Book Chapter

Role of Intrageneric Competition in the Performance of Trichoderma Based Biofungicides

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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 intrageneric 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 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.

Keywords

Trichoderma; Secondary Metabolite; Exudate; Antagonism; Phytopathogens

phytopathogenic fungi, although these features are strongly influenced by environmental conditions [2, 9, 10, 11, 12].

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

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. *Trichodermas* 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 intragenetic 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 gL⁻¹ casein digest (Difco, 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, KH₂PO₄, K₂HPO₄ and MgSO₄ at 10, 12, 0.5, 0.5, 0.5 and 0.25 gL⁻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 ($\approx 5 \times 10^5$ 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 gL⁻¹, respectively) was overlaid, then centrally inoculated with small mycelium block ($\approx 2 \times 2$ 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 (d_{T24} and d_{T48}), 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 (d_{C24} and d_{C48}): Inhibition rate (%) = $100 - [100 \cdot (d_{T48} - d_{T24}) / (d_{C48} - d_{C24})]$. 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 $\times$ 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 anti-fungal activity of *Trichoderma* and sensitivity responses of test strains following [22]. The SCA separates the basic data matrix into two parts; 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 *Trichodermas* released to medium. The responses of phytopathogenic fungi to *Trichoderma* metabolites were imported of earlier publication [7] for comparative analysis.

Microsoft Office Excel 2003 statistical functions

(Microsoft, Redmond, USA) and modules of Statistica5 program (StatSoft, Tulsa, USA) were used for calculations. 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

No.	Producers (P) <i>Trichoderma</i> and <i>Hypocrea</i> species ^a	Targeted (T) strains ^b							
		S ^c	T01	T03	T04	T05	T06	T07	T08
1	<i>T. aureoviride</i> Rifai	H01	-16	-60	83	17	69	62	82
2	<i>T. citrinoviride</i> Bisset	L01	20	80	83	81	86	80	74
3	<i>T. longibrachiatum</i> Rifai	L02	9	86	80	-46	58	73	92
4	<i>T. pseudokoningii</i> Rifai	L03	23	78	88	70	84	81	92
5	<i>H. jecorina</i> Berkeley & Broome	L04	100	74	38	63	30	66	-2
6	<i>T. reesei</i> Simmons	L05	19	81	55	-35	70	73	58
7	<i>T. ghanense</i> Doi, Abe et Sugiyama	L06	75	79	97	69	91	89	74
8	<i>T. parceramosum</i> (Fabrea)	L07	70	90	-30	89	-10	38	85
9	<i>T. parceramosum</i> Bisset	L08	59	91	78	-41	76	85	85
10	<i>T. saturnisporum</i> Hammil	L09	81	23	46	13	60	73	69
11	<i>T. piluliferum</i> Webster & Rifai	P01	43	42	35	-2	32	81	36
12	<i>T. polysporum</i> (Link ex Persoon) Rifai	P02	14	57	-7	-29	-14	50	29
13	<i>T. hamatum</i> (Bonorden) Bainier	P03	-24	43	100	40	26	53	100
14	<i>T. harzianum</i> Rifai	P04	64	83	63	41	93	80	79
15	<i>T. harzianum</i> T/26	P05	89	91	77	72	70	76	60
16	<i>T. harzianum</i> (Rhytisma)	P06	21	75	-32	66	62	100	-30
17	<i>T. harzianum</i> Rifai (RS)	P07	68	58	86	45	81	88	79
18	<i>T. longipilis</i> Bisset (26)	P08	36	24	-6	72	36	50	65
19	<i>T. minutisporum</i> Bisset (27)	P09	69	88	78	78	68	68	54
20	<i>T. strictipile</i> Bisset	P10	9	20	7	0	37	3	-10
21	<i>T. tomentosum</i> Bisset	P11	-6	7	42	9	58	51	40
22	<i>T. virens</i> (Miller, Giddens & Foster) von Arx	P12	9	-6	52	-53	50	49	50
23	<i>T. atroviride</i> Karsten	T01	27	30	70	9	69	69	75
24	<i>T. koningii</i> Oudemans	T03	48	-13	71	72	78	76	69
25	<i>T. strigosum</i> Bisset (28)	T04	80	71	30	20	65	68	67
26	<i>T. viride</i> Persoon ex Gray	T05	34	52	69	68	82	77	84
27	<i>H. muroiana</i> Hino et Katsumoto	T06	-4	1	80	11	80	90	96
28	<i>H. muroiana</i> IAM 12502	T07	-78	37	29	-20	10	-16	30
29	<i>H. muroiana</i> IAM 12507	T08	52	63	42	11	48	59	7
30	<i>T. spirale</i> Bisset	N01	24	65	92	15	32	100	92
Stimulation (S) ^d			5	3	4	6	2	1	2
Inhibition (I)			25	26	26	22	28	28	27
No effect (NI)			0	1	0	2	0	1	1
LSD _{0.05}			6.9 (F _{PT} =23.22, p<0.05)						

