Development of new forms of biopreparations with Trichoderma strains on wooden compounds

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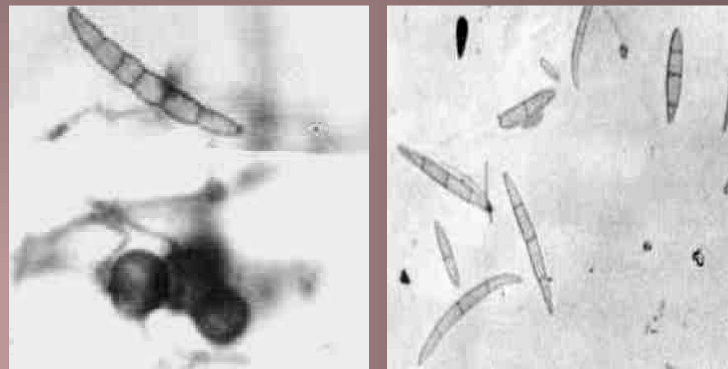
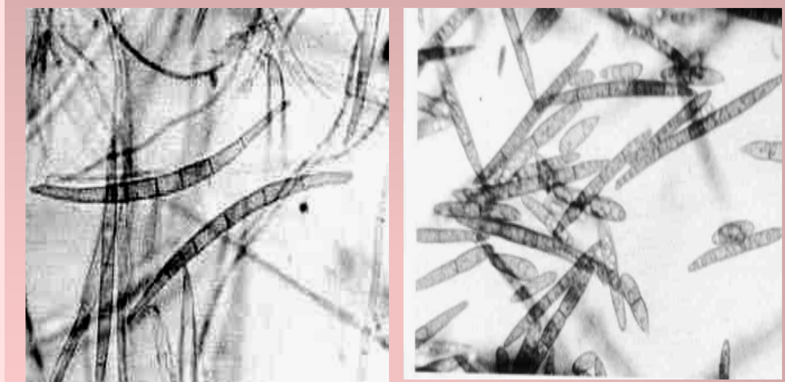
The main cause of forest nurseries diseases and epiphytotics in Siberia are plant pathogenic fungi. Seedling mortality if often 20-30% and in unfavorable years, can exceed 85%. Siberian soils are rich in humus and have pH levels which are favourable for annual recurrence of diseases. The highest losses of coniferous seedlings are caused by damping-off by *Fusarium sporotrichioides* Sherb. *Fusarium chlamydosporum* Wollenw. & Reinking *Fusarium avenaceum* (Fr.) Sacc. *Fusarium heterosporum* Nees.

Fusarium sporotrichioides Sherb



Fusarium chlamydosporum
Wollenw. & Reinking



Fusarium avenaceum (Fr.) Sacc.

Fusarium heterosporum Nees

The frequency of occurrence of Trichoderma genus in Central Siberia.

Species	Frequency of occurrence in soil	Density of population in soil	Quantity of isolates from needle
	Zone of South taiga		
T. koningii	45,6	33,4	0
T. viride	57,0	30,7	0
Trichoderma sp -31	0	0	3
Trichoderma sp.-30	0	0	0
All Trichoderma species	68,2	64,5	3
	Zone grassy forest with forest-steppe island		
T. asperellum	13,6	9,4	2
T. harzianum	18,0	15,7	3
T. koningii	16,0	4,8	0
T. virens	8,4	7,5	3
T. viride	46,5	14,7	0
All Trichoderma species	66,6	38,0	8
	Zone of real insular steppe		
T. asperellum	54,0	34,6	2
T. harzianum	30,0	20,6	4
T. virens	12,6	17,0	2
T. viride	55,0	24,6	0
All Trichoderma species	62,5	59,5	8
	Zone of mountanios forest		
T. asperellum	38,0	33,5	3
T. harzianum	46,0	36,8	3
T. koningii	17,5	27,0	0
T. virens	10,4	14,3	1
T. viride	62,5	25,7	0
All Trichoderma species	75,0	54,0	4

- From a collection of native isolates of Trichoderma, 197 isolates were selected for further testing (*T.asperellum, T.viride, T.harzianum, T.koningii, T.virens*). as to their antagonistic activity against the key *Fusarium* pathogens. Results from such assays reduced the potential candidates to 15 aboriginal strains of *T. asperellum*. Strains providing the best control under laboratory conditions were further evaluated in small field plot tests in 5 forest nurseries. Strain Mg–97 was found to provide 65 % control under high diseases pressure and 85 % control under moderate disease conditions. This strain was submitted to the Russian Collection of Industrial Microorganisms
- (F-765) and protected by a patent


