most important olive-growing country of the southern Mediterranean region; over 30% of its cultivated land is dedicated to olive growing (at present 1.68 million ha) since the 8th century BC. Like all foods, olives and oil quality begin from field. Fungal microbiomes through their interactions with olives trees could have an impact on oil quality.

Aim: During this work we were interested on: i) the study of the fungal microbiomes from olives carposphere, and ii) identifying the volatile aromatic compounds produced by fungi that could influence olive oil quality.

Materials & methods

1. Sampling regions

The sampling country belong to the south of the mediterranean basin: Tunisia (Fig. 1).

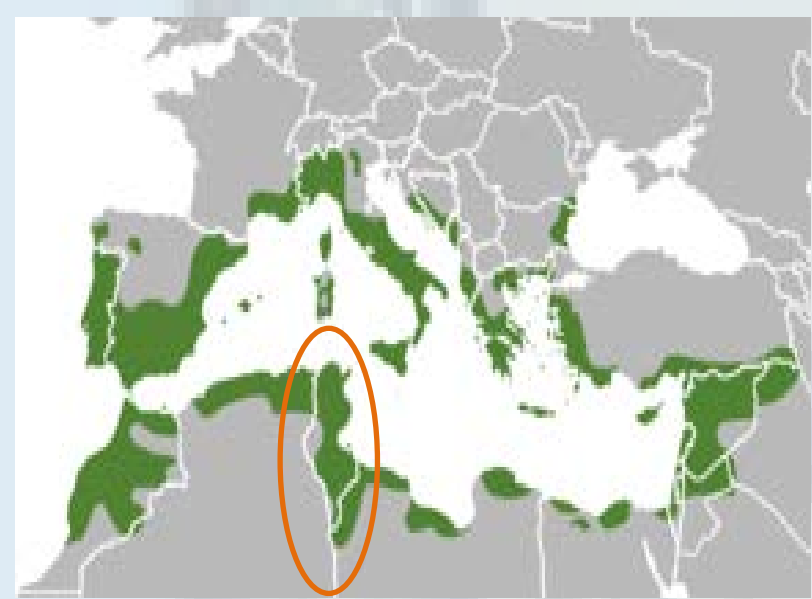


Fig 1: Sampling country, regions and fields

Fifteen fields from five climatic regions were sampled. Five samples were collected from each field by following the two diagonals.

2. Fungal isolation

Five olives were directly plated on potatoe dextrose agar (PDA) (Difco) medium in Petri dishes (Fig. 2). All plates were incubated for 7 days at 30 °C, after which all fungal genera were isolated and preserved in distilled water until identification.

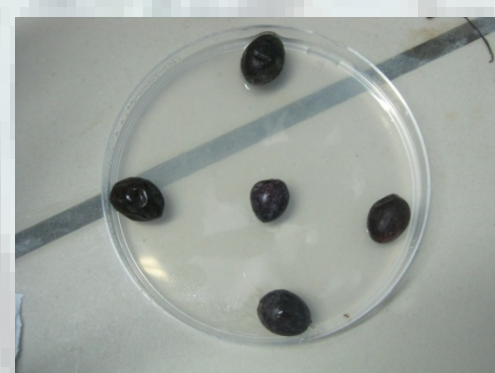


Fig 2: Plated olives on PDA medium

3. Fungal identification

3.1. Morphological identification

A preliminary fungal identification was carried out using taxonomic schemes based on morphological characters.

3.2. Molecular identification

Genomic DNA of fungi was extracted by the SDS protocol and was used for PCR amplification and sequencing of the internal transcribed spacer (ITS) region of the rRNA gene.

4. Fungal cultures

Twenty eight fungi isolates from olives were chosen to be tested for their aromatic profiles. Each fungus was cultured on 0.5 mL PDA medium for four days at 25 °C in 1.5 mL Eppendorff tubes.

5. Volatile molecules extraction and identification

A commercially available fiber, 50/30 μm DVB/CAR/PDMS (No. 57348-U), was used to sample the headspace of Eppendorffs with fungal cultures (Fig. 3). After equilibration time, the fiber was exposed to the headspace for 45 min. Then, the fiber was withdrawn into the needle and transferred to the injection port of the GC-MS system. GC-EIMS analyses were performed with a Varian CP-3800 gas-chromatograph equipped with a DB-5 capillary column (30 m x 0.25 mm; coating thickness 0.25 μm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions: injector and transfer line temperatures 220 and 240°C respectively; oven temperature programmed from 60°C to 240°C at 3°C/min; carrier gas helium at 1 mL/min; splitless injection.