^a= Names of species correspond to nomenclature of ISTE [41]. According to morphometric parameters anamorph of *Hypocrea muroiana* strains IAM 12502 and IAM 12507 are of *Trichoderma* section.

^b= Strains of *Trichoderma* section tested in dual cultures.

^c= Sections: H=*Hypocrea*, T=*Trichoderma*, L=*Longibrachiatum*, P=*Pachybasium*.

^d= 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

Producers ^a (P)				Targeted (T) strains ^b												G ^f
No.	Code ^c	O ^d	S ^e	P01	P02	P03	P04	P05	P06	P07	P08	P09	P10	P11	P12	
1	B-318	B	H01	100	100	66	53	71	73	29	67	76	39	78	17	29
2	B-311	S	L01	100	74	62	76	80	81	81	85	76	92	85	80	22
3	B-313	S	L02	80	88	67	58	77	75	65	77	80	64	83	77	32
4	B-312	S	L03	58	64	61	58	81	86	78	82	83	73	86	39	23
5	B-301	B	L04	79	100	65	58	100	36	7	54	18	49	68	100	30
6	B-314	I	L05	100	73	32	-48	65	58	59	66	54	74	53	38	28
7	B-305	S	L06	82	82	76	45	96	83	86	92	91	96	79	71	23
8	B-328	Dr	L07	100	100	83	73	20	-113	24	65	73	-50	-13	87	27
9	B-315	I	L08	100	72	20	54	74	80	65	61	59	89	74	78	28
10	B-316	S	L09	67	56	60	-19	60	15	58	54	71	62	38	65	32
11	B-306	S	P01	53	71	48	36	34	37	46	55	59	43	66	30	32
12	B-307	S	P02	53	57	50	-89	0	7	0	18	50	39	11	54	27
13	B-308	S	P03	69	60	26	38	100	93	37	60	83	43	90	-9	24
14	B-309	S	P04	100	90	74	50	74	85	83	59	58	77	84	79	38
15	B-075	B	P05	91	93	83	74	80	74	85	65	75	63	76	89	24
16	B-325	Ra	P06	82	88	78	59	65	73	69	41	74	60	70	47	23
17	B-317	Rs	P07	76	65	66	45	65	67	87	83	90	51	62	65	26
18	B-338	B	P08	55	53	49	9	-4	-33	16	4	4	-65	17	-2	16
19	B-339	B	P09	100	90	76	58	75	72	73	50	62	71	83	76	14
20	B-332	B	P10	78	72	50	42	37	60	51	41	59	55	55	30	29
21	B-331	B	P11	30	32	37	17	-18	32	2	57	56	15	63	0	29
22	B-310	S	P12	89	92	50	34	41	20	3	39	63	41	41	-2	27
23	B-320	S	T01	75	64	64	-7	50	37	35	27	51	65	44	50	26
24	B-322	S	T03	63	100	56	55	53	72	46	44	70	60	52	31	30
25	B-340	S	T04	76	89	67	45	53	63	67	63	49	71	68	45	23
26	B-319	S	T05	75	78	62	-17	75	82	80	77	74	88	84	73	21
27	B-302	Le	T06	46	46	19	-1	3	80	91	82	88	80	86	-2	24
28	B-303	Am	T07	77	64	63	44	1	24	24	26	70	48	49	-7	21
29	B-304	Le	T08	72	77	61	12	31	58	65	72	40	59	55	30	32
30	B-333	S	N01	48	72	60	38	-1	77	59	90	78	67	51	38	24
Stimulation (S) ^g				0	0	0	5	2	2	0	0	0	2	1	5	
Inhibition (I)				30	30	30	24	25	28	29	30	30	28	29	24	
No effect (NI)				0	0	0	1	3	0	1	0	0	0	0	1	
LSD _{0.05}				3.9 (F _{ET} =19.0, p<0.05)												

