Potential of *Trichoderma* spp. and *Pinus sylvestris* L. bark extracts as biocontrol agents against fungal pathogens residing in the *Botryosphaeriales*

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Starting hypothesis:
- Waste as a resource and source -

Starting material:
- Scots pine (*Pinus sylvestris* L.) bark -

Sawmill industry as a bark waste generator

Bark as a resource:

- Substrate formulations, soil conditioners, plant protection, health and industrial products; application in bioremediation;
- Eco-friendly alternatives to agrochemicals.

Bark as a source:

- Secondary metabolites;
- Beneficial microorganisms;
- *Trichoderma* sp.

Starting material:
- Scots pine (*Pinus sylvestris* L.) bark -

Coniferous bark as a component of commercial substrates

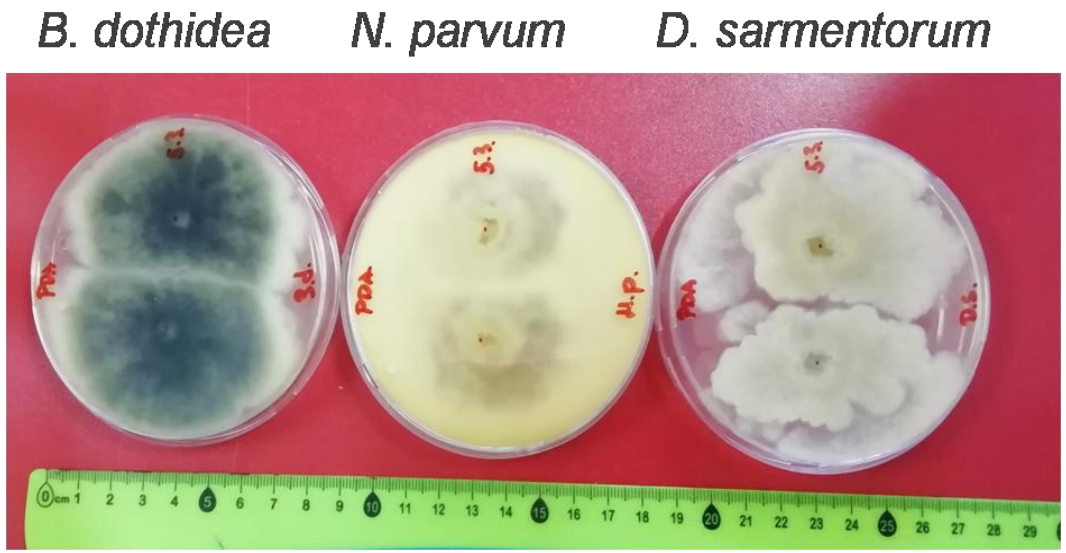
- Increases water supply;
- Increases nutrients availability;
- Antifungal, antibacterial, insecticidal effects

  are results of:

1. Chemical components;
2. Microbial communities.

Objectives:

- ❖ Isolation of *Trichoderma* spp. from Scots pine bark;
- ❖ Evaluation of the antifungal activity of Scots pine bark water extracts;
- ❖ Evaluation of the antifungal activity of *Trichoderma* spp. *in vitro*.



Plant pathogens (Ascomycota: *Botryosphaeriales*)

- *Botryosphaeria dothidea*;
- *Dothiorella sarmentorum*, and
- *Neofusicoccum parvum*.

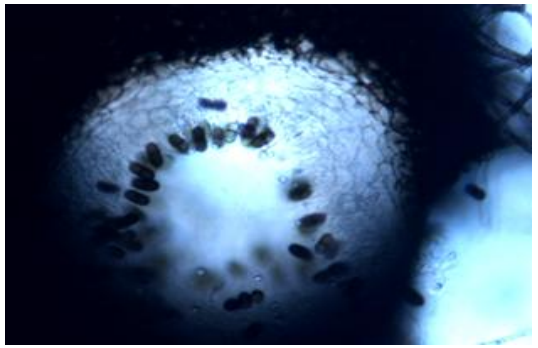
Starting material:
- *Botryosphaeriales* -

Botryosphaeriales (Ascomycota)

- Distributed worldwide on various tree species;
- Endophytes, latent pathogens, pathogens and saprophytes;
- Urban environments.