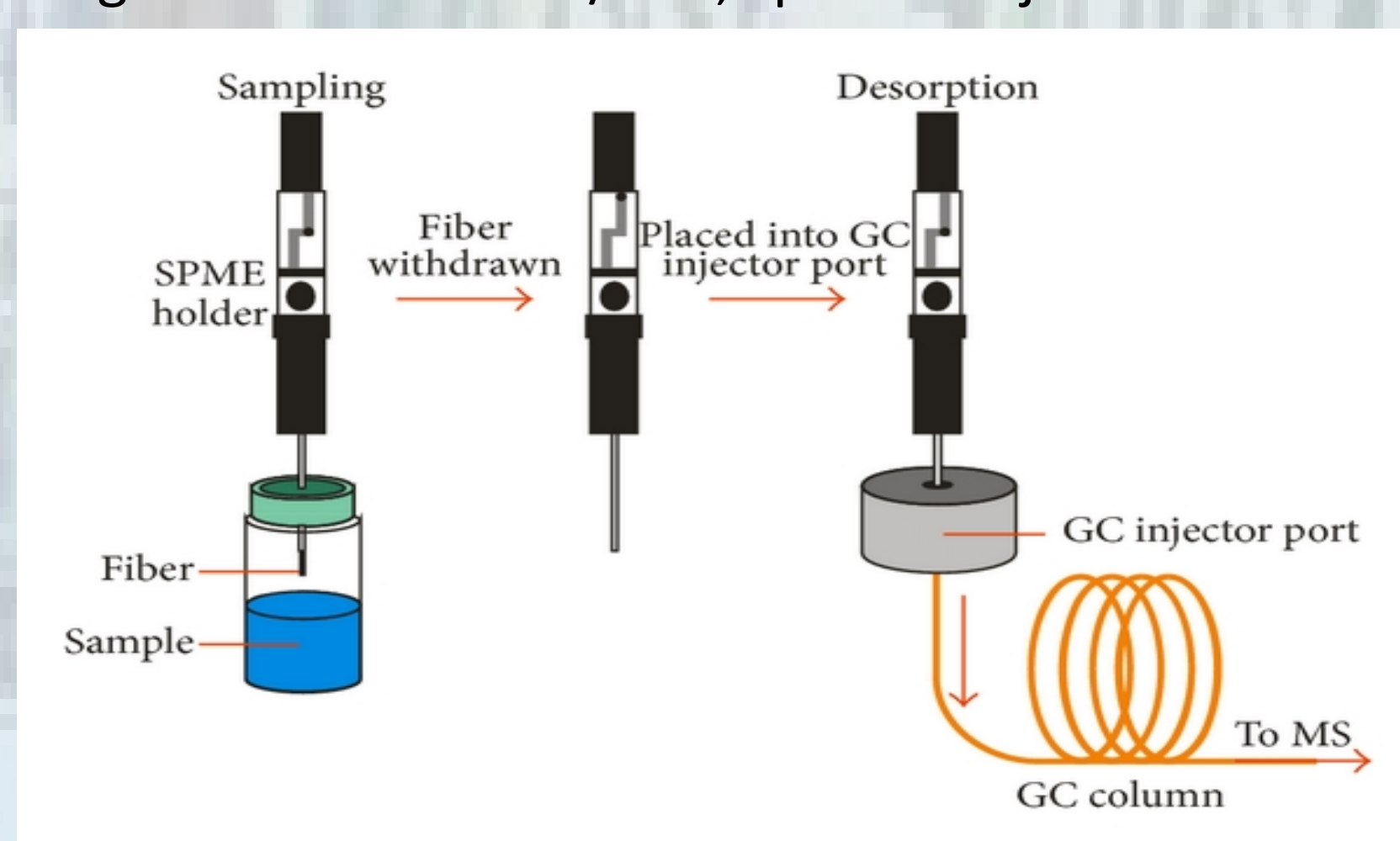


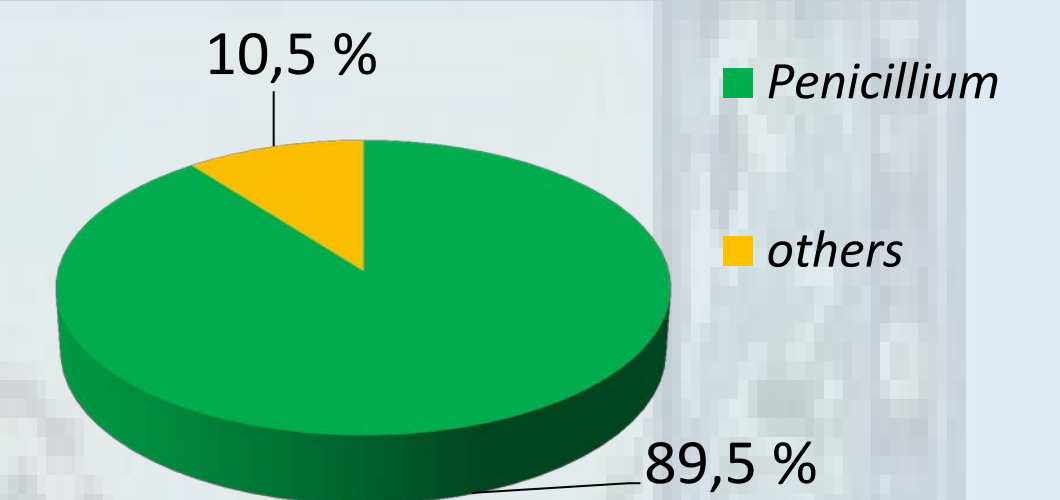
Fig 3: SPME GC/MS System

Results

1. Mycobiota of olives

Fungal flora from the fields had a large diversity, but it was dominated by *Penicillium* genus (Fig. 4).

