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TRICHODERMA VIRIDE PERS. – EXPERIMENTAL MODEL FOR BIOLOGICAL AND BIOTECHNOLOGICAL INVESTIGATIONS OF MYCROMYCETA WITH IMPORTANCE IN OBTAINING PLANT PROTECTION BIOPRODUCTS

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Abstract: The technological process for obtaining plant protection bioproducts contains 2 main phases: (i) biomass biosynthesis of microorganisms in a culture medium, available for industrialization and (ii) biomass conditioning of microorganism, the antagonistic micromycetes, respectively. For this type of activities it is essential to establish biological development parameters: (i) the optimum composition of the liquid culture medium for development of the fungus under aerobiotic conditions and (ii) the optimal parameters of biosynthesis in the studied medium. The biomass biosynthesis technology is discontinuous, of cascade type, and develops several phases: (1) preparing of the laboratory inoculum, (2) preparing of the fungal pure culture in Erlenmeyer bottles, (3) industrial (simulated) multiplication in the aired and agitated liquid medium.
This paper presents some experimental aspects referring to: 1 – Characterization of the biologically active *T. viride* isolates, establishing and verifying of their biological thresholds; 2 – Evaluation and experimental verifying of the mass multiplication ability of antagonistic *T. viride* fungi on the culture media in order to select the optimum industrial culture substrate (medium); 3 – Biochimical characterization of *T. viride* isolates by electrophoretic analysis of their protein profile; 4 – Evaluation of the *T. viride* biological activity of *T. viride* isolates against phytopathogenic fungi with high practical importance: *Fusarium graminearum* Schwabe (T. *Gibberella zeae* (Schwein.) Petch), *F. culmorum* (W. G. Sm.) Sacc., *Pythium ultimum* Trow, *Botrytis cinerea* Pers., *Sclerotinia sclerotiorum* (Lib.) de Bary, *Alternaria* spp. [*A. alternata* (Fr.) Keissl., *Alternaria radicina* Meier, Drechsler and E. D. Eddy (*Stemphylium radicinum* (Meier, Drechsler and E. D. Eddy) Neerg.)] etc.; 5 – Processing of technological scheme for obtaining plant protection preparates based on biologically active isolates of *T. viride*.

Key words: antagonistic micromycetes, *Trichoderma viride*, electrophoretic analysis, plant protection bioproducts

Introduction

Trichoderma viride Pers., Neues Mag. Bot. **1**: 92 (1794), syn. *T. lignorum* (Tode) Harz, *Linig. Hyph.* 29 (1871) belonging to *Hypocreaceae* Family, *Hypocreales* Order, *Hypocreomycetidae* Subclass, *Sordariomycetes* Class, *Ascomycota* Phyllum, *Regnum* Fungi [15, 16, 36], is one of the most studied as a fungus with importance in biotechnology [2, 7, 10, 17, 18, 19, 20, 21, 26, 28, 29, 30, 31, 34, 35 a.o.]. It is an appropriate experimental model of mycoparasitic fungus for obtaining bioproducts with plant protection importance [4, 6, 9, 11, 14].

The aim of this paper was the development of the technology for obtaining plant protection bioproducts based on *T. viride* as an experimental model for the autochtonous biotechnology.

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The objectives of the research were the following: 1 – Characterization of the biologically active *T. viride* isolates, establishing and verifying of their biological thresholds; 2 – Evaluation and experimental verifying of the mass multiplication ability of antagonistic *T. viride* fungi on the culture media in order to select the optimum industrial culture substrate (medium); 3 – Biochemical characterization of *T. viride* isolates by electrophoretic analysis of their protein profile; 4 – Evaluation of the biological activity of *T. viride* isolates against phytopathogenic fungi with high practical importance; 5 – Processing of technological phases for obtaining plant protection products based on biologically active isolates of *T. viride*.

Material and methods

As biological material there were used 5 isolates of *T. viride* (Td_5, Td_{35}, Td_{45}, Td_{49},Td_{50}) and isolates of the following 8 phytopathogenic fungi: *Fusarium graminearum* Schwabe (T. *Gibberella zeae* (Schwein.) Petch), *F. culmorum* (W. G. Sm.) Sacc., *Pythium ultimum* Trow, *Botrytis cinerea* Pers., *Sclerotinia sclerotiorum* (Lib.) de Bary, *Alternaria* spp. [*A. alternata* (Fr.) Keissl., *Alternaria radicina* Meier, Drechsler and E. D. Eddy (*Stemphylium radicinum* (Meier, Drechsler and E. D. Eddy) Neerg.), all cultures obtained, isolated and preserved by the first author.

1. For the characterization of the biologically active *T. viride* isolates, 7 different solid culture media (Fig. 1), 17 sources of carbon (Fig. 2) and 18 of nitrogen (Figs. 3-4), 16 initial pH values of culture media (Fig. 5), 19 temperatures have been performed (Fig.6).

Fungal growth have been evaluated by measuring the colony diameter (3-5 replicates/variant) every day and using for graphs the data after 2 and 6 days. The last day of experiment, the 6th day, was the day when the *T. viride* colony covered the whole surface of the Petri plate of 10 cm diameter. The sporulation of tested fungus was appreciated by macroscopical analysis following the microscopical analysis [3, 22, 23, 24, 26, 27, 28, 29, 31].

2. For selecting the optimum submerged culture medium, there were tested 9 media containing intermediate products or residual ones from the food industry (Fig. 7) and and 4 liquid media (a-d) (tables 1-3) [25, 26, 27, 28, 29, 31].

3. For the biochemical characterization of *T. viride* isolates the SDS-PAGE analysis was used to reveal protein bands and to determine protein molecular mass. Investigations have been performed by Laemmli vertical method, in MINI-PROTEAN II (BIO-RAD), using poliacrylamide gel as a migration substrate [5].