РОССИЙСКАЯ ФЕДЕРАЦИЯ

ПАТЕНТ

НА ИЗОБРЕТЕНИЕ

№ 2171580

Российским агентством по патентам и товарным знакам на основании Патентного закона Российской Федерации, введенного в действие 14 октября 1992 года, выдан настоящий патент на изобретение

ШТАММ ГРИБА Trichoderma sp. МГ-97, ИСПОЛЬЗУЕМЫЙ ДЛЯ ЗАЩИТЫ СЕЯНЦЕВ ХВОЙНЫХ ОТ ФУЗАРИОЗОВ

Патентообладатель(ли):

Красноярский государственный университет

по заявке № 99126556, дата поступления: 17.12.1999

Приоритет от 17.12.1999

Автор(ы) изобретения:

см. на обороте

Патент действует на всей территории Российской Федерации в течение 20 лет с 17 декабря 1999 г. при условии своевременной уплаты пошлины за поддержание патента в силе

Зарегистрирован в Государственном реестре изобретений Российской Федерации

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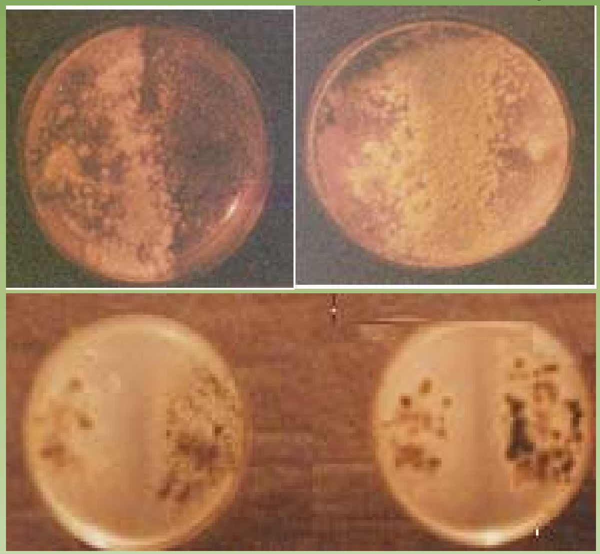
Генеральный директор

А.Д. Корчагин

Different culture-morfological groups in population of *Trichoderma asperellum* Samuels (from left to right – I – IV groupe)

Monitoring of the monospore clones of 15 wild isolates have shown high heterogeneity with respect to culture-morphological properties, radial growth and the intensity of sporulation. These properties have a great importance for selection of strains to be used for creation of biopesticides. With respect to these properties, all isolates can be split into four different groups.

