Heterodera schachtii biocontrol using *Pleurotus ostreatus* mycelium – practical solutions for field applications

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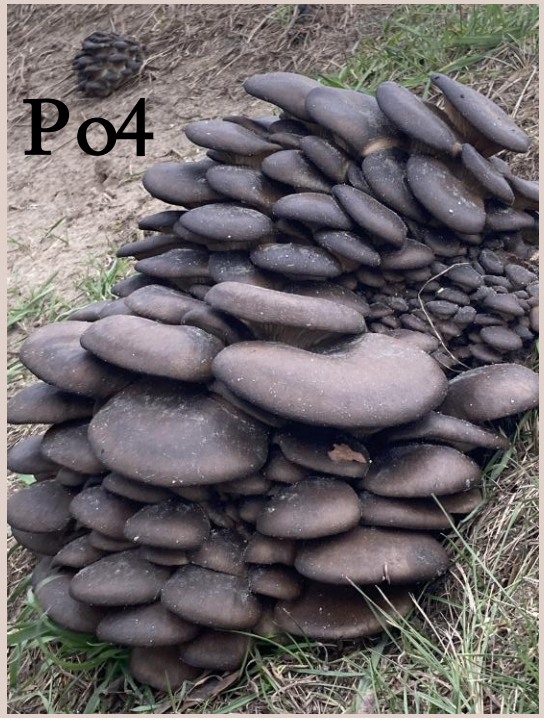
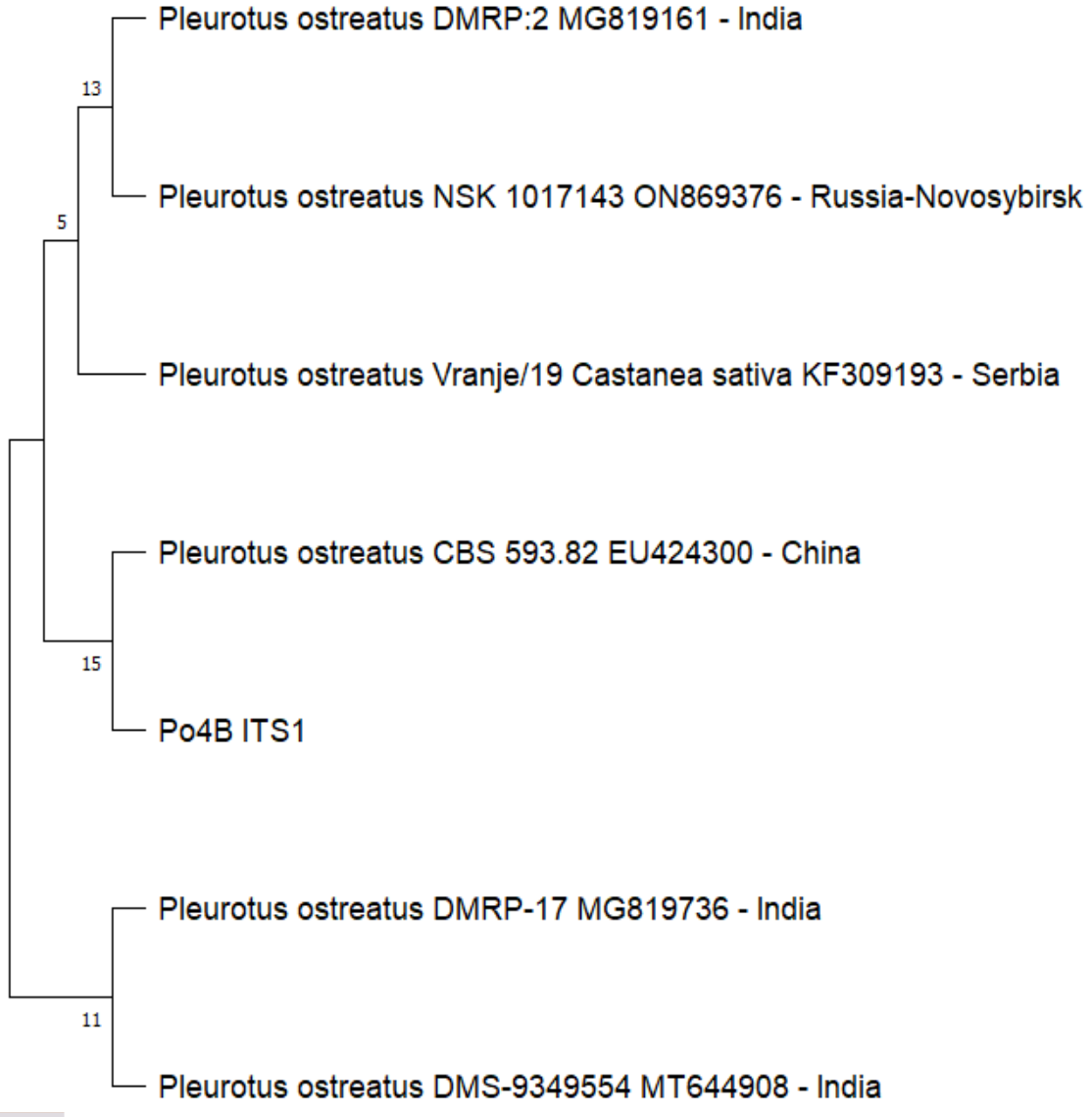
The maternal mycelia, homokaryotic and heterokaryotic progenies

- 3 strains of maternal *Pleurotus ostreatus* mycelia designed as Po1, Po2 and Po4.

Mycelia isolation.

Mycelia identification.



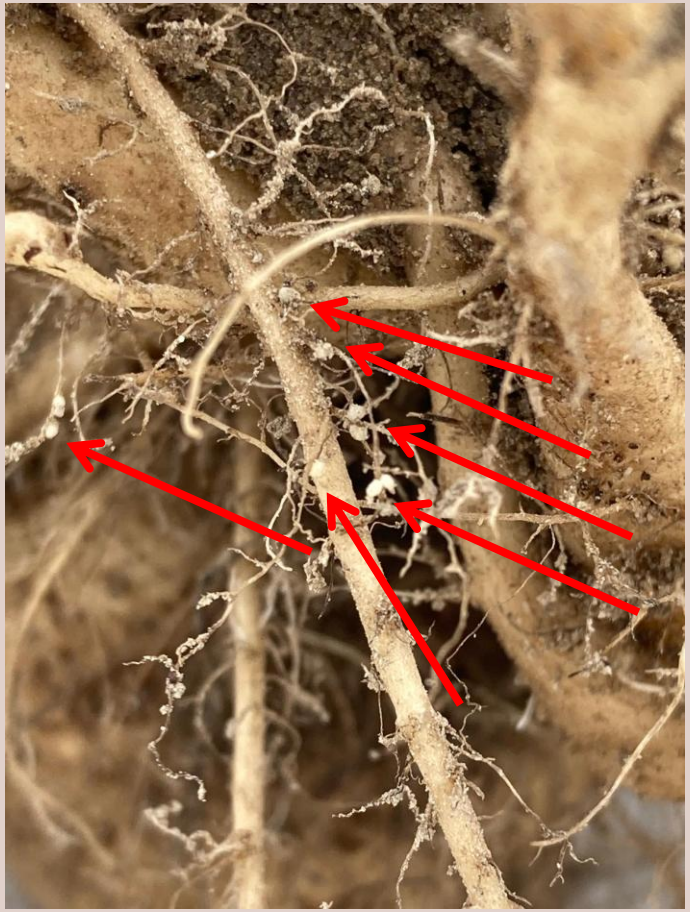


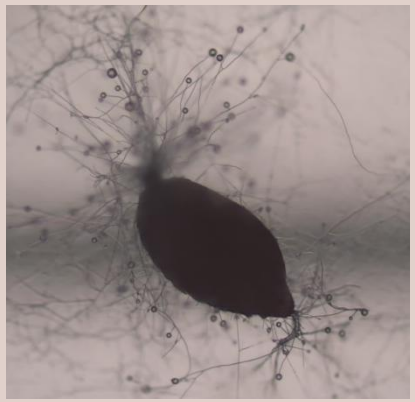
Model organisms

Heterodera schachtii (Wild strains)

Caenorhabditis elegans N2

[Wild strain obtained from Caenorhabditis Genetics Center (CGC) University of Minnesota (USA)]





Source: https://cgc.umn.edu/what-is-c-elegans

The maternal mycelia, homokaryotic and heterokaryotic progenies

Mushrooms growing.

