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Characterization of *Diaporthe toxica* associated with lupin beans: growth, spore production, phomopsin-A and alkaloid biosynthesis

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INTRODUCTION & AIM

The interest in vegetable proteins, namely those derived from legumes, recently increased due to the growing demand for new nutritious food sources. Among legumes, lupin emerges as major player for its great nutritional profile, which includes a high protein content and a plethora of bioactive compounds. Unfortunately, lupin also represents a concern both for its antinutritional factors, consisting of quinolizidine alkaloids (QA), and for the potential presence of the endophytic, phytopathogenic, mycotoxin-producing fungal species *Diaporthe toxica*. The main mycotoxin produced by this species, phomopsin-A (PHO-A), has been recognized as potentially harmful for humans (EFSA, 2012).

Up to date, only limited or outdated scientific evidence exists about *D. toxica*, PHO-A production and the relationship between this fungus and QA. Consequently, this study aims at characterizing *D. toxica* and its metabolic pathways in different environmental conditions.

METHOD

Different agar media (Oat Flake Medium (OFM), Potato Dextrose Agar (PDA), Yeast extract, Peptone, Dextrose (YPD), Malt Extract Agar (MEA), Water Agar (WA) and WA with lupin beans) were inoculated by using an 8 mm mycelial plug of a 5-day-old *D. toxica* culture, and then incubated at 25.0 ± 0.1 °C for 21 days. Fungal growth was evaluated by means of diameter growth measurements, and the spore production was confirmed by optical microscopy. In addition, lupin beans were inoculated following 3 different protocols, and PHO-A and QA were quantified over time. Fungal load was determined by microbiological sampling, PHO-A by µSPE extraction followed by UHPLC-MS/MS analysis, and alkaloids by HPLC-MS/MS.

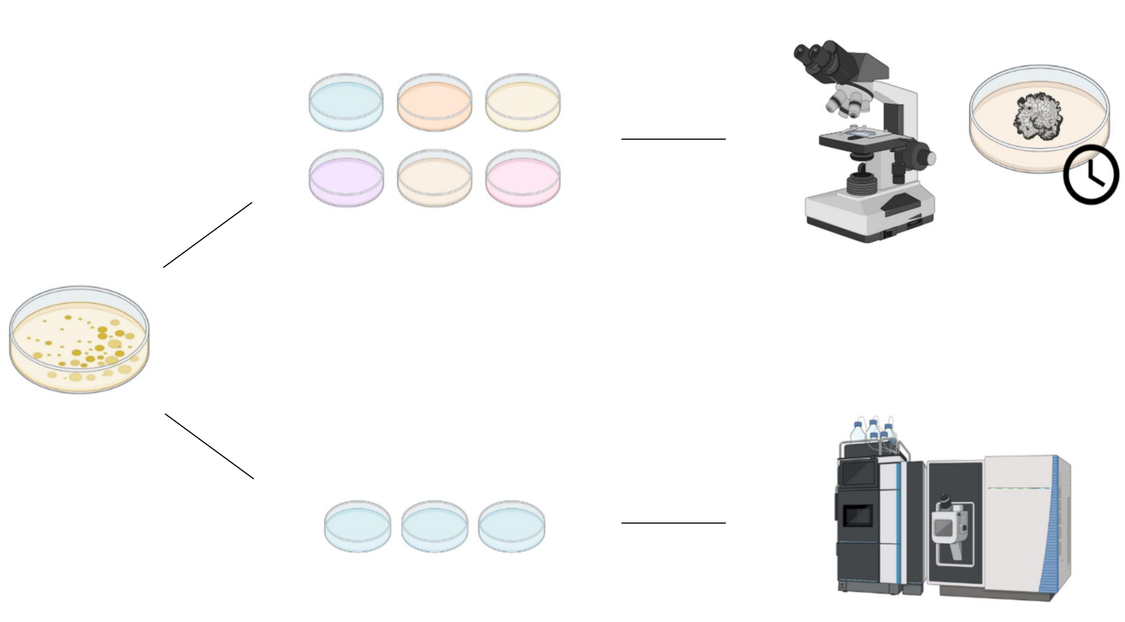

Figure 1. Materials and methods for growth dynamics and spore observation, PHO-A and QA quantification

