Role of Intragenic Competition in the Performance of Trichoderma Based Biofungicides

*1Gyula Oros, 2Zoltán Naár
1Plant Protection Institute Hungarian Academy of Sciences, 1025 Budapest, P.O.Box 102 Hungary
2NARIC Food Science Research Institute, 1022 Budapest, Herman Ottó út 15. Hungary

Abstract

The goal of this study was to examine whether Trichoderma species might have potential as a biological control agent against other Trichoderma spp. possibly residing in the local environment. Metabolites of thirty Trichoderma isolates were screened for growth moderating activity against test fungi in dual cultures using vertical diffusion technique. Most of different Trichoderma isolates as producers demonstrated strain dependent growth moderating activities either stimulating or completely inhibiting the exposed fungi, while some isolates showed no activity.

The examined Trichoderma spp. showed high natural variation in sensitivity responses to metabolites released to the medium by other Trichoderma strains, depending on traits related to their adaptation to ecological niches, thus the suspected mycoparasitic strains significantly differed of those habiting in soil.

The released complex set of metabolites with broad intragenic spectrum of growth moderating activity exhibited broad spectrum of activity against phytopathogenic fungi as well, however, the strength of the effect could not be predicted.

Few common factors were revealed comparing growth responses of Trichoderma and phytopathogens (their possible hosts) to released metabolites by examinded Trichoderma strains. Seemingly, some genus specific traits regulate the character of intragenic connections among various Trichoderma strains, and these properties might influence the performance of Trichoderma based eubiotic preparations in pest management practices.

Keywords

Trichoderma; Secondary Metabolite; Exudate; Antagonism; Phytopathogens

Introduction

Since discovery of antagonistic properties of Trichoderma [1] and first exploration of this feature for controlling phytopathogenic fungi [1] large Trichoderma based industry has been developed, and diverse ways of their use have been patented as well as several hundred Trichoderma based preparations have been commercialized to prevent yield losses caused by phytopathogenic microbes [2, 3, 4, 5, 6, 7, 8]. Nowadays biology of this genus is intensively studied, and examination of numerous strains revealed that the antagonism is a common property of Trichoderma species which usually can parasitize the phytopathogenic fungi, although these features are strongly influenced by environmental conditions [2, 9, 10, 11, 12].
The mycofungicides - mostly selected strains of *T. harzianum* and *T. viride* - perform well both in laboratory and model applications, but sometimes are less effective in the field where strains must tolerate a wide range of climatic, edaphic and biotic factors [9, 10, 11, 13, 14, 15, 16]. The compatibility to pest management and the ecological fitness of strains can be improved through genetic manipulations [17] and use of appropriate formulations [7].

Fungal biological control agents have several mechanisms of action that allow them to control pathogens, including mycoparasitism, competition for nutrients and the induction of plant host defenses. *Trichoderma* are able to secrete a wide complement of depolymerases and a plethora of secondary metabolites into their vicinity [18], and all these biogenic molecules take part in performance of biopreparates used in pest management practices [19, 20]. Optimization of their properties makes necessary for the adequate selection of appropriate species for the pest control [7, 21]. Hoping to promote further work in the field of development of mycofungicides here we share promising results obtained in model experiments about intrageneric competition studying growth responses of antagonistic *Trichoderma* to their metabolites released into the medium.

### Material and Methods

#### Fungi

The fungi listed in Table 1 were maintained on potato dextrose agar slants at 22 – 25°C (CM0139B, OXOID, Basingstoke) amended with 2 g L⁻¹ casein digest (Difeo, Detroit, USA), vitamins (pyridoxine HCl, thiamin HCl, riboflavin and nicotinamide at 1.0, 10.0, 1.0 and 20.0 mg L⁻¹, respectively) mineral salts (KCl, KHPO₄, K₂HPO₄ and MgSO₄ at 10, 12, 0.5, 0.5 and 0.25 g L⁻¹, respectively). Except *Hypocrea jecorina* IAM 14456 (B-301), *H. muroiana* IAM 12501 (B-302), IAM 12502 (B-303), IAM 12507 (B-307), and *Trichoderma citrinoviride* CBS25.5 (B-311), *T. ghanense* IAM 13109T (B-305), *T. piluliferum* IMI 15209 (B-306), *T. reesei* IMI 45548 (B-314) and *T. saturnisporum* IMI 177881 (B-316) all strains were isolated from various sources in Hungary and deposited in The Mycology Collection (WDCM824) of the Plant Protection Institute, Hungarian Academy of Sciences (Budapest, Hungary).

#### Biological Tests

**Toxicity tests:** The conidia of *Trichoderma* were washed up with sterile distilled water containing 0.05% Tween 20 of 8 days old colonies grown up on milk agar (fat free fresh cow milk solidified with 2 g Agar No.1) to produce conidia for inoculation of agar plates. The suspension of conidia (≈ 5 × 10⁸ cell mL⁻¹) was mixed up with potato dextrose agar (45 - 50°C) prepared as above (1 + 10 mL) and dispensed into Petri dishes (90 mm). After 10-12 hours incubation 20 mL of Trypton B containing agar solution (2 and 10 g L⁻¹, respectively) was over layered, than centrally inoculated with small mycelium block (≈2×2 mm) cut of the edge of 2-4 days old colony of target strain grown up on PDA as above at 22 - 24°C. Diameter of colonies was measured 24 and 48 hours later (dT24 and dT48), and the rate of growth was expressed as a difference between diameters measured, and expressed in percent of diameters of fungi grown on *Trichoderma* free medium (dC24 and dC48): Inhibition rate (%) = 100 – [100*(dC48 – dT48)/(dC48 – dC24)]. Negative values mean stimulation of fungal growth.