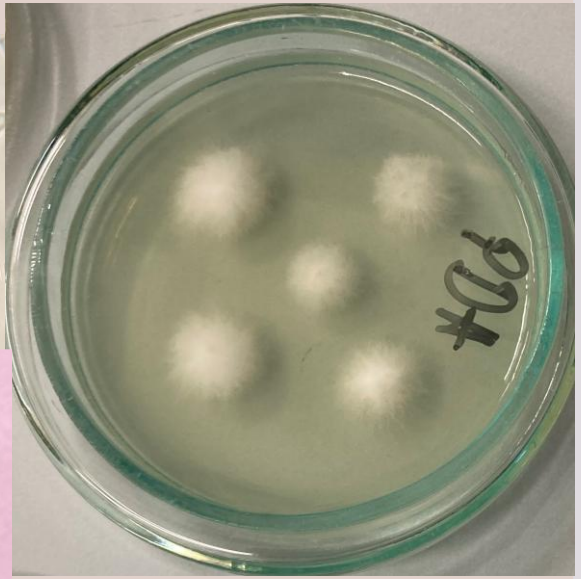
Basidiospores collection.

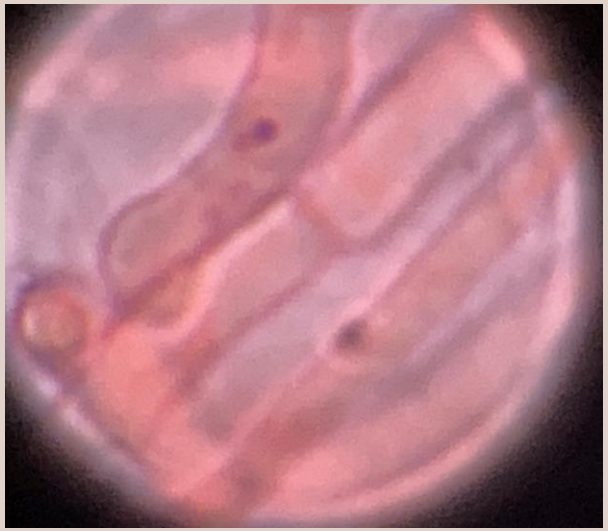
Binucleate basidiospores.

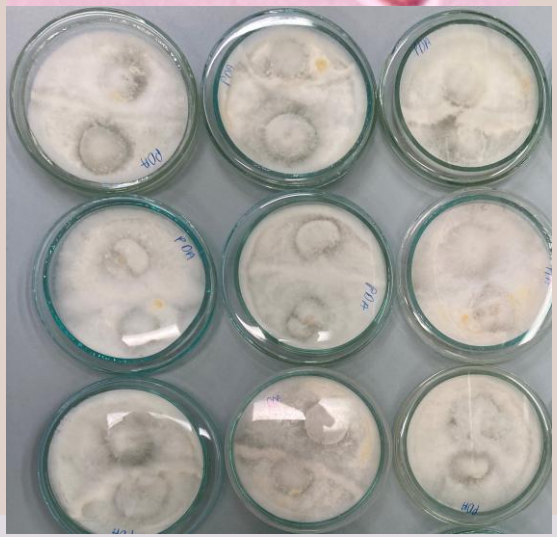
Monokaryotic/ Homokaryotic mycelia growing.

Crossing homokaryons and obtaining new heterokaryons.

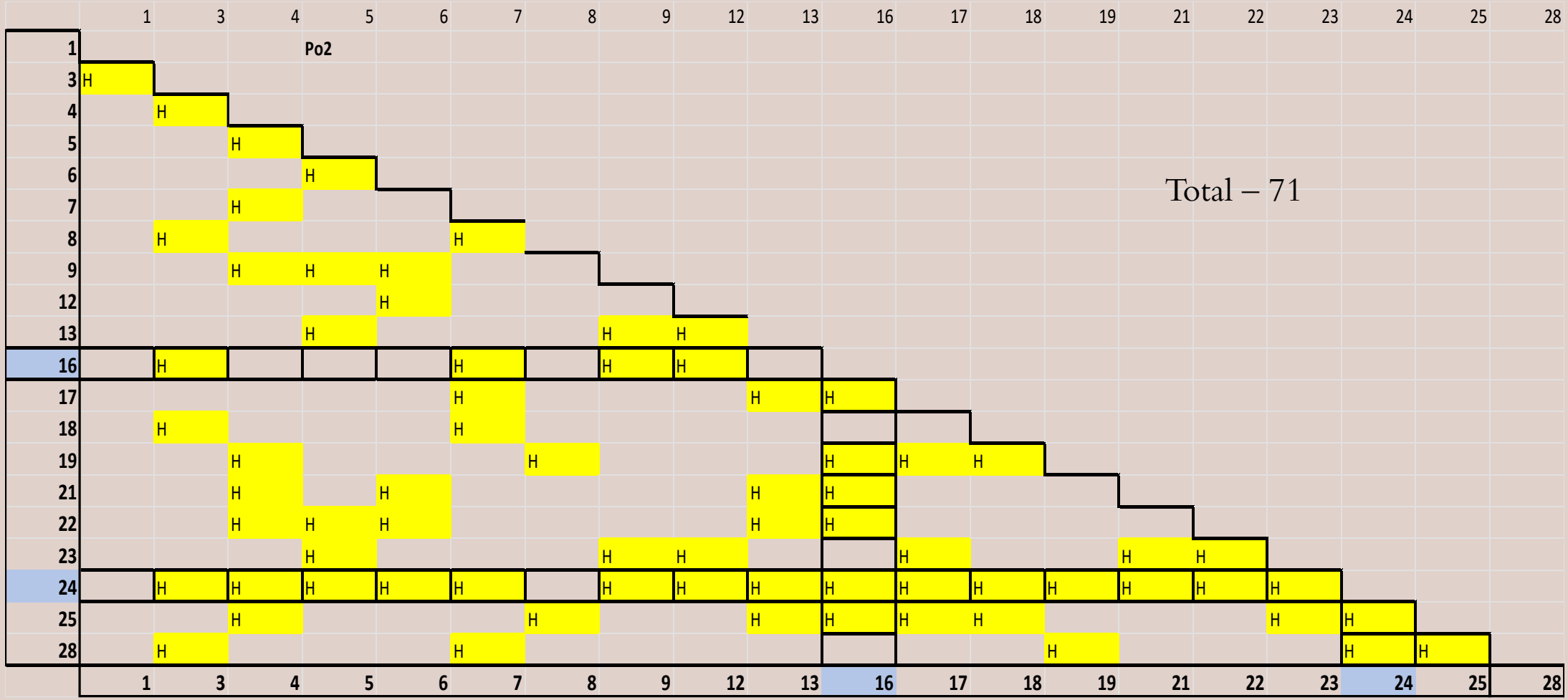
Heterokaryons obtained by „Buller crossing”



