INTRODUCTION

A considerable amount of research reported the synthesis of volatiles compound by fungi which impact their morphogenesis, filamentation, pathogenesis as well as various other functions (Jorge, 2015). Among them, the major compound 1-octen-3-ol is a self-inhibitor of Penicillium paneum by the "crowding effect" phenomenon. In addition, 1-octen-3-ol induced the microcyclic conidiation in P. paneum, suggesting an additional role as a factor leading the fungal colony to spore production (Raina et al., 2012). Recently, 1-octen-3-ol, 3-octanol and 3-octanone were recognized as signalling molecules which induce conidiation in Trichoderma spp. (Nemcovic et al., 2008). The formation of 1-octen-3-ol (a C₈ alcohol) is simultaneously accompanied by the formation of 1-octanone and 1-octanol (a C₈ aldehyde 10-oxo-trans-δ-decenoic acid (ODA) (Mau et al., 1992), which was reported to stimulate mycelial growth, stipe lengthening and fruiting bodies growth of yeast extract). When cultivated in solid conidiation medium, 12 g of agar was added to the liquid conidiation medium before sterilization (at 121°C for 20 min).

Cultural conditions on soluble substrates

The inoculum of P. canescens was cultivated at 30°C and 150 RPM in 250 ml Erlenmeyer flasks containing 100 ml of sterile dextrose-based medium (20 g/l of dextrose, 10 g/l of casein peptone and 10 g/l of yeast extract) or sterile liquid conidiation medium (2 g/l of dextrose and 2 g/l of yeast extract). When cultivated in solid conidiation medium, 12 g of agar was added to the liquid conidiation medium before sterilization (at 121°C for 20 min).

Cultural conditions on insoluble substrates

Solid state fermentation: it was performed in 250 ml Erlenmeyer flasks containing 9 g of 1 mm soya oil cake and casein peptone at 0.75% (W/V) in 20 ml of distilled water. The medium was autoclaved (121°C for 20 min) and inoculated after cooling, by 15, 150 and 1500 µl suspension equivalent to 10⁵, 10⁶, 10⁷ spores/g of soya oil cake, respectively. The fermentation was then performed at 30°C under static conditions for three, seven and twelve days.

Submerged fermentation: it was performed in 250 ml Erlenmeyer flasks containing 9 g of 1 mm wheat bran, 100 ml of a nutritive solution composed by yeast extract at 0.5% (W/V), (NH₄)₂SO₄ at 0.2% (W/V), Na₂HPO₄·2H₂O at 1% (W/V), KCl at 0.05% (W/V) and MgSO₄·7H₂O at 0.015% (W/V). The medium was autoclaved (121°C for 20 min) and inoculated after cooling at 105ºC/ml by 0.15 ml spore suspension. The fermentation was then performed at 30°C and 150 rpm for three, seven and twelve days with constant agitation.

2.5. Enzyme extraction

After fermentation, the fermented solid soya oil cake was complemented with distilled water at 75% (W/V) to obtain a liquid mixture. This mixture and the submerged fermented medium were separately centrifuged at 10000 RPM for 15 minutes at 4°C using a Avanti TM J-25 I centrifuge (Beckman, Palo Alto, USA) to remove the residues. These extract solutions were filtrated through a folded filter (α = 150 mm, Ref. No. 10311645) (Schleilcher and Schuell, Dassel, Germany) and the resulting clear supernatant filtrate was used as the enzyme source.

Enzymes assays

The Endo-β-(1, 4)-xylanase activity was measured using 1% birchwood xylan (X0502, EC 232-760-6) (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) (Bailey et al., 1992).

The β-galactosidase activity was measured by the extent of hydrolysis of o-nitrophenyl-beta-galactopyranoside (ONPG) (Gouats et al., 2007).

Assays of fresh and dried matter of P. canescens

The fresh matter of P. canescens was obtained after filtration through a Sупer 450 membrane ( Pall Corporation, USA) with 0.20 µm pore...
size of the biomass issued from two days culture of *P. canescens* on dextrose-based medium or liquid conidiation medium (supplemented or not by pure 1-Octen-3-ol). The dried matter was obtained after drying the fresh matter for 48 hours at 105°C.

### Isolation of volatiles compounds by microdistillation-extraction procedure

The volatile compounds from culture filtrates of *P. canescens* were isolated by microdistillation extraction procedure according to Renata (2004) with ether-pentane (1:1, V/V) as the extraction reagent (8). Isolation of volatile compounds was performed with a Likens-Nickerson apparatus (extraction time: 90 min) for 10 ml of *P. canescens* culture filtrates and 5 ml of the microdistillation-extracted volatiles were injected by a PTFE-faced silicone fibre (Supelco, Bellefonte, PA) into a GC (Agilent Technologies, Eindhoven, Netherlands) recorded to an Agilent 5973 mass spectrometer for the identification and measurement of 1-octen-3-ol. The recovery of added 1-octen-3-ol was 75%.