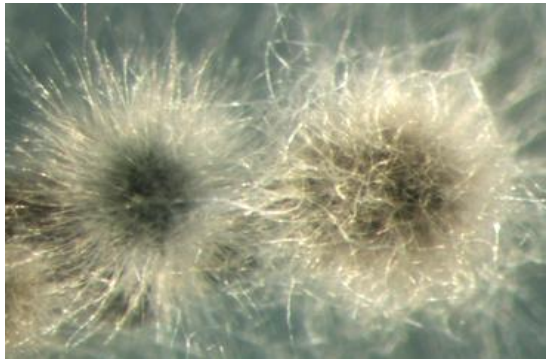
B. dothidea

Branch die-back of *Pseudotsuga menziesii*

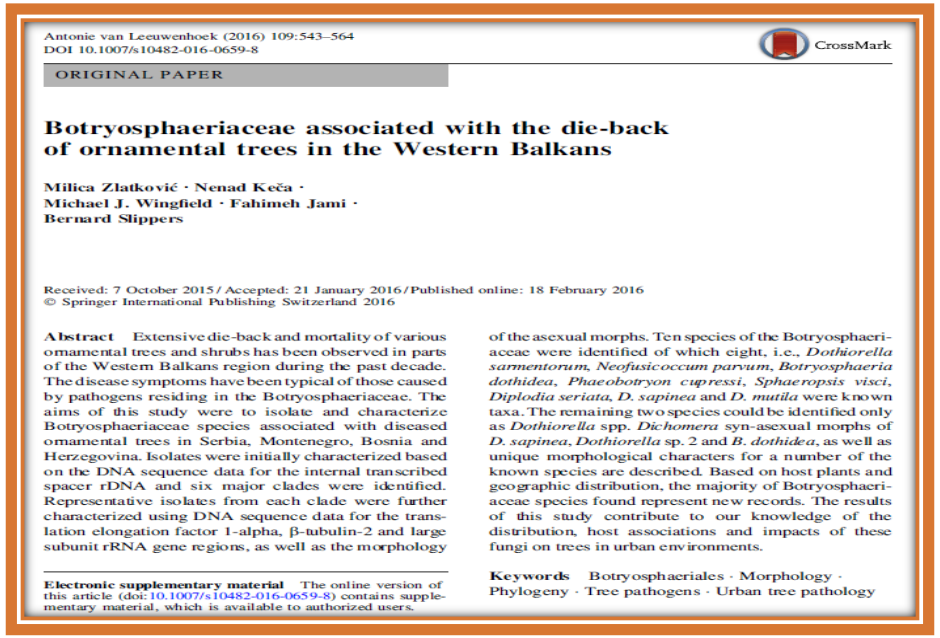


D. sarmentorum

Canker- *Pseudotsuga menziesii*



N. parvum



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CrossMark

ORIGINAL PAPER

Botryosphaeriaceae associated with the die-back of ornamental trees in the Western Balkans

Milica Zlatković · Nenad Keča · Michael J. Wingfield · Fahimeh Jami · Bernard Slippers

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Abstract Extensive die-back and mortality of various ornamental trees and shrubs has been observed in parts of the Western Balkans region during the past decade. The disease symptoms have been typical of those caused by pathogens residing in the Botryosphaeriaceae. The aims of this study were to isolate and characterize Botryosphaeriaceae species associated with diseased ornamental trees in Serbia, Montenegro, Bosnia and Herzegovina. Isolates were initially characterized based on the DNA sequence data for the internal transcribed spacer rDNA and six major clades were identified. Representative isolates from each clade were further characterized using DNA sequence data for the translation elongation factor 1-alpha, β-tubulin-2 and large subunit rRNA gene regions, as well as the morphology of the asexual morphs. Ten species of the Botryosphaeriaceae were identified of which eight, i.e., *Dothiorella sarmentorum*, *Neofusicoccum parvum*, *Botryosphaeria dothidea*, *Phaeobotryon cupressi*, *Sphaeropsis visci*, *Diplodia seriata*, *D. sapinea* and *D. mutila* were known taxa. The remaining two species could be identified only as *Dothiorella* spp. *Dichomera* syn-asexual morphs of *D. sapinea*, *Dothiorella* sp. 2 and *B. dothidea*, as well as unique morphological characters for a number of the known species are described. Based on host plants and geographic distribution, the majority of Botryosphaeriaceae species found represent new records. The results of this study contribute to our knowledge of the distribution, host associations and impacts of these fungi on trees in urban environments.

Electronic supplementary material The online version of this article (doi:10.1007/s10482-016-0659-8) contains supplementary material, which is available to authorized users.

Keywords Botryosphaeriales · Morphology · Phylogeny · Tree pathogens · Urban tree pathology

Starting material:
- *Botryosphaeriales* -

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ORIGINAL ARTICLE

WILEY Forest Pathology

Host specificity of co-infecting Botryosphaeriaceae on ornamental and forest trees in the Western Balkans

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Summary
The Botryosphaeriaceae is a diverse family of endophytes and fungal pathogens of mainly woody plants. We considered the host range and distribution of these fungi by sampling diseased ornamental and forest trees and shrubs in Serbia, Montenegro, Bosnia and Herzegovina, spanning a Mediterranean and a Continental climatic region. In total, ten Botryosphaeriaceae species were identified in the Western Balkans and with the exception of *Sphaeropsis visci* and *Phaeobotryon cupressi*, which occurred on one host, all the species had a broader host range. *Phaeobotryon cupressi* was found only in the Mediterranean region and *S. visci*, *Dothiorella* sp., *Dothiorella sarmentorum* and *Diplodia seriata* were present only in the Continental region. Pathogenicity tests were conducted on a variety of hosts from which the Botryosphaeriaceae species were isolated. These included leaves and/or stems of seedlings of 21 hosts, and cut leaves and/or branches of six hosts. Moreover, stems of seedlings of *Chamaecyparis lawsoniana*, *Cedrus deodara*, *Picea omorika*, *Pinus patula* and *Eucalyptus grandis* were inoculated as hosts from which some or all of the Botryosphaeriaceae species used for inoculation were not isolated. Inoculations showed that the majority of these fungi could also co-infect hosts other than those from which they were isolated. The results suggest that most of the species have broad host ranges and can potentially cause disease on a broad range of tree species under certain conditions.