4. For the appreciation of biological activity of *T. viride* isolates it was used the method of dual cultures [12]. Evaluation of antagonistic activity of *T. viride* isolates has been done by calculating of x coefficient from the ratio between inner radius (i) and outer radius (e) of the phytopathogenic test-fungi (A) and the antagonistic ones (B) (*T. viride*), after the formula X = iA/iB x eB/eA. When x=1, there is no influence between two fungi; when x<1, the antagonism is as strong as the value is lower, more closed with yero value; when x>1, the tested isolate prove no antagonism [31].

5. Biotechnological parameters for production of the bioproducts based on *T. viride* strain was followed according to the general literature for microbial plant protection products' biosynthesis and formulation [8, 13, 14, 18, 21, 32, 33].

Results and discussion

1 – Characterization of the biologically active *T. viride* isolates, establishing and verifying of their biological thresholds.

1.1. Development of *T. viride* on different solid media. Among the 6 tested solid culture media, the most favourables for the development (growth and sporulation) of *T. viride*, isolate Td_{50}, were: Weindling, Warcup, Malt agar extract and PDA (Fig. 1). The diameter of fungal colony have had values between 2.82-5.2 cm, after 2 days, and between 8.54-9.0 cm, after 6 days, respectivelly. On these media the fungal sporulation was excellent.

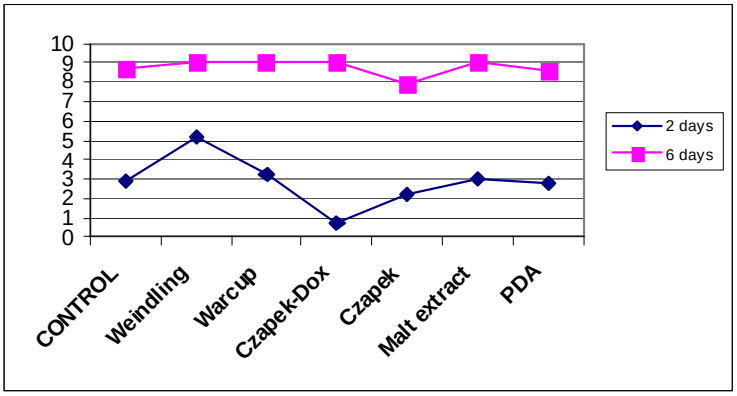

Fig. 1. Growth of *Trichoderma viride*, isolate Td_{50}, on different solid culture media, evaluated by the diameter of fungal colony

1.2. Development of *T. viride* on solid Weindling medium (WG) with different carbon sources. (Fig. 2). Among monosaccharides, the most favourable for growth and sporulation of *T. viride* were: mannite, fructose (levulose), D-ribose, D-galactose, D-mannose, D-dextrose (glucose), the fungal colony having between 4.8-5.833 cm in diameter, after 2 days, and 9.0 cm, after 6 days. Sporulation of *T. viride* was abundant on medium variants containing the mentioned monosaccharides. The poorest development of fungus was found on the medium with D-sorbitol/sorbite, fungal colony having 2.333 cm diameter after 2 days and only 4.433 cm after 6 days.

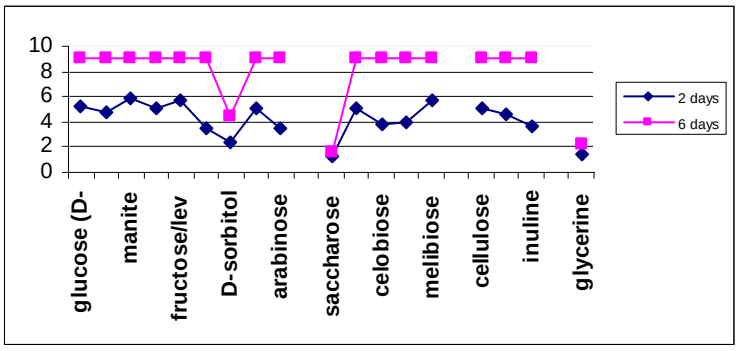

Fig. 2. Growth of *Trichoderma viride*, isolate Td_{50}, on solid culture Weindling medium (WG) containing different carbon sources, evaluated by the diameter of fungal colony

Among disaccharides, the most suitable for the *T. viride* development were melibiose and maltose (diameter of colonies between 5.057 and 5.733 cm after 2 days, and 9.0 cm after 6 days, respectively), followed by lactose and celobiose (diameter between 3.833 and 3.933 cm, after 2 days, and 9.0 cm after 6 days). The most inadequate for the fungal development was saccharose, with the values of 1.2 cm diameter, after 2 days and 1.533 cm, after 6 days.

In the group of polysaccharides, the most favourable was cellulose (5.067 cm diameter after 2 days), followed by starch and inuline (3.633-4.567 cm diameter after 2 days and 9.0 cm after 6 days). The lowest development of *T. viride* has been performed on the medium containing glycerine, fungal colony measuring only 1.4 cm diameter after 2 days and 2.3 cm after 6 days).

1.3. Development of *T. viride* on solid Weindling medium (WG) with different nitrogen sources. The cultivation of *T. viride* on Czapek medium containing different sources of organic and mineral nitrogen (Figs. 3-4) showed that peptone and the aminoacids DL-leucine, L-cystine, DL-citruline, DL-nor-leucine were the most favourable for fungal development (Fig. 3).

