Scientific Research Publishing

American Journal of Plant Sciences, 2017, 8, 113-125
http://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

Mycofungicide: *Trichoderma* Based Preparation for Foliar Applications

Gyula Oros[1], Zoltán Naár[2]

[1]Plant Protection Institute HAS, Budapest, Hungary
[2]NARIC Food Research Institute, Budapest, Hungary
Email: gyula.oros@gmail.com, z.naar@crfri.hu

How to cite this paper: Oros, G. and Naár, Z. (2017) Mycofungicide: *Trichoderma* Based Preparation for Foliar Applications. *American Journal of Plant Sciences*, **8**, 113-125.
http://dx.doi.org/10.4236/ajps.2017.82009

Received: November 24, 2016
Accepted: January 16, 2017
Published: January 19, 2017

Abstract

The *Trichoderma* based emulsifiable mycofungicide for controlling foliar diseases lessened the yield loss to economically acceptable level with significant increase of the quality of product. The amount of phylloplane originated *T. harzianum* and *T. parceramosum* strains containing liquid formulation, to be applied as leaf spray, might be reduced in two order of magnitude as compared to the solid preparations to achieve the same effect. Both sensitivity of 13 phytopathogenic fungi to antifungal properties of toxic substances released by 32 *Trichoderma* strains and their susceptibility to the same were examined during development of new mycofungicide. Both toxin production of *Trichodermas* and the sensitivity of target fungi varied within large limits, being *Pythium irregulare* the most, while *Phytophthora infestans* and *Macrophomina phaseolina* the less tolerant. The sensitivity responses of fungi to toxins correlated to their susceptibility to antagonists. The spectrum of antagonists of pathogenic fungus or targets of *Trichoderma strain* proved to be unpredictable. Conidia of *Trichoderma* strains in liquid paraffin (LP) of pharmaceutical quality (LP PQ) survived over 2 years. However, in commercial LP the shelf life of them significantly decreased in strain dependent manner, and the presence of emulsifiers selectively reduced the survival rate as well. The LP PQ was not phytotoxic in therapeutic doses, but commercial LP proved to be toxic when applied as leaf spray independently on the emulsifiers. Both fungitoxic and phytotoxic contaminants of commercial LP could be eliminated with activated carbon.

Keywords

Mycofungicide, *Trichoderma*, Rosa, *Diplocarpon*, Pepper, *Phytophthora*

1. Introduction

The observation of antagonistic properties *T. virens* [1] promoted efforts to ex-

plore this feature for controlling phytopathogenic fungi [2]. Nowadays biology of this genus is intensively studied and various strains are used in diverse fields of human practices [3] [4] [5]. Examination of numerous strains revealed that the antagonism is a common property of *Trichoderma* species and they usually can parasite the phytopathogenic fungi [3] [6] [7] [8], although these features are strongly influenced by environmental conditions [9] [10]. Since discovery of Weidling [1] large *Trichoderma* based industry has been developed, and diverse ways of their use have been patented (**Table 1**) as well as several hundred *Trichoderma* based preparations have been commercialized to prevent yield losses caused by phytopathogenic microbes [11]. These 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 [10] [12] [13] [14]. We should remark that some *T. harzianum* strains seemingly are in reality *T. asperellum*, thus the taxonomic position of strains needs verification applying recent molecular methods [15].

About four fifths of commercialized *Trichoderma* formulations are wettable powders or granules containing dried propagules (conidia and chlamydospores)

Table 1. Number of patents applied on the use of various *Trichodermas.*

Trichoderma spp.	Section[a]	Clade	A[b]	B
asperellum	P	13-hamatum	30	9
atroviride	T	12-viride	52	19
citrinoviride	L	14-longibrachiatum	14	0
gamsii	?	?	2	0
ghanense	L	14-longibrachiatum	8	0
hamatum	P	13-hamatum	88	31
harzianum	P	1-harzianum	1170	108
jecorina	L	14-longibrachiatum	306	7
koningii	T	12-viride	748	27
longibrachiatum	L	14-longibrachiatum	1007	12
parceramosum	L	14-longibrachiatum	93	0
piluliferum	P	9-pachybasioides	3	1
polysporum	P	9-pachybasioides	122	7
pseudokoningii	L	14-longibrachiatum	93	13
reesei	L	14-longibrachiatum	2136	28
saturnisporum	L	14-longibrachiatum	40	1
tomentosum	P	14-longibrachiatum	8	0
virens	P	2-virens	227	62
All together	3	5	7240	246