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SHORT COMMUNICATION

WILEY Forest Pathology

Shot hole disease on *Prunus laurocerasus* caused by *Neofusicoccum parvum* in Serbia

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Summary
Between 2010 and 2014, symptoms of a shot hole disease were observed on cherry laurel (*Prunus laurocerasus* L.) trees and shrubs in parks and other public plantings in Belgrade, Serbia. Ten symptomatic leaves were collected from each of the diseased plants and the associated fungus isolated and identified using multigene phylogenetic analyses and asexual morphological characters. The pathogen was identified as *Neofusicoccum parvum*. The same symptoms were produced when the pathogen was inoculated on test plants. To the best of our knowledge, this is the first report of *N. parvum* causing shot hole disease on *P. laurocerasus*.

Prunus laurocerasus shot hole disease

Material and methods

Extracts preparation:

Bark was:

- Ground with a mill;
- Shifted through 0.5 mm sieve;
- Dried at 50ºC.

Solvent:

- Distilled water;
- Bark powder : solvent ratio 1:10.

The extraction conditions:

Solvent	Distilled water	Distilled water
Temperature regime	80	120
Pressure	/	1.5 bar
Extraction Time	120 min	20 min

Extraction yields:

- 4.10% at 80°C;
- 5.14% at 120°C.

Material and methods

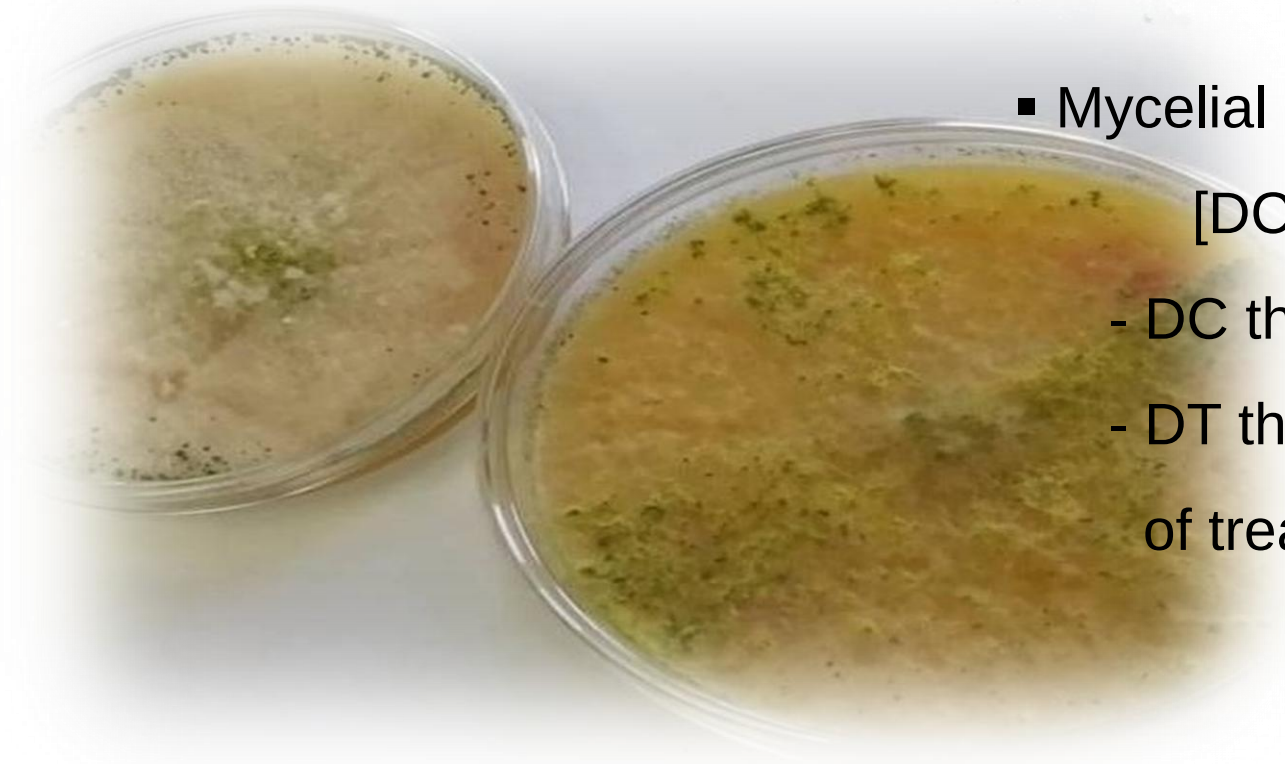
Antifungal activity assay of chemical components of pine bark

- PDA supplemented with 20 and 30% of water bark extracts;
- The effects on *Trichoderma* spp. and plant pathogens mycelial growth;
- Mycelial growth inhibition (%) =

$$[DC- DT] / DC] \times 100$$

- DC the average diameters of control;
- DT the average diameters of fungal colony of treatment.

