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The inhibitor effect of some vegetal extracts on the fungal strains of the *Alternaria sp*. and *Fusarium sp.*

OANA MIRONAS*, C. POHRIB*, RODICA MATEESCU*, ELENA BOCA, STEFANA JURCOANE***

**University of Agronomical Sciences and Veterinary Medicine, Faculty of Biotechnology, 59 Bd. Marasti, Bucharest, Romania*

***Biotehnos S. A.,Bucharest, Romania*

Abstract

The antifungal effect was tested for some vegetal extracts of: sage, lavender, hyssop, horseradish, garlic, black mustard, fennel, tomatoes. The fungus strains used - Alternaria tenuis, Alternaria porri, Fusarium oxysporum – have been isolated from samples of pepper, and tomatoes. Two methods have been used to test the vegetal extracts: the first method added in the culture medium the vegetal extract in proportion of 4% v/v, to the culture medium followed by inoculation of the fungus and observation of its development; the second testing method consisted in allowing the fungus to develop on Petri plates, followed by forming gauderies in which a certain amount of vegetal extract was dripped and ended by measurement of the inhibition zones. The specific medium of growth and development for the fungal strains subjected to testing is the Czapek solid medium.

The antifungal effect of these vegetal extracts was evaluated one, two and twelve months after preparation.

The best results were obtained with the solanum and fennel extracts; the inhibitor effect of this extracts was the same after twelve months.

*The inhibitor effect was studied also by evaluating the vegetal extracts as preventive treatment for the vegetable seeds**, in the green house.***

The experiment was carried out on cucumber seeds treated before the inoculation in the green house. The procedure was the following: 20 seeds of cucumber have been treated for 24 hours with 2,5ml solution of vegetal extract 4% (2,4ml distillated water + 0,1ml vegetal extract). Subsequently 2 seeds/vegetable-pot were planted in the green house.

*The samples were compared to 7 blanks (**M**) in which 2 untreated seeds/vegetable-pot were inoculated. The coming up phase was observed for 3 weeks.*

*The best result was obtained by treating the seeds with solanum extract (prepared in 2003). In this case the percentage of come up plants has doubled, comparatively with **M.** After 15 days from the inoculation the best percentage of come up plants was 95% for the cucumber seeds treated with the solanum extract comparatively with the untreated seeds to which the percentage of come up plants was 40%. The plants treated with fennel extract have also encounteres a high percentage, 80%.*

These experiments have proven that vegetal extracts and especially the solanum and fennel extracts have a powerful antifungal effect on the tested fungal strains-Alternaria tenuis, Alternaria porri, Fusarium oxysporum.

Keywords: antifungal, *Alternaria tenuis, Alternaria porri, Fusarium oxysporum*, vegetal extract.

Introduction

The biological control of different phytopathogenic fungi started with the discovery of the antagonistic features of *Trichoderma sp.I*, encouraging results being obtained by means of *in vitro* and *in vivo* experiments. Regarding the use of vegetal extracts as biocontrol agents against the phytopathogene fungus results are poorer.

Materials and Methods

1. Tested vegetal extracts

The testing of the antifungal effect has been carried out on a range of vegetal extracts conditioned as liquids obtained within Biotehnos S.A. from the following plants: sage, lavender, hyssop, horseradish, garlic, black mustard, fennel, tomatoes.

2. Used microorganisms

Three fungal strains have been used - *Alternaria tenuis, Alternaria porri, Fusarium oxysporum* - abbreviated as Alt.1, Alt2, Fus., isolated from samples of pepper, and tomatoes. Pure cultures on specific mediums have been obtained by means of the usual mycological methods.

3. The culture medium

The specific medium of growth and development for the fungal strains subjected to testing is the Czapek solid medium, with the following composition:

Component	Quantity [g]
sucrose	30,0
NaNO3↓	3,0
Mg SO4↓	0,5
KCl	0,5
FeSO4↓	0,01
K2PO4↓	1,0
Agar-agar	20,0
Distillated water	→1000 ml

The correction of pH (6,5 ± 0,1) is done with NaOH solution 40%. The resulted medium is sterilized for 20 minutes.