[a]Sections: L = Longibrachiatum, P = Pachybasium, T = Trichoderma. [b]The number of cases where the species was mentioned (A) and patents (B) of preparations used for biocontrol in database of US Patent Office since 1790.

of a given concentration to be mixed with water or diluted with suitable solid material prior to use [16], and applied by diverse methods [11]. The rate of use varies between 50 - 300 kg·ha^{-1} that is realistic for soil applications of homemade products to be used in small plots [16] but hinders both their wholesale marketing and expansive use in big farms. The narrow compatibility of eubiotic biocides with pesticides containing synthetic active ingredients also limits their integration into recent pest management programs with special regard to their application against canopy threatening pathogens, where the use of modern synthetic pesticides is inevitable due to the required high sureness of the protective effect (ornamental plants, leafy vegetables, fruits etc.). In the cases of integrated use the sensitivity of the antagonist to inert ingredients (surfactants, liquid or solid carriers, etc.) can also be a restricting factor [14]. Nevertheless, the liquid formulations containing *Trichoderm*a propagules has great advantages thus we started to develop an eubiotic preparate based on organic carrier that might open a possibility to decrease the specific rate of use and the foliar application of such biofungicide. The response of phytopathogenic fungi to toxic compounds released by *Trichoderma* strains of various taxonomic position as well as the survival of *Trichoderma* conidia in liquid preparations were examined in model experiments for selection of appropriate strain useable in biocontrol practices [17].

Here we present our experiences of the development and promising results hoping to promote further work in this field.

2. Material and Methods

2.1. Fungi

The *fungi* listed in **Table 2** were maintained on potato dextrose agar slants at 22˚C - 25˚C (CM0139B, OXOID, Basingstoke) amended with 2 g·L^{-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, KH_2PO_4, K_2HPO_4 and $MgSO_4$ at 10, 12, 0.5, 0.5, 0.5 and 0.5 g·L^{-1}, respectively). All strains were from the Mycology Collection (WDCM824) of the Plant Protection Institute, Hungarian Academy of Sciences (Budapest, Hungary).

2.2. 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 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˚C - 50˚C) prepared as above (1 + 10 mL) and dispensed into Petri dishes (90 mm ∅). After 16 hours incubation 10 mL of Trypton B containing agar solution (2 and 10 g·L^{-1}, respectively) was over layered than centrally inoculated with mycelium disc (3.5 mm ∅) cut of three days old colony of phytopathogenic fungi grown up on PDA as above at 22˚C - 24˚C. Response of *Pythium* and *Phytophthora* was tested on Green Pea Agar prepared as described earlier [18] at 15˚C - 18˚C. Diameter of

Table 2. (a) Sensitivity response of fungi to *Trichoderma* toxins; (b) Sensitivity response of fungi to *Trichoderma* toxins.

(a)