### Data Analysis

Fisher’s test was applied to evaluate significance of differences between variants at p = 0.05 level. The basic data matrix (30 producer × 30 target strains) comprising response values expressed in percents of growth inhibition (Table 1) was transformed into probit values and subsequently analyzed with multivariate statistical methods. Potency Mapping (PM) and Spectral Component Analysis (SCA) were employed to disclose differences between both antifungal activity of *Trichoderma* and sensitivity responses of test strains following [22]. The SCA separates the basic data matrix into two part; the first is a vector proportional to overall strength of responses (PM), while the second is a matrix of spectral components (Spectral Map, SPM) characterizing the spectrum of activity or sensitivity. The Principal Component Analysis (PCA) was applied to elucidate the number of factors affecting the selective response of target fungi to toxic principles [23]. Cluster Analysis (CA) was carried out to reveal relationship among spectrum of activities of *Trichoderma* strains as spectral variables. The Canonical Correlation analysis (CCA) and Principal Component Regression Analysis were used to reveal similarities in response of taxonomic groups to metabolites of *Trichoderma* released to medium. The repercusions of phytopathogenic fungi to *Trichoderma* metabolites were imported of earlier publication [7] for comparative analysis.

Microsoft Office Excel 2003 statistical functions
The graphical presentation of data analysis was uniformly edited in Microsoft Office Power Point 2003.

Table 1A: Sensitivity responses (%) of *Trichoderma* strains in dual cultures

<table>
<thead>
<tr>
<th>No.</th>
<th>Producers (P) <em>Trichoderma</em> and <em>Hypocrea</em> species&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Targeted (T) strains&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td><em>T. aureoviride</em> Rifai</td>
<td>H01</td>
</tr>
<tr>
<td>2</td>
<td><em>T. citrinoviride</em> Bisset</td>
<td>L01</td>
</tr>
<tr>
<td>3</td>
<td><em>T. longibrachiatum</em> Rifai</td>
<td>L02</td>
</tr>
<tr>
<td>4</td>
<td><em>T. pseudokoningii</em> Rifai</td>
<td>L03</td>
</tr>
<tr>
<td>5</td>
<td><em>H. jecorina</em> Berkeley &amp; Broome</td>
<td>L04</td>
</tr>
<tr>
<td>6</td>
<td><em>T. reesei</em> Simmons</td>
<td>L05</td>
</tr>
<tr>
<td>7</td>
<td><em>T. ghanense</em> Doi, Abe et Sugiyma</td>
<td>L06</td>
</tr>
<tr>
<td>8</td>
<td><em>T. parceramosum</em> (Fabrea)</td>
<td>L07</td>
</tr>
<tr>
<td>9</td>
<td><em>T. parceramosum</em> Bisset</td>
<td>L08</td>
</tr>
<tr>
<td>10</td>
<td><em>T. saturnisporum</em> Hammil</td>
<td>L09</td>
</tr>
<tr>
<td>11</td>
<td><em>T. pililferum</em> Webster &amp; Rifai</td>
<td>P01</td>
</tr>
<tr>
<td>12</td>
<td><em>T. polysporum</em> (Link ex Persoon) Rifai</td>
<td>P02</td>
</tr>
<tr>
<td>13</td>
<td><em>T. hamatum</em> (Bonord) Bainer</td>
<td>P03</td>
</tr>
<tr>
<td>14</td>
<td><em>T. harzianum</em> Rifai</td>
<td>P04</td>
</tr>
<tr>
<td>15</td>
<td><em>T. harzianum</em> T/26</td>
<td>P05</td>
</tr>
<tr>
<td>16</td>
<td><em>T. harzianum</em> (Rhytizma)</td>
<td>P06</td>
</tr>
<tr>
<td>17</td>
<td><em>T. harzianum</em> Rifai (RS)</td>
<td>P07</td>
</tr>
<tr>
<td>18</td>
<td><em>T. longipilis</em> Bisset (26)</td>
<td>P08</td>
</tr>
<tr>
<td>19</td>
<td><em>T. minutisporum</em> Bisset (27)</td>
<td>P09</td>
</tr>
<tr>
<td>20</td>
<td><em>T. strictipile</em> Bisset</td>
<td>P10</td>
</tr>
<tr>
<td>21</td>
<td><em>T. tomentosum</em> Bisset</td>
<td>P11</td>
</tr>
<tr>
<td>22</td>
<td><em>T. viriens</em> (Miller;Giddens &amp; Foster) von Arx</td>
<td>P12</td>
</tr>
<tr>
<td>23</td>
<td><em>T. atroviride</em> Karsten</td>
<td>T01</td>
</tr>
<tr>
<td>24</td>
<td><em>T. koningii</em> Oudemans</td>
<td>T03</td>
</tr>
<tr>
<td>25</td>
<td><em>T. strigosum</em> Bisset (28)</td>
<td>T04</td>
</tr>
<tr>
<td>26</td>
<td><em>T. viride</em> Persoon ex Gray</td>
<td>T05</td>
</tr>
<tr>
<td>27</td>
<td><em>H. muroiana</em> Hino et Katsumoto</td>
<td>T06</td>
</tr>
<tr>
<td>28</td>
<td><em>H. muroiana</em> IAM 12502</td>
<td>T07</td>
</tr>
<tr>
<td>29</td>
<td><em>H. muroiana</em> IAM 12507</td>
<td>T08</td>
</tr>
<tr>
<td>30</td>
<td><em>T. spirale</em> Bisset</td>
<td>N01</td>
</tr>
<tr>
<td></td>
<td>Inhibition (I)</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>No effect (NI)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>LSD&lt;sub&gt;min&lt;/sub&gt;</td>
<td>6.9</td>
</tr>
</tbody>
</table>

<sup>a</sup> Names of species correspond to nomenclature of ISTH [41]. According to morphometric parameters anamorph of *Hypocrea muroiana* strains IAM 12502 and IAM 12507 are of *Trichoderma* section.

<sup>b</sup> Strains of *Trichoderma* section tested in dual cultures.

<sup>c</sup> Sections: H=Hypocreanum, T=Trichoderma, L=Longibrachiatum, P=Pachybasium.