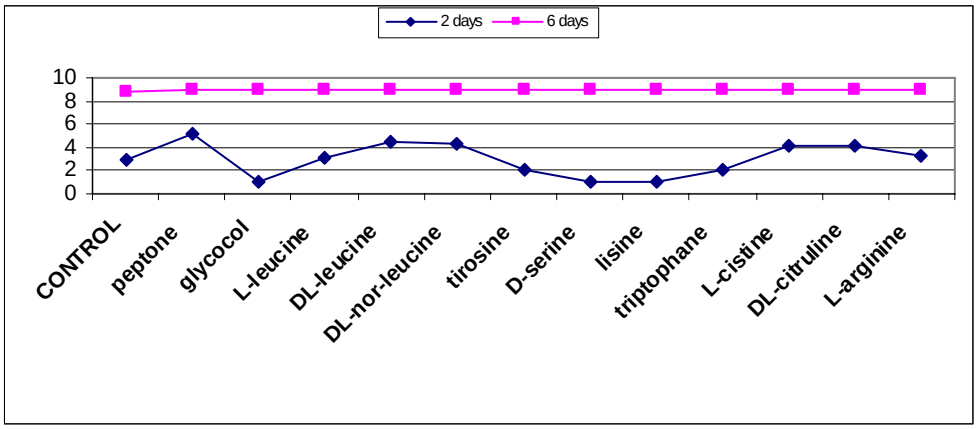

Fig. 3. Growth of *Trichoderma viride*, isolate Td_{50}, on solid Weindling culture medium containing different nitrogen sources (aminoacids), evaluated by the diameter of fungal colony

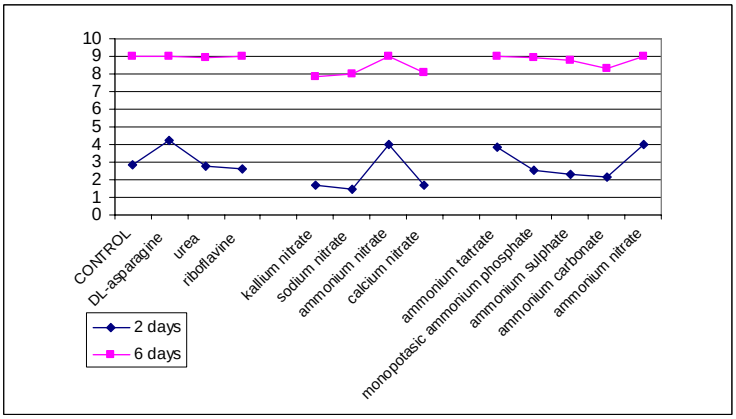

Fig. 4. Growth of *Trichoderma viride*, isolate Td_{50}, on solid culture Weindling medium containing different nitrogen sources (salts, amides, vitamins), evaluated by the diameter of fungal colony

Fungal sporulation was very good on media with: peptone, lysine, tryptophan, DL-asparagine, urea and ammonium salts, and good on media with glycocol, tyrosine, DL-citruline and riboflavin.

1.4. Development of *T. viride* on solid Weindling medium (WG) with different initial values of pH. The best growth of the *T. viride* colonies was recorded in acid medium (pH 4.0-5.5), with a diameter of 6.32 until 7.66 cm after 2 days and 9.0 after 6 days and the poorest one in highly alkaline medium with pH 13.0, and the diameter of the *T. viride* colony of 3.24 cm after 2 days and 5.42 cm after 6 days (Fig. 5).

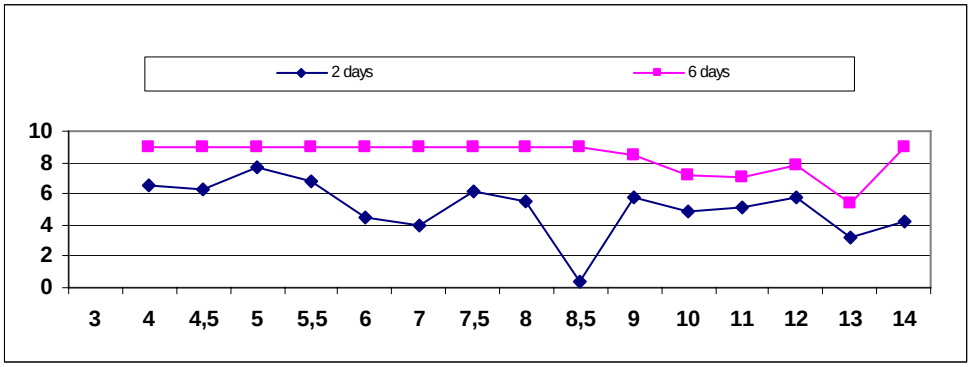

Fig. 5. Growth of *Trichoderma viride*, isolate Td_{50}, on solid Weindling culture medium with different initial values of pH, evaluated by the diameter of fungal colony.

The sporulation of *T. viride* was highest on acid medium, and the poorest on highly alkaline medium (pH 9.0-13.0).

1.5. Development of *T. viride* on solid Weindling (WG) medium under the different temperatures. The optimum growth and sporulation of *T. viride* was the range between 24 and 32ºC (Fig. 6).

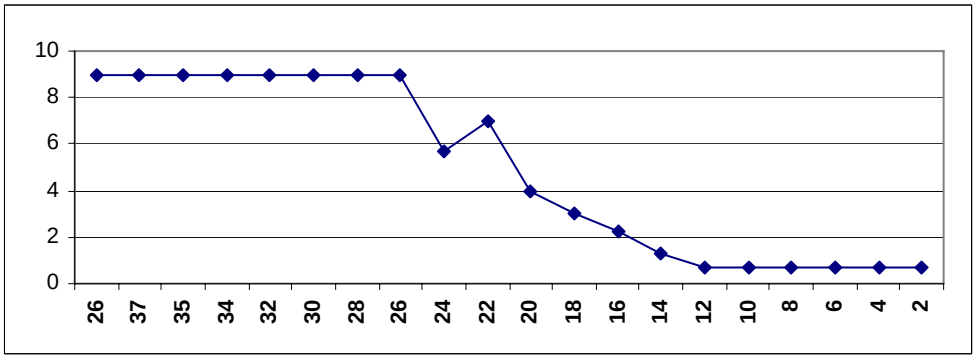

Fig. 6. Growth of *Trichoderma viride*, isolate Td_{50}, on solid Weindling culture medium at different values of temperature, evaluated by the diameter of fungal colony, after 2 days.