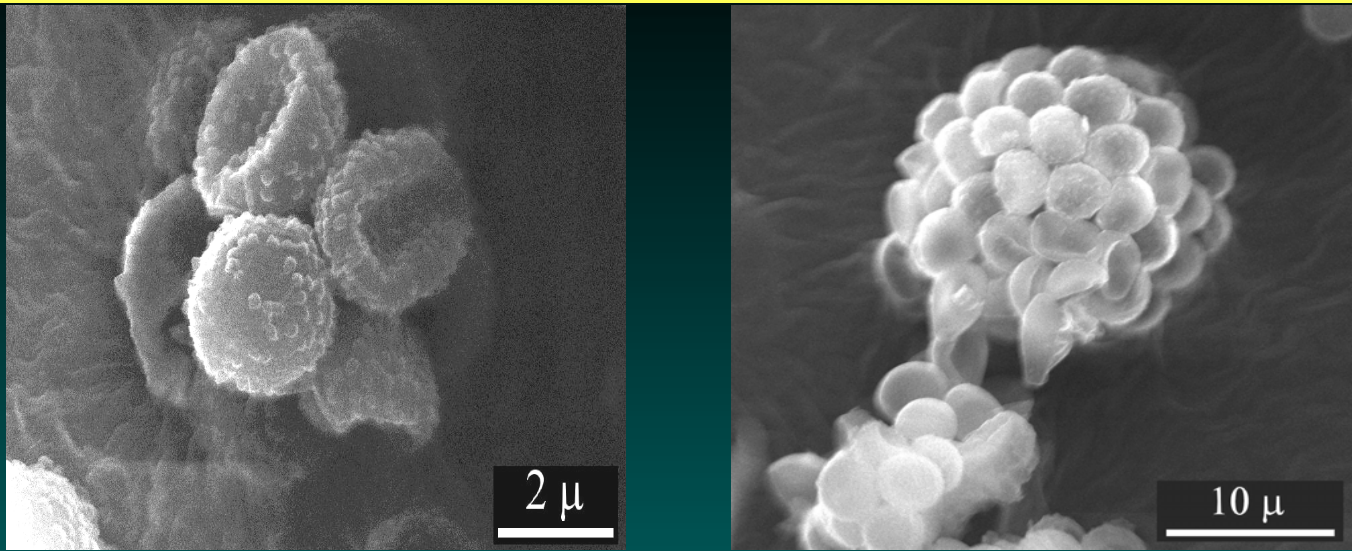
antibiotic activity



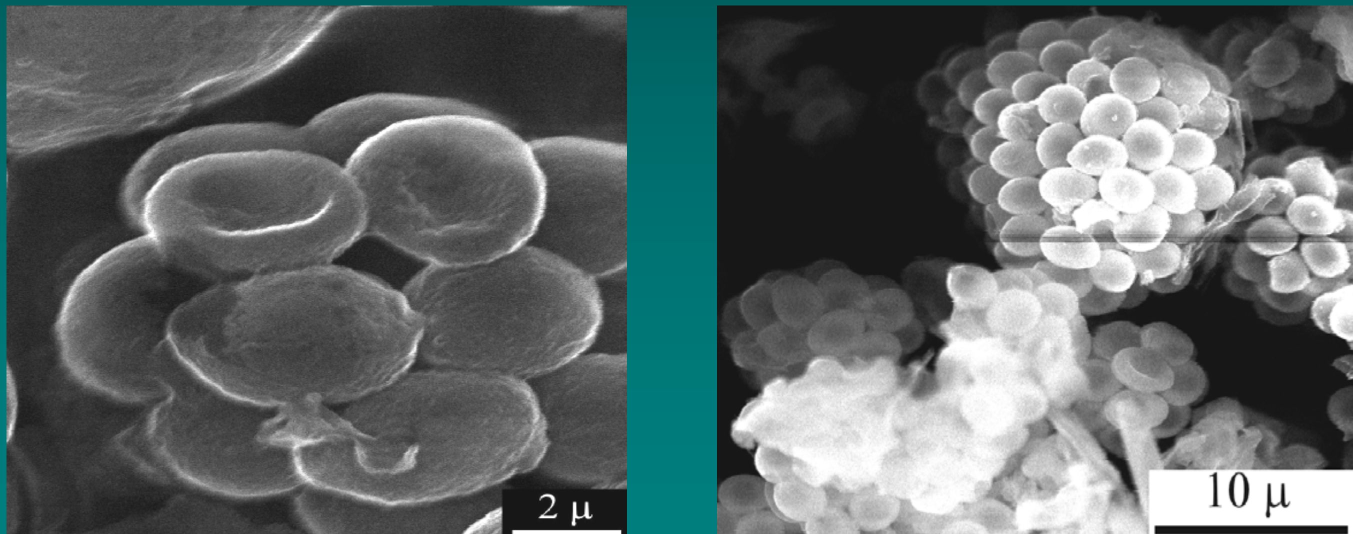
Vegetative compatibility

The investigated monospore strains of T. asperellum proved to be heterogeneous with respect to antibiotic activity as regarding Fusarium species. The maximal antibiotic activity was observed for the clones of the second group which proved to be the most productive and maintained the initial culture-morphological capacity in a series of passages.