<sup>d</sup> Type of type of action: Number of producers stimulating (S), completely inhibiting (I) or not influencing (NI) the mycelial growth of the given strain.
Table 1B

<table>
<thead>
<tr>
<th>No.</th>
<th>Codea</th>
<th>Strain</th>
<th>Producersb (P)</th>
<th>Targeted (T) strainsb</th>
<th>Gc</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>B-318</td>
<td>B</td>
<td>B-311</td>
<td>100 100 66 53 71 73 29 67 76 39 78 17 29</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>B-311</td>
<td>S</td>
<td>L01</td>
<td>100 74 62 76 80 81 85 76 92 85 80 22</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>B-313</td>
<td>S</td>
<td>L02</td>
<td>80 88 67 58 77 75 65 77 80 64 83 77 32</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>B-312</td>
<td>S</td>
<td>L03</td>
<td>58 64 61 58 81 86 78 82 83 73 86 39 23</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>B-301</td>
<td>B</td>
<td>L04</td>
<td>79 100 65 58 100 36 7 54 18 49 68 100 30</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>B-314</td>
<td>I</td>
<td>L05</td>
<td>100 73 32 -48 65 58 59 66 54 74 53 38 28</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>B-305</td>
<td>S</td>
<td>L06</td>
<td>82 82 76 45 96 83 86 92 91 96 79 71 23</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>B-328</td>
<td>Dr</td>
<td>L07</td>
<td>100 100 83 73 20 -113 24 65 73 -50 -13 87 27</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>B-315</td>
<td>I</td>
<td>L08</td>
<td>100 72 20 54 74 80 -113 65 61 59 89 74 78 28</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>B-316</td>
<td>S</td>
<td>L09</td>
<td>67 56 60 -19 60 15 58 54 71 62 38 65 32</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>B-306</td>
<td>S</td>
<td>P01</td>
<td>63 71 48 36 34 37 46 55 59 43 66 30 32</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>B-307</td>
<td>S</td>
<td>P02</td>
<td>53 57 50 -89 0 7 0 18 50 39 11 54 27</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>B-308</td>
<td>S</td>
<td>P03</td>
<td>69 60 26 38 100 93 37 60 83 43 90 -9 24</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>B-309</td>
<td>S</td>
<td>P04</td>
<td>100 90 74 50 74 85 83 59 58 77 84 79 38</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>B-075</td>
<td>B</td>
<td>P05</td>
<td>91 93 83 74 80 74 85 65 75 63 76 89 24</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>B-325</td>
<td>Ra</td>
<td>P06</td>
<td>82 88 78 59 65 73 69 41 74 60 70 47 23</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>B-317</td>
<td>Rs</td>
<td>P07</td>
<td>76 65 66 45 65 67 87 83 90 51 62 65 26</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>B-338</td>
<td>B</td>
<td>P08</td>
<td>55 53 49 9 -4 -33 16 4 4 -65 17 -2 16</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>B-339</td>
<td>B</td>
<td>P09</td>
<td>100 90 76 58 75 72 73 50 62 71 83 76 14</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>B-332</td>
<td>B</td>
<td>P10</td>
<td>78 72 50 42 37 60 51 41 59 55 55 30 29</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>B-333</td>
<td>B</td>
<td>P11</td>
<td>30 32 37 17 -18 32 2 57 56 15 63 0 29</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>B-310</td>
<td>S</td>
<td>P12</td>
<td>89 92 50 34 41 20 3 39 63 41 41 -2 27</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>B-320</td>
<td>S</td>
<td>T01</td>
<td>75 64 64 -7 50 37 35 27 51 65 44 50 26</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>B-322</td>
<td>S</td>
<td>T02</td>
<td>63 100 56 55 53 72 46 44 70 60 52 31 30</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>B-340</td>
<td>S</td>
<td>T04</td>
<td>76 89 67 45 53 63 67 63 49 71 68 45 23</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>B-319</td>
<td>S</td>
<td>T05</td>
<td>75 78 62 -17 75 82 80 77 74 88 84 73 21</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>B-302</td>
<td>Le</td>
<td>T06</td>
<td>46 46 19 -1 3 80 91 82 88 80 86 -2 24</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>B-303</td>
<td>Am</td>
<td>T07</td>
<td>77 64 63 44 1 24 24 26 70 48 49 -7 21</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>B-304</td>
<td>Le</td>
<td>T08</td>
<td>72 77 61 12 31 58 65 72 40 59 55 30 32</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>B-333</td>
<td>S</td>
<td>N01</td>
<td>48 72 60 38 -1 77 59 90 78 67 51 38 24</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stimulation (S)</th>
<th>Inhibition (I)</th>
<th>No effect (NI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 0 0 0 0 0 1</td>
<td>0 0 0 1</td>
<td>0 0 0 0 0 1</td>
</tr>
</tbody>
</table>

LSD0.05 = 3.9 (Fp=19.0, p<0.05)

---

*a= Strains of Trichoderma and Hypocreia species.
*b= The strains of Pachybasium section tested in dual cultures.
*c= Codes are accession numbers of strains in Mycology Collection (WDCM824).
*d= Origin: K=bark, T=soil, I=industrial strain, Am= Armillaria mellea (Vahl.) P. Kumm., Le= Lentinula edodes (Berrk.) Pergler, Dr= stroma of Diplocarpon roseae F.A. Wolf, Rs= pseudoscleritium of Rhizoctonia solani Kühn, Ra= Rhytisma acerinum (Pers.) Fr.
*e= Sections: H=Hypocreanum, T= Trichoderma, L= Longibrachiatum, P= Pachybasium.
*f= Radial growth (mm/day) measured on glucose agar amended with Trypton B (5 g L-1).
*g= Type of type of action: Number of producers stimulating (S), completely inhibiting (I) or not influencing (NI) the mycelial growth of the given strain.
Table 1C