The temperatures ranging between 2 and 12ºC do not allow fungal growth and sporulation, between 14 and 18ºC growth but not sporulation was promoted, whereas

between 20-22ºC both growth and sporulation were favoured. At 37ºC the growth and sporulation of the *T. viride*, isolate Td_{50}, were very well.

Based on these investigations, it was possible to be established the optimal biological parameters for the development of *T. viride*, isolate Td_{50}: (i) solid culture media: Weindling, Warcup, Malt extract agar, PDA; (ii) carbon sources: monosaccharides mannite, fructose, ribose, glucose, manose; (iii) nitrogen sources: peptone, aminoacids DL-leucine, L-cistine, DL-citruline, DL-nor-leucine, ammonium nitrate and tartrates salts; (iv) pH initial values of culture media: 4.0-5.5 (for growth), 4.0-8.5 (for biomass); (v) temperatures: optimum 26ºC, for growth 24-32ºC, for sporulation 20-22ºC.

2 – Evaluation and experimental verifying of the mass multiplication ability of antagonistic *T. viride* fungi on the culture media in order to select the optimum industrial culture substrate (medium).

In the submerged cultivation of *T. viride* on nine liquid media, some of which contained intermediate products from the food commodity industry, the best growth was obtained on beer must (mB), on the Weindling medium with the imported chemically pure glucose (WG) or with indigenous food glucose (Wg) and on the MPB medium (Fig. 7.).

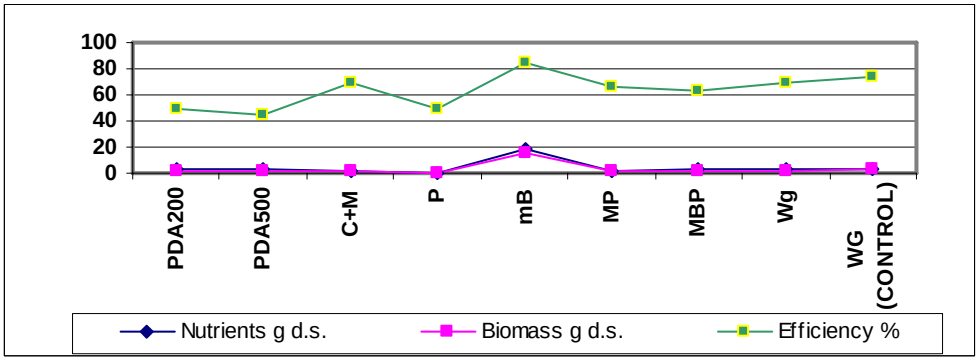

Fig. 7. Accumulation of biomass (g. dry substance/ d.s.) and efficiency (%) of culture liquid media by *Trichoderma viride*, isolate Td_{50}

On these media the sporulation and the titre of conidia and chlamidospores were high. The highest quantity of dry biomass was obtained and a better use (70.0-84.863%) of the nutrients from the medium was recorded. Data of optic density of the *T. viride* liquid cultures confirm this (Fig. 8.).

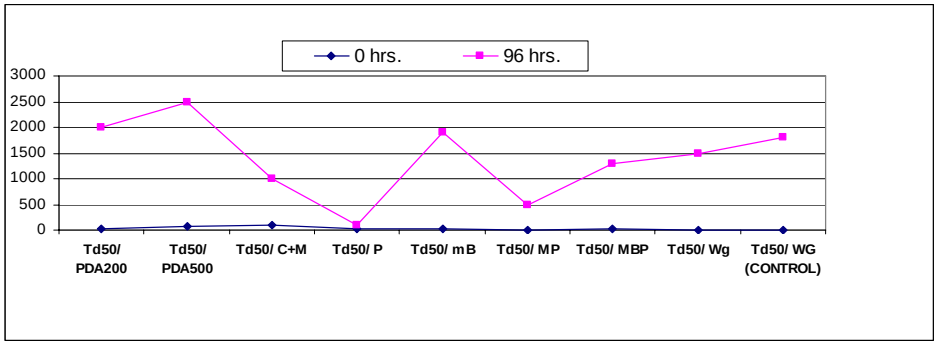

Fig. 8. Optic density of *Trichoderma viride*, isolate Td_{50}, cultivated on different liquid agitated media

Biomass accumulation in the cultures grown in liquid Weindling medium with different initial pH was the highest at pH 4.5-5.5 and the lowest in alkaline (pH 7.5-13.0) and highly acid (pH1.0-2.0) media (Fig. 9.).

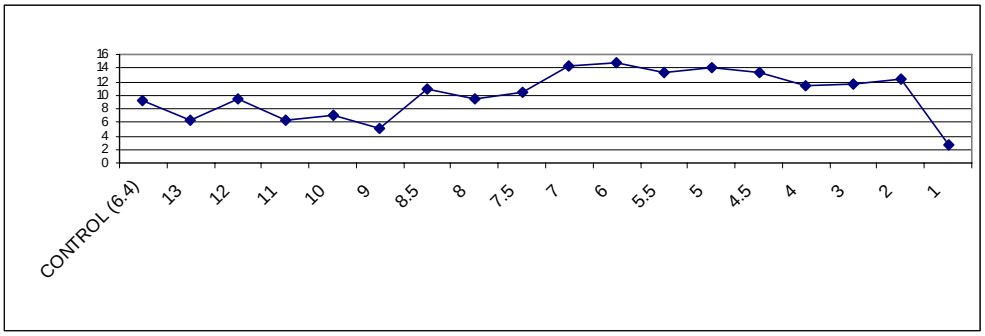

Fig. 9. *Trichoderma viride* biomass (cg) accumulated in Weindling medium with different pH values, after 21 days

A Weindling medium with initial pH between 4.0 and 6.4 and an inoculum rich in conidia ($1x10^8$ – $1x10^5$ conidia/ml) which ensure a fast development of both conidia and chlamidospores in the culture are most favourable for the submerged cultivation of *T. viride*. Based on these investigations we can favour the obtaining of a mixed biological product of phytosanitary utilization containing also conidia and chlamidospores besides mycelium of *T. viride* by maintaining the appropriate cultivation parameters.