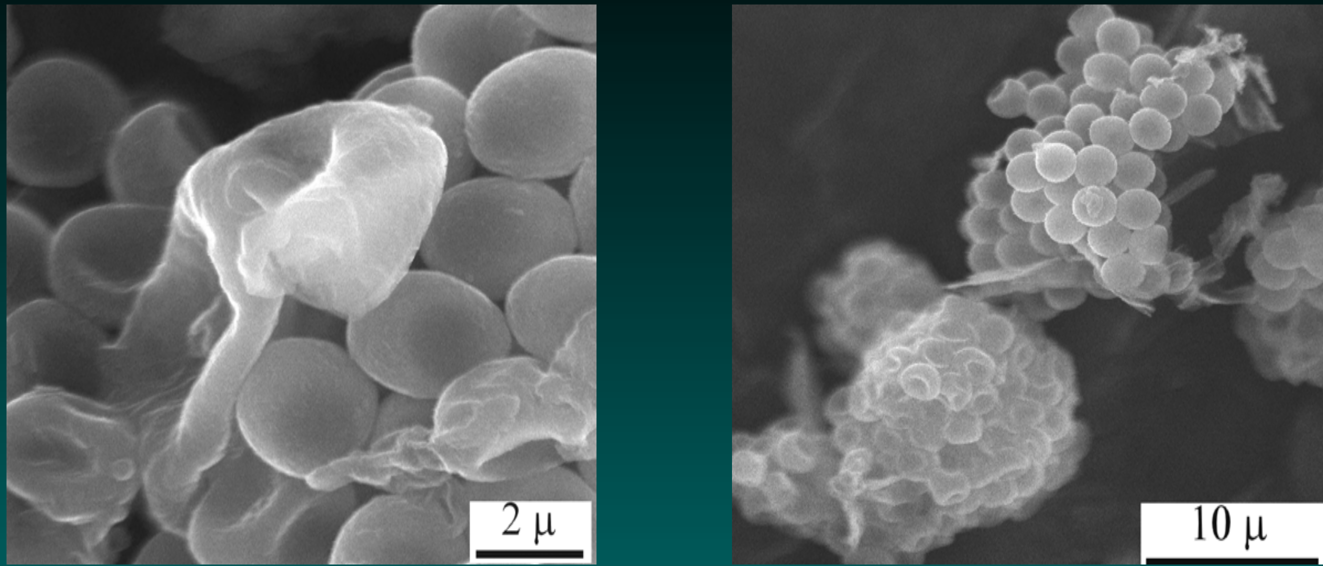
Vegetative compatibility of the isolates is in correspondence with these groups. The monospore isolates belonging to second group showed incompatible interaction with clones from the other groups. This data was used as a basis for further selection of isolates within the given group. And the results of these research was the selection 5 monospore strains: MG-97/6, 48/5, MO, M-99/5, K-12.