4. Analysis and control

For the testing of the antagonist activity of vegetal extracts two methods have been used:

a) The method of growing the fungal strains subjected to testing on Petri plates containing Czapek medium with agar and vegetal extract in proportion of 4% v/v (24 ml medium : 1ml vegetal extract).

The sterile Czapek medium cooled to 60ºC is aseptically distributed in bottles to which the vegetal extract (4%) is subsequently added. The content of each bottle is poured into sterile Petri plates and is inoculated with the test fungal strain on surface followed by statically thermostat incubation (30ºC). The blank **(M)** should be without inhibiting vegetal extract.

b) The diffuzimetric method in Petri plates, using the technique of gauderies in solid medium.

The sterile Czapek medium cooled to 48ºC is aseptically distributed in sterile Petri plates and is inoculated with the test fungal strain. For the inoculation, from the test fungal culture, suspensions are made in sterile distillated water containing 10^9 germs/ml.

In each plate prepared as above, gauderies are made in the center and in each of them a precise volume of vegetal extract is dripped. The plates are kept 18 hours at room temperature and then they are incubated into a thermostat (31ºC), statically. The **M** should be without gaudery.

The inhibitor effect of vegetal extracts is determined over 10 days of measuring, every 24 hours, as well as the diameter of the inhibition zones (mm) surrounding the gauderies.

Results and Discussions

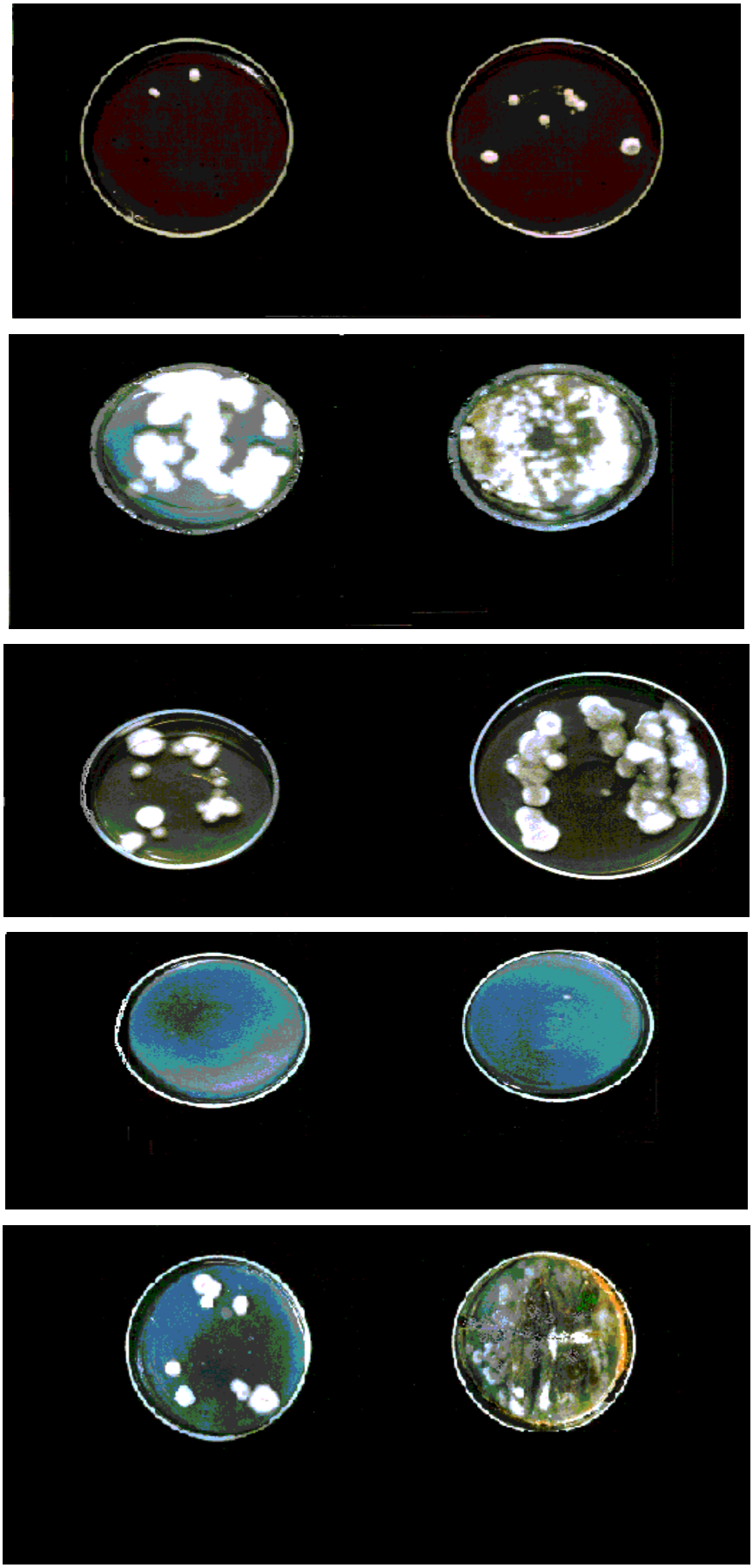
Assessment of the antifungal effect of some vegetal extracts one month after preparation, on the *Alternaria tenuis* and *Alternaria porri* fungus

