MICROSOIL - Investigation of Alternative Test Methods to Correctly Assess the Impact of Plant Protection Products, Biocides and Pharmaceuticals on Soil Microorganisms

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Introduction and Aim

Mycorrhizal fungi play a fundamental role in terrestrial ecosystems[1]. Through root symbiosis with terrestrial plants, they have important functions for plant growth like e.g. nutrient exchange[2]. Due to these positive effects on the host plant, the symbiosis has a far-reaching ecological significance[3]. However, studies have shown that arbuscular mycorrhizal fungi (AMF) are sensitive to e.g. pesticides[2]. Therefore, it is important to consider these key organisms in an environmental risk assessment.

The aim of this study is to investigate effects of chemicals on the spore germination (ISO 10832) of AMF (*Funneliformis mosseae*) in different substrates and to prove the suitability of this test system for the regulation of chemicals.

Benefits of the symbiosis

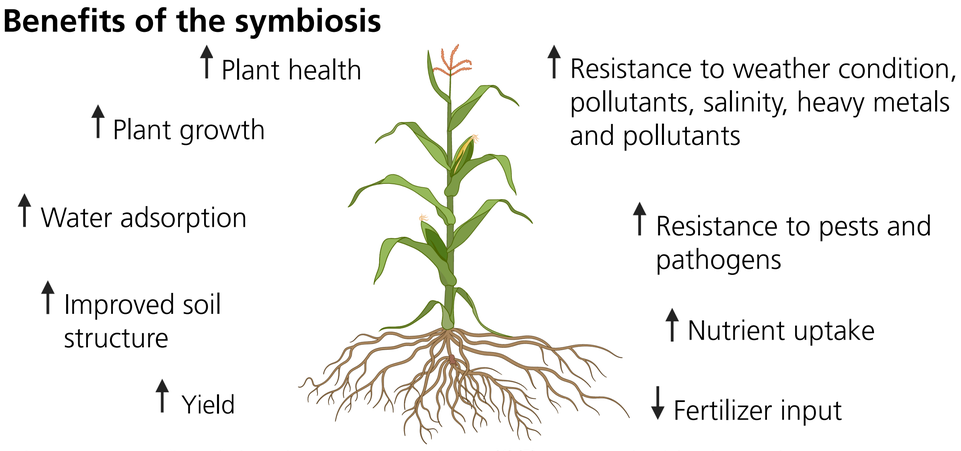

Figure 1: Benefits of the plant- AMF symbiosis[4],[5] . Created with BioRender.com