Material and methods



Antifungal activity assay of biological component of pine bark

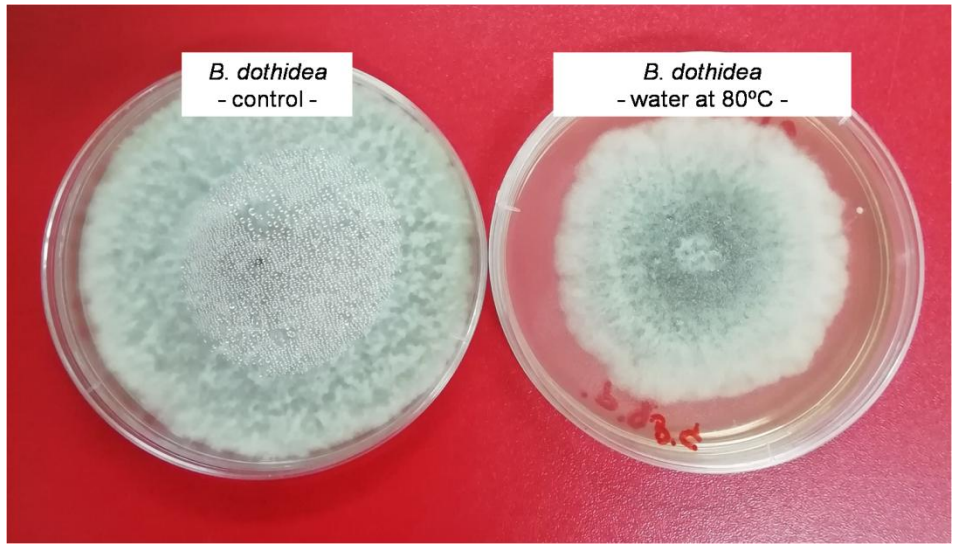
- The effects of *Trichoderma* spp. on plant pathogens mycelial growth;

- Mycelial growth inhibition (%) =

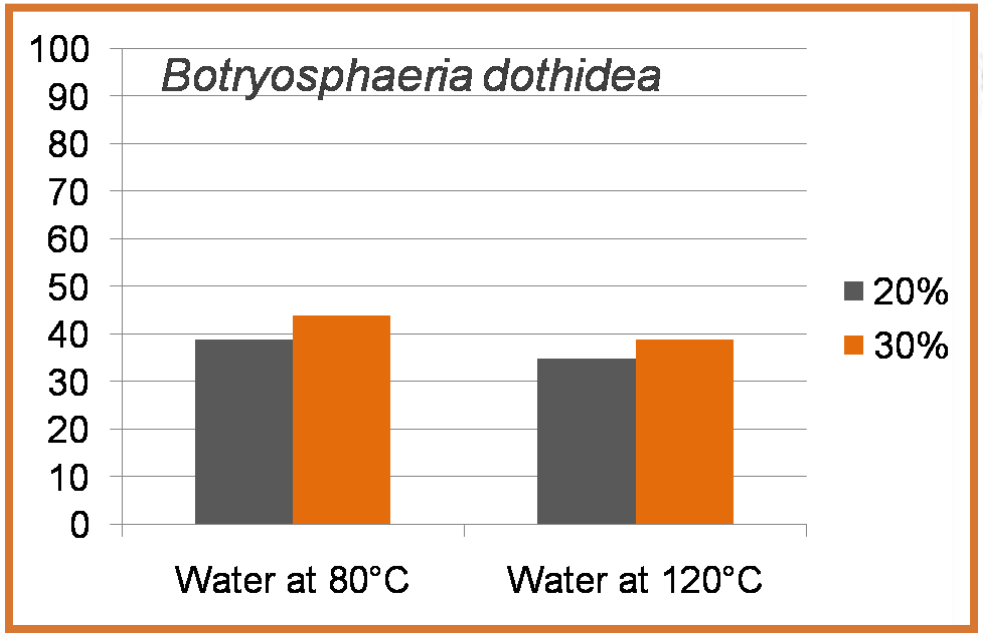
  [DC- DT] / DC] × 100

  - DC the average diameters of control;
  - DT the average diameters of fungal colony of treatment.

Results
- Antifungal activity -

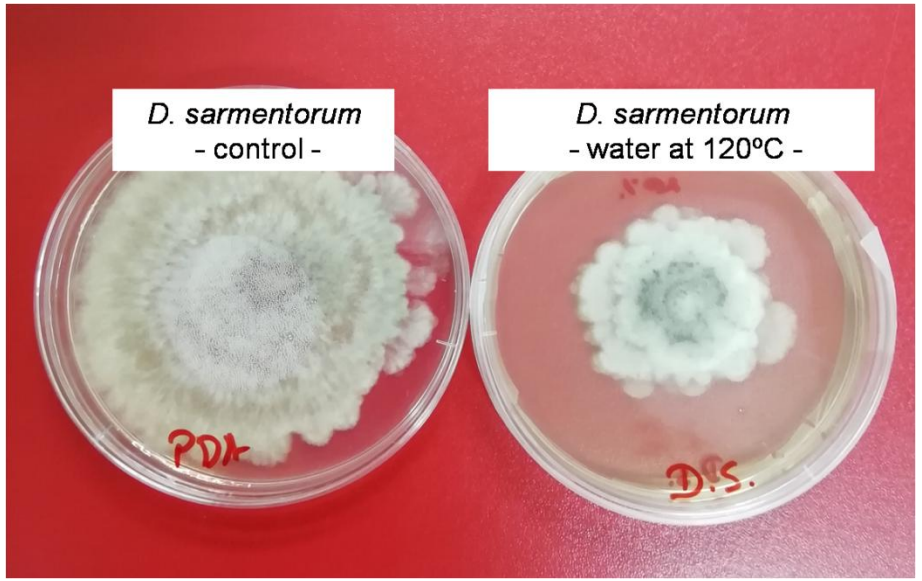


Mycelia growth inhibition by pine bark water extracts (%)