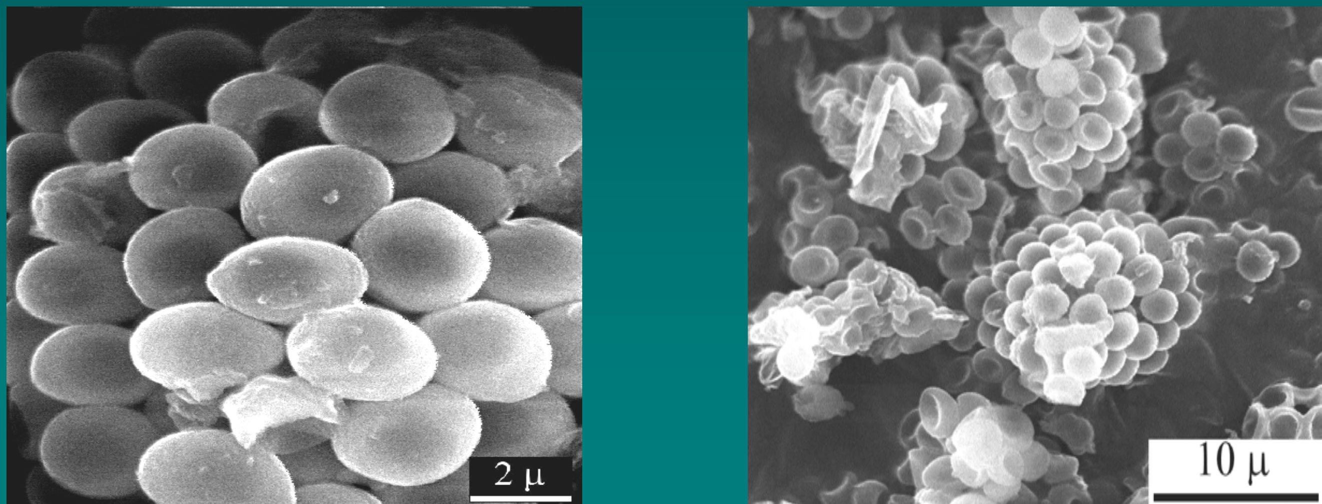
M -97/6 *Trichoderma asperellum*



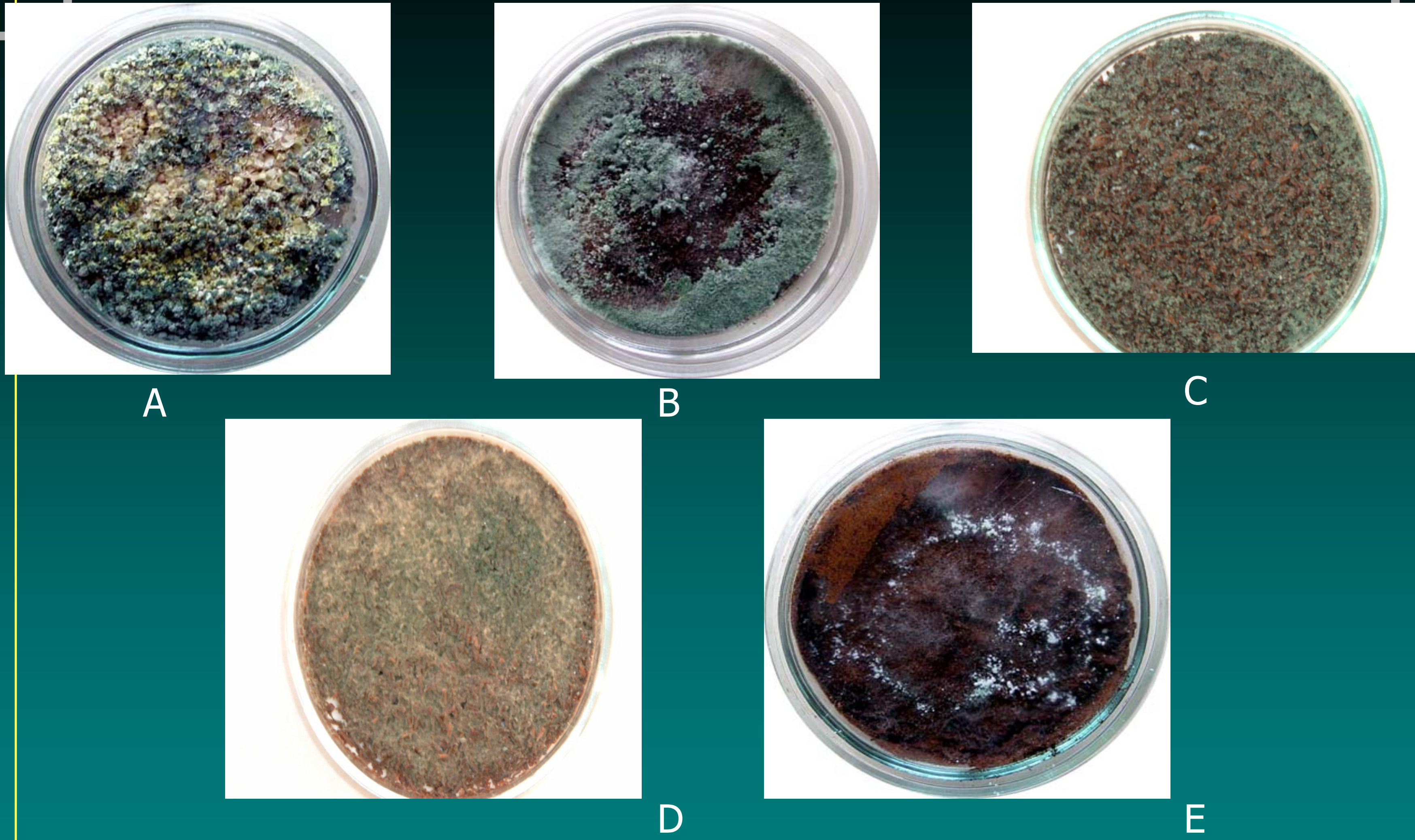
K-12 *Trichoderma asperellum*



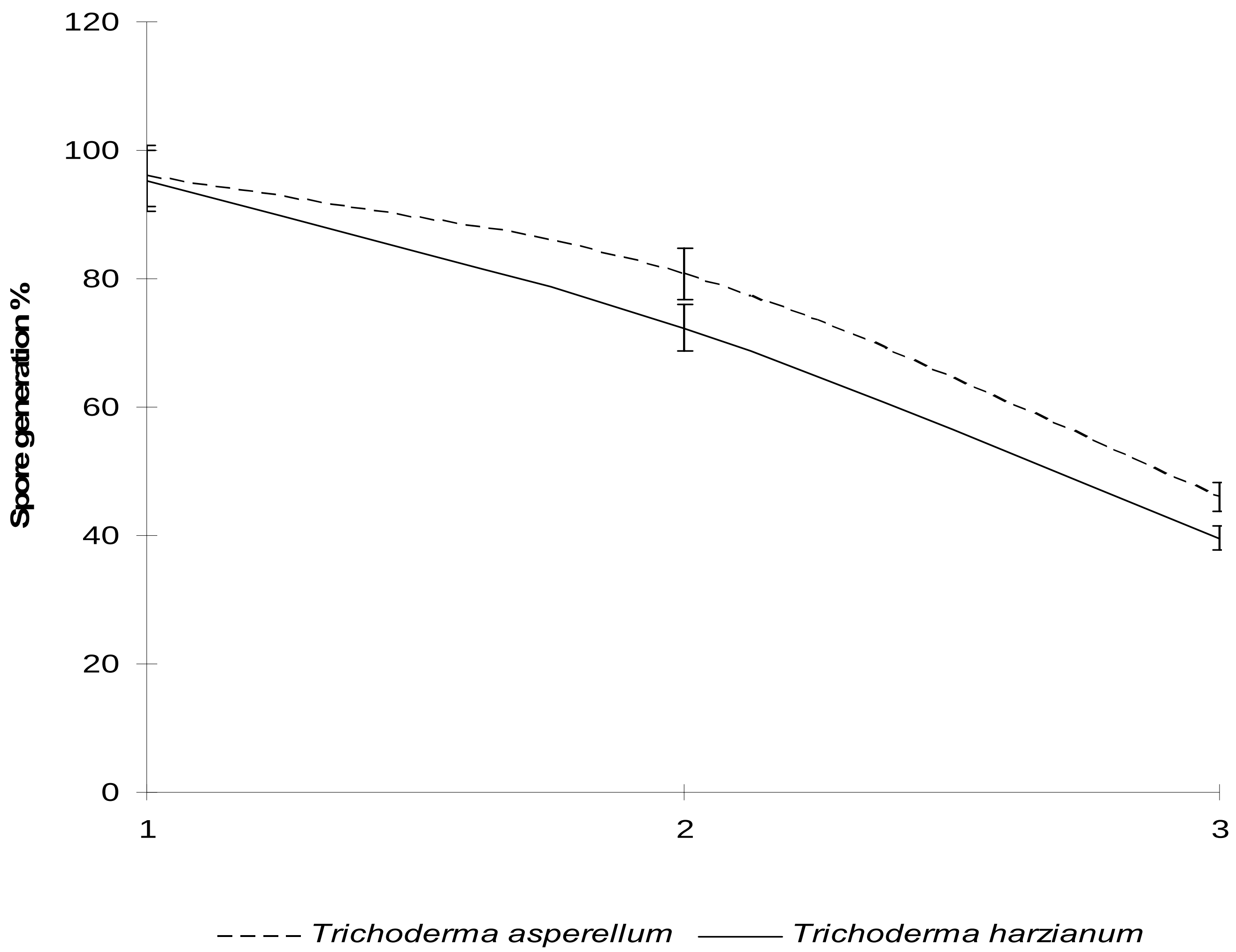
MO *Trichoderma hamatum*



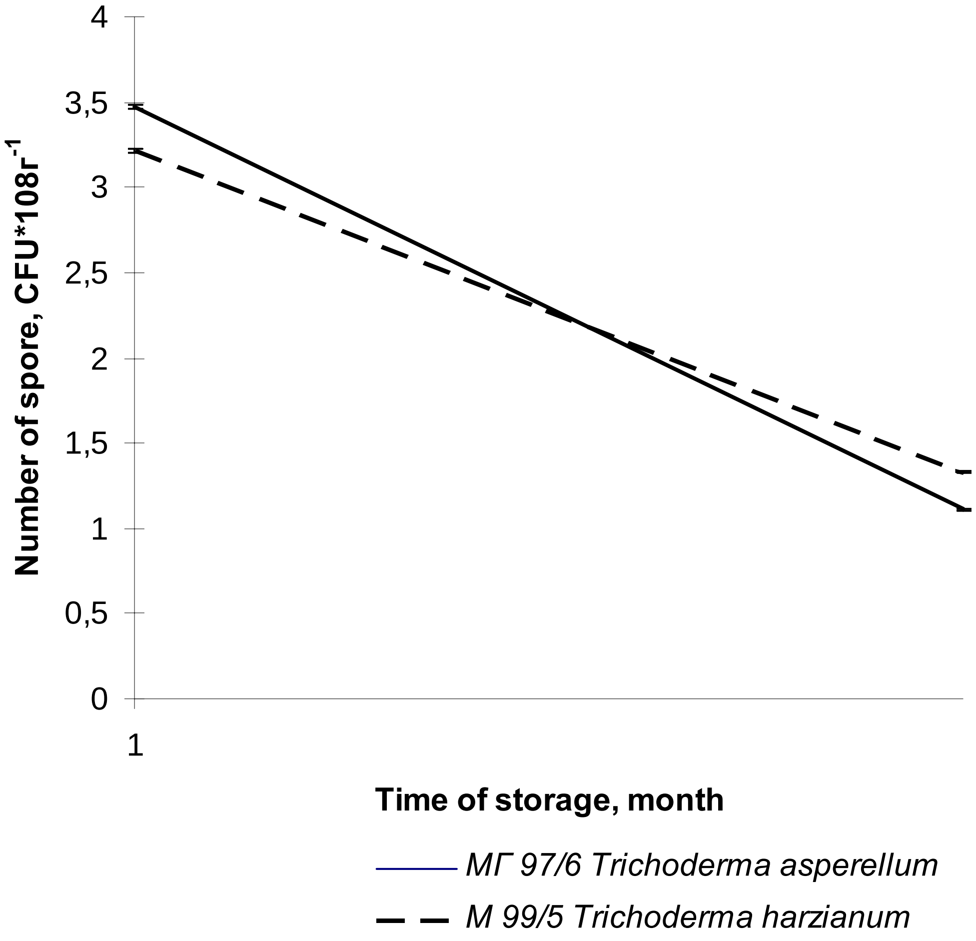
M 99/5 *Trichoderma harzianum*



Growth of strain MG 97/6 *Trichoderma asperellum on* larch bark (A), spruce bark (B), spruce bark after