^a= Strains of *Trichoderma* and *Hypocrea* 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* (Berk.) Pegler, Dr= stroma of *Diplocarpon roseae* F.A. Wolf, Rs= pseudoscleritium of *Rhizoctonia solani* Kühn, Ra=*Rhizyctima acerinum* (Pers.) Fr.^e= Sections: H=Hypocrea, T=Trichoderma, L=Longibrachiatum, P=Pachybasium.^f= Radial growth (mm/day) measured on glucose agar amended with Trypton B (5 gL⁻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

Producers ^a (P)			Targeted (T) strains ^b												Response ^c		
No.	Code ^d	S ^e	L01	L02	L03	L04	L05	L06	L07	L08	L09	H01	N01	I	ST	NE	
1	B-318	H01	-17	55	71	49	-53	75	53	72	34	-110	64	2	5	0	
2	B-311	L01	35	83	70	64	31	79	86	50	67	-70	89	1	1	0	
3	B-313	L02	50	10	2	61	27	65	84	67	75	-100	62	0	2	0	
4	B-312	L03	38	38	1	69	48	93	88	56	47	38	71	0	0	0	
5	B-301	L04	91	40	61	100	29	69	50	100	88	50	27	6	1	0	
6	B-314	L05	-31	10	-50	45	-69	61	63	-1	44	31	75	1	6	0	
7	B-305	L06	46	13	81	97	62	59	100	72	75	79	92	1	0	0	
8	B-328	L07	96	67	96	-110	87	88	-34	11	100	81	31	3	7	0	
9	B-315	L08	21	49	83	74	0	37	70	63	59	-57	63	1	2	1	
10	B-316	L09	40	92	69	52	-8	60	73	2	69	79	73	0	2	0	
11	B-306	P01	53	20	38	63	38	26	41	61	63	22	54	0	1	0	
12	B-307	P02	-11	50	21	-54	-11	11	18	21	64	-14	19	0	8	2	
13	B-308	P03	-12	28	-97	53	-43	100	97	7	-17	-86	60	4	7	0	
14	B-309	P04	63	88	66	74	60	73	74	71	93	69	77	1	0	0	
15	B-075	P05	96	82	83	64	83	85	76	90	93	78	82	0	0	0	
16	B-325	P06	76	66	-35	45	63	76	59	68	74	48	33	1	3	0	
17	B-317	P07	77	36	36	74	66	82	49	54	87	49	64	0	0	0	
18	B-338	P08	67	65	36	13	-3	25	0	4	46	30	7	0	6	1	
19	B-339	P09	100	80	72	79	90	82	74	87	88	79	82	2	0	0	
20	B-332	P10	54	48	-3	47	47	3	52	2	59	35	55	0	2	1	
21	B-331	P11	44	48	-5	3	43	18	56	28	56	40	50	0	3	1	
22	B-310	P12	33	27	-50	44	6	41	27	16	72	-13	20	0	5	0	
23	B-320	T01	23	49	43	51	9	31	40	41	73	-86	28	0	2	0	
24	B-322	T03	-1	66	77	66	-22	50	62	58	58	-8	46	1	4	0	
25	B-340	T04	11	73	38	70	60	67	49	65	30	71	60	0	0	0	
26	B-319	T05	25	15	-7	77	52	66	76	82	78	27	76	0	2	0	
27	B-302	T06	55	-30	-30	84	4	88	91	-16	39	8	84	0	6	0	
28	B-303	T07	32	-30	-37	71	26	35	71	-9	63	47	29	0	7	0	
29	B-304	T08	57	-14	43	48	20	50	43	51	70	44	59	0	1	0	
30	B-333	N01	36	0	-1	29	51	57	92	43	61	-3	69	1	3	1	
Stimulation (S) ^f			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 (NI)			1	1	3	0	1	0	1	3	0	0	0				
LSD _{0.05}			5.1 (F _{pT} =21.9, p<0.05)										5.9	4.0			

