

SEQUENCES IN BIOLOGICAL PEST CONTROL OF PHYTOPATOGENIC AGENTS FOR APPLE TREES

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Abstract: The exclusion of organic synthesis chemical products from the organic agriculture involves the necessity to find other kinds of products for pests and diseases control but without diminution of the quality and quantity production.

The objective of our research was to test “*in vitro*” the influence of some water plant extracts on the apple scab conidia germination and to test the efficiency of some biological products made from *Trichoderma harzianum* and *Bacillus subtilis* on the control of rugosis cancer apple trees in the field.

The results showed that the majority of water plant extracts totally inhibited *Fusicladium dendriticum* conidia germination. About the rugosis cancer control made with biological products, the best results were obtained by using, in the same time, two studied species of micro-organisms: *Trichoderma harzianum* and *Bacillus subtilis*. It is known that the best products for rugosis cancer control are made from copper, but after the petals fall it is preferred to use some biological products because they do not produce phytotoxicity like the copper products.

Introduction

The biological pest control for the crop plants remains a base component of the ecological agriculture that gained larger proportions in our country. From the most aimed fruit growing species, we mention the apple tree, species in which we propose and by our experiments, to bring improvements in the control strategy of two mycoses of major importance: (*Venturia inaequalis* f.c., *Fusicladium dendriticum*) and the rugosis cancer of trees in apple tree (*Diaporthe pernicioso* f.c. *Phomopsis mali*).

Regarding the apple scab, a series of liquid plant extracts were *in vitro* tested for identifying conidia germination, and for the rugosis cancer, some biological products were tested in the field.

The experiments regarding the influence of some plant extracts on the germination of apple scab conidia were made by Gilliver (1987) in USA and by Minoiu, Maxim and Bobeș (1995) in Romania. Although some tests were done regarding the usage of plant extracts in combats, it must be taken into consideration the different strains of apple scab that exist in certain areas of our country and on the entire globe.

The chemical composition of the plants is also important depending on their provenience.

This *in vitro* stage control has to be followed by field tests on the extracts that establish their efficiency in natural conditions.

The rugosis cancer is one of the diseases with economical importance that contributes to the decline of apple tree plantations. The disease manifests on all aerial organs, on the branches and leaves like a soft and brown mould. In the advanced stages of the disease we can see the presence of a numerous withered multiyear branches that considerable diminish the fruit production and accelerate the decline of the trees [3].

Maxim (1998) revealed the negative effect of the rugosis cancer on the quantity and quality of the infected apple trees. So, the total medium production of fruits was with 21,1% lower on the infected trees compared to *Phomopsis mali* free trees. The production for I quality fruits was with 28,2% reduced on the infected trees compared to the healthy ones.

Materials and Methods

For the anti-fungal properties evaluation of some plant extracts were used some plant species from the spontaneous flora and cultivated plants: *Armoracia rusticana*, *Daucus carota*, *Urtica dioica*, *Primula officinalis*, *Juglans regia*, *Cannabis sativa*, *Equisetum arvense*.

Leaves were mashed and mixed up with water in 1:5 proportion. From the horse radish was used mashed rhizome. The mixture was left for 48 hours to macerate. Secondary infections on the apple scab leaves were harvested from the orchard, from the Golden delicious variety.

The culture media were made with Agar noble 2%, Bacto peptones 1%, saccharose 20% and were divided in Petri recipients. 5 ml from each liquid extract were added to the culture medium, left for 10 minutes and afterwards removed. On the medium there were made inoculations with *Fusicladium dendriticum* conidia. Observations were done after 24 and 72 hours with a light microscope.

For the control of apple tree rugosis cancer (*Phomopsis mali*) 5 variants were organized each year (2002-2003) on trees from Starkrimson variety with generalized infections of rugosis cancer. The sprays were done with the manual pump, from the beginning of the vegetative period until the beginning of June. In 2002, five treatments were done and in 2003 a number of 7 phytosanitary treatments were done. The microbiological product Trichodex 25 WP was used, based on *Trichoderma harzianum*, antagonist species for some fungi which proved to be hyperparasitical (Baicu, Șesan, 1996). For *Bacillus subtilis* variants were used 30 and 49 strains, obtained at the Research Institute for Plant Protection Bucharest.

The entire experiment was placed in a 21-year-old intensive orchard with grafted trees on the rootstock MM 106. Observations were done in the beginning of September by the noting system used in plant protection with 6 notation classes for the intensity of the attack.

Results and Discussions

The data show that the majority of the liquid plant extracts (*Armoracia rusticana*, *Daucus carota*, *Primula officinalis*, *Juglans regia*, *Cannabis sativa*, *Equisetum arvense*) had a maximum efficacy against the germination of *Fusicladium dendriticum* conidia for the apple trees. After 3 days from the spores inoculation, their germination was zero (Table 1).

In case of the stinging nettle (*Urtica dioica*) the proportion of the germinated conidia was 5-8% after 24 hours and after 3 days it overcame to 40-45%. We specify that the filaments of infection were short and very short in the presence of the macerate from this plant.

