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CHANGES IN MORPHOLOGY AND GROWTH RATE OF *FUSARIUM SOLANI* COLONIES EXPOSED TO VOLATILE COMPOUNDS SYNTHESIZED BY WOOD-ROTTING BASIDIOMYCETES

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Abstract: This study aims to determine the effects of the volatile metabolites synthesized by 53 species of wood-rotting basidiomycetes on the morphology and growth rate of *Fusarium solani* colonies. The fungi were cultivated in bi-compartmented Petri dishes. For every combination 4 different plates were prepared as well as a control Petri dish containing only *Fusarium solani*. The species were cultivated on PFMEA (potato flakes malt extract agar) and kept for 5 days at 25°C and further, the test plates were compared with the control, regarding the general aspect of *Fusarium solani* colony, pigmentation and differences in growth rate. The observations revealed that the volatiles synthesized by 42 species of wood-rotting basidiomycetes evidently influenced the development of the phytopathogenic species. The volatiles of *Neofavolus alveolaris* inhibited the most the growth of *Fusarium solani*. The GC-MS analysis of the volatile profile of *Neofavolus alveolaris* revealed the presence of compounds such as: 3-methyl-3-buten-1-ol, 2-methyl-1-butanol, 2-methyl-1-propanol, 3-methyl-1-butanol, 1-octen-3-ol and benzaldehyde.

Keywords: *Fusarium solani*, growth inhibition, morphological changes, volatile metabolites, wood-rotting basidiomycetes.

Introduction

Fungi are a group of organisms that managed to developed specific adaptations which allow them to colonize different habitats and use trophic resources unavailable or hardly accessible to other species. Wood-rotting basidiomycetes form a particular ecological and functional group of higher fungi that due to an impressive enzymatic system can break down complex and resistant polymers such as cellulose or lignin [SCHMIDT, 2006]. In order to colonize a substrate, wood-rotting basidiomycetes must compete with other species. For that, they developed different repellent strategies, many of them involving the synthesis and secretion of secondary metabolites with antimicrobial properties that chemically signal the presence of a species on a certain substrate and inhibit the growth and development of other organisms.

The unique properties of the fungal enzymes and secondary metabolites are harnessed in various biotechnological processes [LORENZEN & ANKE, 1998], in the pharmacological industry (antibiotics, antibacterial and antifungal compounds, immunostimulators, antioxidants), cosmetics and perfumery (especially alcohols and terpenes) and agriculture (biopesticides). One particular class of secondary metabolites produced by wood-rotting basidiomycetes includes volatile organic compounds (VOC),

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lipophilic molecules that can easily penetrate the cellular membranes inhibiting, stimulating or disrupting the biological processes [WHEATLEY, 2002]. Due to their volatility, these metabolites have a wide range of action permitting the information to be precisely and exactly sent at various distances. The quantity and quality of these volatiles depends on several factors, including: media composition and pH [WHEATLEY, 2002], temperature and water content [TRONSMO & DENNIS, 1978], presence of other organisms [HYNES & al. 2006], age and stage of development [FÄLDT & al. 1999; LEE & al. 2015]. Fungal volatiles are not produced as sole molecules, but as complex mixtures of alcohols, ketones, aldehydes, terpenes, esters with various ecological functions [HUNG & al. 2015], such as intra- and inter-specific communication or insect, mites or herbivores attractants or repellents [JONSELL & NORDLANDER, 1995; RUESS & LUSSENHOP, 2005; BODDY & JONES, 2008; THAKEOW & el. 2008; DRILLING & DETTNER, 2009; ROHLFS & CHURCHILL, 2011].

This study aims to identify how the volatiles synthesized by 53 species of wood-rotting basidiomycetes influence the morphology and growth rate of *Fusarium solani* colonies. Moreover, after establishing which species of basidiomycetes inhibits the most the phytopathogen's growth, using a GC-MS analysis, the basidiomycete's volatile profile will be determined. By evaluating the inhibitory potential of the volatile metabolites, it is possible to understand the functioning mechanism of these compounds and finding the right circumstances in which they are produced and exhibit their maximum activity.