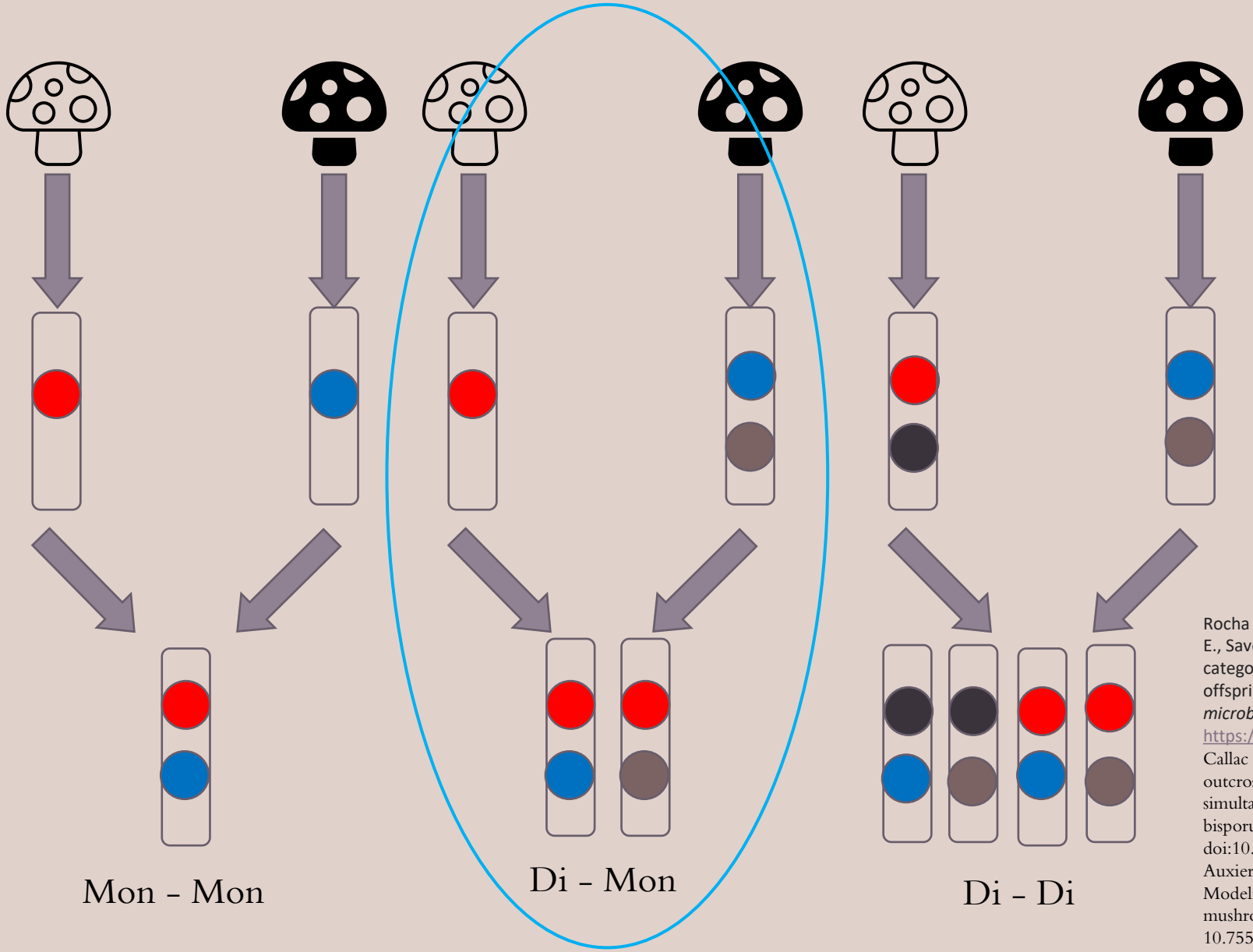




Crossing effectivity for Po2 strain



Heterogeneity by „di-mon" mating types



Buller's phenomenon (1931) – refers to fungi exhibiting independent dikaryotic, saprotrophic life stages, mainly of Basidiomycota phylum.

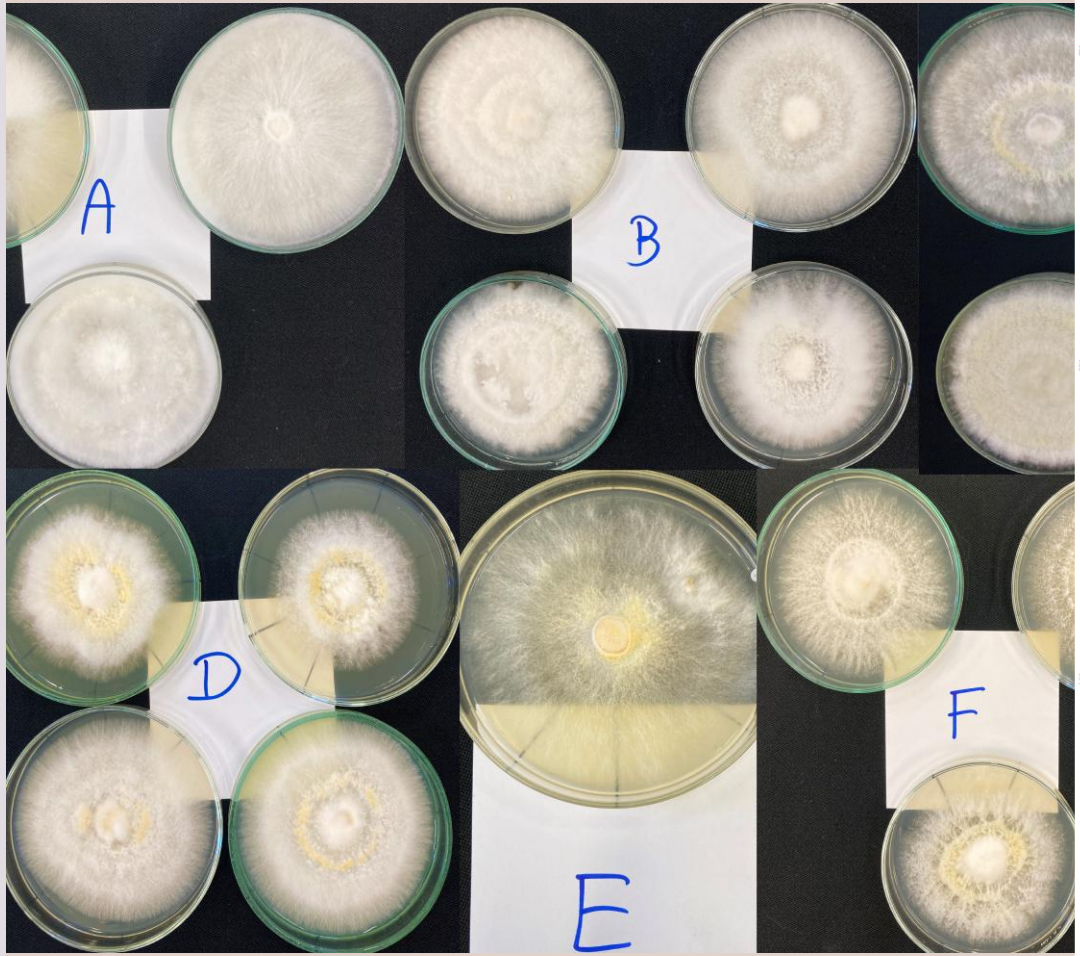
Rocha de Brito, M., Foulongne-Oriol, M., Moinard, M., Souza Dias, E., Savoie, J. M., & Callac, P. (2016). Spore behaviors reveal a category of mating-competent infertile heterokaryons in the offspring of the medicinal fungus Agaricus subrufescens. *Applied microbiology and biotechnology*, *100*(2), 781–796. https://doi.org/10.1007/s00253-015-7070-7

Callac P, Spataro C, Caille A, Imbernon M. Evidence for outcrossing via the Buller phenomenon in a substrate simultaneously inoculated with spores and mycelium of Agaricus bisporus. *Appl Environ Microbiol.* 2006;72(4):2366-2372. doi:10.1128/AEM.72.4.2366-2372.2006

Auxier, Benjamin & Czárán, Tamás & Aanen, Duur. (2022). Modeling the consequences of the dikaryotic life cycle of mushroom-forming fungi on genomic conflict. eLife. 11. 10.7554/eLife.75917.