Trichoderma and *Hypocrea* species	C[a]	Test Fungi[b]						
		A	B	C	D	E	F	G
T. aureoviride	B-318	43	69	84	63	97	52	8
T. citrinoviride	B-311	83	78	62	100	81	76	-13
T. longibrachiatum	B-313	88	90	96	91	76	88	55
T. pseudokoningii	B-312	38	83	11	74	68	84	29
H. jecorina	B-301	40	164	18	100	82	12	40
T. reesei	B-314	30	73	44	88	64	32	-25
T. ghanense	B-305	83	88	98	100	56	80	38
T. parceramosum	B-328	98	73	85	92	96	56	50
T. parceramosum	B-315	77	80	85	90	92	40	50
T. saturnisporum	B-316	78	45	62	100	70	80	45
T. piluliferum	B-306	70	89	80	100	97	28	53
T. polysporum	B-307	83	86	82	100	97	88	50
T. hamatum	B-308	22	40	0	60	66	0	13
T. harzianum	B-309	90	88	98	100	97	68	50
T. harzianum	B-075	78	84	98	100	95	64	48
T. harzianum	B-325	78	80	80	100	95	64	49
T. harzianum	B-408	92	90	100	100	97	80	50
T. harzianum	B-317	100	83	85	100	85	76	53
T. longipile	B-338	53	84	76	76	100	100	100
T. minutisporum	B-339	63	95	98	100	100	100	100
T. strictipile	B-332	80	84	91	93	100	100	40
T. tomentosum	B-331	85	93	95	94	100	100	49
T. virens	B-310	20	40	25	82	96	28	50
T. atroviride	B-320	97	93	96	94	92	60	50
T. atroviride	B-396	95	93	100	96	92	68	50
T. koningii	B-322	13	38	5	64	81	4	3
T. strigosum	B-340	82	88	78	89	100	100	100
T. viride	B-319	78	83	38	88	83	68	−25
H. muroiana	B-302	85	76	87	100	90	32	15
H. muroiana	B-303	85	76	80	100	87	60	3
H. muroiana	B-304	83	79	84	100	89	68	11
T. spirale	B-333	73	84	95	93	100	100	28

[a]Codes are accession numbers of strains in Mycology Collection. [b]Test fungi: A = *Pleospora tarda* E.G. Simmons, B = *Alternaria solani* Sorauer, C = *Cochliobolus carbonum* R.R. Nelson, D = *Macrophomina phaseolina* (Tassi) Goid., E = *Sclerotinia sclerotiorum* (Lib.) deBary, F = *Verticillium albo-atrum* Reinke et Berthold, G = *Fusarium venenatum* Nierenberg.

(b)

N	*Trichoderma* and *Hypocrea* species	O[a]	S[b]	Test Fungi[b]					
				H	I	J	K	L	M
1	*T. aureoviride*	K	H01	0	63	28	0	0	23
2	*T. citrinoviride*	T	L01	47	100	95	0	64	68
3	*T. longibrachiatum*	T	L02	9	100	100	95	95	91
4	*T. pseudokoningii*	T	L03	100	76	100	32	0	0
5	*Hypocrea jecorina*	K	L04	0	92	0	0	0	0
6	*T. reesei*	Ind	L05	0	11	100	98	31	77
7	*T. ghanense*	T	L06	0	100	100	100	100	85
8	*T. parceramosum*	*Dr*	L07	0	82	0	12	0	0
9	*T. parceramosum*	Ind	L08	7	100	100	98	73	45
10	*T. saturnisporum*	T	L09	0	100	92	0	7	77
11	*T. piluliferum*	T	P01	9	100	95	62	100	85
12	*T. polysporum*	T	P02	14	92	100	100	72	82
13	*T. hamatum*	T	P03	0	0	0	0	0	0
14	*T. harzianum*	T	P04	14	100	100	100	100	92
16	*T. harzianum*	K	P05	13	58	100	35	100	95
17	*T. harzianum*	*Ra*	P06	0	95	96	0	28	48
18	*T. harzianum*	*Ra*	P13	21	100	100	100	93	95
19	*T. harzianum*	*Rs*	P07	3	87	100	95	85	95
20	*T. longipile*	K	P08	3	100	99	23	100	78
21	*T. minutisporum*	K	P09	0	100	100	100	0	100
22	*T. strictipile*	K	P10	0	100	72	8	24	42
23	*T. tomentosum*	K	P11	0	84	68	35	60	74
24	*T. virens*	T	P12	100	76	0	0	0	0
25	*T. atroviride*	T	T01	27	100	100	0	75	71
26	*T. atroviride*	*Cl*	T02	0	100	100	78	87	89
27	*T. koningii*	T	T03	0	47	0	0	31	0
28	*T. strigosum*	T	T04	0	95	7	48	73	48
29	*T. viride*	T	T05	0	82	0	100	0	8
30	*H. muroiana*	*Le*	T06	14	100	89	86	60	77
31	*H. muroiana*	*Am*	T07	0	100	53	0	0	11
32	*H. muroiana*	Le	T08	0	100	33	0	0	35
33	*T. spirale*	T	N01	100	100	95	28	91	54