The quantification of 1-octen-3-ol was calculated on the basis of a calibration curve (0.014-0.4185 mg/10 ml) with a coefficient of correlation r² (0.995). After the analysis of the culture medium, this compound was also identified and quantified by gas chromatography-mass spectrometry analysis with high solvatochromic index. The gas chromatography-mass spectrometry analysis were expressed as the percentage of concentration and inducting conidiation in *P. canescens* (Raina et al., 2012). Quorum sensing molecules Multiciclic acid and related derivatives Butyrolactone induced the morphological changes in *Penicillium cyclopium* (Roncal et al., 2002). Quorums sensing molecules 1-Octen-3-ol also reduced the size of virulence of the fungus, which inducing conidiation and morphological transitions in *Aspergillus nidulans* (Roncal et al., 2002). Gas chromatography analysis

A GC system 6890N gas chromatograph (Agilent Technologies, Eindhoven, Netherlands) coupled to an Agilent 5973 mass selective detector was used for the analyses. The compounds were separated on a DB-264 capillary column (30 m, 0.25 mm i.d., film thickness 1.4 µm) (J&W Scientific, California, USA). Helium was used as carrier gas with a linear velocity of 27.3 cm/s. The gas chromatography oven temperature programme began when the fibre was inserted and held at 38°C for 13 min, ramped to 110°C at 3°C per min, then to 150 at 4°C per min and to 210°C at 10°C per min and finally, held at 210°C for 5 min. The total run time was 58 min and the gas chromatograph mass-spectrometer interface was maintained at 240°C. Mass spectra were obtained by electron impact at 70 eV, and data were acquired across the range of 29-400 uma. The compounds sorbed by the fibre were desorbed from the injection port of the gas chromatograph (Agilent 6890 N) for 6 min at 220°C with the purge valve off (splitless mode). After, the fibre was heated to 220°C for an additional 25 min to avoid an analyte carry-over between samples. So, no compounds were present when the fibre was reinserted.

### Effect of 1-Octen-3-ol on isotropic growth of *P. canescens* and on the beta-galactosidase and endo-beta(1,4)-xylanase production

The diluted 1-Octen-3-ol (in distilled water) was aseptically filtered (sterile syringe filter, 0.45 µm cellulose acetate) (VWR, Leuven, Belgium) and then added to the sterile culture of *P. canescens*. After the addition of 1-Octen-3-ol, the enzymatic activities (endo-beta(1,4)-xylanase and beta-galactosidase) of the fresh and dried matter, the *P. canescens* pellets size (diameter) were determined and compared to the controls without addition of 1-Octen-3-ol.

### Data Analysis

Each test was done in triplicate. The average value was validated when the variation between tests values were less than 8.0%. The results of gas chromatography-mass spectrometry analysis were expressed as means of three replicates for each experiment point.

### RESULTS AND DISCUSSION

In all the *P. canescens* culture filtrates, 1-Octen-3-ol represented more than 90% of the total area of the volatiles compounds extracted by the solid phase micro-extraction analyses (Figure 1). The production was influenced by the mycelia matter and the initial inoculum level (Figure II). During the 7th and 12th day, the growth of *P. canescens* was finished and the culture media were poor, causing the volatilization of the 1-Octen-3-ol maybe due to the agitation of the flasks, excepted for the cultures in the SSF where with 10^6 spores/g of soya oil cake as inoculum inoculon, the *Penicillium* strain grew until the 12th day in parallel to the increase of the level of 1-Octen-3-ol.

In parallel to the production of 1-Octen-3-ol, *P. canescens* growing on insoluble substrates in mycelial filament, produced important level of extracellular endo-beta(1,4)-xylanase and beta-galactosidase (Figure III). The production increased to the initial inoculum level whereas these decreased weak when the strain adopted a visual pellet form on soluble substrates (glucose, xylose, sucrose...). Many studies described a relationship between the production of hemicellulolytic enzymes and beta-galactosidase and the fungal metabolic changes (Raina et al., 2012). This effect maybe due to the agitation of the flasks, excepted for the cultures in the SSF where with 10^6 spores/g of soya oil cake as initial inoculum level whereas these 1-Octen-3-ol levels were too low compared to the minimal inhibitory level (5859 µg/ml of 1-Octen-3-ol of soya oil cake) which reduced the productions of 1-Octen-3-ol and 10-oxo-trans-8-decenoic acid (ODA) which inducing conidiation and morphological transitions in *P. canescens* (Raina et al., 2012). The effect of 1-Octen-3-ol on isotropic growth of *P. canescens* and on the beta-galactosidase and endo-beta(1,4)-xylanase production in parallel to the production of 1-Octen-3-ol was confirmed within the range of 0.990-0.998.