Material and methods

Fungal strains

The 53 species of wood-rotting basidiomycetes used in this study were collected from Romanian natural habitats and isolated within the Research Laboratory for Fungi with application in ecological reconstruction, Faculty of Biology, "Alexandru Ioan Cuza" University of Iaşi and are now part this laboratory's scientific collection. The phytopathogenic species *Fusarium solani* (Mart.) Sacc. was isolated from potato (*Solanum tuberosum*) tubercles. All fungi are maintained on malt extract agar 30% at 4 ºC.

Fungal screening

The effects of the volatile metabolites synthesized by the species of wood-rotting basidiomycetes on *F. solani* colonies were observed using the bi-compartmented Petri dishes in such way that the two mycelia didn't come into contact and the results was only due to the volatile compounds, as described in previous papers [PETRE & al. 2017]. The medium used in the screening activity was PFMEA (potato flakes malt extract agar): 20 $g \times l^{-1}$ potato flakes, 5 $g \times l^{-1}$ malt extract, 5 $g \times l^{-1}$ glucose, 15 $g \times l^{-1}$ agar [PETRE & al. 2017].

Every species of wood-rotting basidiomycete was inoculated in one compartment of the plate and the plant pathogen in the other. The plates were wrapped in two layers of Parafilm and incubated in the dark at 25 ºC, for 5 days. Four replicates were used for every combination. The control plate contained only the plant pathogenic species inoculated in one of the compartments. After 5 days, the test plates were compared with the control regarding the general morphology of *F. solani* colonies, pigmentation and inhibition of fungal growth. The inhibitory percentage was calculated for every plate: $IP=[C-T] \times 100/C$, where C represents the diameter of the control colony and T represents the diameter of the colony exposed to the VOC synthesized by the test fungi [NIDIRY & BABU, 2005]. The

medium inhibitory percentage (IP_{med}) was calculated as the average value of all four replicates inhibitory percentages. The first three species with the highest inhibition percentage were further tested on two different media: MEA (malt extract agar): 30 $g \times l^{-1}$ malt extract, 20 $g \times l^{-1}$ agar and KM media: 20 $g \times l^{-1}$ glucose, 2 $g \times l^{-1}$ peptone, 2 $g \times l^{-1}$ yeast extract, 0.25 $g \times l^{-1}$ KH_2PO_4; 0.25 $g \times l^{-1}$ $MgSO_4 \times 7\ H_2O$ [KAWABE & MORITA, 1993] in which 20 $g \times l^{-1}$ agar were added. This step was performed in order to determine if the media composition is influencing the production of antifungal volatiles. The species with the highest IP was selected for the analysis of volatile compounds produced *in vitro*.

Solid-Phase Extraction

Neofavolus alveolaris, species which showed the highest IP when tested *in vitro* was cultivated on a liquid medium containing 20 $g \times l^{-1}$ glucose, 2 $g \times l^{-1}$ peptone, 2 $g \times l^{-1}$ yeast extract, 0.25 $g \times l^{-1}$ KH_2PO_4; 0.25 $g \times l^{-1}$ $MgSO_4 \times 7\ H_2O$ [KAWABE & MORITA, 1993] and incubated in the dark at 25 ºC. After 25 days the surface culture of the wood-rotting basidiomycete was homogenized; 10 ml of homogenate was filtered and after mixed with 20 ml of pure water and 1 µl of 4-hydroxy-4-methyl-2-pentanone was added as an internal standard. The mixture was extracted on LiChrolut cartridges-EN (40-120 µm) 100 mg (bottom); RP-18 (40-63 µm) 200 mg (top) (Merck Millipore). These cartridges were previously conditioned with 2×6 ml *n*-hexane, 2×6 mL dichloromethane, 2×6 ml acetone, 2×6 ml methanol and 2×6 ml pure water allowing each solvent to pass completely before adding the next conditioning solvent. On the same filter used for the homogenate, 10 ml of pure water mixed with 1 g NaCl, 1 g Na_2SO_4 and 1 g of KH_2PO_4 were also passed in order to increase the ionic strength, thus facilitating the extraction of the compounds from the remaining biomass. The filtrate was later passed over the same cartridges. The SPE cartridges were completely dried using compressed air and later placed in a desiccator at 600 mbar for 24 h under a gentle nitrogen flow. Next, the cartridges were eluted with 1.5 ml of *n*-hexane, dichloromethane, acetone and acetonitrile respectively and eluate was further dried on anhydrous sodium sulfate [PETRE & al. 2017]. The extraction experiment was performed in duplicate. The eluents were collected in separate vials and analyzed by gas-chromatography with mass spectrometer detection (GC-MS).