The results obtained from the assessment of the antifungal effect of some vegetal extracts one month after preparation through the method of growing fungal strains *Alternaria tenuis* and *Alternaria porri* on Petri plates containing Czapek medium with agar and vegetal extract (4%) are presented in (**Table 1**) (- inhibition, +development) and (**Figure 1**).

Table 1. The assessment of the antifungal effect of vegetal extracts (4%) one month after the preparation, on the *Alternaria tenuis* and *Alternaria porri* fungus

Sample no.	Type of extract	Thermostating		The antifungal effect	
		Temp.(ºC)	Time(days)	Alt.1	Alt.2
1	Sage extract	30	3	-	-
2	Lavender extract	30	3	-	-
3	Hyssop extract	30	3	-	-
4	Fennel modified extract	30	3	-	-
5	Black mustard modified extract	30	3	-	-
M		30	3	-	-
1	Sage extract	30	5	-	-
2	Lavender extract	30	5	+	+
3	Hyssop extract	30	5	+	+
4	Fennel modified extract	30	5	-	-
5	Black mustard modified extract	30	5	+	+
M		30	5	+	+

In table 1. one can notice that the lavender, hyssop and the black mustard modified extracts (4%) do not have antifungal effect on *Alternaria tenuis* and *Alternaria porri* fungus.



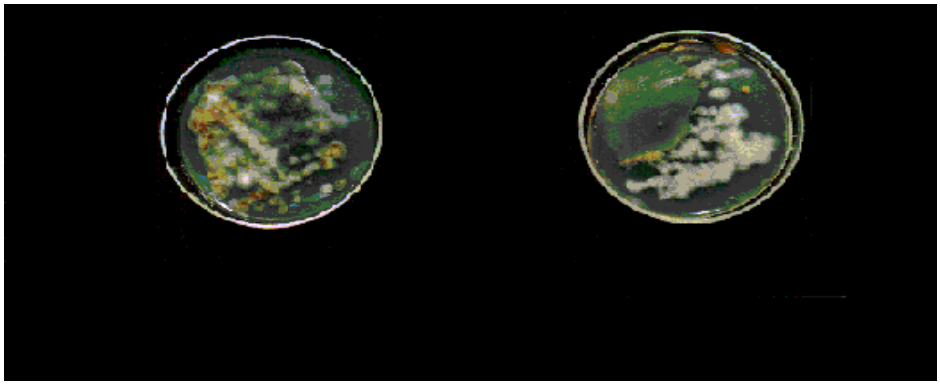


Figure 1. The antifungal effect of vegetal extracts (4%) one month after preparation, on the *Alternaria tenuis* and *Alternaria porri* fungus

Assessment of the antifungal effect of some vegetal extracts two months after preparation, on the *Alternaria tenuis* fungus