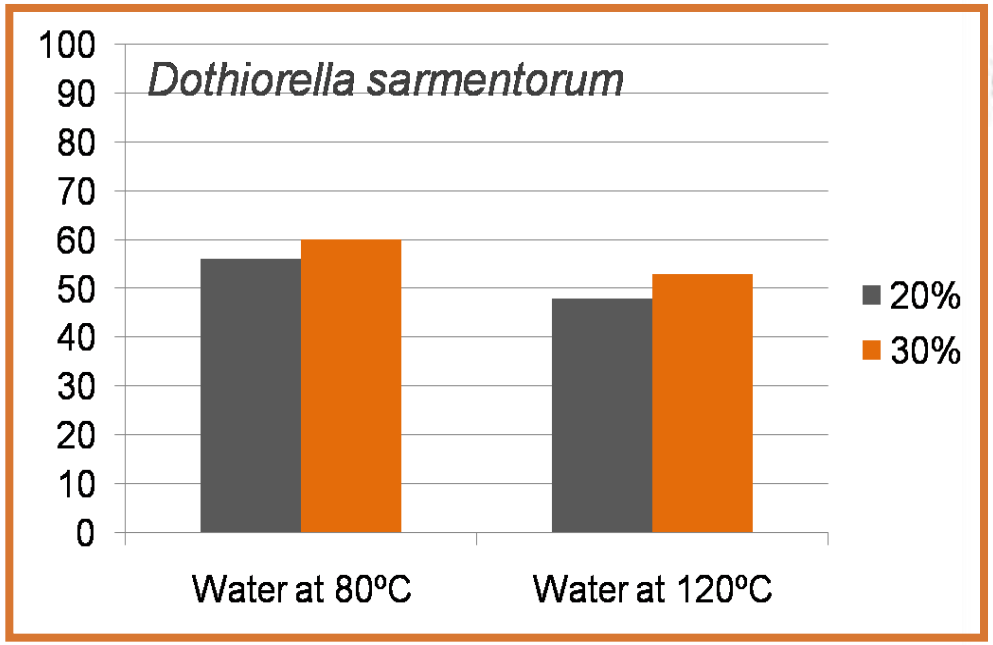


- Little toxic;
- The highest inhibition was 39% in 20% extract;
- The highest inhibition was 44% in 30% extract.

Results
- Antifungal activity -



Mycelia growth inhibition by pine bark water extracts (%)



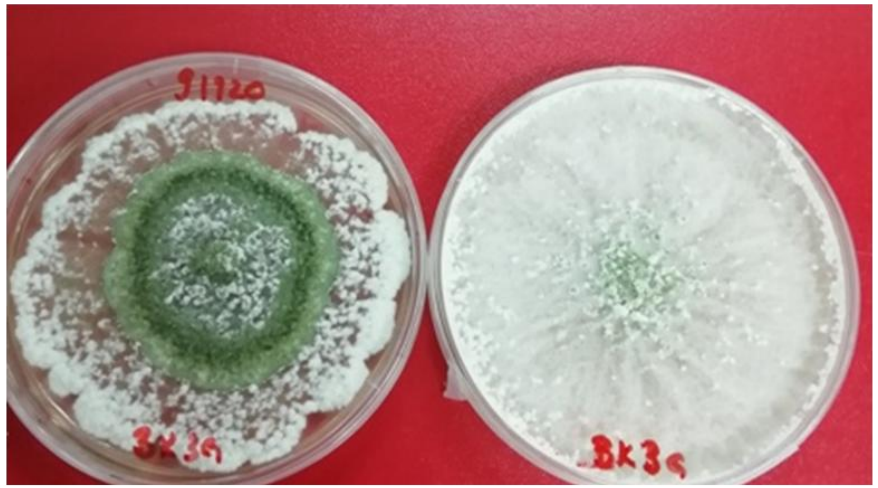
- Moderate toxic;
- The highest inhibition was 56% in 20% extract;
- The highest inhibition was 60% in 30% extract.