GC-MS analysis

The GC-MS analysis of *N. alveolaris* extracts was done on a Shimadzu GC-MS 2010 equipped with a ZB WAXplus capillary column (10 m × 0.1 mm × 0.1 µm) operated in split mode injection (split ratio 1/10), as described in PETRE & al. (2017): the GC oven temperature was set from 35 ºC for 5 minutes, with an increase of 5 ºC/min to 220 ºC and hold for 5 minutes, with a total analysis time of 47 minutes. Helium was used as carried gas, with a total flow of 15.9 ml/min, column flow of 0.9 ml/min and a purge flow of 6 ml/min. The MS ionization source was operated in electron impact mode (EI) with the EI source temperature set at 200 ºC. The full scan mass-spectrums were acquired at every 0.1 seconds (equivalent with 5000 a.m.u.), between 30-500 Da (m/z).

Results and discussions

For this research 53 species of wood-rotting basidiomycetes were studied in order to determine the effects of their volatile metabolites on the morphology and growth rate of *F. solani* colonies. On the control plate, *F. solani* covered the compartment in 8 days. The colony was heterogeneous with areas where the aerial mycelium was well developed, white and downy, alternating with areas with submersed, pinkish mycelium (Figure 1).

Following the experiment, in 80% of the cases the morphology of the phytopathogen's colony was different from the one in the control plate (Figure 2-6), indicating that the volatile compounds synthesized by the wood-rotting basidiomycetes influenced the development of the mycelium (Table 1).

Moreover, in 9.5% of the cases, the aerial mycelium of *F. solani* was intensively pigmented unlike the control colony and in 30% of the cases, the mycelium was white, lacking the pigmentation.

All these observations underline the relation between the synthesis of volatile compounds and pigments during fungal interactions. The correlation between the productions of these categories of metabolites was recorded by literature [GRIFFITH & al. 1994; BRUCE & al. 2003; WALD & al. 2004; HYNES & al. 2006] and it was described as happening both prior and after the direct contact of the mycelia.

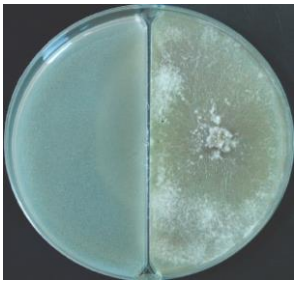

Figure 1. *F. solani* - control plate (PFMEA)

Figure 2. *L. arcularius* - *F. solani* (PFMEA)

Figure 3. *H. fasciculare* - *F. solani* (PFMEA)

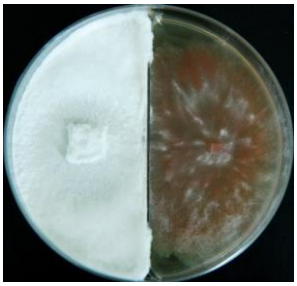

Figure 4. *L. betulinus* - *F. solani* (PFMEA)

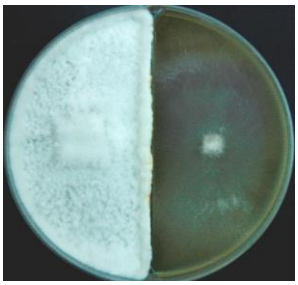

Figure 5. *N. alveolaris* – *F. solani* (PFMEA)