Fig 4: Fungal population description



The remaining species were (Fig. 5):

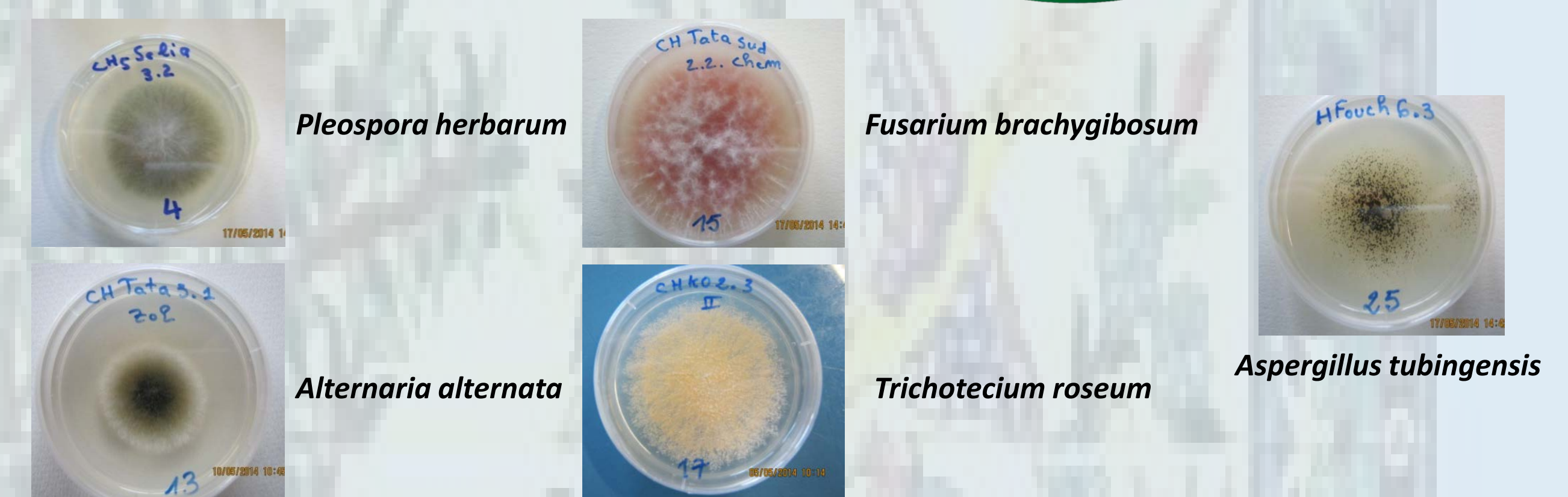


Fig 5: Fungal diversity of olives

Three species of *Penicillium* were identified (Fig. 6):