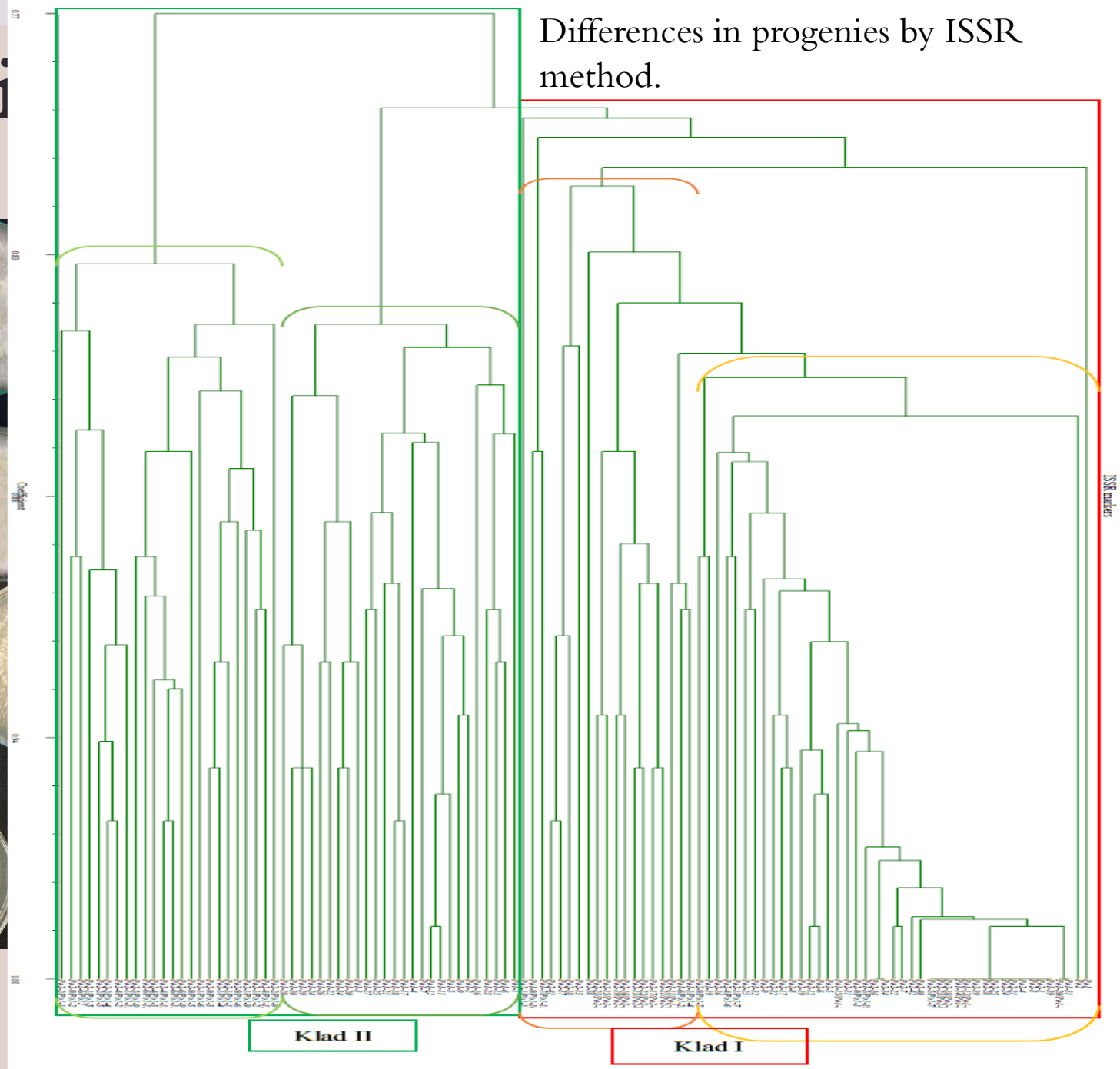
Differences in progeni

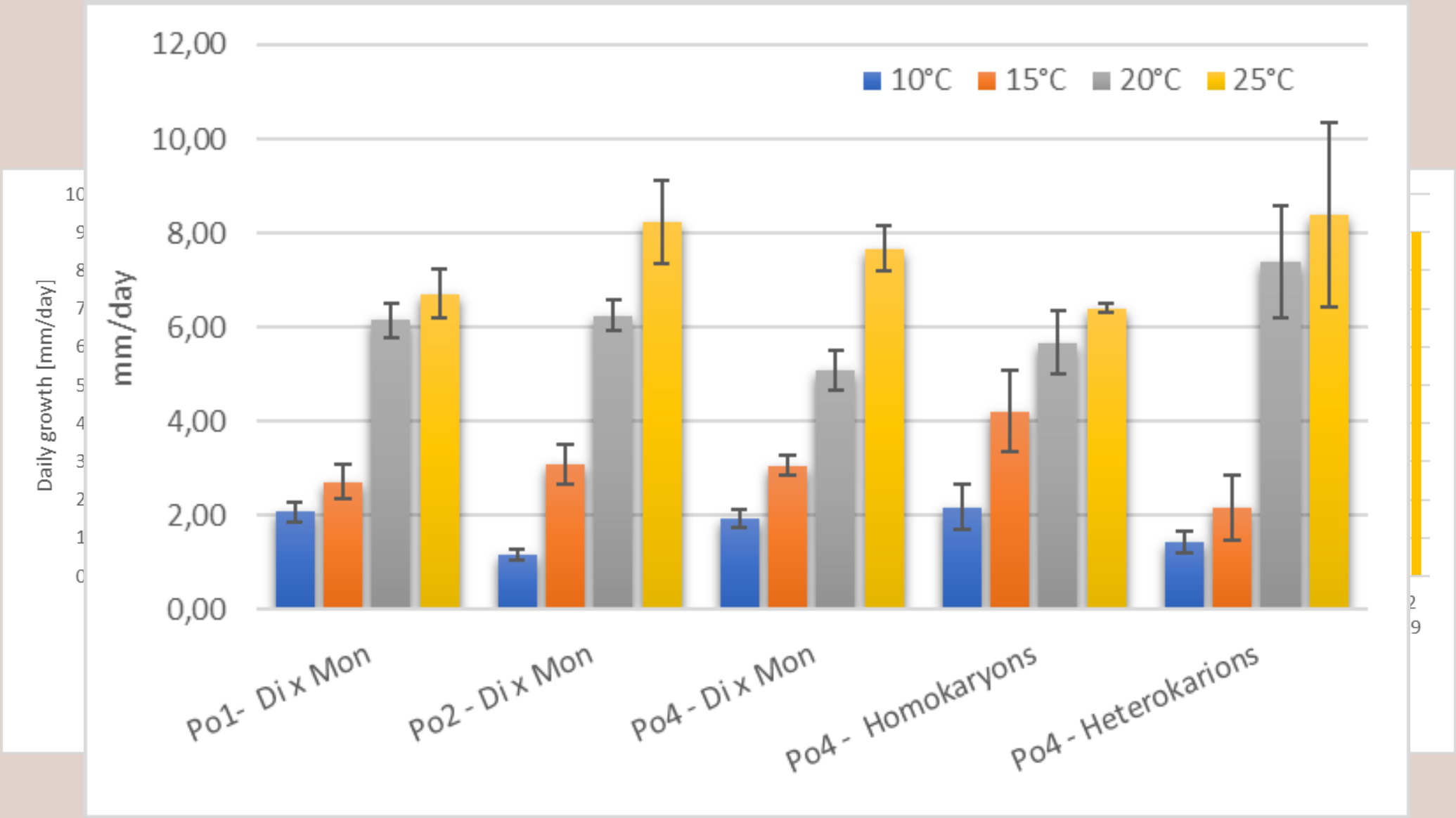
Mycelia morphology in 20°C



Dendrograms were made by the UPGMA method using NTSYS software.

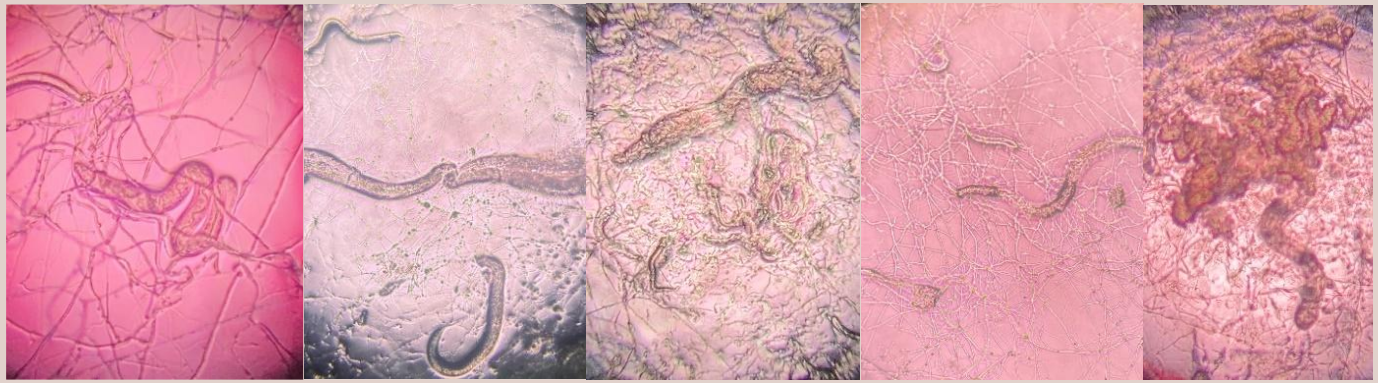
Differences in progenies by ISSR method.



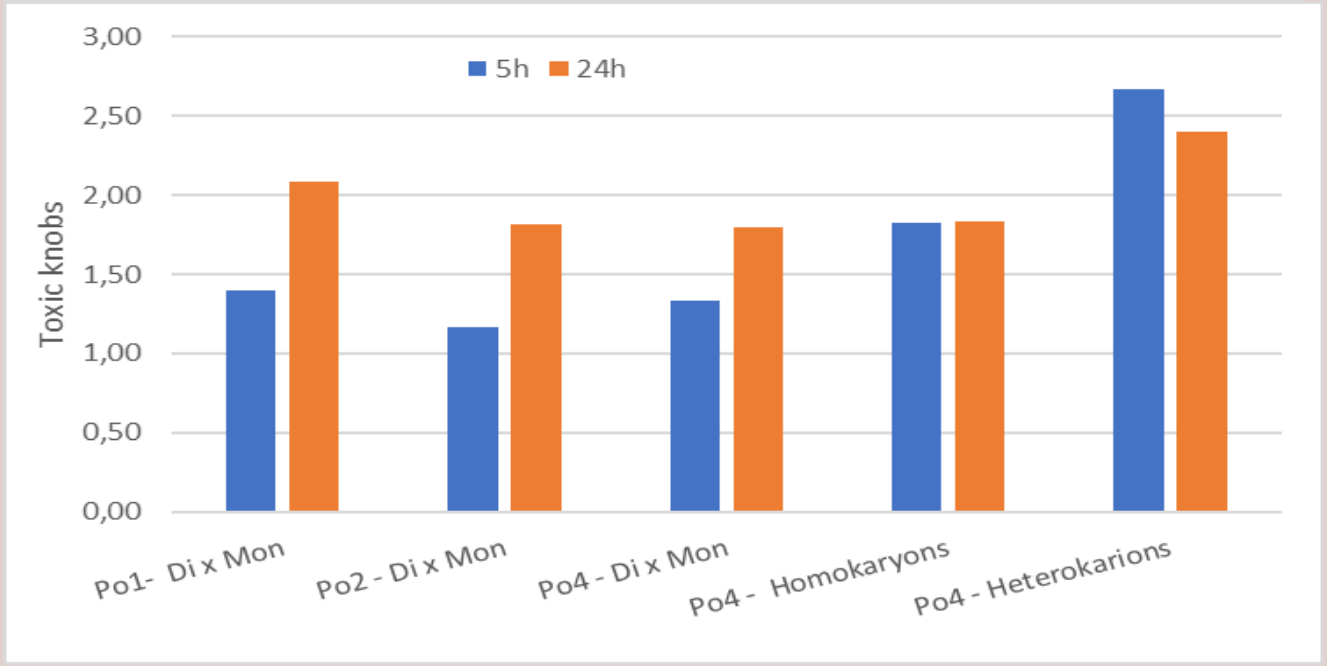


Strains differed in growth speed. Usually it took 1,5-2 weeks to overgrow a Petri dish. According to he growth ability, mainly at 15°C, mycelia were selected for future tests.