<table>
<thead>
<tr>
<th>Producers (P)</th>
<th>Targeted (T) strains</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>Code</td>
<td>S</td>
</tr>
<tr>
<td>1</td>
<td>B-318</td>
<td>H01</td>
</tr>
<tr>
<td>2</td>
<td>B-311</td>
<td>L01</td>
</tr>
<tr>
<td>3</td>
<td>B-313</td>
<td>L02</td>
</tr>
<tr>
<td>4</td>
<td>B-312</td>
<td>L03</td>
</tr>
<tr>
<td>5</td>
<td>B-301</td>
<td>L04</td>
</tr>
<tr>
<td>6</td>
<td>B-314</td>
<td>L05</td>
</tr>
<tr>
<td>7</td>
<td>B-305</td>
<td>L06</td>
</tr>
<tr>
<td>8</td>
<td>B-328</td>
<td>L07</td>
</tr>
<tr>
<td>9</td>
<td>B-315</td>
<td>L08</td>
</tr>
<tr>
<td>10</td>
<td>B-316</td>
<td>L09</td>
</tr>
<tr>
<td>11</td>
<td>B-306</td>
<td>P01</td>
</tr>
<tr>
<td>12</td>
<td>B-307</td>
<td>P02</td>
</tr>
<tr>
<td>13</td>
<td>B-308</td>
<td>P03</td>
</tr>
<tr>
<td>14</td>
<td>B-309</td>
<td>P04</td>
</tr>
<tr>
<td>15</td>
<td>B-075</td>
<td>P05</td>
</tr>
<tr>
<td>16</td>
<td>B-325</td>
<td>P06</td>
</tr>
<tr>
<td>17</td>
<td>B-317</td>
<td>P07</td>
</tr>
<tr>
<td>18</td>
<td>B-338</td>
<td>P08</td>
</tr>
<tr>
<td>19</td>
<td>B-339</td>
<td>P09</td>
</tr>
<tr>
<td>20</td>
<td>B-332</td>
<td>P10</td>
</tr>
<tr>
<td>21</td>
<td>B-331</td>
<td>P11</td>
</tr>
<tr>
<td>22</td>
<td>B-310</td>
<td>P12</td>
</tr>
<tr>
<td>23</td>
<td>B-320</td>
<td>T01</td>
</tr>
<tr>
<td>24</td>
<td>B-322</td>
<td>T03</td>
</tr>
<tr>
<td>25</td>
<td>B-340</td>
<td>T04</td>
</tr>
<tr>
<td>26</td>
<td>B-319</td>
<td>T05</td>
</tr>
<tr>
<td>27</td>
<td>B-302</td>
<td>T06</td>
</tr>
<tr>
<td>28</td>
<td>B-303</td>
<td>T07</td>
</tr>
<tr>
<td>29</td>
<td>B-304</td>
<td>T08</td>
</tr>
<tr>
<td>30</td>
<td>B-333</td>
<td>N01</td>
</tr>
</tbody>
</table>

Stimulation (S): 4 3 9 2 7 0 1 2 1 10 0
Inhibition (I): 25 26 18 28 22 30 28 25 29 20 30
No effect (NE): 1 1 3 0 1 0 1 3 0 0 0

LSD_{0.05} = 5.1 (F_{T}^2=21.9, p<0.05)

a = Strains of Trichoderma and Hypocrea species.
b = The strains of Longibrachiatum and Hypocrea sections tested in dual cultures. The T. spirale has not been classified yet into any section according to Bisset et al. [41].
c = Type of response: Number of targeted strains by producer: stimulated (S), completely inhibited (I) or unaffected (NE) mycelial growth.
d = Codes are accession numbers of strains in Mycology Collection.
e = Sections: H=Hypocreanum, T=Trichoderma, L=Longibrachiatum, P=Pachybasium, N=no lineage.
f = Type of type of action: Number of producers stimulating (S), completely inhibiting (I) or not influencing (NE) the mycelial growth of the given strain.
Results

Transparency of plates completely vanished after 10-12 hours of pouring the conidial suspension, and majority of Trichoderma strains started to break through the agar layer between subsequent 48-72 hours, than the surface of plates was covered with a mycelial mat in control plates within one day. The inoculation with mycelial blocks of target strains delayed this break through for 1-2 days in strain dependent manner indicating the presence of mutual interaction of paired strains. However, this effect of inoculants was not possible to quantify in the experimental model applied due to high variation, moreover, the target strains rapidly overgrew the producer around the inoculum. Thus we did not follow the observation after five days of incubation, and concentrated on the reaction of test fungi to metabolites excreted and diffused through the agar layers up to development of mycelial mat of producers on the surface. The growth intensity of test strains varied between 14-38 mm/day in control T. polysporum growing least and T. ghanense most rapidly. In general, the strains isolated of soil tended to grow more intensely than those from bark or fungal bodies (28, 25 and 24 mm/day as averages, respectively).

The reproducibility of experiments was acceptable, the Trichoderma strains as responding targets grew near synchronously supporting the reliability of measurements ($F_{\text{replications}} = 1.49 < F_{0.05} = 2.30; N=900$), and the overall LSD$_{0.05}$ between pairs (target T versus producer P, N=900) was 4.8 % ($F_{P}=22.1, p <0.05$). Nevertheless, in some cases (L4→L6, L7→P10, L7→T4, P6→P10, P6→P11, P7→N1, P8→N1, P11→N1, P12→T1) surpassed $r^2=0.5$ showing the low similarity. Nevertheless, some trends were revealed between sensitivity responses to released metabolites and the origin of targeted strains (Figure 1). In general, the stimulative effect of metabolites of bark isolates was more prominent, however, the strength of effect did not relate to the frequency of this phenomenon. For example, compare reactions of soil strains to metabolites of producers isolated of bark or fungal bodies.

The principal component analysis applied to elucidate relationships among exposed Trichoderma strains revealed nine hypothetical (i.e., background) variables that explained overwhelming majority of total variance with only 13.22 % loss of information (Table 2). Three major factors comprised 53% of total variation determining their selective response (25, 18 and 10 percent) to released metabolites by germinating conidia. All but two (L1 and L5) strains as variables have high loadings only in one PC, the majority in either first or second PCs, indicating that they showed similar reactions regulated by one main factor when their strenght and spectrum of sensitivity to toxic metabolites are taken into consideration simultaneously. Meanwhile the majority of effects (>50%) influenced by factors of the responses of strains originated of fungal bodies and bark were compressed into the first PC, those ruling the response of soil originated and industrial strains were compressed into the second PC. Indeed, plotting the targeted strains as variables by two major PCs (Figure 2) clear separation of strains by their origin was unveiled. Except T08 (H. muroiana IAM 12507) being an outlayer, the strains from either fungal bodies or bark separated strictly of soil originated ones independently of their taxonomic position or potential sensitivities. Thus, the most tolerant L03 (T. pseudokoningii) to metabolites of bark isolates positioned near to least tolerant P02 (T. polysporum). Results of the analysis of spectral map are not detailed here as the strains clustered according to the their origin like Figure 2, thus no other patterns have been revealed.