Results
- Antifungal activity -

Mycelia growth inhibition by pine bark water extracts (%)

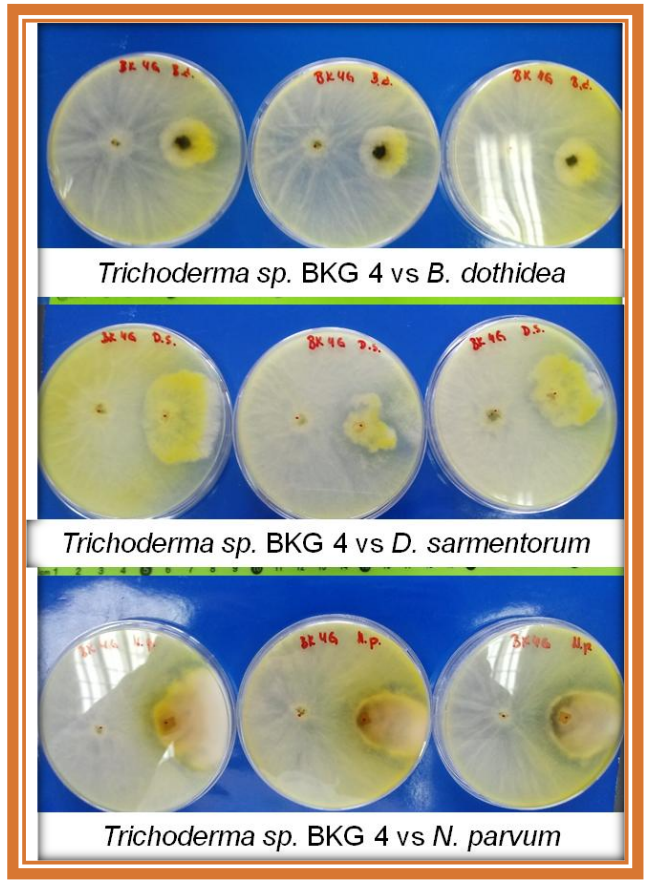
No inhibition of radial growth was noted.

- *Neofusicoccum parvum* - sparse aerial mycelium;
- *Trichoderma* sp. BKG 3 - increased sporulation;
- *Trichoderma* sp. BKG 4 - increased sporulation.

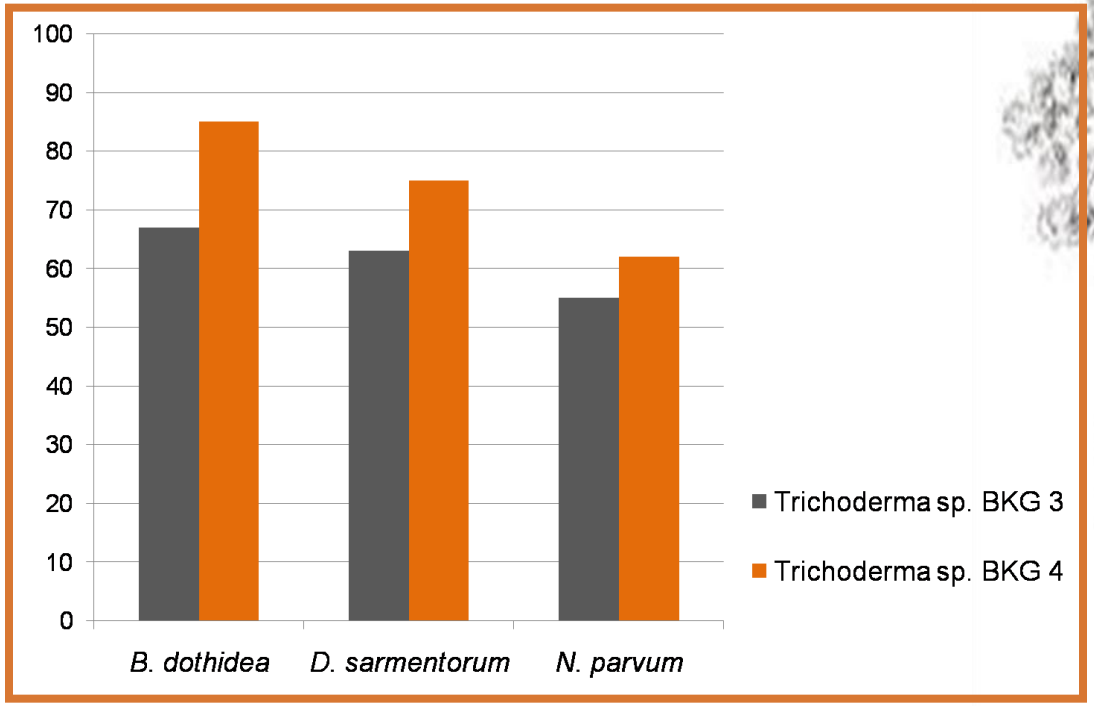


Water at 120 °C *Trichoderma* BKG 3

Results
-*Trichoderma* spp. *vs* pathogens -



Mycelia growth inhibition by *Trichoderma* spp. (%)