From the experiements on submerged cultivation 72 and 96 houres, we have obtained superior results on conidia an chlamidospores titre in the variant of 96 houres of cultivation and at the temperatures between 24 – 30^{o}C.

For the checking of fungal cultivation on the liquid aired medium and aerated with carriers (simulation of industrial process) 4 variant of media have been performed (table 1), the evaluation being based on the efficacy of producing biomass.

Tab. 1. Efficacy expressed by biomass of *Trichoderma viride*, isolate Td_{50}, obtained in different tested liquid media**

Culture media	Wet biomass (g/l)	Dry biomass (g/l)
medium variant a)	9.82 ± 0.54	10.75 ± 0.54
medium variant b)	10.75 ± 1.41	4.04 ± 0.43
medium variant c)	8.64 ± 1.16	2.86 ± 0.37
mediumvariant d)	12.53 ± 0.95	4.88 ± 0.32

* average of least 3 determinations; ** the composition of culture media a), b), c), d) are under the process of a patent

The best results have been performed on the medium with the formula no. 4 (medium d), which is, also, very appropriate being a medium with a low content of ingredients.

These results are in accord with our previous results which mentioned glucose as an optimum carbon source for *T. viride* cultivation [1, 3].

The yeast extract from the medium composition/formula is essential, in general, for the cultivation of microorganisms. This ingredient offers some growing factors, like vitamins and aminoacids, as well as some organic nitrogen compounds with high

bioavailability. Yeast extract is the ingredient which ensure high efficacy in obtaining of *T. viride* biomass.

In the table 2 there were results presenting the influence of temperature on the fungal growth in the medium d) in bioreactor.

Tab. 2. The influence of temperature on the growth of *Trichoderma viride*, isolate Td_{50}, in aired and agitated medium d), evaluated by fungal biomass

Temperature (^{o} C)	Wet biomass* (g/l)	Dry biomasa* (g/l)
22.5	11.47 ± 1.04	4.12 ± 0.39
25.0	12.72 ± 1.66	4.87 ± 0.61
27.5	11.35 ± 0.87	3.39 ± 0.27
30.0	10.75 ± 1.42	3.42 ± 0.46

* average of least 3 determinations

The results showed that *T. viride* is a mesophylous to cryophylous fungus, having the optimum temperature for development at 25^{o}C. These data are in accord with the literature [25].

The effect of aeration on the biosynthesis efficacy of *T. viride* has been performed in the same medium d) and the data are presented in the Tab. 3.

Tab. 3. The effect of airation on the the biosynthesis efficacy of *Trichoderma viride*, isolate Td_{50}, in aired and agitated medium d), evaluated by fungal biomass

Airation rate (l air/l medium/minute)	Wet biomass* (g/l)	Dry biomass* (g/l)
0.50	10.23 ± 1.37	3.42 ± 0.42
0.75	11.35 ± 1.08	3.94 ± 0.37
1.00	12.41 ± 1.67	4.42 ± 0.54
1.25	12.67 ± 1.22	4.58 ± 0.48
1.50	11.88 ± 1.16	3.87 ± 0.35

* average of least 3 determinations

Our results show that a high rate of aeration did not significantly promote the biomass accumulation in the liquid media tested. The optimal rates of aeration are between 0.75 and 1.25 l air/l medium /minute, results which are in accord with similar data from the biosynthesis literature. Based on these results, in practice is recommended the minimal rate of aeration (0.75 l air/l medium/minute), which ensure the efficiency of biosynthesis, due to energy consumption reduction.

For the manufacturing of plant protection bioproducts have been selected strain Td_{49} and Td_{50} of *Trichoderma viride*. On the investigated parameters these strains shown equilibrated characteristics, combining high biological activity with rapid production of biomass on liquid medium (aerated, with carriers) and good survival during the formulation process. These strains were deposited for patent purposes on NCAIM (international depositary authority) and the patent applications were submitted to OSIM.

In conclusion, the parameters for submerged cultivation of *Trichoderma viride* are presented in the Tab. 4.

Tab. 4. Parameters of submerged cultivation of the fungus *Trichoderma viride*

Parameters	Values
Weindling culture medium	variant Wg (indigenous food glucose)
Time of cultivation	4 days (96 houres)
Temperature of culture medium Optimum temperature	$24 – 30^0$ C $24 – 28^0$ C
Reaction of culture medium (pH)	Acid until low acid (pH 4.0 – 6.4)
Titre of inoculum	$1x10^5 – 1x10^8$ conidia/ml medium
Optimal airation	0.75-1.25 l air/l medium/ minute
Viability of biomass	6-7 monthes

3 – Biochemical characterization of *T. viride* isolates by electrophoretic analysis of their protein profile (Tab. 5) reveled that:

3.1. Proteins with MM 7, 8, 10, 12, 14, 35, 56, 60 kD represent characteristics for genus and species, being well differentiated;

3.2. Within the same species the quantitative differences appeared at the level of proteins with MM of 29, 45, 87, 89 kD and are determinated by provenance of various isolates belonging to the same species;

3.3. Isolates Td49 and Td50 proved strong similarities having a common provenance; both isolates proved a high antagonistic capacity against the test-pathogens studied.

Tab. 5 Biochemical characterization of some *Trichoderma viride* isolates based on protein bands separated[z]

MM/kD	7	8	10	12	14	29	35	45	56	60	89	97
Rf	0.98	0.93	0.89	0.84	0.78	0.55	0.49	0.44	0.33	0.31	0.18	0. 15
Td_{35}	++	+	+	++	++	-	++	-	+	+	-	-
Td_{45}	++	++	±	++	++	±	±	±	+	±	±	±
Td_{49}	++	++	++	++	++	±	++	+	+	++	++	++
Td_{50}	++	++	++	++	++	±	++	+	+	++	±	±
Td_5 (ctr)	+	++	++	++	++	-	+	-	+	+	-	-

z = band intensity; '-' = no band; '±' = weak band; '+' = intense band; '++' = very intense band; ctr = control

These results are the first step in a future research conducted to obtain a molecular characterization of our *T. viride* collection of isolates selected as biological control agents of some practically important plant pathogens.