[a]Origin: K = bark, T = soil, Ind= industrial, *Dr* = stroma of *Diplocarpon rosae* F.A. Wolf, *Ra* = stroma of *Rhytisma acerium* (Pers.) Fr., *Cl* = *Fusarium oxysporum* Schltdl. infected *Chionodoxa lucillae* Boiss. bulb, *Rs*= pseudosclerotium of *Rhizoctonia solani* Kühn, *Le* = *Lentinula edodes* (Berk.) Pegler, *Am* = rhizomorph of *Armillaria mellea* (Vahl) P. Kumm. [b]Sections: H = Hypocreanum, L = Longibrachiatum, P = Pachybasium, T = Trichoderma, N = No lineage lineage, according to International Subcomission on Trichoderma and Hypocrea Taxonomy. [c]Test fungi: H = *Pythium irregulare* Buism., I = *Phytophthora infestans* (Mont.) deBary., J = *Waitea circinata* Warcup et P.H.B., K = *Rhizoctonia solani* Kühn. AG-3, L = *Rhizoctonia solani* Kühn. AG-4, M = *Rhizoctonia solani* Kühn. AG-2.

colonies was measured 24 and 48 hours later (dT_{24} and dT_{48}), 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 (dC_{24} and dC_{48}): Inhibition rate (%) = 100 − [100*(dT_{48} − dT_{24})/(dC_{48} − dC_{24})]. Negative values mean stimulation of fungal growth.

Storage life test: The conidia of *Trichoderma* were washed up with paraffin oil (pharmacological quality) of 8 days old colonies grown up on milk agar then suspension was mixed up with 250 mg of dry $MgSO_4$ and 10 min later shaken up and filtered through glass filter (G-2). The conidia were separated of paraffin oil in centrifuge (5 min, 2000 rpm) and the sediment suspended subsequently with appropriate carrier material to achieve concentration 10^8 cell·mL^{-1} or 10^9 cell·g^{-1} than the prepared formulations were stored at usual conditions for pesticides. During first two week samples were taken each day and later weekly and the number of living cell was counted by ten and two-fold dilution series technique.

Preparation of eubiotic formulations was carried out following recipes described in examples of the patent application HPO 0800405 [17].

Treatment of plants: Watery suspensions or suspo-emulsions were made of formulations at appropriate concentrations and sprayed run off by usual manner. Their effect was evaluated either by measuring changes in yield or health state of plants treated.

Survival of Trichoderma in canopy was examined washing the surface of leaves and counting the number of propagules by ten and two-fold dilution series technique on Askew and Lang medium [19].

Detecting pathogens on pepper field was carried out by traditional manners: Disease symptoms were evaluated as well as samples of plant organs were taken and surveyed under dissecting microscope. The zoosporangia formed on leaves were transmitted onto the potato discs (cv. Desirée) to detect the presence of *P. infestans*. The selective media were used to reveal presence of *Fusarium* [20], *Macrophomina* [21], *Pythium* [22] and *Rhizoctonia* [23] in root necks, where the respective microbicides were replaced with metalaxyl (100 mg·L^{-1}), carbendazim (50 mg·L^{-1}), triadimefon (20 mg·L^{-1}) and kanamycin (10 mg·L^{-1}) to depress the respective microbes presented in samples. Propamocarb (100 mg·L^{-1}) was used for differentiation between *Pythium* and *Phytophthora*.