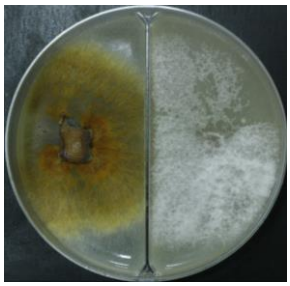

Figure 6. *P. igniarius* - *F. solani* (PFMEA)

Table 1. General aspect of *F. solani* colonies on the test plates

Species	IP_{med}	The morphology of *F. solani* colony on the test plates (PFMEA) compared with the control colony	Pigmentation of *F. solani* colony
Plicaturopsis crispa (Pers.) D.A. Reid	5%	poorly developed, heterogeneous aerial mycelium, submerged mycelium with hyaline hyphae	-
Crucibulum laeve (Huds.) Kambly	1.76%	unchanged	-
Cyathus striatus (Huds.) Willd.	5%	poorly developed, heterogeneous aerial mycelium, submerged mycelium with hyaline hyphae	+
Crepidotus applanatus (Pers.) P. Kumm.	3.23%	mycelium with concentric development: rings of dense, aerial hyphae around the inoculum point, submerged mycelium with marginal hyaline hyphae	+
Megacollybia platyphylla (Pers.) Kotl. & Pouzar	2.64%	homogeneous, lax aerial mycelium	+
Panellus stipticus (Bull.) P. Karst.	2.35%	homogeneous, lax aerial mycelium	+
Gymnopus dryophilus (Bull.) Murill.	2.05%	unchanged	+
Flammulina velutipes (Curtis) Singer	4.41%	unchanged	+
Hymenopellis radicata (Relhan) R. H. Petersen	4.41%	unchanged	+
Mucidula mucida (Schrad.) Pat.	7.64%	aerial mycelium relatively dense around the inoculum point and lax towards the margins	+
Psathyrella candolleana (Fr.) Maire	1.47%	aerial mycelium developed only around the inoculum point, submerged mycelium with hyaline hyphae towards the margins	-
Schizophyllum commune Fr.	4.7%	heterogeneous, lax aerial mycelium, submerged mycelium with hyaline hyphae towards the margins	+
Gymnopilus junonius (Fr.) P.D. Orton	5%	aerial mycelium absent, submerged mycelium with hyaline hyphae	+
Hypholoma fasciculare (Huds.) P. Kumm.	6.76%	well developed, dense aerial mycelium with a ring of hyaline hyphae around the inoculum point	+
Hypholoma lateritium (Schaeff.) P. Kumm.	2.94%	unchanged	+
Pholiota aurivella (Batsch) P. Kumm.	5%	heterogeneous aerial mycelium with concentric development	+
Auricularia mesenterica (Dicks.) Pers.	1.76%	relatively homogeneous, lax aerial mycelium	++
Inonotus hispidus	2.64%	relatively homogeneous, lax aerial mycelium	-