RESULTS & DISCUSSION

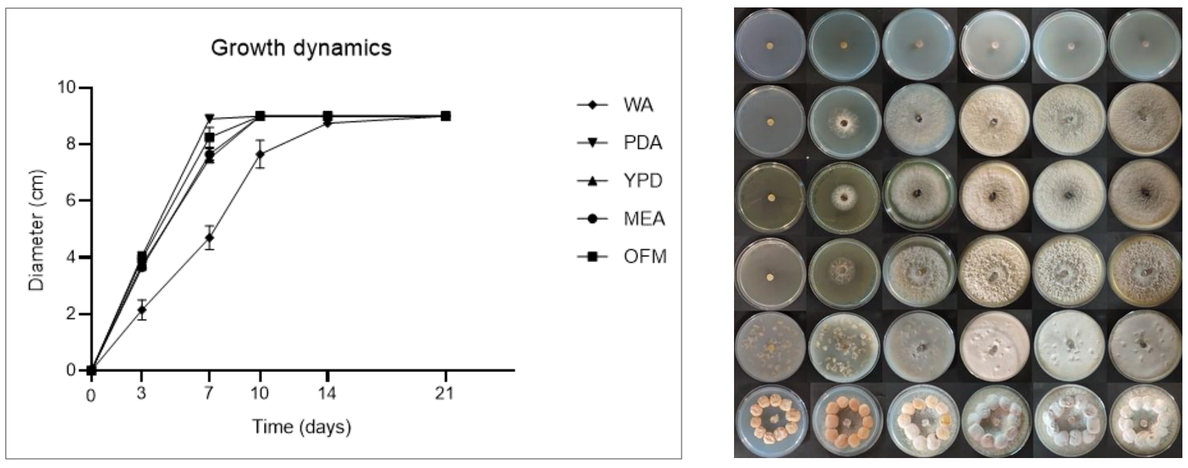

Figure 2. Growth dynamics observation by measurement (left) and hyphae morphology (right)

Growth data of *D. toxica* demonstrated a great adaptation of this species to the different media. In fact, it could grow also in low-nutrient substrates (WA), though at different rates. Nonetheless, spore production was only achieved on lupin seeds, thus demonstrating that spore formation requires specific conditions.

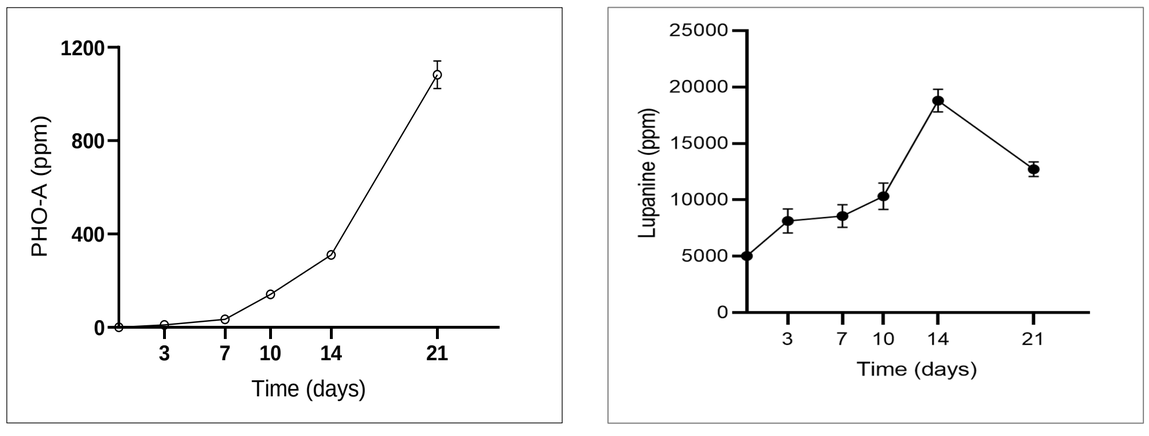

Figure 3. PHO-A (left) and QA (right) concentration overtime.

Lupins at a_w equal to 0.98, with the inoculum spread above the seeds, were found to be the best substrate for PHO-A production. Evidently, this medium resembles the conditions encountered during lupin cultivation, which can be considered the most critical moment of lupin production process, due to the possible PHO-A formation. Furthermore, on this substrate, *D. toxica* also produced QA (in the figure, only lupanine is reported), and this finding had never been reported for this species, although it has been observed in other endophytic fungal species. Therefore, this outcome must be taken in proper consideration.

CONCLUSION

As demonstrated by this study, *D. toxica* is a resilient fungal species that can produce considerable levels of PHO-A and QA. Therefore, this feature should be considered in the risk assessment and deserves further research in the future.

FUTURE WORK / REFERENCES

Novel strategies to counteract the production of PHO-A and reduce QA concentrations are under evaluation, particularly non-thermal technologies that can help achieving good detoxification without affecting the physical, chemical and sensory characteristics of lupin beans.