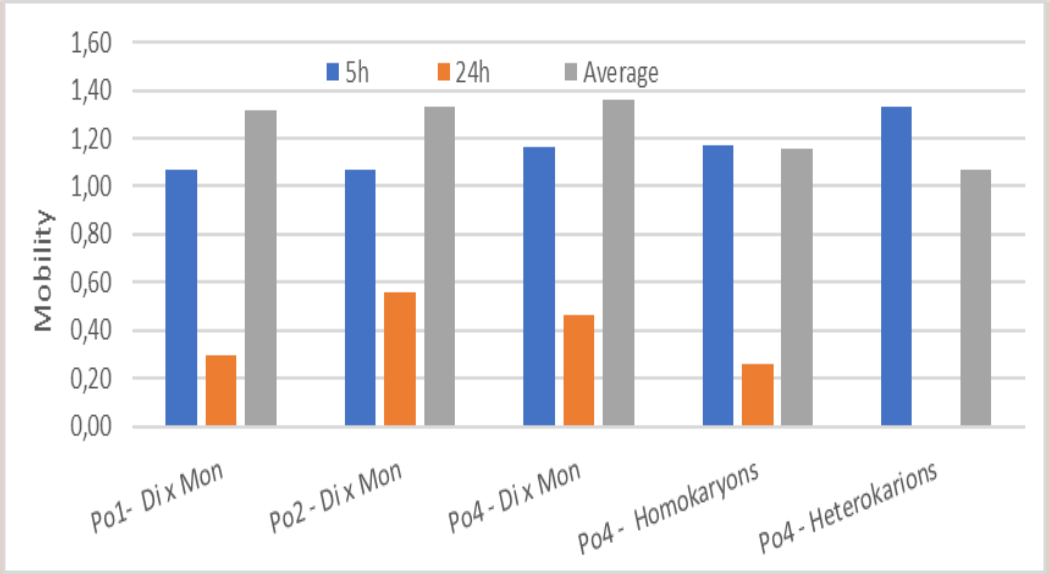
Toxicity to *Caenorhabditis elegans*



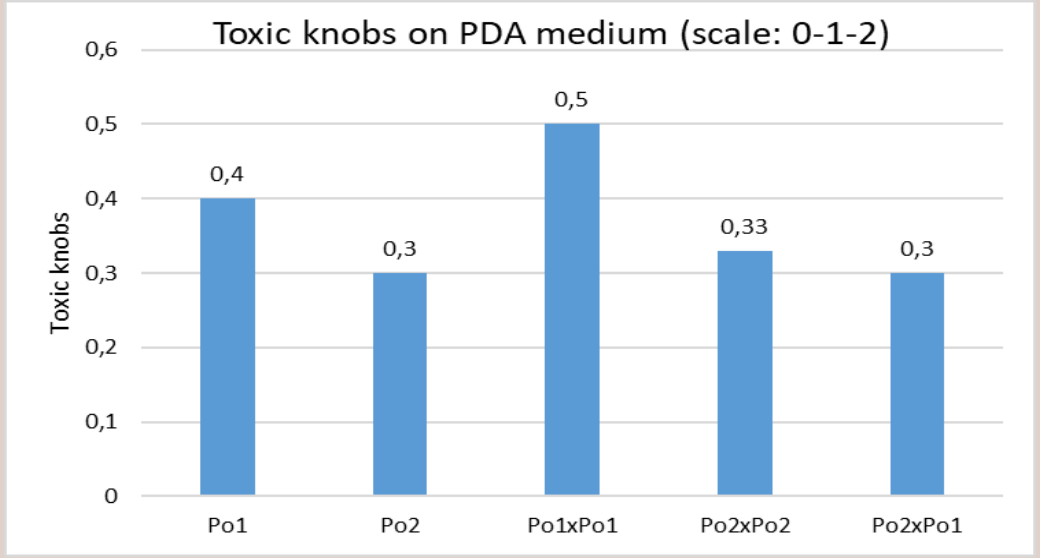
Various stages of nematodes' body digestion by *P. ostreatus*



P. ostreatus toxic knobs on Water Agar medium without nematode presence [Control] ((scale: 0-3; 3- means the highest knobs frequency)

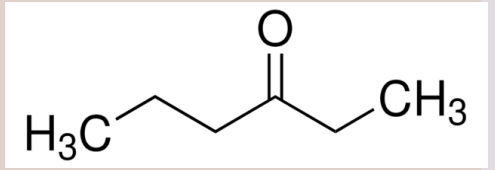


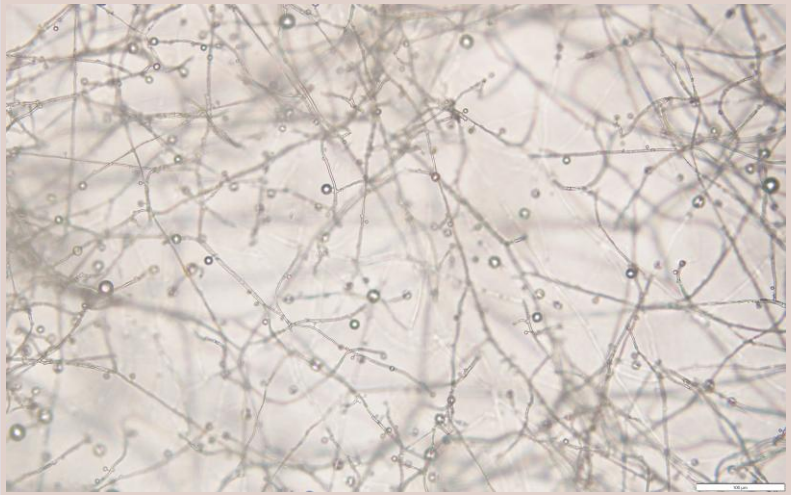
Caenorhabditis elegans mobility under the *P. ostreatus* influence (scale 0-3,) 0-means death, 3-full mobility

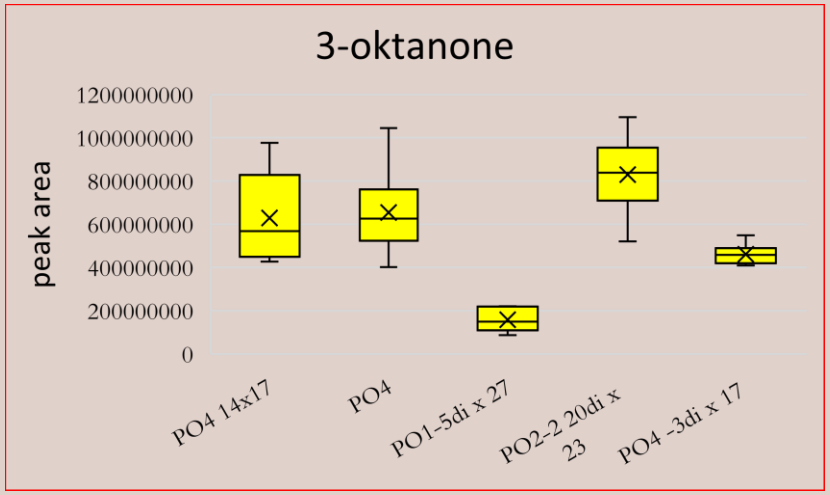


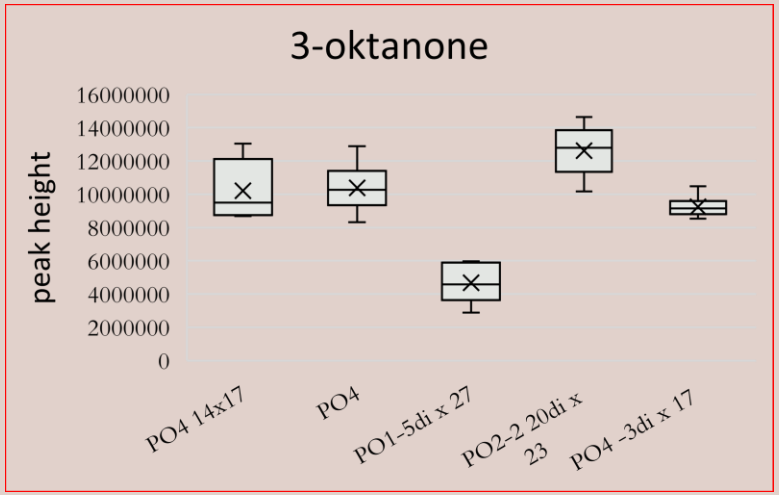
P. ostreatus toxic knobs on PDA medium without nematode *C. elegans* presence (scale 0-2)

How *Pleurotus ostreatus* fights against nematodes?