(Bull.) P. Karst.			
Phellinus igniarius (L.) Quél.	5.29%	well developed, heterogeneous aerial mycelium, with lax and dense areas	+
Phellinus pomaceus (Pers.) Maire	5.29%	aerial mycelium dense around the inoculum point and lax towards the margins	+
Daedalea quercina (L.) Pers.	5.58%	heterogeneous, adpressed aerial mycelium	-
Fomitopsis pinicola (Sw.) P. Karst.	5.29%	aerial mycelium with concentric development	+
Fomitopsis betulina (Bull.) B. K. Cui, M. L. Han & Y. C. Dai	3.82%	homogeneous, dense, well developed aerial mycelium	-
Ganoderma adspersum (Schulzer) Donk	2.94%	poorly developed aerial mycelium, submerged mycelium with hyaline hyphae	-
Ganoderma applanatum (Pers.) Pat.	5%	aerial mycelium dense around the inoculum point and lax towards the margins	++
Meripilus giganteus (Pers.) P. Karst.	2.64%	poorly developed aerial mycelium, submerged mycelium with hyaline hyphae	-
Bjerkandera adusta (Willd.) P. Karst.	1.47%	aerial mycelium developed only around the inoculum point, submerged mycelium with hyaline hyphae towards the margins	-
Bjerkandera fumosa (Pers.) P. Karst.	5.58%	poorly developed aerial mycelium around the inoculum point, submerged mycelia with hyaline hyphae	+
Phlebia radiata Fr.	3.82%	well developed, dense, downy aerial mycelium	+
Coriolopsis gallica (Fr.) Ryvarden	1.47%	unchanged	+
Daedaleopsis confragosa (Bolton) J. Schröt.	3.82%	aerial mycelium with concentric development	+
Daedaleopsis tricolor (Bull.) Bondartsev & Singer	4.11%	aerial mycelium with concentric development	+
Fomes fomentarius (L.) Fr.	2.94%	poorly developed aerial mycelium, submerged mycelium with hyaline hyphae	+
Lentinus tigrinus (Bull.) Fr.	5.58%	relatively homogeneous, dense, downy aerial mycelium	+
Lenzites betulinus (L.) Fr.	7.94%	relatively homogeneous, dense, downy aerial mycelium	-
Neofavolus alveolaris (DC.) Sotome & T. Hatt.	9.11%	poorly developed aerial mycelium around the inoculum point, submerged mycelium with hyaline hyphae	+
Panus neostrigosus Drechsler-Santos & Wartchow	5.29%	aerial mycelium with concentric development	+
Lentinus arcularius (Batsch.) Zmitr.	6.76%	well developed, homogeneous, dense aerial mycelium	-
Picipes melanopus (Pers.) Zmitr. & Kovalenko	5%	unchanged	+

Cerioporus squamosus (Huds.) Quél.	2.64%	unchanged	++
Cerioporus varius (Pers.) Zmitr. & Kovalenko	4.41%	unchanged	-
Picipes badius (Pers.) Zmitr. & Kovalenko	7.64%	well developed, homogeneous, downy aerial mycelium	+
Skeletocutis alutacea (J. Lowe) Jean Keller	1.47%	well developed, homogeneous, downy aerial mycelium	-
Trametes gibbosa (Pers.) Fr.	3.82%	aerial mycelium with concentric development	+
Trametes hirsuta (Wulfen) Lloyd	1.47%	well developed, dense, downy aerial mycelium around the inoculum point, submerged mycelium towards the margins	-
Trametes ochracea (Pers.) Gilb. & Ryvarden	4.11%	unchanged	+
Trametes pubescens (Schumach.) Pilát	2.94%	poorly developed aerial mycelium only around the inoculum point, submerged mycelium with hyaline hyphae	-
Trametes suaveolens (L.) Fr.	5.58%	downy aerial mycelium developed towards the margins, submerged mycelium with hyaline hyphae around the inoculum point	-
Trametes trogii Berk.	3.82%	well developed, homogeneous, downy aerial mycelium	+
Trametes versicolor (L.) Lloyd	3.82%	unchanged	++
Hericium coralloides (Scop.) Pers.	3.82%	heterogeneous aerial mycelium with concentric development	+
Stereum hirsutum (Willd.) Pers.	5%	poorly developed aerial mycelium around the inoculum point, submerged mycelium with hyaline hyphae	+
Stereum subtomentosum Pouzar	5%	heterogeneous aerial mycelium with concentric development	++

(-) unpigmented *F. solani* colony; (+) pigmented *F. solani* colony resembling the control; (++) intensively pigmented *F. solani* colony.

Following the exposure to the volatiles synthesized by the basidiomycetes, not only the morphology and pigmentation of *F. solani* colonies were influenced, but also the growth rate of the mycelia.

High IPs were observed for the volatiles synthesized by *P. badius*, *L. betulinus*, *L. arcularius*, *M. mucida* and *H. fasciculare*, while low IPs were calculated for species such as: *T. hirsuta*, *S. alutacea*, *B. adusta*, *C. gallica*, *C. leave*, *P. candolleana* and *A. mesenterica*.

The highest IPs were recorded for the volatiles synthesized by *M. mucida*, *L. betulinus* and *N. alveolaris*. When tested on MEA and KM media, the IPs for these three species were different compared with the ones recorded on PFMEA (Table 2).