2.3. Data Analysis

Fisher's test was applied to evaluate significance of differences between variants at $p = 0.05$ level. The experimental data were analyzed with multivariate statistical methods where the basic data matrix (32 *Trichoderma* × 13 test fungi) was transformed into probit values. Potency Mapping (PM) and Spectral Component Analysis (SCA) were employed to disclose differences between both antifungal activity of *Trichodermas* and sensitivity responses of test species following Lewi [24]. The SCA separates the basic data matrix into two part; the first is a vector proportional to overall strength of response (PM), while the second is a matrix of spectral components (SPM) characterizing the spectrum of activity or sensitivity.

The Principal Component Analysis was applied to demonstrate the potential number of factors affecting the selective response of target fungi to toxic principles [24]. Cluster Analysis (CA) was carried out to reveal relationship among spectrum of activities of *Trichoderma* strains as spectral variables.

3. Results

The *Trichoderma* strains broke throw the agar layer after 72 hours in majority of cases, thus the growth of test fungi was altered only by metabolites excreted and diffused throw the agar layer. The growth inhibition rates are compiled in Table 2. The reproducibility of experiments was good, which means, both test fungi and *Trichoderma* strains grew near synchronously supporting the reliability of measurements ($F_{replication} = 1.91 < F_{0,05} = 2.30$).

3.1. Antifungal Activity of Metabolites Released by *Trichoderma* Strains

The strength of antifungal activity of *Trichoderma* metabolites varied in strain dependent manner, and differences between strains of the same species were in the level of differences between strains of strains of various sections (Figure 1).

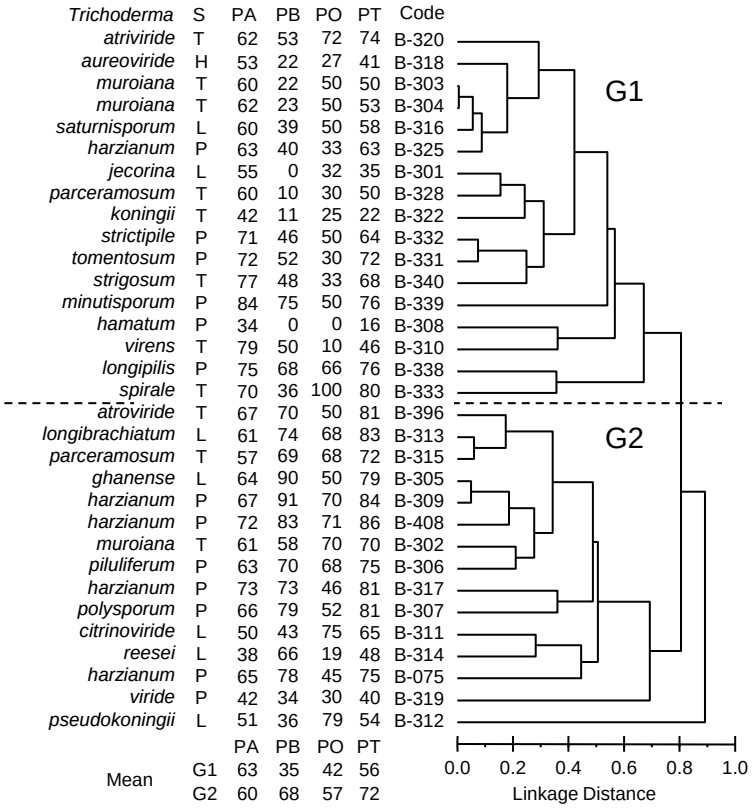

Figure 1. Cluster-diagram of the interrelationships between *Trichoderma* strains on the base of the antifungal efficacy of their metabolites released. Unweighted pair group average method based on Pearson's correlation coefficients of spectral component variables (*Trichoderma* strains) was applied to construct the clusterogram. The codes refer to strains as listed in Table 2. S = see Table 2. PA, PB and PO are potential activities against asco-, basidio- and oomycetaceous fungi, respectively. PT is the overall potential activity.