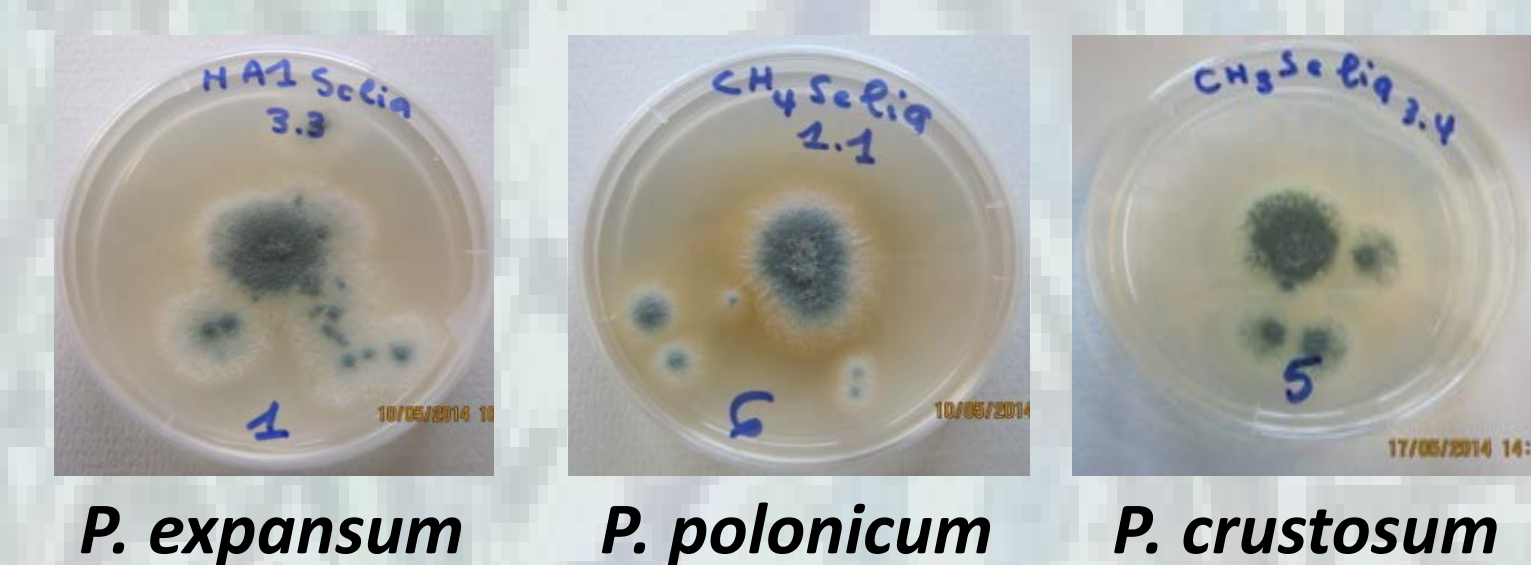


Fig 6: *Penicillium* species identified in olives

2. Penicillium aromatic profiles

Volatile organic compounds (VOCs) from *Penicillium* isolates were analysed. Three particular profiles were found and they were species-specific (Fig. 7). Principle component analysis was done and specific patterns were confirmed.