For all three species the inhibition percentages were higher on the KM medium, fact that underlines the influence that the media composition has in the synthesis of the fungal volatiles [NORRMAN, 1971a, 1971b; BJURMAN, 1999].

Table 2. IP_{med} of the volatiles synthesized by *M. mucida*, *L. betulinus* and *N. alveolaris* on three culture media

Species	IP_{med} + SEM		
	PFMEA	MEA	KM
Mucidula mucida	7.64% ± 0.33%	10% ±0.33%	10.29% ± 0.29%
Lenzites betulinus	7.94% ± 0.56%	7.35% ± 0.56%	8% ± 0.29%
Neofavolus alveolaris	9.11% ± 0,29%	10.29% ± 0.29%	11.47% ± 0.29%

Compared to the other three media, KM had a greater complexity. The carbon source was represented by glucose (20 $g \times l^{-1}$), in the highest quantity, the nitrogen source by peptone (2 $g \times l^{-1}$), a mixture of amino acids rich in glutamic acid, proline, leucine aspartic acid and lysine, yeast extract (2 $g \times l^{-1}$) and several other essential elements such as potassium and phosphorus (0.25 $g \times l^{-1}$ KH_2PO_4), magnesium and sulfur (0.25 $g \times l^{-1}$ $MgSO_4 \cdot 7H_2O$), sodium and chloride (yeast extract with 0.5% NaCl).

The volatiles produced by *N. alveolaris* showed the highest IPs when cultivated on all three media, with a maximum on KM, thus being the species selected for the GC-MS analysis. By our knowledge this is the first study focused on the antifungal potential of the volatiles synthesized by this species.

Due to the large number of peaks present in the recorded mass-chromatograms of each analyzed final extract, we could not use only the comparison with NIST 2.0 mass-spectra database combined with retention times from previous literature in the field as acceptance criteria in order to declare a certain compound as present in our samples. In order to identify the classes of organic compounds synthesized by tested wood-rotting basidiomycete species, we applied some additional acceptance criteria described as described in PETRE et al. (2017). Since we tested each sample in duplicate against a control sample, we subtracted the results obtained for control samples from the chromatograms of each replicate. The results variability between replicates for the remaining identified compounds after blank-subtraction was further estimated (as relative standard deviation (RSD, %) between the results obtained for duplicate samples). Only results showing the lowest variability (RSD<30%) were later accepted as positively identified compounds in tested samples.

The highest number of compounds was identified within the dichloromethane fraction, while the lowest number was observed in the acetonitrile fraction. Table 3 presents data on the identified classes of organic compounds isolated from the culture fluids of *N. alveolaris* after elution with different solvents: *n*-hexane, dichloromethane (DCM), acetone and acetonitrile (AcN) together with their retention times (RT, minutes) recorded in specified GC elution conditions. Compounds marked with "*" were declared present in the analyzed samples.

One of the most abundant volatile compound synthesized by many fungi, 1-octen-3-ol was present in the culture fluid of *N. alveolaris* being previously recorded in other wood-rotting basidiomycetes [BERGER & al. 1986a; RAPIOR & al. 1996; ZIEGENBEIN & al. 2010], being responsible for the earthy / mushroom-like aroma [VENKATESHWARLU & al. 1999] and also acting as a signaling agent in the interspecific communication [THAKEOW & al. 2008].

Table 3. Volatile compounds isolated from *N. alveolaris*

Compound	RT (min)	Fraction (elution solvent)			
		***n*-hexane**	**DCM**	**Acetone**	**AcN**
Alcohols					
(R)-2-butanol	1,94		*		
(S)-2-octanol	5,59		*		
1-butanol	4,01		*		
1-octen-3-ol	13,4	*	*	*	
1-propanol	2,06		*		
2-hexanol	6,81		*		
2-methyl-1-butanol	6,19	*			
2-methyl-1-butanol	6,29			*	
2-methyl-1-propanol	2,84		*	*	
3-hexanol	5,97		*		
3-methyl-1-butanol	6,24		*	*	
3-methyl-3-buten-1-ol	7,31	*			
3-methyl-3-buten-1-ol	7,3		*		
4-methyl-2-pentanol	5,28		*	*	
Ketones					
2-hexanone	2,48		*		
3-hexanone	2,12		*		
3-penten-2-one	3,43			*	
4,6-dimethyl-2-heptanone	7,07	*			
4,6-dimethyl-2-heptanone	7,06		*		
4-hydroxy-4-methyl-2-pentanone	10,28		*	*	
4-methyl-3-penten-2-one	3,32		*	*	
Other					
Benzaldehyde	14,2	*	*		
Esther	10,75		*		
Phenol	30,95	*			