### Solid phase microextraction

The extraction of headspace volatile compounds was done with a Solid phase microextraction device (Supelco, Inc. Bellefonte, PA) using a B-85 PDMS/divinylbenzene/divinylsiloxane fibre (Supelco, Bellefonte, PA) (Blueprints 52759-U). Before the analysis, the fibre was preconditioned in the injection port of the gas chromatograph at 300°C for 1 h. The culture filtrates of *P. canescens* (2 ml) were inserted into 10 ml headspace vial and sealed with a PTFE-faced silicone septum (Supelco, Inc. Bellefonte, PA). The Solid phase microextraction fibre was exposed to the headspace of the vial while maintaining the sample at 30°C with constant stirring during 30 min.

The compounds absorbed by the fibre were identified and quantified by gas chromatography analysis with high solvatochromic index. The gas chromatography-mass spectrometry analysis were expressed as the percentage of concentration and inducting conidiation in *P. canescens* (Raina et al., 2012). Quorum sensing molecules Multiciclic acid and related derivatives Butyrolactone induced the morphological changes in *Penicillium cyclopium* (Roncal et al., 2002). Quorums sensing molecules 1-Octen-3-ol also reduced the size of virulence of the fungus, which inducing conidiation and morphological transitions in *Aspergillus nidulans* (Roncal et al., 2002). Gas chromatography analysis

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Table 1. Diameter measured from 48 hours of the growth zone at 30°C of the fungi after 16 hours of action of 1-octen-3-ol

<table>
<thead>
<tr>
<th>Fungi at 10^6 spores/ml of LSM</th>
<th>Control</th>
<th>Control + 4.2 mg/ml of 1-octen-3-ol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.5 ± 0.1</td>
<td>1 ± 0.1</td>
</tr>
<tr>
<td>Penicillium canescens</td>
<td>2.4 ± 0.2</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>Fusarium oxysporum</td>
<td>1 ± 0.1</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>Penicillium restrictum</td>
<td>2 ± 0.2</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>0.9 ± 0.1</td>
<td>0.6 ± 0.5</td>
</tr>
<tr>
<td>Aspergillus awamori</td>
<td>2.2 ± 0.2</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>Botrytis cinerea</td>
<td>1 ± 0.1</td>
<td>0.9 ± 0.1</td>
</tr>
</tbody>
</table>

whereas 10^5 spores/ml as initial inoculums, which induced the productions of 11 1 and 17 1 µg of 1-octen-3-ol/ml on dextrose-based medium and submerged fermentation media, respectively. In spite of its antifungal activity, a wide selection of fungi such Penicillium, Aspergillus, Alternaria and Fusarium species have been shown to produce this secondary metabolite (Chitarra et al., 2004) which caused the restriction of the mycelial growth of P. canescens, Fusarium oxysporum, Botrytis cinerea, Cladosporium cucunciunum, Aspergillus awamori, Penicillium restrictum, and Aspergillus niger, respectively (Table I).

Plants infected by fungi emit 1-octen-3-ol, which induced their defensive fungal mechanism. Thus, treated with 1-octen-3-ol, the defensive genes of Arabidopsis plants (turned on by wounding or ethylene/jasmonic acid signalling) were stimulated to respond to the infection. The treatment also enhanced resistance of the plant against Botrytis cinerea. It would seem that 1-octen-3-ol may be a signal by which Arabidopis perceives the presence of a fungal pathogen. Some insects synthesized also this molecule during their search for a palatable fungus (Kishimoto et al., 2007). 1-octen-3-ol gets an antibacterial activity and can inhibit the growth of insects (Sawahata et al., 2008). In all cases, the action of the compound is dose-dependant and reversible (Wood et al., 2001). In addition of this extracellular volatile compound, P. canescens synthesises extracellular non volatiles compounds such griseofulvin, curvulolic acid, a fungitoxic extrolite and antibacterial substance named Sch 642305 and an antibiotic named canescin (Brian et al., 1953). These all secondary metabolites could aid the strain P. canescens in its attack or defensive mechanisms.

CONCLUSIONS

The ubiquitous volatile compound 1-octen-3-ol, could be used as an indicator of the growth of P. canescens. This compound can inhibit the germination of P. canescens conidia. Getting a concentration-dependent effect, the level of 1-octen-3-ol emitted during the cultural conditions is lower to affect the growth of P. canescens and the production of extracellular endo-beta-(1, 4)-xylanase and beta-galactosidase. So, this compound can’t not be considered as a grow regulating substance or a quorum sensing molecule in P. canescens.

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Disclosure statement

No potential conflict of interest was reported by the author.

Financial and proprietary interest: Nil

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Figure 2. At top) Levels produced of 1-octen-3-ol during the growth of P. canescens on 863 (black) and submerged fermentation media (white) with initial inoculum size of 10^5 spores/ml and at underneath). Levels produced of 1-octen-3-ol during the growth of P. canescens on SSF with initial inoculum (Spores/g of soya oil cake): 10^5 (black), 10^6 (blue) and 10^7 (white).

Figure 3. Levels produced of: (At top) endo-beta-(1, 4)-xylanase and (at underneath) beta-galactosidase by P. canescens on SSF with initial inoculum (Spores/g of soya oil cake): 10^5 (black), 10^6 (blue) and 10^7 (white).
REFERENCES