For example, two strains of *T. harzianum* (B305 and B428) isolated of *R. acerinum* exhibited different potential activities against asco-, basidio- and oomycetaceous fungi. *Trichoderma* strains formed two groups (G1 and G2) when clustered applying selective growth responses of test fungi in dual cultures being the group G2 significantly more active ($p = 0.005$). The potential host range of these groups also altered being the strains of G1 group more active against asco- while those of G2 against basidiomycetaceous targets ($p = 0.001$ and 0.08, respectively). However, the subclusters G1 and G2 were formed of strains of various sections, indicating that their taxonomic position did not relate to this groupping.

3.2. Sensitivity Response of Test Fungi to Metabolites of *Trichoderma* Strains

The sensitivity of target species varied within large limits, being the *Pythium irregulare* the most, while *Phytophthora infestans* and *Macrophomina phaseolina* the less tolerant ones among 13 phytopathogenic fungi tested (Table 2). Three major factors comprised 68% of total variation of SPM as revealed by means of PCA determining the selective response of target species (37, 20 and 9 percent). Plotting target species as spectral variables one compact group was formed and some outliers stand apart (Figure 2).

3.3. Survival of *Trichodermas* in Liquid Formulations

The LP PQ was not phytotoxic in therapeutic doses, but commercial LP proved to be harmful when applied as leaf spray independently on the emulsifiers. Both

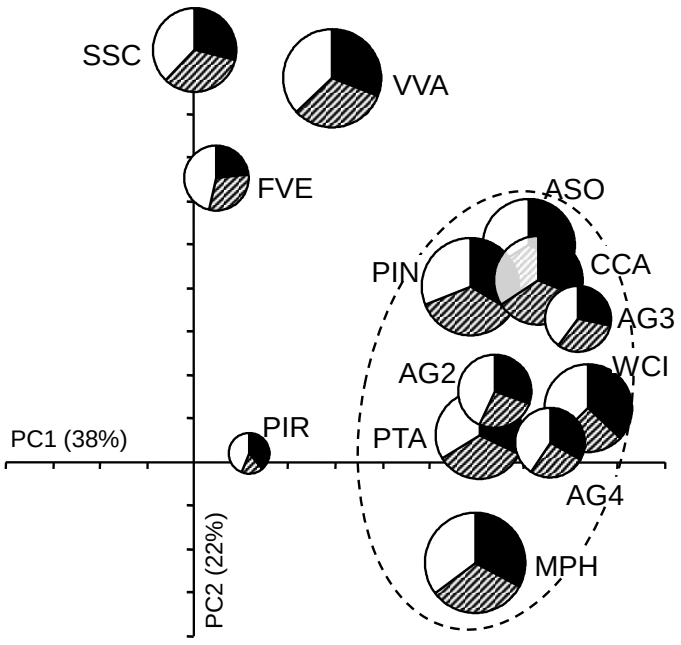

Figure 2. Similarity of test fungi based on their growth responses to toxin composition released by *Trichoderma* strains into the medium. The codes refer to species listed in Table 2. The size of pies is proportional to potential sensitivity to released mycotoxic substances, while the size of white, black and striped sectors relates to sensitivity responses to strains of Pachibasium, Longibrachiatum and Trichoderma sections, respectively.

fungitoxic and phytotoxic contaminants of commercial LP could be eliminated with activated carbon [HPO]. All *Trichoderma* strains examined well tolerated the LP PQ (Table 3). However, their conidia lost vitality rapidly in commercial

Table 3. Tolerance of *Trichoderma* conidia to mineral oil formulations[a].