Response of Trichoderma strains to released metabolites

The growth response of exposed strains varied within large limits ($F_{L}=175.7, p>0.001$) of full inhibition to potent stimulation in pair dependent manner (25 and 86 cases of 900 pairs, resp.), while no reaction occurred in 7 cases (Table 1). The coefficient of variation of response of strains to released metabolites of producer set diverged extremely (c.v.≈23-380% having median=66%); the variation in growth responses of six strains (H01, L03, L05, P04, T01 and T04) surpassed 100%, indicating differences in the spectrum of sensitivity. In this respect the path coefficients only in 13 cases (L1→L5, L4→L7, L4→P6, L4→T6, L7→P6, L7→P10, L7→T4, P6→P10, P6→P11, P7→N1, P8→N1, P11→N1, P12→T1) surpassed $r^2=0.5$ showing the low similarity. Nevertheless, some trends were revealed between sensitivity responses to released metabolites and the origin of targeted strains (Figure 1). In general, the stimulative effect of metabolites of bark isolates was more prominent, however, the strength of effect did not relate to the frequency of this phenomenon. For example, compare reactions of soil strains to metabolites of producers isolated of bark or fungal bodies.

Growth moderating activity of metabolites of Trichoderma strains released to medium

The Trichoderma strains as evaluated by growth moderating activity of their metabolites released into the medium showed marked differences ($F_{L}=177.8, p>0.001$), indicating that they may exert different impacts on Trichoderma in the environment.
**Figure 1:** Influence of metabolites released by *Trichoderma* strains isolated of various sources to their mycelial growth

<table>
<thead>
<tr>
<th>Cases of responding pairs</th>
<th>Origin of target strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fung</td>
</tr>
<tr>
<td>Fungi</td>
<td>5</td>
</tr>
<tr>
<td>Bark</td>
<td>3</td>
</tr>
<tr>
<td>Soil</td>
<td>13</td>
</tr>
<tr>
<td>Industry</td>
<td>2</td>
</tr>
<tr>
<td>Fungi</td>
<td>3</td>
</tr>
<tr>
<td>Bark</td>
<td>4</td>
</tr>
<tr>
<td>Soil</td>
<td>8</td>
</tr>
<tr>
<td>Industry</td>
<td>2</td>
</tr>
<tr>
<td>Fungi</td>
<td>1</td>
</tr>
<tr>
<td>Bark</td>
<td>0</td>
</tr>
<tr>
<td>Soil</td>
<td>20</td>
</tr>
<tr>
<td>Industry</td>
<td>4</td>
</tr>
<tr>
<td>Fungi</td>
<td>0</td>
</tr>
<tr>
<td>Bark</td>
<td>0</td>
</tr>
<tr>
<td>Soil</td>
<td>5</td>
</tr>
<tr>
<td>Industry</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
</tr>
</tbody>
</table>

Abbreviations: O=origin of strains, S=stimulation, I=inhibition, F=full inhibition, N=no effect. Bars on the graph are proportional to response of target strains to metabolites released by producers depending of their origin. The growth response values were calculated of the data of Table 1 applying Potency mapping (Lewi, 1976).

**Figure 2:** Similarity of test strains based on their growth responses to metabolites released by *Trichoderma* strains into the medium

The first major principal components comprise 43% of the total variation of basic data matrix (900 elements). The numeration refers to strains listed in Table 1, where sections are Hypocreanum (H), Longibrachiatum (L), Pachybasium(P), Trichoderma (T) and no lineage (N). The size of pies is proportional to overall potential sensitivity to released metabolites, while the size of black, gray and white sectors relates to sensitivity responses of given strains to metabolites released by the strains isolated of soil, bark and fungal bodies, respectively. Group A comprises vast majority of soil isolates and industrial strains except *T. aureoviride* (H1) and *H. muroiana* (T7), while Goup B those isolated of bark and fungal bodies except *T. ghanense* (L6), *T. strigosum* (T4) and *T. spirale* (N1).
Table 2: Similarities in responses of *Trichoderma* strains as targets to metabolites excreted by antagonistic *Trichoderma* strains