^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 sex 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 g 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 % ($F_{T,P}=22.1$, $p < 0.05$). Nevertheless, in some cases (L4→L9, T4→P2, L8→P7, T6→L6, P11→T5, P10→P9) the variation in responses was higher than 10 %, due to irregular growth (sector formation, extreme stimulation, intense sporulation) in replicates. The cause of this phenomenon needs further studies.

Response of *Trichoderma* strains to released metabolites

The growth response of exposed strains varied within large limits ($F_T=175.7$, $p>0.001$) 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 target 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 strength 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 outlier, 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 their origin like Figure 2, thus no other patterns have been revealed.

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 ($F_p=177.8$, $p>0.001$), 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

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 Hypocrea (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 Group 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

O _b	Strain ^c	Principal Components ^a								
		PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
B	H01	-0.083	-0.358	0.632	0.090	-0.004	0.081	0.261	0.275	0.293
S	L01	0.036	-0.556	0.703	0.041	0.165	-0.039	0.041	-0.137	-0.180
S	L02	-0.117	-0.461	-0.515	-0.190	0.049	0.312	0.070	-0.072	-0.239
S	L03	-0.125	-0.606	-0.375	-0.067	-0.327	-0.083	0.403	-0.139	0.011
B	L04	0.737	0.062	0.154	-0.449	0.297	-0.060	0.315	-0.020	0.076
I	L05	0.206	-0.537	0.643	0.101	0.104	-0.175	-0.281	-0.074	-0.236
S	L06	0.440	-0.029	-0.285	0.594	0.287	0.289	0.017	0.343	-0.025
F	L07	0.833	0.251	0.055	-0.378	-0.060	0.037	-0.080	0.026	0.012
I	L08	0.087	-0.666	-0.319	-0.321	0.275	0.213	-0.238	-0.153	-0.254
S	L09	-0.315	-0.645	0.274	-0.071	-0.291	-0.399	-0.048	-0.244	0.223
S	P01	0.052	-0.337	-0.418	0.294	-0.186	-0.365	0.361	0.068	-0.174
S	P02	-0.170	-0.442	-0.556	0.096	0.343	-0.412	-0.076	0.048	0.271
S	P03	-0.074	-0.752	0.040	0.100	0.161	-0.202	-0.184	0.048	0.095
S	P04	0.156	-0.344	0.024	0.163	0.647	-0.251	-0.034	-0.191	-0.358
B	P05	0.490	-0.284	-0.397	0.034	0.052	-0.226	0.092	0.550	-0.071
F	P06	0.854	0.175	-0.044	-0.396	-0.070	-0.080	-0.158	0.083	0.003
F	P07	0.710	-0.332	0.205	0.292	-0.042	0.045	0.293	-0.044	0.154
B	P08	0.711	-0.260	-0.001	0.373	-0.223	0.006	-0.260	-0.149	0.088
B	P09	0.599	0.045	-0.057	0.488	-0.188	-0.115	-0.461	-0.033	0.084
B	P10	0.800	0.111	-0.029	-0.382	-0.247	-0.171	-0.238	0.128	0.015
B	P11	0.905	-0.046	-0.013	0.078	0.197	0.007	0.036	0.082	-0.121
S	P12	0.137	-0.802	-0.234	-0.225	-0.369	-0.025	-0.126	0.061	0.020
S	T01	-0.091	-0.781	-0.043	-0.335	-0.231	0.180	0.115	0.016	-0.026
S	T03	0.059	-0.359	0.463	0.120	-0.361	0.327	0.100	0.385	-0.410
S	T04	0.837	0.191	-0.101	-0.025	-0.004	-0.133	0.136	-0.211	-0.105
S	T05	0.159	-0.466	0.010	0.286	0.410	0.424	0.058	-0.051	0.381
F	T06	0.761	0.007	0.136	-0.291	0.254	0.020	0.365	-0.223	0.121
F	T07	0.245	-0.386	-0.237	-0.116	-0.078	0.614	-0.231	-0.194	0.182
F	T08	0.191	0.349	-0.193	0.513	-0.285	0.103	0.208	-0.475	-0.221
S	N01	0.821	-0.174	0.032	0.293	-0.330	-0.017	0.084	-0.076	0.084
Expl.Var		7.58	5.47	3.08	2.48	2.02	1.67	1.41	1.25	1.08
Prp.Totl		0.25	0.18	0.10	0.08	0.07	0.06	0.05	0.04	0.036
		Distribution of variation (% of total)								
Soil		8.67	1.58	0.53	2.49	0.54	1.33	1.16	1.08	0.40
Bark		10.38	0.99	1.95	2.47	0.92	0.35	1.71	1.42	0.42
Fungi		6.05	13.22	6.08	2.92	4.99	3.65	1.37	1.57	2.38
Industry		0.17	2.44	1.72	0.38	0.29	0.25	0.45	0.10	0.40