CO_2 extraction (C) spruce bark after ethanol extraction (D) and hydrolysis lignin (E)



120
100
80
60
40
20
0
Spore generation %
1
2
3
Trichoderma asperellum
Trichoderma harzianum

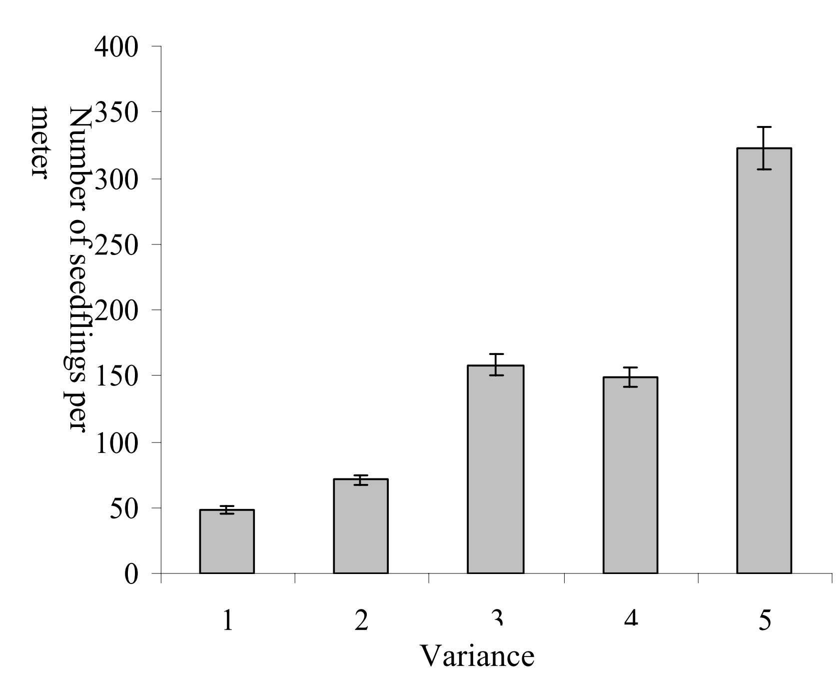

4
3,5
3
2,5
2
1,5
1
0,5
0
1
Number of spore, CFU*108г-1
Time of storage, month
МГ 97/6 Trichoderma asperellum
M 99/5 Trichoderma harzianum

The different form of biopreparation was done for the evaluation in forest nurseries on *Picea obovata* L. seedlings: МГ 97/6 *Trichoderma asperellum* on pine and larch bark after CO_2 extraction (contains 3·108 spores per 1 gramm). Complex biopreparation consist МГ–97/6 *Trichoderma asperellum*, M 99/5 *Trichoderma harzianum*, K-12 *Trichoderma asperellum*, MO *Trichoderma hamatum* (contains 2,5·108 spores per 1 gramm) on pine bark.