Materials and Method

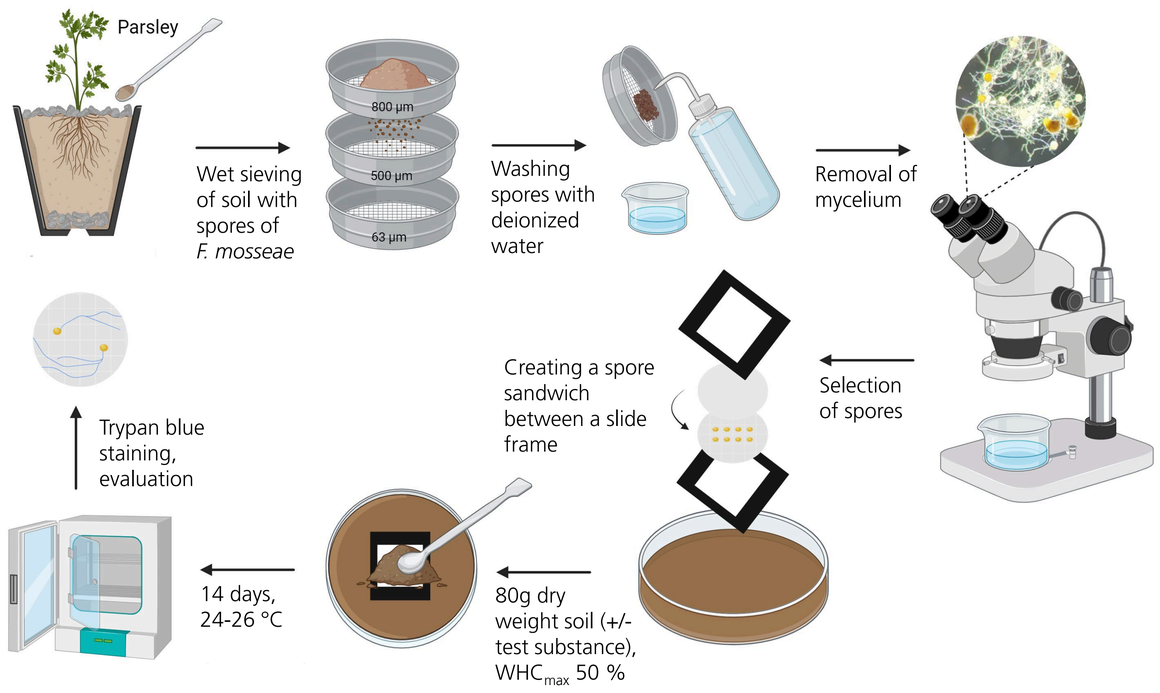

Figure 2: Schematic illustration of the ISO 10832 spore germination test; For the spore germination test two concentrations with six replicates containing 30 spores were used. Created with BioRender.com

Results and Discussion Part 1

Pre-tests

- Following ISO 10832 using a WHC_{max} of 90 %, tests with artificial soil (ISO 11268-1) and the natural soil RefeSol 02A showed no germination. By lowering the WHC_{max} to 75 %, spore germination was achieved for one natural soil (RefeSol 02A) and improved germination in quartz sand:

Soil	WHC_{max} [%]	Average germination rate [%]
Artificial soil	90	0.0
Quartz sand	90	40.4
Quartz sand	75	65.0
Lufa 2.1	75	0.0
RefeSol 02A	90	0.0
RefeSol 02A	75	37.1
RefeSol 04A	75	0.0

Table 1: Pre-tests at 25 °C with different soils: artificial soil (ISO 11268-1:2012; pH 7.3 adjusted with CaCO3), quartz sand (pH 7.6), Lufa 2.1 (loamy sand; pH 4.4), RefeSol 02A (silt loam; pH 6.8) and RefeSol 04A (loamy sand; pH 5.1)

Results and Discussion Part 2

Pre-tests with 5 different soils

- Further lowering of the WHC_{max} to ~ 50 % lead to following results:

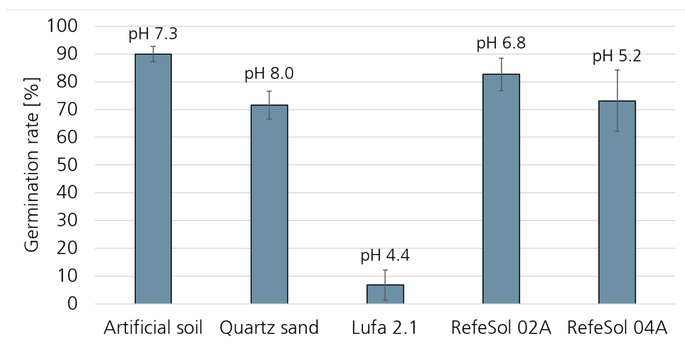

Figure 3: Pre-tests at 25 °C with different soils: artificial soil (pH adjusted with $CaCO_3$; WHC_{max} 55 %), quartz sand (WHC_{max} 60 %), Lufa 2.1 (WHC_{max} 50 %), RefeSol 02A (WHC_{max} 50 %) and RefeSol 04A (WHC_{max} 50 %)

- In contrast to the recommended WHC_{max} of 90 %, a reduced WHC_{max} of 50 % leads to increased spore germination in most tested soils except Lufa 2.1
- RefeSol 02A is suitable for tests with AMF, RefeSol 04A might be used as well
- While elements like Al, Mn, Fe or P can influence the germination, it became clear that a low pH value (4.4) negatively influences the spore germination

Spore germination test with RefeSol 02A (WHC_{max} 50 %) and 5 chemicals

- In accordance with ISO 10832, Benlate (fungicide) was used as a reference substance, showing an inhibition of 8 % at 1 mg/kg and a complete inhibition (100 %) at 10 mg/kg.