The results obtained from the evaluation of the antifungal effect of some vegetal extracts two months after preparation, through the method of growing *Alternaria tenuis* on Petri plaits containing Czapek medium with agar and vegetal extract (4%) are presented in (**Table 2**) (-inhibition, +development):

Table 2. The assessment of the antifungal effect of vegetal extracts (4%) two months after preparation, on the *Alternaria tenuis* fungus

Sample no.	Type of extract	Thermostating		The antifungal effect
		Temp.(ºC)	Time(days)	
1	Sage extract	30	4	-
2	Lavender extract	30	4	+
3	Hyssop extract	30	4	-
4	Fennel modified extract	30	4	-
5	Black mustard modified extract	30	4	-
M		30	4	+
1	Sage extract	30	7	-
2	Lavender extract	30	7	+
3	Hyssop extract	30	7	-
4	Fennel modified extract	30	7	-
5	Black mustard modified extract	30	7	-
M		30	7	+

In table 2. one can notice that the lavender extract (4%) does not have antifungal effect on *Alternaria tenuis* fungus.

Assessment of the antifungal effect of some vegetal extracts twelve months after preparation

a) Assessment of the antifungal effect of some vegetal extracts on the *Alternaria tenuis* fungus

The results obtained upon the assessment evaluation of the antifungal effect of some vegetal extracts twelve months after preparation, through the diffuzimetric method (dripping 20µl/godery) in Petri plates containing Czapek medium with agar on which the *Alternaria tenuis* fungus has incipiently developed are presented in (**Table 3**).

In table 3. one can notice that the horseradish, black mustard and fennel extracts have antifungal effect after 4 days, while for the solanum extract the effect appears later, after 11days. The best results on *Alternaria tenuis* fungus, for the dose of 20µl/godery, were obtained after 10 days with the horseradish and black mustard extracts (the diameter of the inhibition zone being of 30, respectively 40mm) and after11days with the solanum extract (the diameter of the inhibition zone being of 30mm). The garlic extract does not have antifungal effect, for the dose of 20µl/godery, on *Alternaria tenuis* fungus.

Table 3. Assessment of the antifungal effect of vegetal extracts (20µl/godery) twelve months after preparation, on the incipiently developed *Alternaria tenuis* fungus

Sample no.	Type of extract	Thermostating		The diameter of the inhibition zone (mm)
		Temp.(ºC)	Time(days)	
6	Horseradish extract	31	4	4
7	Garlic extract	31	4	0
8	Black mustard extract	31	4	6
9	Fennel extract	31	4	10
10	Solanum extract	31	4	0
M		31	4	0
6	Horseradish extract	31	10	30
7	Garlic extract	31	10	0
8	Black mustard extract	31	10	40
9	Fennel extract	31	11	20
10	Solanum extract	31	11	30
M		31	11	0

b) Assessment of the antifungal effect of some vegetal extracts on the *Alternaria porri* fungus

The antifungal effect of vegetal extract twelve months after preparation was tested through the diffuzimetric method (dripping 20, 50, 200 µl/godery) on Petri plates containing Czapek medium with agar on which the *Alternaria porri* fungus has incipiently developed. It has been observed that for this fungus species a higher quantity of vegetal extract is needed to produce the inhibition. The results obtained for the dose of 200 µl/godery are presented in (**Table 4**).

Table 4. Assessment of the antifungal effect of vegetal extracts (200µl/godery) twelve months after preparation, on the incipiently developed *Alternaria porri* fungus

Sample no.	Type of extract	Thermostating		The diameter of the inhibition zone (mm)
		Temp.(ºC)	Time(days)	
6	Horseradish extract	31	10	20
8	Black mustard extract	31	10	13
9	Fennel extract	31	10	0
10	Solanum extract	31	10	30
M		31	10	0

In table 4. one can notice that the horseradish, black mustard and solanum extracts have antifungal effect after 10 days. The best results on *Alternaria porri* fungus, for the dose of 200µl/godery, were obtained after 10 days with the horseradish and solanum extracts (the diameter of the inhibition zone being of 20, respectively 30mm). The fennel extract does not have antifungal effect for the dose of 200µl/godery on *Alternaria porri* fungus.

c) Assessment of the antifungal effect of some vegetal extracts on the *Fusarium oxysporum* fungus

The antifungal effect of vegetal extract twelve months after preparation has been tested through the diffuzimetric method (dripping 100, 200µl/godery) on Petri plates containing Czapek medium with agar on which the *Fusarium oxysporum* fungus has incipiently developed.