<table>
<thead>
<tr>
<th>O</th>
<th>Strain</th>
<th>Principal Components</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PC1</td>
</tr>
<tr>
<td>B</td>
<td>H01</td>
<td>-0.083</td>
</tr>
<tr>
<td>S</td>
<td>L01</td>
<td>0.036</td>
</tr>
<tr>
<td>S</td>
<td>L02</td>
<td>-0.117</td>
</tr>
<tr>
<td>S</td>
<td>L03</td>
<td>-0.125</td>
</tr>
<tr>
<td>B</td>
<td>L04</td>
<td>0.737</td>
</tr>
<tr>
<td>I</td>
<td>L05</td>
<td>0.206</td>
</tr>
<tr>
<td>S</td>
<td>L06</td>
<td>0.440</td>
</tr>
<tr>
<td>F</td>
<td>L07</td>
<td>0.833</td>
</tr>
<tr>
<td>I</td>
<td>L08</td>
<td>0.087</td>
</tr>
<tr>
<td>S</td>
<td>L09</td>
<td>-0.315</td>
</tr>
<tr>
<td>S</td>
<td>P01</td>
<td>0.052</td>
</tr>
<tr>
<td>S</td>
<td>P02</td>
<td>-0.170</td>
</tr>
<tr>
<td>S</td>
<td>P03</td>
<td>-0.074</td>
</tr>
<tr>
<td>S</td>
<td>P04</td>
<td>0.156</td>
</tr>
<tr>
<td>B</td>
<td>P05</td>
<td>0.490</td>
</tr>
<tr>
<td>F</td>
<td>P06</td>
<td>0.854</td>
</tr>
<tr>
<td>F</td>
<td>P07</td>
<td>0.710</td>
</tr>
<tr>
<td>B</td>
<td>P08</td>
<td>0.711</td>
</tr>
<tr>
<td>B</td>
<td>P09</td>
<td>0.599</td>
</tr>
<tr>
<td>B</td>
<td>P10</td>
<td>0.800</td>
</tr>
<tr>
<td>B</td>
<td>P11</td>
<td>0.905</td>
</tr>
<tr>
<td>S</td>
<td>P12</td>
<td>0.137</td>
</tr>
<tr>
<td>S</td>
<td>T01</td>
<td>-0.091</td>
</tr>
<tr>
<td>S</td>
<td>T03</td>
<td>0.059</td>
</tr>
<tr>
<td>S</td>
<td>T04</td>
<td>0.837</td>
</tr>
<tr>
<td>S</td>
<td>T05</td>
<td>0.159</td>
</tr>
<tr>
<td>F</td>
<td>T06</td>
<td>0.761</td>
</tr>
<tr>
<td>F</td>
<td>T07</td>
<td>0.245</td>
</tr>
<tr>
<td>F</td>
<td>T08</td>
<td>0.191</td>
</tr>
<tr>
<td>S</td>
<td>N01</td>
<td>0.821</td>
</tr>
<tr>
<td>Expl. Var</td>
<td>7.58</td>
<td>5.47</td>
</tr>
<tr>
<td>Prp. Totl</td>
<td>0.25</td>
<td>0.18</td>
</tr>
</tbody>
</table>

*Expl. Var* = Principal Components calculated of the data of Table 1 after probit transformation. The bold marked loadings have relevant weight in determination of the behavior of variables concerned.

*Strains originates (O) of fungal bodies (F), bark (B), soil (S) and industry (I).*

*Codes of strains correspond to those given in Table 1.*
The overall potential activity of metabolite complex of *Trichoderma* varied within large limits being *T. ghanense* (L6) 15 times more active than *T. polysporum* (P2). The growth promoting effect (stimulation) that expressed mainly in the case of targeted industrial strains was seemingly not related to either taxonomic position or origin of producers (Figure 3). The inhibitory effect was in most cases less pronounced against strains isolated of soil, although a general rule can not be formulated as in some cases reverse relation was revealed; for example metabolites of *H. jecorina* (L4) and *T. longipilis* (T8) were two times more active against soil isolates than those isolated of fungal bodies. Nevertheless, a group of potent inhibitors (PA>50%) can be distinguished (L1, L3, L4, L6, P4, P5, P7, P9), while metabolites of some strains (P2, P8, T7) exhibited comparatively low overall activity (<15%) against *Trichoderma* strains.

**Figure 3:** Cluster-diagram of the interrelationships between *Trichoderma* strains on the base of the antifungal efficacy of their metabolites released.

The names of *Trichoderma* and *Hypocrea* strains refer to those listed in Table 1.

The clusterogram was constructed applying Unweighted Pair Group Average method based on Pearson’s correlation coefficients of spectral component variables (*Trichoderma* strains). The spectral map was calculated of inhibitory activities (Table 1) against targeted *Trichoderma* strains after probit transformation [22]. Dotted lines separate subclusters of superclusters G1 and G2 revealed at as minimum as P=5%.

Abbreviations: Sec = sections, H = Hypocreanum, L = Longibrachiatum, P = Pachybasiurn, T = Trichoderma, N = No lineage, according to International Subcommission on *Trichoderma* and *Hypocrea* Taxonomy [41], for numeration see Table 1. The S, B, F are potential activities of strains isolated of soil, bark and fungal bodies, while I relates to industrial strains. Ec and En are potential activities against ecto- and endotrophic phytopathogenic fungi imported of our previous publication [7]. Negative values mean growth stimulation.
The PCA revealed eight background variables explaining 83% of total variation, however, contrary to factors related to growth responses no pattern could be recognized in distribution of information within PCs, although ten of fifteen strains of soil as variables had high loadings (>0.5) in the first PC (Table of loadings is not shown). Six strains as variables (L6, L7, P1, P4, T3, T8) have not significantly dominant (pcl<0.5) loadings in any PC, while other six have high loadings (pcl>0.5) in two PCs (L3, L5, L8, P6, P9, T6), and loadings of eighteen strains dominate one of first three PCs, demonstrating presence of numerous strain specific factors in regulation of the antagonistic effect when the strength and spectrum of activity of toxic metabolites released are taken into consideration simultaneously.

The activity spectra of strains as producers showed great differences; the path coefficients only in eight cases (L1~L2, L1~T1, L2~L8, L3~P3, L3~T6, L5~L9, T3~H1, T6~T7) surpassed 0.5 (50%) that means the similarity in this respect is lower then in sensitivity responses. *Trichoderma* strains as producers formed two groups (G1 and G2, p < 0.005) when clustered applying their selective growth moderating activities in dual cultures (Figure 3). The potential host ranges of these megaclusters seemingly differ at high extent, however, the differences among single strains are more striking then between averages of G1 and G2 groups. For example, *T. aureoviride* (H1) and *T. koningii* (T3) or *T. reesei* (L5) and *T. parceramosum* (L9) with significantly different strength but similar spectrum of activities formed compact pairs. The taxonomic relationships seemingly take also minimal role in this respect, for example *T. aureoviride* (H1) and *T. koningii* (T3) having highly similar spectrum of activity form a strict subcluster. Seemingly, the traits related to ecological adaptation play also negligible role in regulation of the composition of metabolites released into the medium, for example strains P7 and P6 of *T. harzianum* isolated of fungal bodies were clustered in G1 and G2 reflecting significant differences in their host range. The fact, that subclusters of G1 and G2 were formed of strains of various sections as well as different origin (p < 0.01) indicates that neither their taxonomic position nor former ecological adaptation relate to these groupings.