4 – Evaluation of the biological activity of *T. viride* isolates against phytopathogenic fungi with high practical importance has been performed by dual cultures of 7 phytopathogenic fungi (*Fusarium graminearum*, *F. culmorum*, *Pythium ultimum*, *Alternaria alternata*, *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Stemphylium radicinum*) and 4 antagonistic *T. viride* isolates (Tab. 6).

Tab. 6 Evaluation of the antagonistic activity of some isolates of *Trichoderma viride*, by the x coefficient, calculated after Jouan and others (1964)

Trichoderma viride	*Fusarium graminearum*	*Fusarium culmorum*	*Pythium ultimum*	*Alternaria alternata*
Td_{35}	0.25	0.40	0.24	0.40
Td_{45}	0.78	0.86	0.72	0.86
Td_{49}	0.28	0.38	0.48	0.90
Td_{50}	0.30	0.38	0.36	0.42
Td_5 (control)	0.55	0.39	0.54	0.54
	Botrytis cinerea (Bc.1)	*B. cinerea* (Bc.2)	*Sclerotinia sclerotiorum*	*Stemphylium radicinum*
Td_{35}	0.44	0.42	0.22	0.35
Td_{45}	0.70	0.80	0.48	0.52
Td_{49}	0.62	0.58	0.76	0.90
Td_{50}	0.35	0.38	0.54	0.75
Td_5 (control)	0.89	0.92	0.45	0.30

The antagonistic ability of tested *T. viride* isolates was different against these plant pathogens; the most active was Td_{35}, followed the others: $Td_{35} > Td_{49} > Td_{50} > Td_{45} > Td_5$.

5 – Processing of technological scheme for obtaining plant protection preparates based on biologically active isolates of *T. viride*.

Based on all performed investigations, *T. viride*, isolates Td_{49} and Td_{50}, have been selected for the manufacturing of a bioproduct of plant protection, these isolates being under the procedure for obtaining a patent.

The technological process for obtaining plant protection bioproducts (Fig. 10-11.) contains 2 main phases: (i) biomass biosynthesis of microorganisms in a culture medium, available for industrialization and (ii) biomass conditioning of microorganism, the antagonistic micromycete, respectively. For this type of activities it is essential to establish biological development parameters: (i) the optimum composition of the liquid culture medium for development of the fungus under aerobiotic conditions and (ii) the optimal parameters of biosynthesis in the studied medium.

The biomass biosynthesis technology is discontinuous, of cascade type, and develops several phases: (1) preparing of the laboratory inoculum, (2) preparing of the fungal pure culture in Erlenmeyer bottles, (3) industrial (simulated) multiplication in the aired and agitated liquid medium.

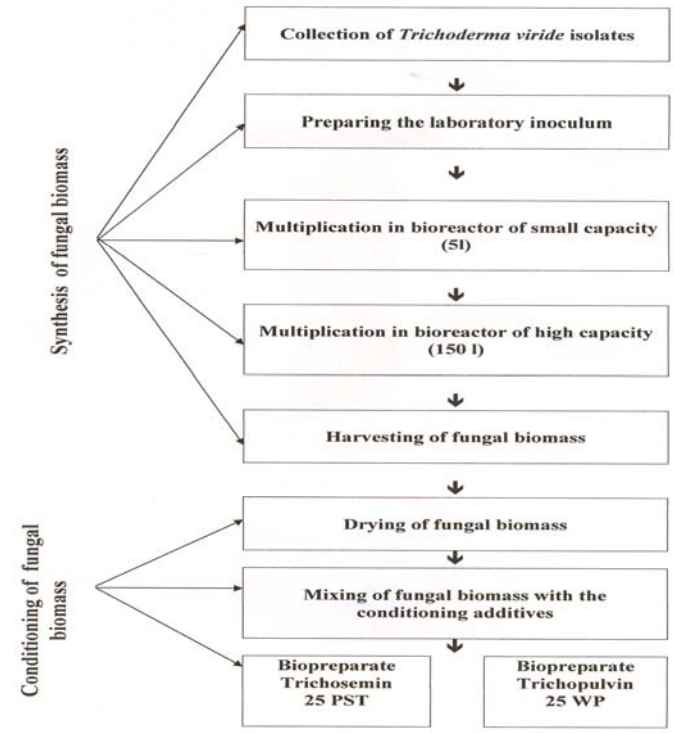

Fig. 10. Technological scheme for obtaining plant protection bioproducts based on *Trichoderma viride* [1, 27, 29]