Strains[b]		Survival of preparations[b] (days)				
Code	Sect	A	B	C	D	E
318	H	>360	12 - 66	12 - 66	7 - 31	11 - 38
311	L	>360	10 - 52	7 - 45	3 - 45	7 - 45
313	L	>360	9 - 17	5 - 12	1 - 2	1 - 4
312	L	>360	9 - 17	9 - 17	1 - 4	1 - 5
301	L	>360	12 - 66	12 - 66	5 - 38	7 - 38
314	L	>360	10 - 52	10 - 45	2 - 45	5 - 45
305	L	>360	17 - 180	17 - 180	4 - 57	7 - 57
328	L	>360	>360	>360	360 - >360	360 - >360
315	L	>360	52 - 80	52 - 80	17 - 38	17 - 38
316	L	>360	9 - 45	7 - 38	1 - 5	3 - 5
306	P	>360	10 - 52	10 - 45	2 - 45	5 - 45
307	P	>360	17 - 52	17 - 45	8 - 17	8 - 17
308	P	>360	10 - 52	10 - 52	5 - 52	7 - 52
309	P	>360	9 - 17	8 - 14	1 - 2	2 - 6
075	P	>360	12 - 66	12 - 66	8 - 12	8 - 17
325	P	>360	360 - >360	360 - >360	180 - 360	180 - 360
408	P	>360	360 - >360	360 - >360	180 - >360	180 - >360
317	P	>360	>360	>360	>360	>360
338	P	>360	17 - 180	17 - 180	17 - 31	17 - 31
339	P	>360	12 - 66	12 - 66	6 - 10	6 - 10
332	P	>360	>360	>360	180 - 360	180 - 360
331	P	>360	52 - 80	52 - 80	17 - 38	17 - 45
310	P	>360	180-360	180-360	45 - 66	45 - 66
320	T	>360	>360	>360	360 - >360	360 - >360
396	T	>360	52 - 80	45 - 80	45 - 80	45 - 80
322	T	>360	10 - 52	9 - 59	6 - 17	8 - 17
340	T	>360	10 - 24	10 - 24	6 - 9	6 - 9
319	T	>360	9 - 17	9 - 17	1 - 4	2 - 6
302	T	>360	52 - 80	52 - 80	17 - 66	24 - 66
303	T	>360	6 - 9	6 - 9	1 - 4	1 - 4
304	T	>360	7 - 24	7 - 24	3 - 5	3 - 5
333	N	>360	12 - 66	12 - 66	10 - 38	10 - 38

[a]Composition of preparations tested: A = Paraffin oil PH V., B and C = Paraffin oil, Atloxplus 300F (Uniquema), Tween 20 (Reanal), Hostaphat KLM (Clariant, Swiss), Alcanol-polyglycol-ether (EO:15) (Clariant), lecithin (Merck), (D = Vektafid (Rogátor Kft., Hungary), E = Spraypover (Fine Agrochemicals Ltd., UK). [b]Codes and sections: see Table 2. [c]Limits of time (days) requested for decrease to 100 living cells per ml or destruction of all conidia in the preparation, respectively.

pesticides even in cases, when the active ingredient did not possess fungicidal activity due to toxicity of carriers and surfactants [14] or impurities of liquid carriers. We have tested the shelf life of *Trichoderma* strains storing their conidia in liquid formulations that meet requirements of application (**Table 3**). The surfactants heavily and strain dependent manner influenced the survival of conidia. Nevertheless, some strains exhibited high tolerance, and these were selected for field applications.

3.4. Results of Foliar Applications

The liquid formulations were significantly more efficient against rose black spot disease either evaluated by incidence of spots or distribution in canopy, with special regard to *T. harzianum* strains (**Table 4**). The strains of phylloplane origin also proved to be more efficient, independently of their taxonomic position, even in solid formulations. However, only two of them were as active as the chemical control (combination of Benlate and Tilt in doses recommended by producers). The amount of liquid formulations to be applied as leaf spray could be reduced in two order of magnitude as compared to the solid preparations to achieve the same effect. The application of optimized liquid preparation based on phylloplane originated *T. harzianum* that exhibited high activity against some pepper diseases lessened the yield loss to economically acceptable level with significant increase of the quality of product (**Table 5**). The plants were wilted mainly by *Macrophomina* infection, *Rhizoctonia* and *Fusarium* were presented in few suppressed plants as well. The *Alternaria* infection was sporadic, while *Phytophtora*, *Pythium* and TSPW occurred only in control plots. The soil treatment of frames with P408 wettable powder significantly enhanced the quality of fruits.

Table 4. Efficacy of biopreparations against rose black spot disease.