**Figure 4:** Potential activity of *Trichoderma* metabolites against diverse groups of fungi

The potential growth moderating effect of *Trichoderma* strains (N=30) as producers of biactive metabolites as evaluated by growth responses of exposed *Trichoderma* strains (Table 1) and phytopathogenic fungi (data imported of Oros and Naár, 2017) applying Potency Mapping technique (Lewi, 1976) after probit transformation. MT and MP are medians of respective groups. The codes of producer strains assigned to sections Hypocreanum (H), Longibrachianum (L), Pacybasium (P) and Trichoderma (T) correspond to those in Table 1.
Similarities in growth moderating activities of *Trichoderma* strains against various fungal genera

The *Trichoderma* strains tested as producers exhibited higher strength of growth moderating activity against selected phytopathogenic fungi than against *Trichoderma* as potential antagonists having medians 65 and 25%-s, respectively. Seemingly, no are relationships between strength of activities expressed to these groups as it well demonstrated on distribution graph (Figure 4). For example, *T. polysporum* (P2) having been highly active against phytopathogens proved to be near ineffective against tested set of *Trichoderma*. No patterns on the bivariate plot of potential activities against these two groups could be revealed ($R_{TF} = 0.275$, $p>0.1$).

The comparative analysis of growth moderating activities revealed certain similarities in spectrum of sensitivities (SS) between fungi of similar ecological adaptation (Table 3, Matrix A) as evaluated by means of CCA. The coefficients of first roots showed significant similarity but industrial strains among targeted *Trichoderma* and vascular pathogens. Interestingly, the SS of ectoparasitic fungi showed significant similarity to that of soil habiting *Trichoderma* ($R^2=0.809$). The strength of reaction of various fungal groups showed similarity only in the case of two groups of phytopathogens ($R^2=0.513$). However, the spectrum of activities (SPM) of metabolites against these two groups of possible targets showed certain similarity. Relatively high correlation ($R^2=0.706$) was demonstrated comparing two sets of data with CCA (Figure 5). The more active metabolite complex against *Trichoderma* acts probably ($p<0.01$) more actively against phytopathogens as well. However, the more detailed analysis revealed that only minor part of factors relates in both cases. Thus, the correlation among background variables was significant in the case of pairing limited number of principal components of two SPMs comprising 10 vs 44 and 7 vs 15 % of total variation (*Trichoderma* and phytopathogens, respectively).

One can conclude that the strenght of activity expressed to a single *Trichoderma* strain can not be predicted, while the strenght of antagonistic effect measured on a single phytopathogenic fungus may orient on the expected effect to an other one. Furthermore, it can be expected that the antagonistic strain with either narrow or wide spectrum of activity against vascular pathogens may express the same effect on local *Trichoderma* complex residing in treated area (phylloplan, spermosphere, rhizosphere, soil, or other surfaces).

Discussion

*Trichoderma* species are important participants of agroecosystems that are complex environments exhibiting intricate interactions between biotic and abiotic factors [15]. Currently over 100 anamorphs have been classified in soils [24, 25, 26], furthermore, certain *Trichoderma* species are known as colonizers of natural and artificial substrata, endophytes of plants and facultative pathogens of humans, demonstrating a high adaptability to various ecological niches [27]. This group of fungi has remarkably diverse metabolism and catabolic activities as well as produces a plethora of secondary metabolites (SMs) that influence wide variety of biological functions of targeted organisms [28, 29] playing pivotal roles in chemical defense and communication, and their antibiotic abilities have been demonstrated in biocontrol applications [19, 30]. Genome analysis of mycoparasitic species illustrated the richness of genes encoding compounds required to kill other fungi [17, 31]. The expression of these traits can be downregulated during interaction with targeted host [32, 33, 34]. Although, numerous SM gene clusters have been revealed in *Trichoderma* genomes, deeper understanding of the metabolic pathways leading to SMs is still needed further studies with special regard in mycotrophic strains [28]. Based on results of the model applied in our work the strength and spectrum of either growth moderating activity of excreted metabolites or growth response to them could be separated. In both aspects, high divergence was revealed in activities of the examined set of 30 *Trichoderma* strains (anamorphs of 18 *Hypocrea* species).

The strength of growth moderating activity of excreted complex of molecules varied in strain dependent manner when *Trichoderma* were targeted, and no pattern manifested according to either taxonomic position or traits related to adaptation to their original localities (Table 3, Matrix A), contrary to their spectrum of activity. In the latter case three main factors explained the *Trichoderma* strains as producers which showed sensibilities by their origin (Table 3, Matrix B) when evaluated by means of Canonic Correlation Analysis with exception of two industrial strains (L5 and L8). These two can be connected to soil originated strains only ($R^2=0.831$, $p<0.001$). All strains as variables but L7 and P8 have high loadings in first two spectral components comprising 73% of total variation of the spectral map indicating few qualitative differences in composition of exudates, meanwhile the quantity of produced molecules varied seemingly in strain dependent manner.
### Table 3: Similarity of strength of growth modulation and spectrum of activity of *Trichoderma* metabolites against fungi originated of various ecological niches

<table>
<thead>
<tr>
<th></th>
<th>Matrix A</th>
<th>Bark</th>
<th>Fungi</th>
<th>Soil</th>
<th>Industry</th>
<th>Surface</th>
<th>Vascular</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trichoderma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bark</td>
<td>0.364</td>
<td>0.121</td>
<td>0.088</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fungal bodies</td>
<td>0.944</td>
<td>0.012</td>
<td>0.006</td>
<td>0.001</td>
<td>0.038</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil</td>
<td>0.892</td>
<td>0.955</td>
<td>0.466</td>
<td>0.093</td>
<td>0.071</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Industry</td>
<td>0.261</td>
<td>0.323</td>
<td>0.831</td>
<td></td>
<td></td>
<td>0.187</td>
<td>0.074</td>
</tr>
<tr>
<td><strong>Pathogens</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface</td>
<td>0.397</td>
<td>0.265</td>
<td>0.809</td>
<td>0.185</td>
<td></td>
<td></td>
<td>0.513</td>
</tr>
<tr>
<td>Vascular</td>
<td>0.624</td>
<td>0.729</td>
<td>0.942</td>
<td>0.182</td>
<td>0.376</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of strains</td>
<td>7</td>
<td>6</td>
<td>15</td>
<td>2</td>
<td>4</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

Path coefficients R²>0.5 (< P=0.1%) are marked with bold numbers. Matrix A (under diagonal) and Matrix B (over diagonal) comprise path coefficients of relationships between spectrum of activity and potential activity values (strength of action) to various taxonomic groups, respectively.