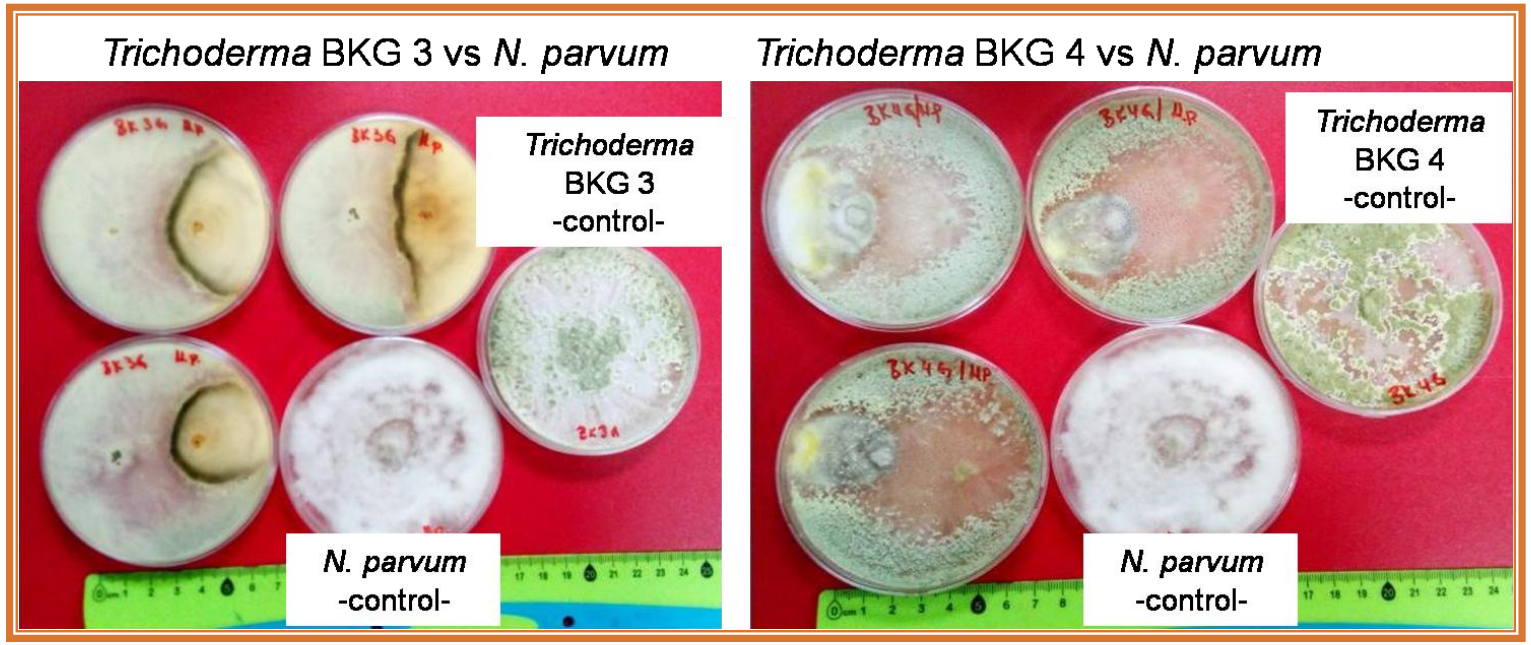


- *Trichoderma* sp. BKG 4 expressed very high inhibition of *B. dothidea* and *D. sarmentorum*;
- Overgrow with replacement of *B. dothidea* and *D. sarmentorum.*

Results
-*Trichoderma* spp. *vs* pathogens -

Mycelia growth inhibition by *Trichoderma* sp.

- All *Trichoderma* strains exhibited moderate inhibition of *N. parvum*;
- Deadlock at a distance;
- The accumulation of dark pigment.