Complex biopreparation

МГ 97/6 *Trichoderma asperellum* on larch bark after CO2 extraction

МГ 97/6 *Trichoderma asperellum* on pine bark after CO2 extraction

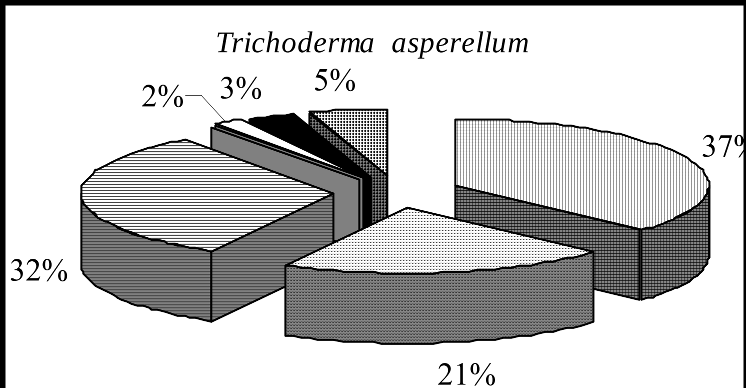


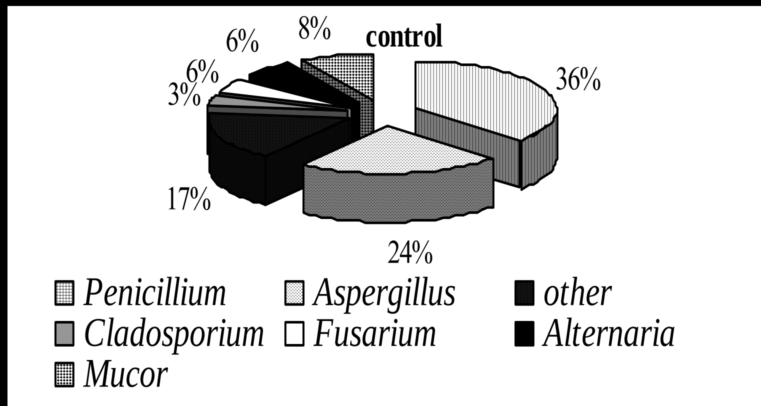
During the period 2004-2005, plots treated with the biopesticide had between 75 – 80% healthy seedlings in control plots. The preparations maintained a viable fungal concentration of 106 cfu/g soil during the first year of application to the seedling and this prevented disease development all year.The results shown that the treatments of seeds and seedlings of *Picea obovata* L. could increase the amount of healthy seedlings *Picea obovata* L. in compare with control variant: pure spore treatment in 1,6 times; biopreparation on larch bark in 4 times; biopreparation on spruce bark after CO2 in 3,4 times. The maximum of percent of healthy seedlins was in variant with 4 strains MΓ–97/6 *Trichoderma asperellum*, M 99/5 *Trichoderma harzianum*, K-12 *Trichoderma asperellum*, MO *Trichoderma hamatum*. It was in 8,5 times more in compare with control variant.

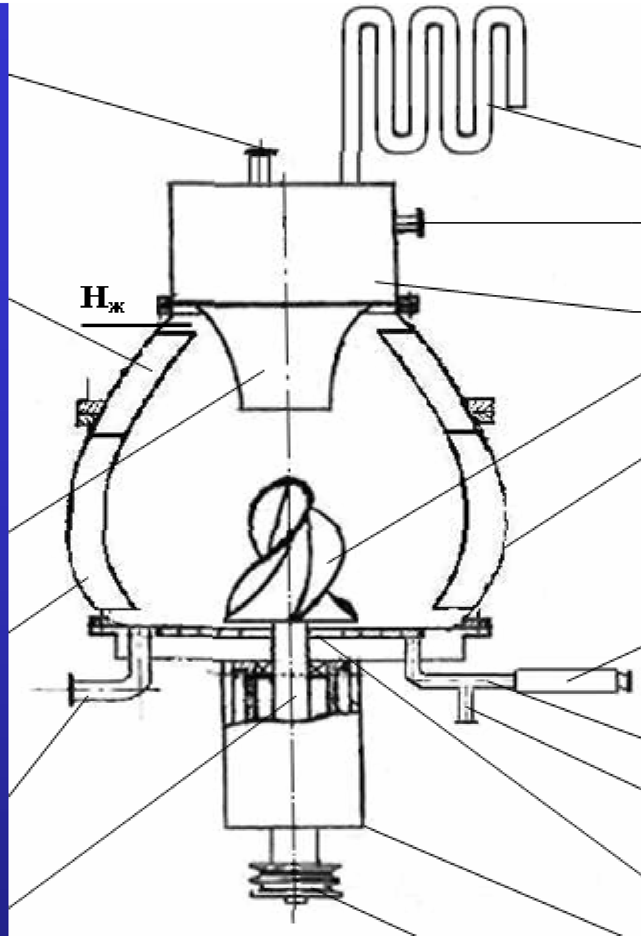
Treatment of Larix sibirica seedlings Trichoderma asperellum with organic compounds

1- control, 2 –pure spore preparation, 3 – MG-97 with pine bark bark after CO2 extraction, 4 – MG-97 with hydrolysis lignin, 5 – different Trichoderma strain with pine bark (MG-97, K-12, MO and GT-4)

Correlation (%) of main genera of micromycetes in rhizosphere of *Picea obovata* L. seedlings







For increasing the density of population in biopreparation was developed the uniquied solid imerrsive technology which allowed mixing of the substrates and oxygen. This construction is not harm the mycelium of growing culture.

Using this technology we optimized the growth and spore production of strain *T. asperellum* MG-97 on larch barck and released the new product “Trichodermin-P”. The product contains 8.5-9 x 109 spores and the products of wooden hydrolysis.

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