**Figure 5:** Similarities in spectrum of sensitivities of diverse fungal groups to metabolite complex released to the medium by germinating conidia of *Trichoderma* strains

The codes of producer strains assigned to sections Hypocreanum (H), Longibrachiatum (L), Pacybasium (P) and Trichoderma (T) refer to those in Table 1. The opened and closed circles, while triangles and opened squares mark strains isolated of bark and fungal bodies, industrial strains and those of soil, respectively. The regression of trend line of the first root by means of Canonic Correlation of spectral components: Y_{Pathogens} = 0.831*X_{Trichoderma} (R² = 0.6905, p<0.01).
The sensitivity responses also varied in strain dependent manner to released metabolites, and no pattern was revealed when the strength or mode of alterations in growth were evaluated. However, contrary to production of metabolites, significant influence of traits related to adaptation to various ecological niches was manifested (Figure 2). In our model the small sized molecules get to the targeted mycelium more rapidly than macromolecules, thus the first reactions of hyphal tips leading to changes in growth intensity were most probably resulted by their effect. The high number of weighty components indicates the presence of several target sites taking role in regulation of hyphal growth. Some of analogous target sites may be presented in phytopathogenic filamentous fungi as well (Figure 5). However, on our view more species of the latter group have to be included into the comparative studies to identify the potential target sites, as only few PCs with small proportion of information correlated when data sets of responses of Trichoderma and phytopathogenic fungi were compared.

Losses caused by pathogenic microbes urges development of alternative control agents. One approach to discover newer antimicrobial compounds or antagonists is to search for their presence in natural sources. Trichoderma species play major role as biocontrol agents, owing to their capabilities of ameliorating crop-yields by multiple role, such as biopesticide, bioherbicides and plant growth promotion. Information on their use, mechanisms of antagonism and role in plant growth promotion has been well-documented [12]. Even if it is difficult to completely understand the function of their SMs in biological systems, it is important for researchers to attempt to identify as many possible effects associated with the release of any SMs as these molecules represent a prospective tool for pest management.

These studies have also importance for the safe use of Trichoderma based eubiotic preparations in pest management practices, since these agents are alive, their behavior, development and spread may be unpredictable and humans must attempt to foresee possible complications and mitigate any undesired effects. The eubiotic pesticides, being perceived as ‘natural’ and ‘low risk’, are often exempted from the rigorous testing required for chemical pesticides more than one pathogen on a plant. Numerous empirical observations demonstrated, it is often difficult to extrapolate from laboratory experiments to the field to know the exact biocontrol mechanism of an antagonist against a pathogen in vitro might be also be different in vivo.

Trichoderma species are considered to be harmless and spread into the environment improvidently, and several hundred preparation are marketed worldwide [7]. Nevertheless, these species might be very toxic to beneficial microbes and malignant to human Smith [35] put wise to the danger of mass spilling of antagonistic fungi: “opportunist mycoses are fungal diseases of man or other animals caused by fungi which are generally saprobes. They arise when either the fungus propagules are in exceptionally high concentrations or where the subject is compromised, that is when natural immunity is decreased by other diseases or during treatment with drugs such as immunosuppressants, chemotherapeutics or antibiotics.” This statement is right for Trichoderma species as well. The probability of such infections depends on the size of inoculums, i.e., how much is present in the environment. When applied as biocontrol agent, usually big masses of fungal propagula are dispersed into the environment. The risk of mesophilic mycoses caused by Trichodermas to an increasingly immunocompromised human population [14, 36] is an important unresolved issue in the ethics of biofungicides [37, 38, 39, 40]. All Trichoderma strains reported with damage to human belong to Longibrachiatum section, thus, on our opinion there is not advisable to select strains of species of Longibrachiatum section for mass use in biocontrol, but their secondary metabolites deserve attention.

Although, development of the experimental models efficiently predicting influence of bioactive compounds on the performance of living consortia or changes in behaviour of any exposed organism in econiches as well as fate of such compounds in the environment can not be expected soon, discoveries made in this field of research will surely significantly contribute to solution of such complex problems.

Conclusion
The examined Trichoderma showed high natural variation in sensitivity responses to metabolites released to the medium by other Trichoderma strains, and they produce this metabolite complex in strain dependent manner. Their growth responses to exudates depend on traits related to their adaptation to ecological niches, thus the suspected mycopatasitic strains may significantly differ of those habiting in the soil.
The released, in strain dependent manner, complex set of metabolites with broad intrageneric spectrum of growth moderating activity exhibited broad spectrum of activity against phytopathogenic fungi as well.

Few common factors were revealed comparing growth responses of Trichoderma and phytopathogens (their possible hosts) to released metabolites by examined Trichoderma strains. Seemingly, some genus specific traits regulate the character of intrageneric connections among various Trichoderma strains, and these properties might influence the performance of Trichoderma based eubiotic preparations in pest management practices.

The vertical diffusion technique as an experimental model us suitable method for elucidation of the presence of active substances in fungal exudates.

The analysis of chemical composition of the exudates with growth moderating effect can direct to discovery of new antimicrobial substances which might be promising lead compounds for development of new synthetic molecules with antifungal activity.

Acknowledgements

The research work was supported by the Baross Regional Innovation Programme (Grant No. EA07-EA-KOZKFI-2008-0066) and by The National Office for Research and Technology, Grant No. K-67688.

References

biological control on cacao pod production dynamics and black pod disease (Phytophthora megakarya) in Cameroon. Bio Control 44(2): 149-159.