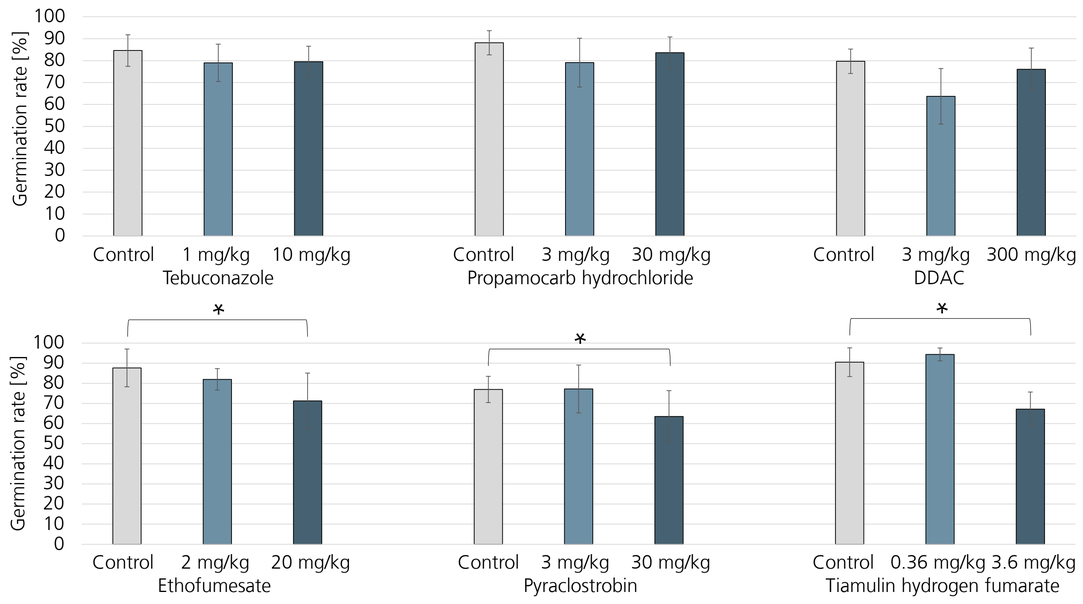

Figure 4: Spore germination test with RefeSol 02A (WHC_{max} of 50 %). Incubation for 14 days at 25.5 °C (temperature was also adapted during the course of pre-tests)

Spore germination test with RefeSol 04A (WHC_{max} 50 %)

- Germination rate was < 23 %
- Further tests are needed

Conclusion and Outlook

- Spore germination tests with adapted experimental conditions (WHC, temperature) on *F. mosseae* in natural soils are possible
- RefeSol 02A is a suitable test soil for spore germination tests
- Lufa 2.1 is not suitable for spore germination tests
- Further experiments on *F. mosseae* in RefeSol 04A are ongoing

Abbreviations: DDAC: Didecyldimethylammonium chloride; PEC: predicted environmental concentration; WHC: water holding capacity

[1] Soka, G.; Ritchie, M. Arbuscular Mycorrhizal Symbiosis and Ecosystem Processes: Prospects for Future Research in Tropical Soils. *Open J. Ecol*. 2014, 04 (01), 11–22. https://doi.org/10.4236/oje.2014.41002.
[2] König, S.; Wagner, S.; Feldmann, F. Taxonomische und funktionelle Diversität arbuskulärer Mykorrhizasymbiosen im Pflanzenbau. *Julius-Kühn-Arch*. 436 123-129 2013, 123–129. https://doi.org/10.5073/JKA.2012.436.019.
[3] Schüßler, A. Struktur, Funktion und Ökologie der arbuskulären Mykorrhiza. *Ökologische Rolle von Pilzen* 2009, 37, S. 97-108. ISSN 0938-5851
[4] S. Trouvelot, L. Bonneau, D. Redecker, D. Van Tuinen, M. Adrian, und D. Wipf, „Arbuscular mycorrhiza symbiosis in viticulture: a review", Agron. Sustain. Dev., Bd. 35, Nr. 4, S. 1449–1467, Okt. 2015, doi: 10.1007/s13593-015-0329-7.
[5] N. Begum u. a., „Role of Arbuscular Mycorrhizal Fungi in Plant Growth Regulation: Implications in Abiotic Stress Tolerance", Front. Plant Sci., Bd. 10, S. 1068, Sep. 2019, doi: 10.3389/fpls.2019.01068.