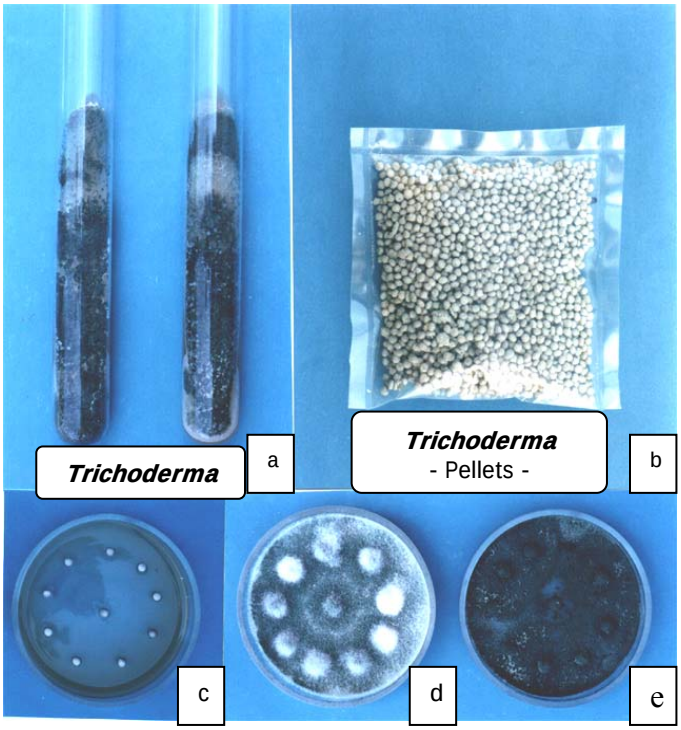

Fig. 11. *Trichoderma viride* bioproduct, in alginate, manufactured at the RIPP Bucharest: a – *T. viride* cultures in tubes on PDA medium (Potato-Dextrose-Agar); b – bioproduct as alginate pellets; c–e – biopreparate/pellets in Petri plates on PDA medium, 0 days (c) and after 3 (d) and 6 days (e) (original)

1. There were established the optimal biological parameters for the development of *T. viride*, isolate Td_{50}: (i) solid culture media: Weindling, Warcup, Malt extract agar, PDA; (ii) carbon sources: monosaccharides mannite, fructose, ribose, glucose, manose; (iii) nitrogen sources: peptone, aminoacids DL-leucine, L-cistine, DL-citruline, DL-nor-leucine, ammonium nitrate and tartrates salts; (iv) pH initial values of culture media: 4.0-5.5 (for growth), 4.0-8.5 (for biomass); (v) temperatures: optimum 26ºC, for growth 24-32ºC, for sporulation 20-22ºC.

2. The parameters for submerged cultivation of *T. viride* are: (i) Weindling medium, variant Wg containing indigenous food glucose; (ii) Period of cultivation – 96 hrs.; (iii) Viability of biomass – 6-7 monthes; (iv) Optimal temperature 25ºC; (v) Optimal pH – 6.0-6.5; (vi) Optimal aeration 0.75-1.25 l air/l medium/ minute, (vii) Viability of biomass – 6-7 monthes.

3. It has been done the first step in a future research conducted to obtain a molecular characterization of our collection of *T. viride* isolates selected as a biological control agents of some practically important plant pathogens. 3.1. Proteins with MM 7, 8, 10, 12, 14, 35, 56, 60 kD represent characteristics for genus and species, being well differentiated; 3.2. Within the same species the quantitative differences appeared at the level of proteins with MM of 29, 45, 87, 89 kD and are determinated by provenance of various isolates belonging to the same species; 3.3. Isolates Td49 and Td50 proved strong similarities having a common provenance; both isolates proved a high antagonistic capacity against the test-pathogens studied.

4. The antagonistic ability of tested *T. viride* isolates was different against plant pathogens; the most active was Td_{35}, followed the others: $Td_{35} > Td_{49} > Td_{50} > Td_{45} > Td_{5}$.

5. It has been established and experimentally verified the technological phases for obtaining plant protection bioproducts based on *T. viride.*

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References

1. BAICU T., ŞESAN T. & OANCEA F. 1998. Compoziţii microbiologice pentru tratarea seminţelor pe bază de *Trichoderma* sp., brevet de invenţie nr. 113.103 din 31. 03. 1998.
2. BENITEZ T., DELGADO-JARANA J., RINCÓN A.M., REY M. & LIMÓN C.M. 1998. Biofungicides: *Trichoderma* as a biocontrol agent against phytopathogenic fungi, *Recent Research Developments in Microbiology*, **63**: 129-150.
3. BONTEA V. & ŞESAN T. 1980. Contribuţii la studiul biologiei unor ciuperci antagoniste. I. Influenţa diferitelor medii de cultură şi a surselor de carbon asupra creşterii şi sporulării ciupercii *Trichoderma viride* Pers. ex Fr., *St. şi Cerc. Biol*., *Biol. Veget*., **32** (2): 165-173.
4. BURGES H.D. 2000. *Formulation of microbial biopesticides. Beneficial microorganisms, nematodes and seed treatments*. Kluwer Academic Publishers Dordrecht / Boston / London: 411 pp.
5. CIURDĂRESCU M., ŞESAN T. E. & OLTEAN E.. 1997. Electrophoretic analysis of some *Trichoderma viride* isolates and mutants, Molecular Approaches in Biological Control, Delémont (Elvetia), 15-18 septembrie 1997; *IOBC wprs Bulletin*, **21** (9): 189-194.
6. COPPING L.G. 1998. *The BioPesticide Manual*, First Edition, British Crop Protection Council: 333 pp.