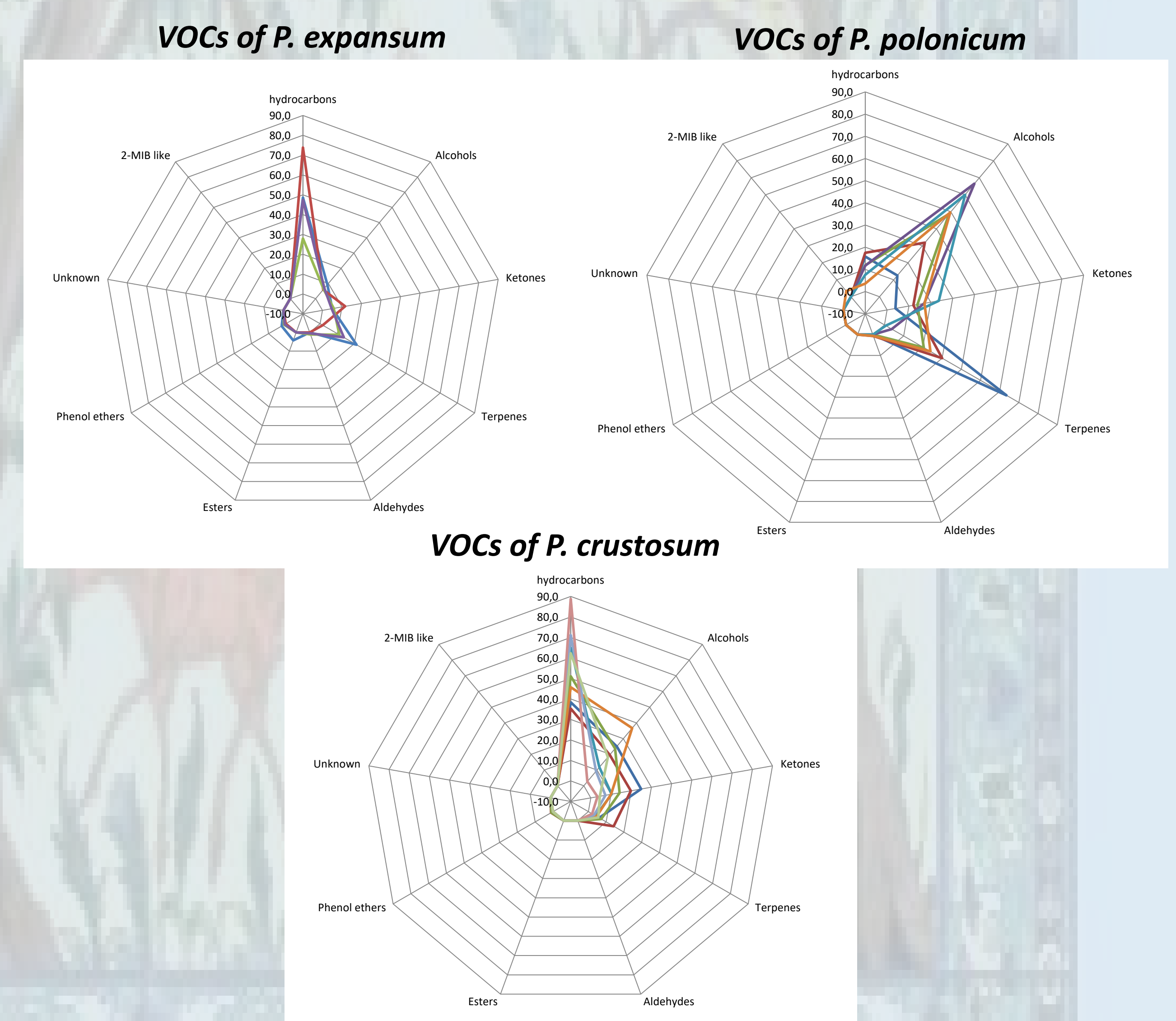


Fig 7: *Penicillium* aromatic profiles

The major VOCs from *Penicillium* are:

- **Styrene:** toxic volatile hydrocarbon
- **1,8-cineole, 1-octen-3-ol, 3-octanol:** oxylipins with musty and earthy characteristics able to induce mycotoxin production
- **3-octanone:** terpene with a eucalyptus herbal camphor odor

Conclusions: during this study, the mycobiota of tunisian olives carposphere was isolated, identified and characterised according to their volatile organic compounds. Mycobiota was dominated by *Penicillium* genera, followed by *Alternaria* and *Pleospora*, and then *Fusarium*, *Trichotecium* and *Aspergillus*. Three *Penicillium* species were identified: *P. expansum*, *P. polonicum* and *P. crustosum*. Aromatic profiles from *Penicillium* were species-specific and were dominated by Styrene, 1,8-cineole, 1-octen-3-ol, 3-octanol, 3-octanone. The effect of those volatiles on olive oil quality is the second part of this work...

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