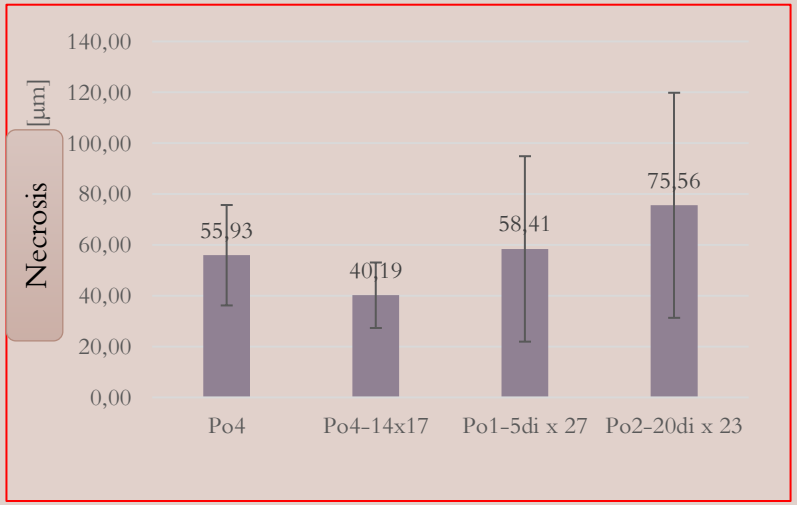


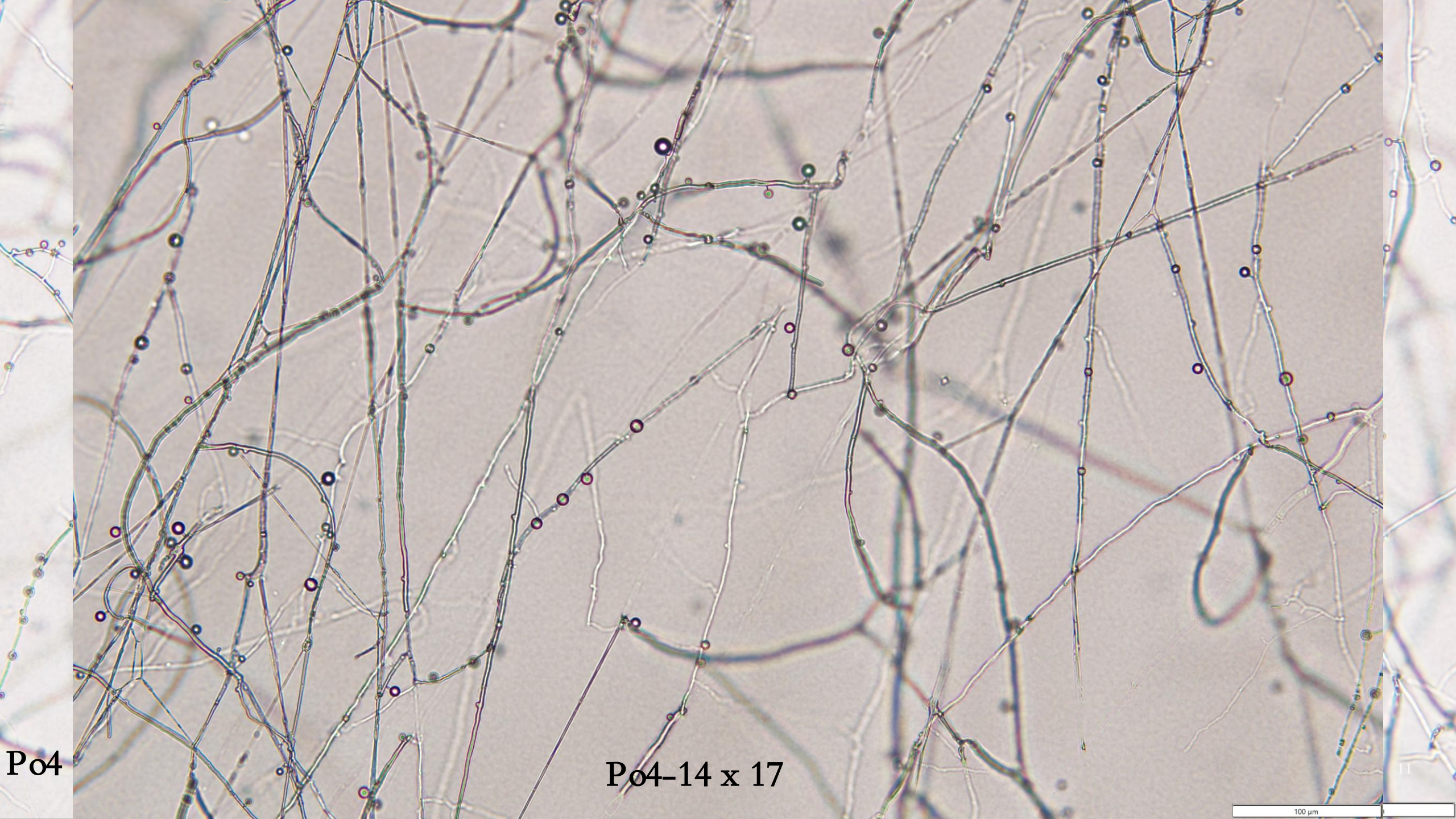






Retention time [min]	Compound	LRIexp	LRIref	Peak surface [%]
17.72	1-Okten-3-ol	986	980	21.83
17.95	3-Oktanon	993	985	8.47
17.64	1-Okten-3-on	984	978	trace
20.46	trans-2-Oktenal	1063	1060	trace
20.75	trans-2-Okten-1-ol	1071	1067	2.33



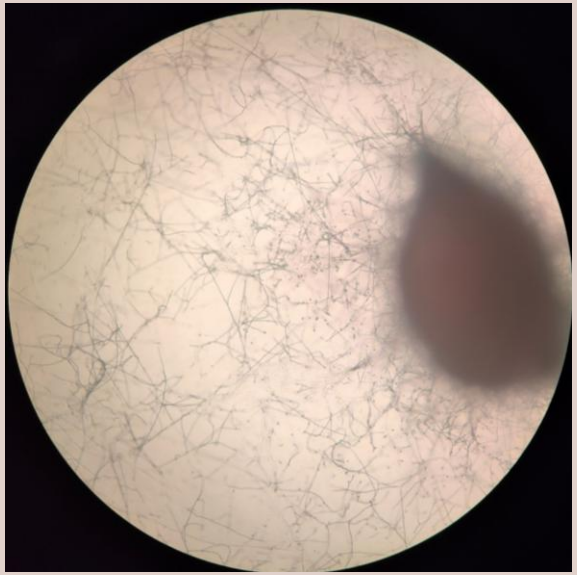

Po4
Po4–14 x 17
100 µm

How Pleurotus ostreatus fights against nematodes?

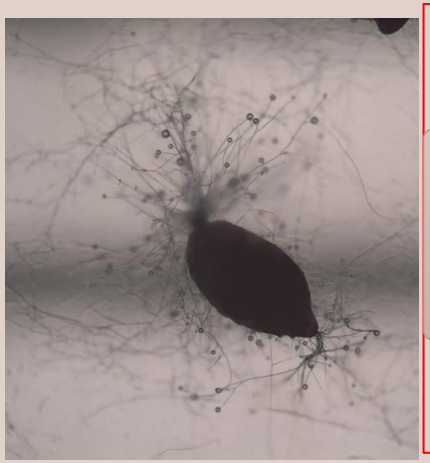
Mechanisms:

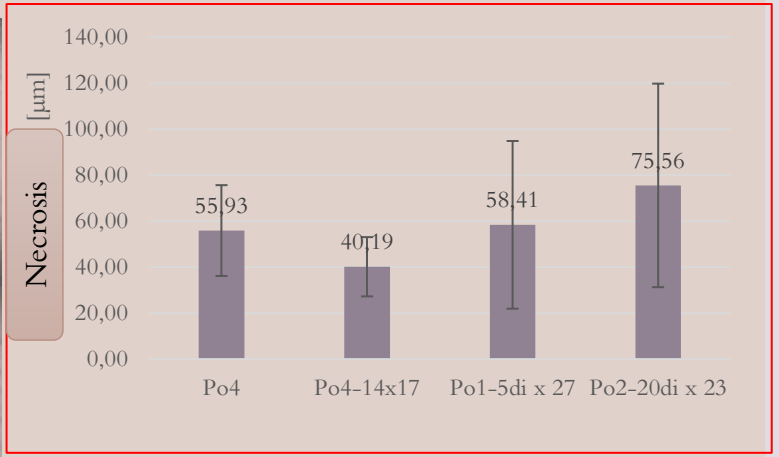
1. Overgrowing

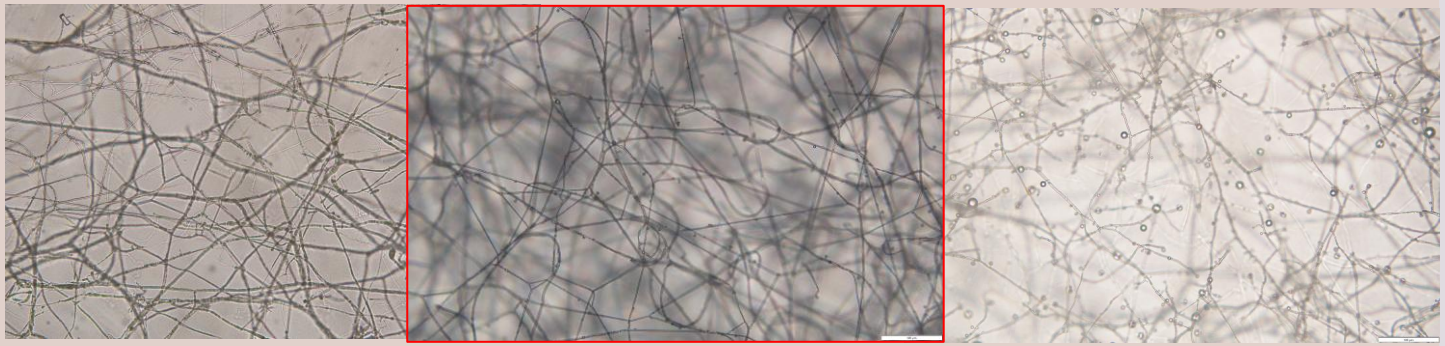
2. Paralyzing



A cyst of *Heterodera schachtii*



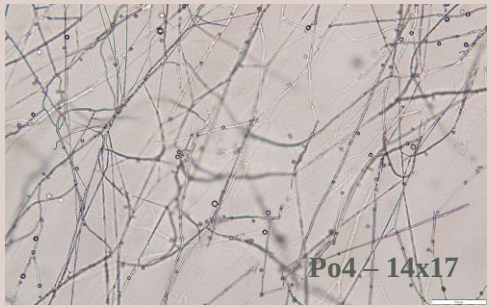


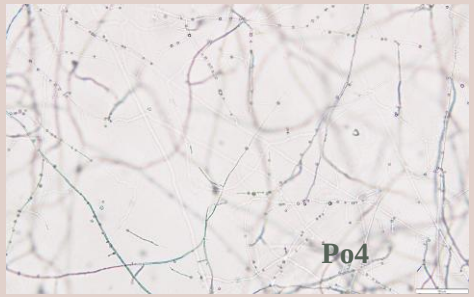


Po4-3di x 17

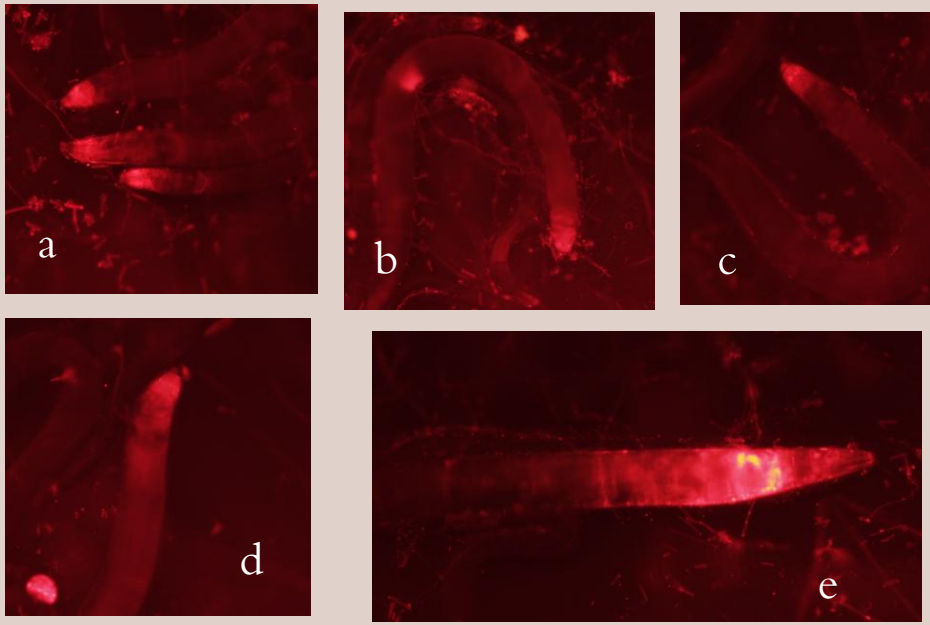
Po1-5di x 27

Po2 -20di x 23





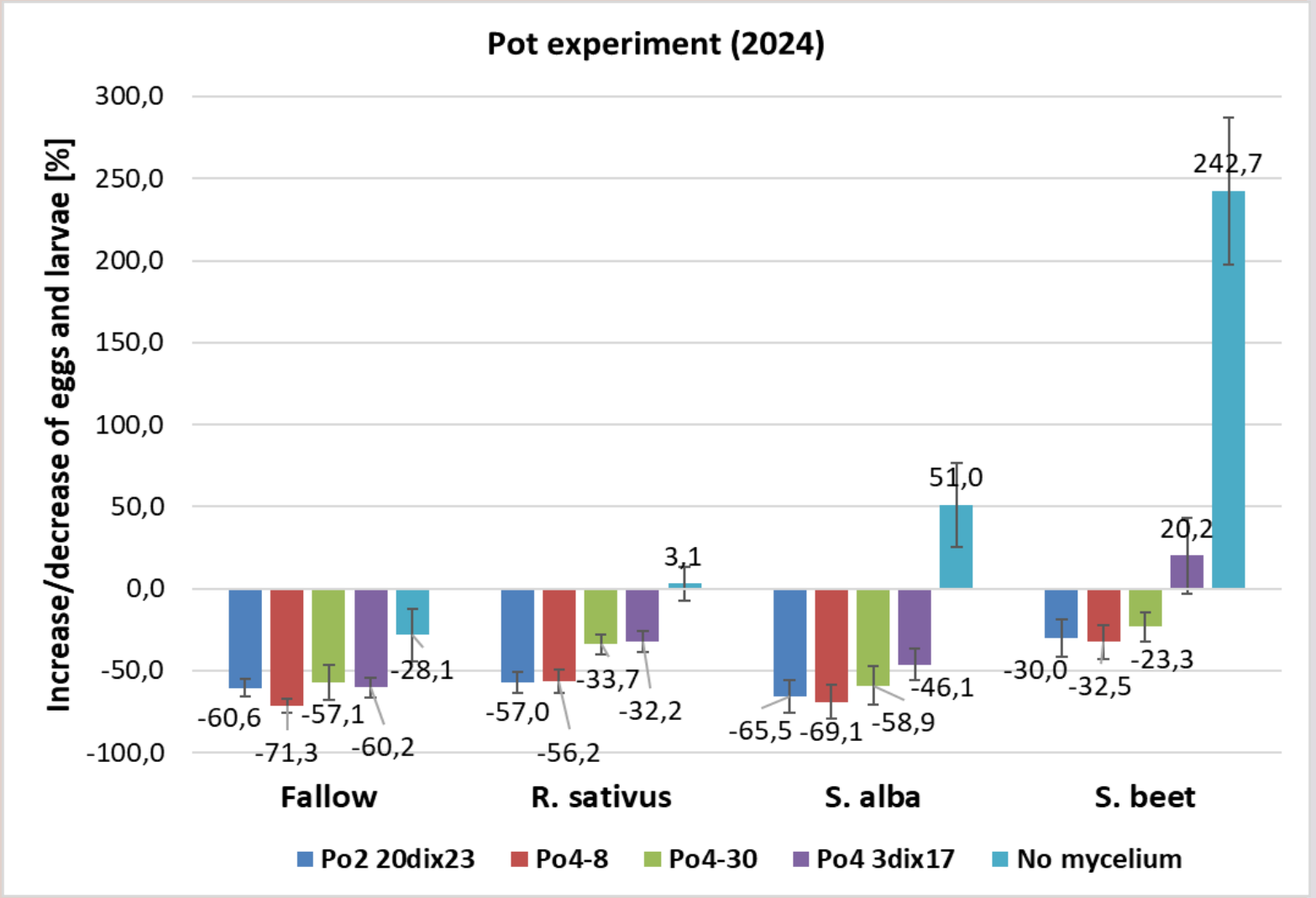
Toxocysts/hyphal knobs on mycelia



Necrosis of *C. elegans* under the *P. ostreatus* influence: a,b,c - Po4-14x17; d – Po4; e – Po1-5di x 27