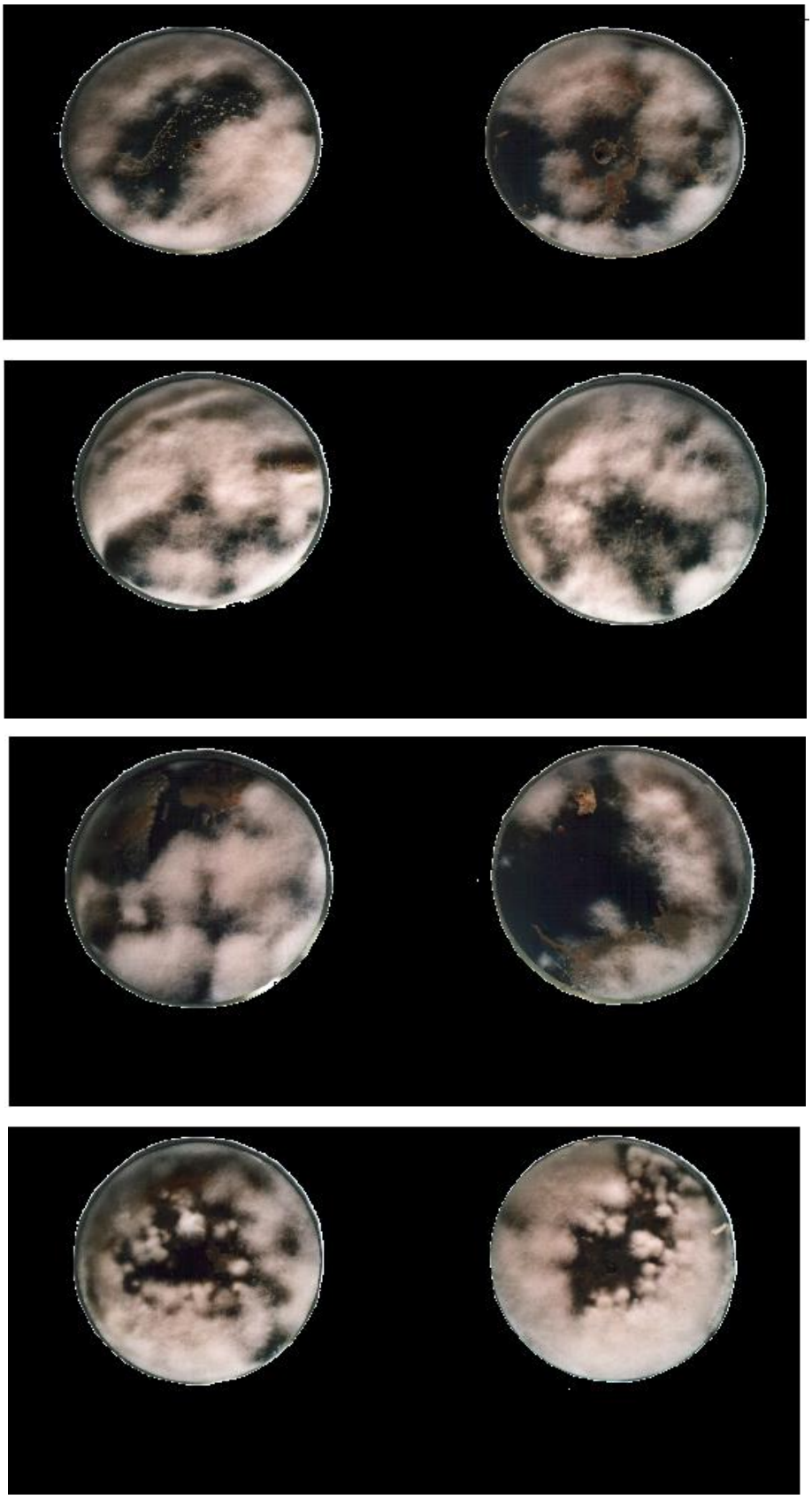
It has been observed that for this species of fungus the dose of 100µl/godery for the horseradish, fennel and solanum extracts is sufficient for the inhibition. The results obtained for the dose of 100 µl/godery, respectively 200µl/godery are presented in (**Table 5**) and (**Figure 1**).