On the dried control culture (without water pellicle), the germination of the conidia wasn't unleashed after 24 hours, but at the second observation the germination process was somewhere between 15 and 20. On the wet control (with water pellicle) after three days, the germinated conidia were 60-65% and the predominant filaments were long. The use of biological products in the control of rugosis cancer in apple trees pointed out their relative low efficacy especially in the singular applied variants. For the Trichodex 25 WP 0.4% variant, the efficiency was between 55.6-56.9% and for *Bacillus subtilis* a little lower (47.2-48.1%). The mixture of Trichodex and *Bacillus subtilis* gave good results. This demonstrates that the two microorganisms (*T. harzianum* and *B. subtilis*) are not antagonists with each other and they can be successfully used together. In this mixture the efficiency oscillated around 70%, with a difference of 10-20% lower than Turdacupral 0.5%. The copper products are the classic fungicides used against phytopathogenic agents that produce cancers to the fruit trees. These can't be used frequently after blooming because they produce rugosis on the fruits that affects the quality aspect of them. In addition, the copper fungicides repeated usage after the falling of the petals can produce serious phytotoxicity phenomenon.

Although the copper products are allowed on the fungicide list destined to ecological agriculture, this alternative of using biological products is extremely benefic. So, on one side it is

possible to maintain the level under a certain attack rate and on the other side to obtain a higher quality of the fruits without rugosis (Table 2).

Table 1: Results regarding the influence of different plant water extracts upon germinated apple scab conidia on agarose medium

Variants	After 24 hours		After three days	
	% germinated conidia	Observations	% germinated conidia	Observations
<i>Armoracia rusticana</i>	0	-	0	-
<i>Daucus carota</i>	0	-	0	-
<i>Urtica dioica</i>	5-8	-	40-45	Short and very short filaments
<i>Primula officinalis</i>	0	-	0	-
<i>Juglans regia</i>	0	-	0	-
<i>Cannabis sativa</i>	0	-	0	-
<i>Equisetum arvense</i>	0	-	0	-
Dried control	0	-	15-20	-
Water control	55-56	-	60-65	15% middle filaments 20% long filaments 25-30% very long filaments

Table 2: Efficacy of some biological products against rugosis cancer of fruit apple trees (*Phomopsis mali*) in Bistrița during 2002-2003

Variants	2002					2003				
	F%	I%	DA%	E%	Observations	F%	I%	DA%	E%	Observations
Trichodex 25 WP – 0,4%	8,0	3,0	0,24	55,6	The data of treatments applied: 16.IV, 23.IV, 30.IV, 9.V, 6.VI The data of visual observations: 03.09	8,5	3,6	0,31	56,9	The data of treatments applied: 1.IV,8.IV,17.IV, 23.IV,05.V,20.V ,03.VI The data of visual observations: 29.08
Trichodex 25 WP – 0,4% + <i>Bacillus subtilis</i>	5,0	3,0	0,15	72,2		6,0	3,5	0,21	70,8	
<i>Bacillus subtilis</i>	8,5	3,3	0,28	48,1		9,3	4,0	0,38	47,2	
Turdacupral 50 – 0,5%	2,0	3,0	0,06	88,9		4,0	3,3	0,13	81,9	
Control	9,0	6,0	0,54	-		11,0	6,5	0,72	-	

Legend: F% = frequency of attack; I% = intensity of attack; DA% = degree of attack; E% = efficacy

Conclusions

1. The majority of the liquid extracts from the plants (*Armoracia rusticana*, *Daucus carota*, *Urtica dioica*, *Primula officinalis*, *Juglans regia*, *Cannabis sativa*, *Equisetum arvense*) totally inhibited “in vitro” germination of the conidia in case of apple tree (*Fusicladium dendriticum*).

2. The best results in the rugosis cancer control in the apple trees (*Diaporthe perniciosa* f.c. *Phomopsis mali*) were obtained by using, in the same time, the two studied species of micro-organisms: *Trichoderma harzianum* and *Bacillus subtilis*.

3. Although the efficiency of the cooper based fungicide in the rugosis cancer combat is superior to the biological products tested, the usage of the last mentioned products is preferred for avoiding the appearance of the rugosis on the fruits and the phytotoxicity phenomenon.

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SECVENȚE ALE COMBATERII BIOLOGICE A UNOR AGENȚI FITOPATOGENI LA MĂR

(Rezumat)

Excluderea produselor organice de sinteză din sistemele de agricultură ecologică impune găsirea de produse fitosanitare și tratamente care să respecte această cerință, pe de o parte, dar să permită obținerea de producții superioare calitativ și cantitativ, pe de altă parte.

Obiectivul cercetărilor noastre a constat în testarea “*in vitro*” a influenței unor extracte apoase de plante asupra germinării conidiilor de rapăn la măr (*Fusicladium dendriticum*) și testarea în livadă a eficacității unor produse biologice pe bază de *Trichoderma harzianum* și *Bacillus subtilis* în combaterea cancerului rugos al pomilor la măr (*Diaporthe perniciosă* f.c. *Phomopsis mali*).

Rezultatele obținute arată că majoritatea extractelor apoase de plante (hrean, morcov sălbatic, ciuboțica cucului, nuc, cânepă sălbatică, coada calului) au inhibat total germinarea conidiilor de *Fusicladium dendriticum*.

În combaterea cancerului rugos al pomilor la măr cu produse biologice, cele mai bune rezultate s-au obținut prin utilizarea concomitentă a două specii de microorganisme luate în studiu (*Trichoderma harzianum* și *Bacillus subtilis*). Deși cele mai eficiente produse împotriva cancerului rugos sunt cele pe bază de cupru, după căderea petalelor este de preferat utilizarea celor biologice, deoarece acestea nu produc rugozitate pe fructe și nici fenomene de fitotoxicitate ca și cele din prima categorie.