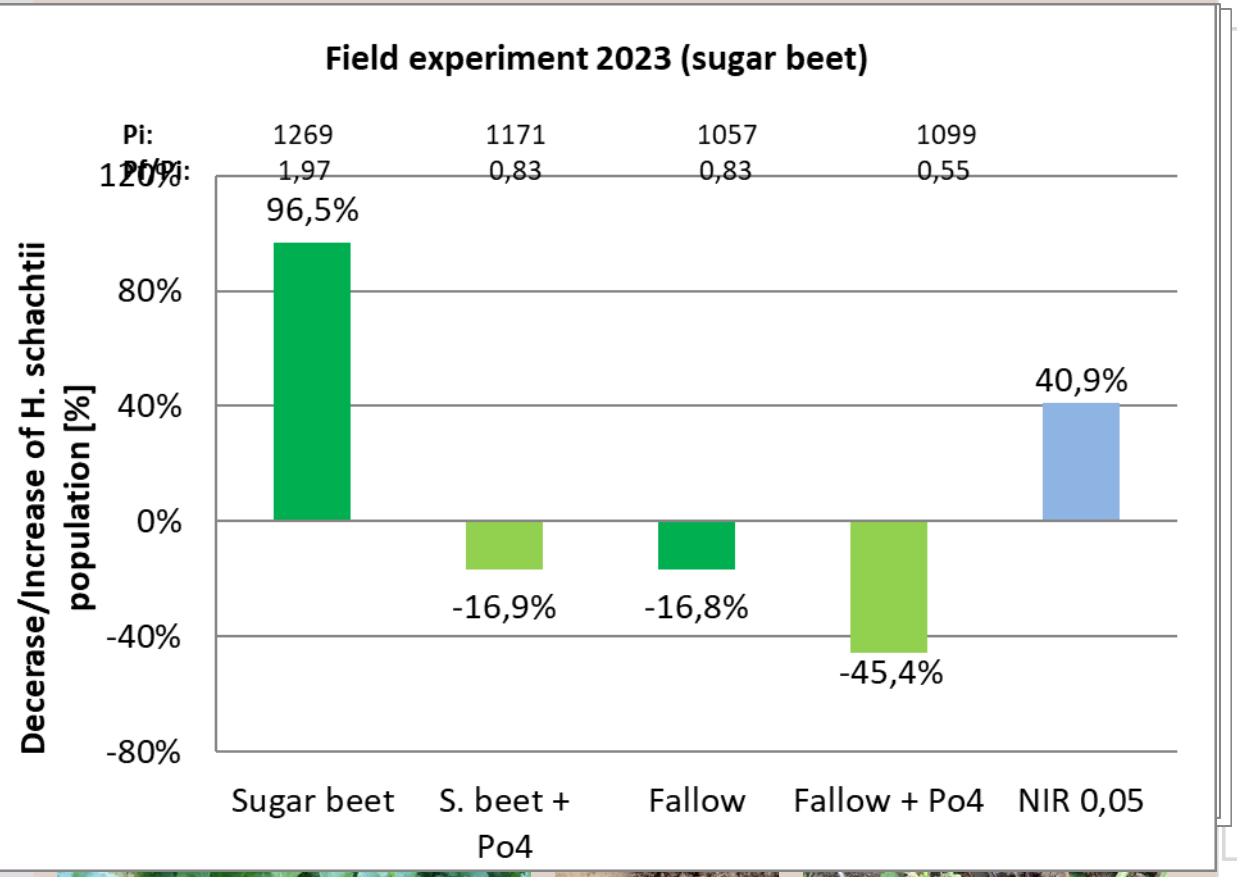
Pot and field g

Pot experiments



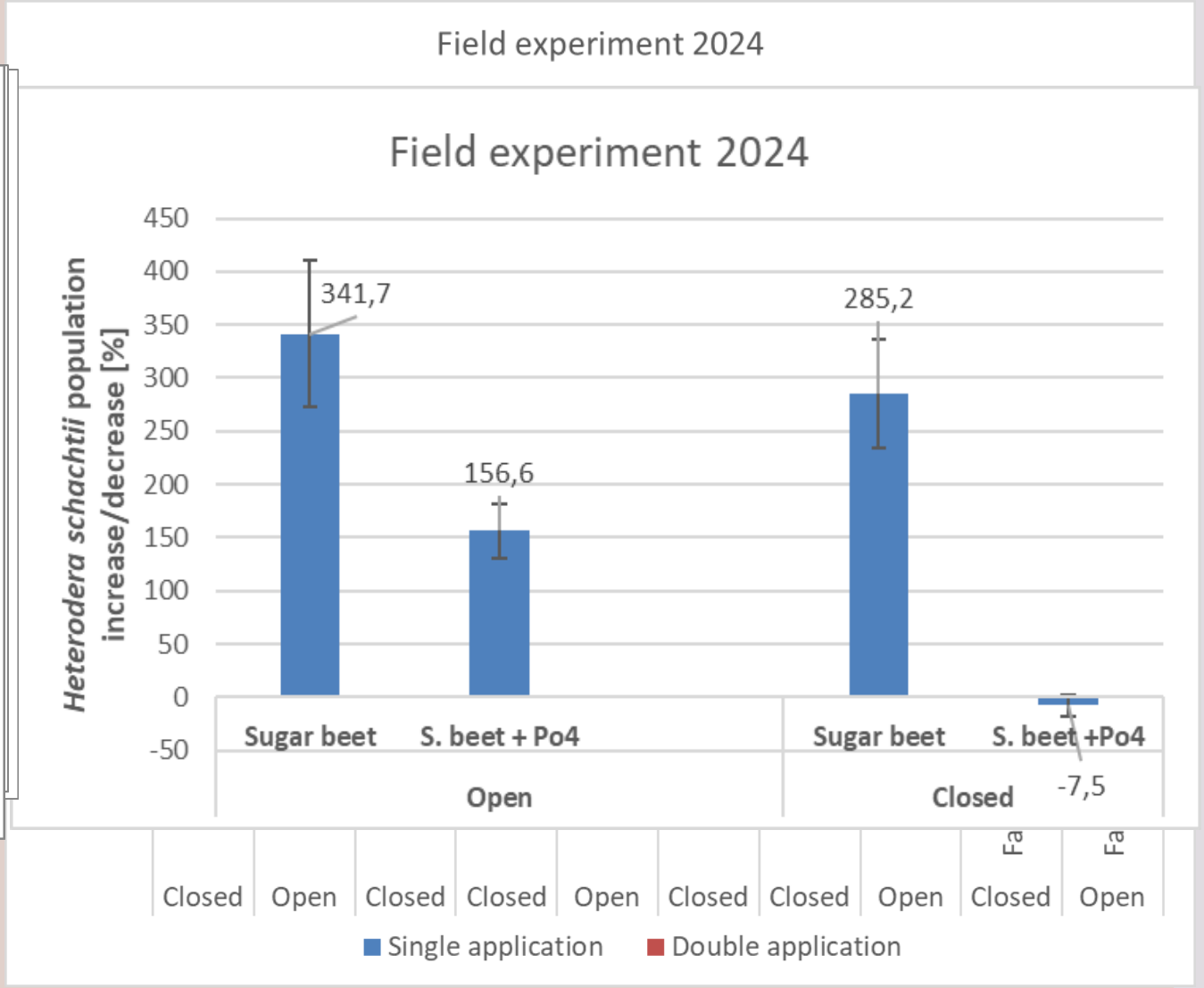
The pot experiment – top: filamentous mycelium of *P. ostreatus* is visible on the soil surface in the pot experiment – confirmation of correct inoculation

Field experiments



Fruits of *Pleurotus ostreatus* among the intercrop *R. sativus* (X.2024)

Symptoms of *H. schachtii* infection on sugar beet root (X.2024)



In 2024 experiments were realized in breeding tents, among which some were covered with foil (Closed), some were not covered (Open).

Crop quality under the mycelium influence

No negative impact of oyster mushroom mycelium on the yield of oil radish and white mustard was observed.

Table 1. Sugar beet yield characteristics

Crop option	Roots	Leaves	Canopy coefficient	Sugar content	Biological sugar yield	K	Na	α-N	Technological sugar content	Technological sugar yield
	[t·ha^{-1}]			[%]	[t·ha^{-1}]	[mmol·kg^{-1}]			[%]	[t·ha^{-1}]
Sugar beet	98,8	33,4	0,34	16,08	15,9	40,6	6,38	16,53	14,04	13,87
Sugar beet + Po4	106,3	43,0	0,40	15,30	16,3	43,1	8,38	16,15	13,21	14,05

Conclusions

Mycelia of *Pleurotus ostreatus* show different levels of nematode killing activity – it is worth choosing the most active ones for further use.

Although the mode of killing ability of *Heterodera schachtii* and *Caenorhabditis elagans* by *Pleurotus ostreatus* mycelia is different, this research presents an interesting way of biological control of phytopathogenic nematodes, independently of their type (free-living or cyst-nematodes).

In our opinion, a final mode of *Pleurotus ostreatus* toxic activity is not defined only by the 3-octanone production, however, 3-octanone is an important toxic compound, and it is probably supported by 1-octen-3-ol.

Mycelia of *Pleurotus ostreatus* present a good alternative for chemical control of phytopathogenic nematodes.