Table 5. Assessment of the antifungal effect of vegetal extracts (100, 200µl/godery) twelve months after preparation, on the incipiently developed *Fusarium oxysporum* fungus.

Sample no.	Type of extract	Dose (µl/godery)	Thermostating		The diameter of the inhibition zone (mm)
			Temp.(ºC)	Time(days)	
6	Horseradish extract	100	31	7	20
		200	31	7	25
8	Black mustard extract	100	31	7	0
		200	31	7	15
9	Fennel extract	100	31	7	40
		200	31	7	50
10	Solanum extract	100	31	7	20
		200	31	7	10
M		-	31	7	0

In table 5. one can notice that the best results on *Fusarium oxysporum* fungus were obtained for the dose of 200µl/godery, after 7 days, especially with the fennel and the

solanum extracts (the diameter of the inhibition zone being of 50, respectively 30mm), but it is also significant the result obtained, for the dose of 100µl/godery, after 7 days, with the fennel extract (the diameter of the inhibition zone being of 40mm).



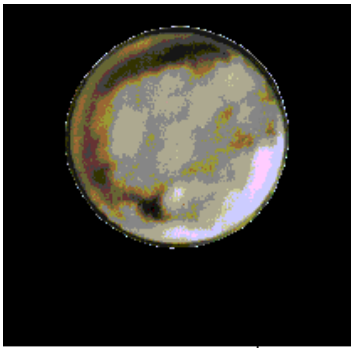


Figure 2. The antifungal effect of vegetal extracts (100, 200µl/godery) twelve months after preparation, on the incipiently developed *Fusarium oxysporum* fungus.

Assessment of the vegetal extracts as preventive treatment for the vegetable seeds, in the green house

The experiment was carried out on cucumber seeds treated before the inoculation in the green house. The procedure was the following: 20 seeds of cucumber have been treated for 24 hours with 2,5ml solution of vegetal extract 4% (2,4ml distillated water + 0,1ml vegetal extract). Subsequently 2 seeds/vegetable pot were planted, in the green house.

The samples were compared to 7 blanks (**M**) in which 2 untreated seeds/vegetable pot were inoculated. The coming up phase was observed for 3 weeks. The results obtained results are presented in (**Table 6**) and (**Figure 3**).

Table 6. Testing the vegetal extracts as preventive treatment of the cucumber seeds, in the green house

Sample no.	Type of extract	Days after the inoculation	Number of come up plants	Percentage of come up plants
6	Horseradish extract	7	1	5
		11	8	40
		14	14	70
8	Black mustard extract	7	2	10
		11	6	30
		14	10	50
9	Fennel extract	7	0	0
		11	13	65
		14	16	80
10.1	Solanum extract (prepared in 2002)	7	2	10
		11	6	30
		14	12	60
10.2	Solanum extract (prepared in 2003)	7	0	25
		11	13	65
		14	19	95
		7	2	10
		11	6	30
		14	8	40

In table 6. one can notice that the best result was obtained by treating the seeds with solanum extract (prepared in 2003). In this case the percentage of come up plants has doubled, comparatively with **M.** After 15 days from the inoculation the best percentage of come up plants was 95% for the cucumber seeds treated with the solanum extract comparatively with the untreated seeds to which the percentage of come up plants was 40%. The plants treated with fennel extract have also encounteres a high percentage, 80%.

Conclusions

These experiments have proven that vegetal extracts and especially the solanum and fennel extracts have a powerful antifungal effect on the tested fungal strains-*Alternaria tenuis*, *Alternaria porri*, *Fusarium oxysporum*. Moreover, the treatment of the cucumber seeds brought us to the conclusions that the vegetal extracts are applicable as antifungal in the vegetable cultures.

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