ABSTRACT

The olive and its derivatives, in particular olive oil, represent one of the most significant agricultural products in the Mediterranean basin. Storage under inadequate conditions poses serious problems concerning fungal contamination, with consequent defects and potential mycotoxin production in olives and olive oils. *Penicillium expansum* represents one of the most significant postharvest pathogens in several fruits, including olives. Not only it causes blue mold but also is one of the most relevant patulin (PAT) producing species of the genus *Penicillium*. The aim of this research was to evaluate the ecophysiological conditions governing growth and PAT production by *P. expansum* strains previously isolated from Tunisian olives. For this purpose, four *P. expansum* isolates were tested in a synthetic medium (Czapek Yeast Autolysate, CYA) and in olive-based medium (OM) for their ability to grow and produce PAT under different temperatures (4 °C, 15°C and 25°C) for 10 and 20 days. The mycotoxin was analysed by HPLC-UV. Results showed that all isolates were able to grow on tested media at different temperatures. Different PAT production profiles were found, showing that at 25 °C *P. expansum* isolates were able to produce PAT on CYA and OM medium. At 15 °C the production of PAT was only detected on CYA medium, while no PAT production was detected at 4 °C for the two media.

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Ecophysiology of *Penicillium expansum* and patulin production in synthetic and olive-based media

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**Introduction:** The olive and its derivatives, in particular olive oil, represent one of the most significant agricultural products in the Mediterranean basin. Storage under inadequate conditions poses serious problems concerning fungal contamination, with consequent defects and potential mycotoxin production in olives and carry-over to olive oils. *Penicillium expansum* represents one of the most significant postharvest pathogens in several fruits, including olives. Not only it causes blue mold but also is one of the most relevant patulin (PAT) producing species of the genus *Penicillium*.

**Objective:** In this study we aimed to evaluate the ecophysiological conditions governing growth and PAT production by *P. expansum* strains previously isolated from Tunisian olives, using olive-based medium as model.

**Materials & Methods**

1. **Fungal strains**
   - Three PAT producing strains of *P. expansum* isolated from Tunisian olives (TUN isolates)
   - One PAT-producing strain of *P. expansum* (MUM 10.175), obtained from Micoteca da Universidade do Minho (MUM), Braga, Portugal, used as control.

2. **Incubation conditions**
   - *P. expansum* strains were inoculated onto CYA (synthetic santard medium) and olive-based medium (OM; olive-puree:water, 1:6) at 25 °C, 15 °C and 4 °C for 20 days.

3. **Measurement of fungal growth**
   - Fungal colonies were measured after 3, 5, 10, 15 and 20 days of incubation. The process was done in triplicate.

4. **PAT evaluation**
   - PAT was extracted after 10 and 20 days of incubation from agar plugs with methanol and quantified by HPLC-UV (λ=276 nm), with a RP-C18 column (150 x 4.6 mm, 5 µm), with isocratic elution in water:methanol (90:10) at 0.8 mL/min.

**Results and Discussion**

**Fungal growth**

- Matrix and temperature had a significant influence on *P. expansum* growth, with growth on OM based on rare and spread synnemata (Fig. 1).
- Optimal growth occurred at 25 °C (Fig. 2).
- Between 10 and 20 days of incubation, fast growth on OM medium was observed.
- Growth at 4°C was significantly reduced.

**PAT production**

- PAT production was affected by nature of matrix, temperature, time of incubation and strain.
- All *P. expansum* strains were able to produce PAT at 15 °C and 25 °C on both media, but no PAT was detected at 4 °C on OM medium (Fig. 3).
- The highest amount of PAT after 10 days was produced by TUN strains at 15 °C in OM (73.1 µg/g), but after 20 days the highest amount was detected on CYA, by the same strains, at 4 °C (180 µg/g).
- PAT production decreased after 10 days of incubation on CYA, while it increased throughout time on OM.

**Conclusions:** Contamination of olives with PAT must be considered a potential risk in the safety plans of the olive producing chain. The olive-based matrix does not seem to be highly adequate for growth and PAT production by *P. expansum* if adequate temperatures throughout storage (refrigeration) are guaranteed. However, if there is an abuse on storage temperature and longevity, PAT can turn into a real risk. Mechanisms of PAT reduction throughout time observed on CYA but not on OM requires further investigation.

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