7. DOMSCH K.H. & GAMS W. 1970. *Fungi in agricultural soils*, Logman Group Ltd London: 290 pp.
8. FRAVEL D.R., CONNICK JR. W.A & LEWIS J.A. 1998. *Production and formulation of biopesticides use to control plant diseases, în Formulation of microbial biopesticides*., ed. Burges, HD., Springer – Kluwer, Academic Publishers Dordrecht, The Netherlands: 187-203.
9. GRANT W., CHANDLER D., GREAVES J., PRINCE G., TATCHELL M. & BAILEY A. 2009. *Biopesticides: pest management and regulation*, CAB International: 256 pp.
10. HAGGAG W.M. & MOHOMED H.A.-L. 2007. Biotechnological aspects of microorganisms used in plant biological control, *World Journal of Agricultural Sciences*, **3** (6): 771-776.
11. HALL F.R. & MENN J.J. 1999. *Biopesticides. Use and delivery*, Humana Press, Totowa, New Jersey: 626 pp.
12. JOUAN B., LEMAIRE J.M. & ARNOUX J. 1964. Éléments d'appréciations des intéractions entre champignons cultivés *in vitro*, *Phytiatrie-Phytopharmacie*, **13**: 185-195.
13. KHACHATOURIANS G.K. 1986. Production and use of biological pest control agents. Trends Biotech. **4**:120-124.
14. KAEWCHAI S., SOYTONG K. & HYDE K.D. 2009. Mycofungicides and fungal biofertilizers, *Fungal Diversity***, 38**: 25-50.
15. KIRK P.M., CANNON P.F., DAVID J.C. & STALPERS J.A. 2001. *Ainsworth and Bisby's Dictionary of the Fungi*, 9th edition, CABI Bioscience, UK: 624 pp.
16. KIRK P.M., CANNON P.F., MINTER D.W. & STALPERS J.A. 2008. Dictionary of Fungi, 10th edition, CAB INTERNATIONAL, UK: 771 pp.
17. KUBICEK C.P. & HARMAN G.E. 1998. *Trichoderma* and *Gliocladium*, vol. 1-2, Taylor & Francis, London: 278 + 393 pp.
18. MONTE E. 2001. Understending *Trichoderma*: between- technology and microbial ecology, *Int. Microbiol*., **4:** 1-4
19. PLOAIE P., ILIESCU H. & OANCEA F. 2007. Dezvoltarea cercetărilor de protecţia plantelor în România – trecut, prezent şi viitor, 353-359, în Hera C. (coordonator), 2007, *Cercetarea ştiinţifică în sprijinul agriculturii*, *ICAR 1927-2007 ASAS*, Ed. Acad. Rom., Buc.: 353-359.
20. RAI M. & BRIDGE P.D. 2009. *Applied mycology*, CAB International: 336 pp.
21. RINCÓN A.M., BENITÉZ T., CODÓN A.C. & MORENO-MATEOS M.A. 2009. Biotechnological aspects of *Trichoderma* spp., în Rai, M., Bridge, P.D. *Applied mycology*, CAB International: 216- 238.
22. ŞESAN T. 1981. Contribuţii la studiul biologiei unor ciuperci antagoniste. II. Influenţa surselor de azot asupra creşterii şi sporulării ciupercii *Trichoderma viride* Pers. ex Fr., *St. şi Cerc. Biol*., *Biol. Veget*., **33** (1): 77-85.
23. ŞESAN T. 1983. Contribuţii la studiul biologiei unor ciuperci antagoniste. III. Influenţa reacţiei mediului asupra creşterii şi sporulării ciupercii *Trichoderma viride* Pers. ex Fr., *St. şi Cerc. Biol*., *Biol. Veget*., **35** (1): 35-43.
24. ŞESAN T. 1984a. Contribuţii la studiul biologiei unor ciuperci antagoniste. IV. Influenţa temperaturii asupra creşterii şi sporulării ciupercii *Trichoderma viride* Pers. ex Fr., *St. şi Cerc. Biol*., *Biol. Veget*., **36** (1): 62-69.
25. ŞESAN T. 1984b. Contribuţii la studiul biologiei unor ciuperci antagoniste. V. Cultivarea ciupercii *Trichoderma viride* Pers. ex Fr. în medii lichide agitate, *St. şi Cerc. Biol*., *Biol. Veget*., **36** (2): 155-160.
26. ŞESAN T. 1985. *Studiul biologic al speciilor de ciuperci cu acţiune antagonistă faţă de unii patogeni ce produc micoze la plante (teză de doctorat), ICEBiol Buc., 198 pp. + 46 planşe.*
27. ŞESAN T. 1986. *Ciuperci cu importanţă practică în combaterea biologică a micozelor plantelor. Trichoderma viride* Pers. ex S. F. Gray, Red. Prop. Tehn. Agr., Buc. 67 pp., 15 pl.
28. ŞESAN T. E. 1992. Bibliografia contribuţiilor româneşti în domeniul combaterii biologice a micozelor plantelor, *Probl. Prot. Plant.* **XX** (1-2): 85-96.
29. ŞESAN T. E. 2000-2002. Proiect RELANSIN 29, Biopreparate pe bază de ciuperci antagoniste destinate protecţiei plantelor agricole.
30. ŞESAN T. E. 2000/ publ. 2001. Combaterea biologică, avantaje, dezavantaje, integrare, Simpozionul dedicat împlinirii a 115 ani de la naşterea Academicianului Gh. Ionescu-Şişeşti, 26 oct. 2000, ASAS, vol. *Priorităţi ale cercetării ştiinţifice în domeniul culturilor de câmp*, Ed. Ceres, Buc.: 143-150.
31. ŞESAN T. E. 2005. Bibliografia românească in domeniul combaterii biologice a micozelor plantelor, în Lucrările celui de al XVI-lea Simpozion Naţional de Micologie, Sinaia, 26-29 august 2004, *Sănătatea plantelor* – Ediţie specială, august 2005: 15-22.

32. TABORSKY V. 1992. Small-scale processing of microbial pesticides, *FAO Agricultural Services Bulletin*, Rome, Italy, **96**: 62-88.
33. TORRES N.V., VASQUES F.A. & VOIT E.O. 2004. *Introduction to the Theory of Metabolic Modeling and Optimization of Biochemical Systems*, în Arora, D.K., Ed.(ed.), Handbook of Fungal Biotechnology (2nd edition), CRC Press/ Marcel Dekker, New York,. USA: 353-366.
34. VERMA M., BRAR S.K., TYAGI R.D., SURAMPALLI R.Y. & VALÉRO J.R. 2007. Antagonistic fungi, *Trichoderma* spp.: Panoply of biological control, *Biochemical Engineering Journal*, **37**: 1-20.
35. VINALE F., SIVASITHAMPARAM K., GHISALBERTI E.L., MARRA ROBERTA, SHERIDAN L.W. & LORITO M. 2008. Trichoderma-plant-pathogen interactions, *Soil Biology and Biochemistry*, **40**: 1-10.
36. * * *. http://www.indexfungorum.org/Index/htm