^a= 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. ^b= Strains originates (O) of fungal bodies (F), bark (B), soil (S) and industry (I). ^c= 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 = Hypocrea, L = Longibrachiatum, P = Pachybasium, T = Trichoderma, N = No lineage 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 ($p < 0.5$) loadings in any PC, while other six have high loadings ($p < 0.5$) 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 than 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 strenght 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 exaple 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 *Trichodermas* 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 *Trichodermas* ($R^2 = 0.809$). 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 strength of activity expressed to a single *Trichoderma* strain can not be predicted, while the strength of antagonistic effect measured on a single phytopathogenic fungus may orient on the expected effect to another 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 sensitivities 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 scivity of *Trichoderma* metabolites against fungi originated of various ecological niches

	Matrix B					
	<i>Trichodermas</i>				Pathogens	
Matrix A	Bark	Fungi	Soil	Industry	Surface	Vascular
<i>Trichodermas</i>						
Bark		0.364	0.121	0.088	<0.001	<0.001
Fungal bodies	0.944		0.012	0.006	0.001	0.038
Soil	0.892	0.955		0.466	0.093	0.071
Industry	0.261	0.323	0.831		0.187	0.074
<i>Pathogens</i>						
Surface	0.397	0.265	0.809	0.185		0.513
Vascular	0.624	0.729	0.942	0.182	0.376	
Number of strains	7	6	15	2	4	9

Path coefficients $R^2>0.5$ ($P=0.1\%$) 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 Hypocreaeanum (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: $Y_{Pathogens} = 0.831 \cdot X_{Trichodermas}$ ($R^2 = 0.6905$, $p<0.01$).

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. The mode of action 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: "opportunistic 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 mycoparasitic 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 is 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.