The specific almond like aroma observed for the *in vitro* cultures of *N. alveolaris* might be attributed to benzaldehyde which is synthesized by many other species of wood-rotting basidiomycetes [BERGER & al. 1986a, 1987; KAWABE & MORITA, 1993; FÄLDT & al. 1999], compound with applications in the cosmetic industry and perfumery [MORATH & al. 2012]. In the analyzed samples we noticed the presence of several alcohols including 2-methyl-1-propanol, 3-methyl-1-butanol and 2-methyl-1-butanol which, as many compounds belonging to the same class, were reported to act at the cell membrane level by increasing its permeability for certain metabolites and ions [INGRAM & BUTTKE, 1984; HEIPIEPER & al. 1994]. Also the compounds 3-methyl-1-butanol and 2-methyl-1-propanol are considered responsible for the antifungal activity of several endophytic fungi such as *Muscodor albus* [STROBEL & al. 2001; EZRA & al. 2004], *Muscodor crispans* [MITCHELL & al. 2010] and *Phomopsis* sp. [SINGH & al. 2011], while 2-methyl-1-butanol was associated with the antifungal activity of *Trichoderma* sp. [WHEATLEY & al. 1997]. The same alcohols were also reported in the extracts of some wood-rotting basidiomycetes [BERGER & al. 1986a, 1986b, 1987; KAWABE & MORITA,

1993; SCHALCHLI & al. 2013]. WANG & al. (2004) isolated from *N. alveolaris* the polypeptide alveolarin with antifungal properties, but by our knowledge there is no record of testing this species volatiles for their antifungal activity.

Conclusions

The study shows that the volatiles synthesized by some species of wood-rotting basidiomycetes can induce noticeable changes in the morphology of *F. solani* colonies.

From the 53 species of basidiomycetes that were tested, only 18% of them synthesize volatiles that didn't influence the morphology of *F. solani*. Moreover, we noticed that the volatile metabolites of species such as: *A. mesenterica*, *G. applanatum*, *P. squamosus*, *S. subtomentosum* and *T. versicolor* increased the production of pigments, resulting in an intense pink coloration of the phytopahtogen's colony, while the volatiles of *C. leave*, *P. candolleana*, *I. hispidus*, *P. betulinus* and *L. arcularius* decreased the pigment synthesis, the phytopathogen's mycelium being white.

The volatiles produced by wood-rotting basidiomycetes also influence the growth rate of *F. solani*. Some of the highest inhibition percentages were recorded for species such as: *N. alveolaris*, *L. betulinus* and *M. mucida*, while the lowest inhibition percentages were calculated for *P. candolleana*, *B. adusta*, *C. gallica*, *S. alutacea* and *T. hirsuta*.

Also, when cultivated on different media, the inhibition percentages for *N. alveolaris*, *L. betulinus* and *M. mucida* varied, the antifungal effect of the volatiles being higher on the richer media, fact that underlines the importance of media composition of the synthesis of volatile metabolites. From all species, on all media, *N. alveolaris* had the highest inhibition percentages against *F. solani*.

Following the GC-MS analysis of *N. alveolaris* extracts we highlighted the major volatile metabolites synthesized by this species which belong mainly to the alcohols and ketones classes. By our knowledge this is the first study that focuses on the influence of volatile metabolites synthesized by *N. alveolaris* on *F. solani*.

The results encourage further research focused on determining the best media composition in order to increase the production of volatile metabolites, on identification of other bioactive molecules and testing the antifungal potential of these compounds against other phytopathogenic species.

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