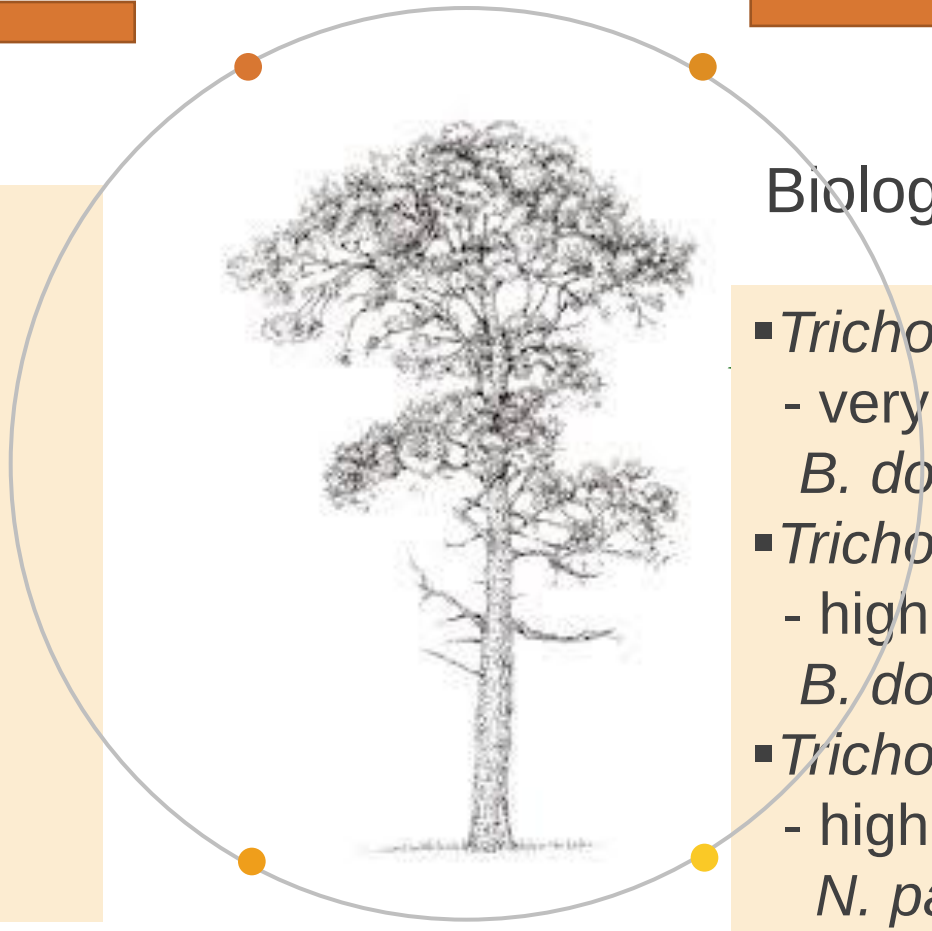


Conclusions

Pine bark as a substrate:
antifungal, antibacterial, insecticidal effects

Chemical component:

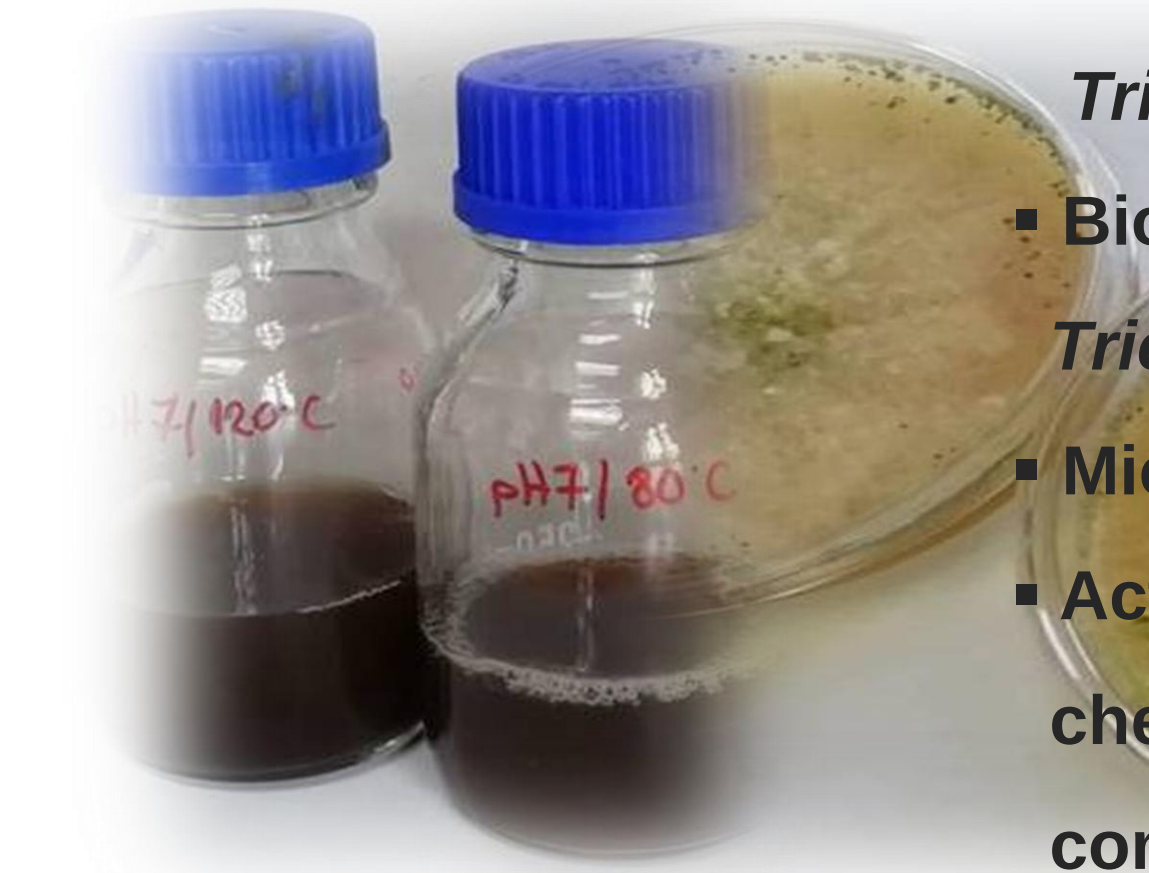
- Bark extracts showed:
  - Little toxic effects on *B. dothidea*;
  - moderately toxic effects on *D. sarmentorum*;
  - *non-toxic effects on N. parvum and Trichoderma spp.*



Biological component:

- *Trichoderma* BKG 4:
  - very high antagonistic activity vs *B. dothidea* and *D. sarmentorum*;
- *Trichoderma* BKG 3:
  - high antagonistic activity vs *B. dothidea* and *D. sarmentorum*;
- *Trichoderma* sp. BKG 4:
  - high antagonistic activity vs *N. parvum.*

Conclusions



The next steps:

- **Extraction optimization;**
- **Molecular identification of *Trichoderma* strains;**
- **Biochemical characterization of *Trichoderma* strains;**
- **Microscopic interactions;**
- **Active matter detection in both, chemical and biological component of pine bark.**


Thank you for the attention