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References

- Weindling R (1932) *Trichoderma lignorum* as a Parasite of Other Soil Fungi. *Phytopathology* 22: 837- 845.
- Papavizas GC (1985) *Trichoderma* and *Gliocladium*: biology, ecology, and potential for biocontrol. *Annu Rev Phytopathol* 23: 23-54.
- Verma M, Satinder K, Brar RD, et al. (2007) Antagonistic fungi, *Trichoderma* spp: Panoply of biological control. *Biochem Eng J* 37: 1–20.
- Naeimi S, Khodaparast SA, Javan-Nikkhah M, et al. (2011) Species pattern and phylogenetic relationships of *Trichoderma* strains in rice fields of Southern Caspian Sea, Iran. *Cereal Res Commun* 39(4): 560-568.
- Cumagun CJR. (2014) Advances in Formulation of *Trichoderma* for Biocontrol. In: *Biotechnology and Biology of Trichoderma* (Editors: Vijai G Gupta, Monika Schmoll, Alfredo Herrera-Estrella, RS Upadhyay, Irina Druzhinina, Maria Tuohy) Chapter 39: 52-531.
- Waghund RR, Shelake RM, Sabalpara AN (2016) *Trichoderma*: A significant fungus for agriculture and environment. *Afr. J. Agric. Res* 11(22): 1952-1965.
- Oros G, Naár Z (2017) Mycofungicide: *Trichoderma* Based Preparation for Foliar Applications. *Am J Plant Sci* 8: 113-125.
- Pandya JR (2017) Petite Prelude: *Trichoderma* and its Significance. *Asian Journal of Applied Science and Technology* 1(6): 69-70.
- Naár Z (2007) Ecological Evaluation of Factors Influencing the Soil Colonization of Antagonistic *Trichoderma* Species. PhD Thesis, Hungarian Academy of Sciences, Budapest
- Esposito E, de Silva M (1998) Systematics and environmental application of the genus *Trichoderma*. *Crit Rev Microbiol* 24: 89-98.
- Atanasova L (2014) Ecophysiology of *Trichoderma* in Genomic Perspective. In: *Biotechnology and Biology of Trichoderma* (Editors: Gupta G V, Schmoll M, Herrera-Estrella A, Upadhyay RS, Druzhinina I, Tuohy M) Chapter 2: 25-40.
- Kashyap PL, Rai P, Srivastava AK, et al. (2017) *Trichoderma* for climate resilient agriculture. *World J Microbiol Biotechnol* 33(8): 155.
- Naár Z, Dobos A (2006) Redundancy Analysis of the Influence of Metal Content and Other Edaphic Parameters on the Coexistence of *Trichoderma* Species. *Appl Ecol Env Res* 4: 113-123.
- Kredics L, Antal Z, Dóczy I, et al. (2003) Clinical importance of the genus *Trichoderma*. A review *Acta Microbiol Immunol Hung* 50: 105–117.
- Bae YS, Knudsen GR (2005) Soil microbial biomass influence on growth and biocontrol efficacy of *Trichoderma harzianum*. *Biol Control* 32(2): 236-242.
- Deberdt P, Mfegue CV, Tondje PR, et al. (2009) Impact of environmental factors, chemical fungicide and

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

17. Mukherjee PK, Horwitz BA, Herrera-Estrella A, et al. (2013) *Trichoderma* Research in the Genomic Era. *Annu Rev Phytopathol* 51: 105-129.

18. Sharma P, Vignesh Kumar P, Ramesh R, et al. (2011) Biocontrol genes from *Trichoderma* species. *Afr J Biotechnol* 10(86): 19898-19907.

19. Szekeres A, Leitgeb B, Kredics L, et al. (2005) Peptaibols and related peptaibiotics in *Trichoderma*. *Acta Microbiol Immunol Hung* 52(2): 137-168.

20. Radjacommaré R, Venkatesan S, Samiyappan R (2010) Biological control of phytopathogenic fungi of vanilla through lytic action of *Trichoderma* species and *Pseudomonas fluorescens*. *Arch Phytopathology Plant Protect* 43: 1-17.

21. Anees M, Tronsmo A, Edel-Hermann V, et al. (2010) Characterization of field isolates of *Trichoderma* antagonistic against *Rhizoctonia solani*. *Fungal Biol* 114(9): 691-701.

22. Lewi PJ (1976) Spectral Mapping, a Technique for Classifying Biological Activity Profiles of Chemical Compounds. *Arzneim Forsch* 26(7): 1295-1300.

23. Mardia KV, Kent JT, Bibby JM (1979) *Multivariate Analysis*, Academic Press, London, 213-254.

24. Jaklitsch WM, Volgmayr H (2015) Biodiversity of *Trichoderma* (Hypocreaceae) in Southern Europe and Macaronesia. *Stud Mycol* 80: 1-87.

25. Saravanakumar K, Yu C, Dou K, et al. (2016) Biodiversity of *Trichoderma* Community in the Tidal Flats and Wetland of Southeastern China. *PLoS ONE* 11(12): e0168020.