Strains in formule		Control[a]		Preparations[b]			
				1/A		2/B	
Code	Sect.	C[c]	A[d]	C	A	C	A
075	P	1 - 13	100	0 - 8	37	0 - 11	98
317	P	0 - 15	97	0 - 3	35	0 - 9	87
325	P	0 - 14	98	0 - 4	12	0 - 11	71
328	L	1 - 11	100	0 - 3	9	0 - 12	47
396	T	1 - 10	100	0 - 1	2	0 - 6	55
408	P	0 - 12	99	0 - 2	4	0 - 10	76
Density of spray		not		2.5×10^3 cell mL^{-1}		5×10^4 cell mL^{-1}	

[a]Control = Paraffin oil with surfactants; [b]Preparations: 1/A = Paraffin oil based preparation proceeded by patent application [17], 2/b Tapioka starch supported preparation containing Lecithin and fatty alkanol-polyglycolether (EO:05); [c]Minimum and maximum number of colonies on a single leaf; [d]A = ratio of infected leaves in the canopy of rose bush.

Table 5. Protective effect of *Trichoderma harzianum* P408 strain on green pepper.

Treatment[c]	Quality Classes[a]						Destroyed[e]
	Extra	I.	II.	III.	Injured	Yield[d]	
Control[b]	6	13	54	16	11	100	21 - 44
OP	5	12	55	13	16	101	25 - 38
TT	6	12	52	15	15	106	23 - 44
TT + OP	5	16	56	9	13	109	19 - 45
TP	10	28	40	11	11	125	17 - 24
OTP	8	25	56	8	4	148	18 - 27
TT + OP	8	26	63	2	2	152	17 - 21
TT + OTP	11	25	60	2	1	171	0 - 6

[a]Meets up the requirements of engrossers. [b]Nursed following traditional rules. [c]Following control programs were carried out: OP = Sprayed with paraffin oil, TT = Preplanting soil treatment with P408 wp (1 g·sqm^{-1}), OTP= Sprayed with liquid *P*408 formule (1 mL·sqm^{-1}), and the combined treatments TT + OP, TT + TP and TT + OTP. [d]The number of fruits harvested. [e]Minimum and maximum number of wilted stools of 250 during the vegetation.

4. Discussion

The present evaluation gave clear indication that the isolates of *T. atroviride*, *T. harzianum* and *T. parceramosum* isolated from bodies of phyllosphere parasiting fungi are strong and virulent antagonists, which can be effectively used in the management of both soil and airborne fungal diseases. Combination of soil application and leaf sprays with *Trichoderma* based biopreparate appears to be the most effective one, however, the increased quality and quantity of the yield in treated pepper plants may be due to the production of plant growth promoters or through indirect stimulation of nutrient uptake as well. The producton of siderophores also should be taken into consideration.

The effect of *Trichoderma* against other plant associated microorganisms, especially against those that are beneficial to crops, should also be investigated.

5. Conclusions

In commercial LP the shelf life of conidia significantly decreased in strain dependent manner, and it was phytotoxic when applied as leaf spray independently on the emulsifiers. Both fungitoxic and phytotoxic contaminants of commercial LP could be eliminated with activated carbon.

The amount of liquid formulation of phylloplane originated *T. atroviride, T. harzianum* and *T. parceramosum* strains to be applied as leaf spray could have been reduced in two order of magnitude as compared to the solid preparations to achieve the same effect against rose black spot.

Even one soil treatment with low level of *Trichoderma* propagules results economically measurable effect on the yield of target plants.

The application of optimized liquid preparation containing carefully selected, phylloplane originated *T. harzianum* strain lessened the yield loss to economically acceptable level with significant increase of the quality of product and re-

sulted impressive increase of financial benefit.

Further studies are requested to reveal factors determining the selective response of target fungi as well as selective action of antagonists.

Acknowledgements

This work was supported by the Hungarian Scientific Research Fund (OTKA) F67908 and by *The National Office for Research and Technology*, Grant No. K67688.

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