26. Siddiquee S (2017) *Practical Handbook of the Biology and Molecular Diversity of Trichoderma from Tropical Regions*. Springer IP AG, Chem, Swiss.

27. Kredics L, Hatvani L, Naeimi S, et al. (2014)

Biodiversity of the Genus *Hypocrea*/*Trichoderma* in Different Habitats. In: *Biotechnology and Biology of Trichoderma* (Editors: Gupta G V, Schmoll M, Herrera-Estrella A, Upadhyay RS, Druzhinina I, Tuohy M) Chapter 1: 3-24.

28. Hermosa R, Elena Cardoza R, Belén Rubio M, et al. (2014) Secondary Metabolism and Antimicrobial Metabolites of *Trichoderma*. In: *Biotechnology and Biology of Trichoderma* (Editors: Gupta VG, Schmoll M, Herrera-Estrella A, Upadhyay RS, Druzhinina I, Tuohy M) Chapter 10: 125-137.

29. Nguyen Q, Ueda K, Kihara J, et al. (2016) Culture Filtrates of *Trichoderma* Isolate H921 Inhibit *Magnaporthe oryzae* Spore Germination and Blast Lesion Formation in Rice. *Adv Microbiol* 6: 521-527.

30. Pandey V, Shukla A, Kumar J (2016) Physiological and Molecular Signalling Involved in Disease Management through *Trichoderma*: An Effective Biocontrol Paradigm. In: *Current trends in disease diagnostics and management practices* (Editors: Kumar P, Gupta VK., Tiwari AK, Kamle M) 317-346.

31. Atanasova L, Le Crom S, Gruber S, et al. (2013) Comparative transcriptomics reveals different strategies of *Trichoderma* mycoparasitism. *BMC Genomics* 14: 121-136.

32. Reithner B, Ibarra-Laclette E, Mach RL, et al. (2011) Identification of Mycoparasitism-Related Genes in *Trichoderma atroviride*. *Appl Environ Microbiol* 77(13): 4361-4370.

33. Shaw S, LeCocq K, Paszkiewicz K, et al. (2016) Transcriptional reprogramming underpins enhanced plant growth promotion by the biocontrol fungus *Trichoderma hamatum* GD12 during antagonistic interactions with *Sclerotinia sclerotiorum* in soil. *Molecular Plant Pathology* 17(9): 1425-1441.

34. Guzmán-Guzmán P, Alamán-Guerte MI, Delaye L, et al. (2017) Identification of effector-like proteins in *Trichoderma* spp And role of hydrophobin in the plant-fungus interaction and mycoparasitism. *BMC Genetics* 18:16-36.

35. Smith JMB (1989) Opportunistic mycoses of man and of the animals. Wallingford, UK, CAB International Mycological Institute, 220 pp.
36. Jacobs F, Byl B, Bourgeois N, et al. (1992) *Trichoderma viride* infection in a liver transplant recipient. *Mycoses* 35(11-12): 301–303.
37. Lockwood JA (1996) The ethics of biological control: undersatnding the moral implications of our most powerful ecological technology. *Agric Human Values* 13(1): 2-19.
38. Repetto R, Baliga SS (1996) Pesticides and the immune system, The public health risks. World Resources Institute, Washington DC, USA, ISBN 1-56973-087-3
39. Samson RA, Hoekstra ES, Frisvad JC et al. (1995) Introduction to food-borne fungi. 5th edn. Baarn/Delft, Netherlands, Centraalbureau voor Schimmelcultures, 322 pp.
40. Ricard J L, Ricard TJ (1997) The ethics of biofungicides – A case study: *Trichoderma harzianum* ATCC 20476 on Elsanta strawberries against *Botrytis cinerea* (gray mold). *Agric Human Values* 14: 251-258.
41. Bissett J, Gams W, Jaklitsch W et al. (2015) Accepted *Trichoderma* names. http://www.ingentaconnect.com/content/ima/imafung/pre-prints/content-j2_Vol6_no2_Article2